



Toxicological profile for Phenyl carbinol

This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.

1. Name of substance and physico-chemical properties

1.1. IUPAC systematic name

Phenylmethanol (PubChem)

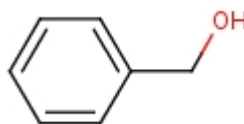
1.2. Synonyms

benzyl alcohol; phenylmethanol; benzenemethanol; phenylcarbinol; benzylalcohol; Benzoyl alcohol; Benzenecarbinol; Hydroxytoluene; Phenylmethyl alcohol; alpha-Toluenol; (Hydroxymethyl)benzene; Phenolcarbinol; Benzal alcohol; alpha-hydroxytoluene; benzylic alcohol; Alcool benzylique; Benzylicum; Methanol, phenyl-; Phenylcarbinolum; hydroxymethylbenzene; Phenyl-Methanol; BENZYL-ALCOHOL; Bentalol; Ulesfia; .alpha.-Hydroxytoluene; Aromatic alcohol; FEMA No. 2137; .alpha.-Toluenol; alcoholum benzylicum; benzenmethanol; Benzylalkohol; Aromatic primary alcohol; Alcohol,benzyl; Methanol benzene; Phenyl Methanol; E-1519; Benzalalcohol; Benzalcohol; Bentalol; EC 202-859-9; (PubChem)

1.3. Molecular formula

C₇H₈O or C₆H₅CH₂OH (PubChem)

1.4. Structural Formula



1.5. Molecular weight (g/mol)

108.14 (PubChem)

1.6. CAS registration number

100-51-6

1.7. Properties

1.7.1. Melting point

(°C): -15.2 (PubChem; -15.5 (EPISuite, 2017); -13 to -16 (ChemSpider)

1.7.2. Boiling point

(°C): 205.3 (PubChem ; EPISuite, 2017); 200-206 (ChemSpider)

1.7.3. Solubility

4.29E+004 mg/L at 25°C (EPISuite, 2017); 33-40 g/L (ChemSpider); 35,000 mg/L at 20°C (HSDB, 2018); 10-50 g/L at 70°F (21°C) (PubChem); 39 g/L at 20°C (GSBL, 2021); 40 g/l at 20°C (GESTIS)

1.7.4. pKa

15.4 (EPISuite, 2017; HSDB, 2018)

1.7.5. Flashpoint

(°C): 93-100 (closed cup), 105 (open cup) (HSDB, 2018); 94-100 (ChemSpider); 93 (Haz-Map, 2020); 101 (closed cup) (IGS, 2021)

1.7.6. Flammability limits (vol/vol%)

Explosion Limit in Air, lower: 1.3 Vol % (IGS, 2021); 1.22 vol.% (GESTIS); upper: 13 Vol.% (IGS, 2021)

1.7.7. (Auto)ignition temperature

(°C): 817 F (436°C) (HSDB, 2018); 435 (IGS, 2021)

1.7.8. Decomposition temperature

(°C): 870 (IGS, 2021)

1.7.9. Stability

Hygroscopic, stable under recommended storage conditions, slowly oxidized to benzaldehyde and benzoic acid on exposure to air; oxidizes slowly, therefore remains stable for long time (HSDB, 2018)

1.7.10. Vapor pressure

0.094 mm Hg at 25°C (EPISuite, 2017); 0.1 mmHg at 68°F (20°C) (PubChem); 0.07 hPa [0.052 mmHg] at 20°C (GESTIS)

1.7.11. log Kow

1.1 (ChemSpider; EPISuite, 2017); 0.593 (ChemSpider); 1.05, 1.43 and 2.83 (SKYE)

2. General information

2.1. Exposure

Probable Routes of Human Exposure:

According to the 2012 TSCA Inventory Update Reporting data, 25 reporting facilities estimate the number of persons reasonably likely to be exposed in their respective industrial manufacturing, processing, or use of benzyl alcohol, may be as low as <10 workers up to the range of 100-499 workers per plant; the data may be greatly underestimated due to confidential business information (CBI) or unknown values(1). [(1) US EPA; Chemical Data Reporting (CDR). Non-confidential 2012 Chemical Data Reporting information on chemical production and use in the United States. Available from, as of Sept 17, 2014: http://java.epa.gov/oppt_chemical_search/ **PEER REVIEWED**

Occupational exposure to benzyl alcohol may occur through inhalation and dermal contact with this compound at workplaces where benzyl alcohol is produced or used. Monitoring and use data indicate that the general population may be exposed to benzyl alcohol via dermal contact with consumer products containing benzyl alcohol and to a lesser extent via inhalation of ambient air, ingestion of food and drinking water(SRC).

Populations at Special Risk:

Premature neonates are at high risk of /benzyl alcohol/ toxicity. [Dart, R.C. (ed). Medical Toxicology. Third Edition, Lippincott Williams & Wilkins. Philadelphia, PA. 2004., p. 1231] **PEER REVIEWED**

As taken from HSDB, 2018

Occurrence in tobacco products:

In the burned part: yes

In tobacco naturally: yes (Stedman, 1968; Lloyd et al. 1976).

Other sources of exposure:

Cosmetics: yes (Cosmetic Bench Ref. 1996; Danish EPA, 2016)

Environment: yes (HSDB, 2018)

Food: yes (Burdock, 2010)

Pharmaceuticals: yes (Martindale, 1999)

Reported uses (ppm): (FEMA, 1994)

Food Category	Usual	Max.	Food Category	Usual	Max.
Alcoholic beverages	28.58	95.24	Gravies	12.00	24.00
Baked goods	236.10	401.80	Hard candy	283.80	357.60
Chewing gum	176.10	1254.00	Meat products	30.00	140.00
Frozen dairy	103.10	244.60	Nonalcoholic beverages	15.13	47.93
Gelatins, puddings	52.51	175.70	Soft candy	105.10	171.20

Reported individual intake from use as a flavouring: 0.2005 mg/kg bw/day.

As taken from Burdock, 2010.

The intake of benzyl alcohol in the EU is estimated to be 13 mg/person/day (EFSA, 2011) or 16 mg/day (WHO Food Additives Series 48), and the intake in the US is estimated to be 17 mg/person/day (WHO Food Additives Series 48).

Benzyl alcohol (CAS RN 100-51-6) is listed as an ingredient (at given concentrations, where specified) in hobby/craft (3-5%), auto (<0.004-10%), commercial/institutional (1-30%), home maintenance (0.1-40%, includes 'old' products), inside the home (0.1-40%), personal care (0-30%, includes 'old' products) and pet care products (<1-70.66%) by the CPID.

Listed as a material used in fragrance compounds by IFRA, the US EPA InertFinder Database (2023) and the Danish EPA (2016).

Used as a perfuming, preservative, solvent and viscosity controlling ingredient in cosmetics in the EU. As taken from CosIng (Cosmetic substances and ingredients database).

"The aim was to describe the exposure to excipients among neonates hospitalised in the neonatal intensive care unit (NICU) of a public hospital in Brasilia, Brazil. This was a retrospective study based on medicines that were prescribed electronically to neonates (≤ 28 days) who were admitted to the NICU of a hospital in Brasilia between January 1 and March 31, 2012. Excipients were identified from the medicine package leaflets and were classified according to toxicity. Seventy-nine infants received a total of 1,303 prescriptions comprising 77 formulations and 70 active drugs. Eighty-six excipients were identified, of which, 9 were harmful excipients (HE) and 48 were potentially harmful excipients (PHE). Almost all the neonates (98.7 %) were exposed to at least one HE and PHE. Preterm neonates ($n = 64$; 1,502 neonate days) presented high risk of exposure to polysorbate 80 (3.26/100 neonate days), sodium hydroxide (3.39), PG (3.19) and propylparaben (3.06). Full-term neonates ($n = 15$; 289 neonate days) presented risks in relation to phenol (4.84), ethanol (3.8) and sodium citrate (3.46). CONCLUSION: Neonates in NICUs in Brazil are exposed to a wide variety of HE and PHE with unpredictable results. Safer alternatives are needed, as well as further studies on the subject." As taken from Souza A et al. 2014. Eur. J. Pediatr. 173, 935-45. PubMed, 2016

"The chemical has reported domestic uses including in:

- adhesives, binding agents;
- bleaching agents;
- cleaning/washing agents;
- colouring agents;
- corrosion inhibitors;
- fillers;
- odour agents;
- surface treatments;
- surface-active agents; and
- paints, lacquers and varnishes

The chemical has reported commercial uses including:

- in absorbents and adsorbents;
- in anti-freezing and anti-static agents;
- in construction and impregnation materials;
- as a fixing agent;
- in lubricants and additives;
- as a solvent and viscosity adjustor;
- in welding and soldering agents;

-in reprographic agents; and

-in photo chemicals and process regulators.

The chemical has reported site-limited uses including as intermediates.

The following non-industrial uses have been identified internationally:

-in food/feedstuff flavourings and nutrients;

-in non-agricultural pesticides and preservatives;-in pharmaceuticals.”

As taken from AICIS, 2016.

Occupational Exposure Limits:

OEL-FINLAND: TWA 10 ppm (45 mg/m³), NOV2011

OEL-RUSSIA: STEL 5 mg/m³, Skin, JUN2003

As taken from RTECS, 2019.

“Benzyl alcohol is used as both an excipient (preservative, solubilising agent) and as an active principal (antiseptic, local anaesthetic).

Benzyl alcohol is used for different purposes:

- preservative (2% in oral or parenteral pharmaceutical preparations, 3% in cosmetics)
- solubilising agent (concentration greater than or equal to 5%)
- disinfectant (10% solutions)
- local anaesthetic (in certain injections, cough remedies, ophthalmic solutions, ointments, dermatological aerosols)

The most common routes of administration are parenteral and to a lesser extend topical.”

As taken from EMA, 2017.

“The dietary exposure to benzyl alcohol (E 1519) from its use as a food additive was calculated based on (1) maximum permitted levels (MPLs) set out in the EU legislation (defined as the regulatory maximum level exposure assessment scenario) and (2) the reported use levels (defined as the refined exposure assessment scenario).

The mean and high exposure estimates in the refined exposure scenarios were maximally 0.27 and 0.81 mg/kg bw per day in toddlers, respectively. Considering the food categories for which the presence of benzyl alcohol (E 1519) can be due to carry over via its authorisation according to Annex III to Reg (EC) No 1333/2008, the exposure estimates to benzyl alcohol (E 1519) were below the ADI of 4 mg/kg bw per day in all population groups. The Panel noted that also the exposure in the regulatory maximum level exposure assessment scenario is below the ADI in all population groups.

In conclusion, based on the toxicological database available, the Panel established an ADI of 4 mg/kg bw per day for benzyl alcohol (E 1519) and concluded that the exposure to benzyl alcohol (E 1519) does not raise a safety concern at the reported uses and use levels.”

As taken from EFSA, 2019

“Used as a preservative, epoxy resin catalyst, and in the photographic developer C-22; [Kanerva, p. 1757] Used as a preservative, solvent, and local anesthetic; [Marks, p. 136] Used as a preservative in drugs for injection; [Kanerva, p. 462-3] Used in flavors and perfumes and as a solvent for inks and dyes; [AIHA]”

Benzyl alcohol (CAS RN 100-51-6) is used as a flavour enhancer, fragrance ingredient, preservative antimicrobial and solvent in non-medicinal natural health products. It has an upper limit toxicity restriction of 5 mg/kg bw/day based on the JECFA ADI, which is expressed as benzoic acid equivalents, and is permitted, when used topically, at up to 10 and 3% in rinse-off and leave-on products, respectively. In general, it must be declared as a medicinal ingredient in throat lozenges when used in daily doses at or above 100 mg/day and in anorectal products in concentrations at or above 1% w/w (Health Canada, 2021).

“The main route of exposure for benzyl alcohol (B) is via the respiratory tract and the skin.”

As taken from GESTIS

“Permitted daily exposure (PDE) values are used by some toxicologists to support the safety qualification of various types of impurities found in a drug substance (DS) or drug product (DP). Permitted daily exposure values are important tools for the toxicologist, but one must be aware of their limitations to ensure that they are used appropriately and effectively in the risk assessment process. First, a toxicologist must always perform a comprehensive analysis of all available animal and human safety data for an impurity, including identifying any data gaps that may exist. Second, if adequate data are available and there are no genotoxicity concerns, an appropriate well-designed repeat-dose toxicity study in animals should be chosen to calculate the PDE. It is important to note that PDE values qualify general systemic toxicity and not necessarily local toleration end points such as irritation and sensitization that are more concentration than dose dependent. In addition, a PDE value calculated from a general toxicity study in animals may not necessarily qualify for reproductive toxicology end points. Lastly, PDE values should never be thought of as analytical limits for or acceptable levels of an impurity in a DS or DP, as this ignores quality considerations. Using safety information from several chemicals as proxy impurities, this article serves as an educational primer to facilitate a better understanding of the development and use of PDE values in the risk assessment process.” As taken from Ball DJ and Beierschmitt WP. 2020. *Int. J. Toxicol.* 39(6), 577-585. PubMed, 2021

“Contact dermatitis linked to cosmetic products is a very common reason for visits to the dermatologist, and in more than half the cases, it is due to an allergic reaction. Fragrances are most often the culprit. The aim of the study was to describe the common fragrance allergens in different categories of cosmetic products available on the European market. We wanted to assess the influence of cosmetic type and distribution channel on the presence of fragrance allergens. There are the allergens whose concentration exceeds 0.001% in leave-on products and 0.01% in rinse-off products. A total of 2044 commercial hygiene, care, and makeup cosmetic products were analyzed to specifically study regulated fragrance allergens. The influence that the product category and its distribution channel (retail stores and specialized stores such as beauty institutes or hairdressers and pharmacy) have on the prevalence of these allergens was evaluated. The Kruskal-Wallis test has been used for statistical data analysis. There is a wide range of fragrance allergens, the most common being limonene (found in about 30% of products tested), linalool (just over a quarter of the products tested), and benzyl alcohol (approximately 16% of the products tested). The average number of allergens found and their nature varies depending on the type of product in question (maximum number for shampoos and oral care with about 70 allergens and minimum number for nail polish and makeup for eyes with fewer than 10 allergens). In the area of hygiene, deodorants and oral hygiene products are particularly noteworthy, the former for their significantly high number of allergens and the latter for their low number. There is also a significant difference between the number of allergens found in eye makeup and foundations. Our results

indicate that the number of regulated fragrance allergens is particularly influenced by the type of products.” As taken from Couteau C et al. 2020. Clin. Rev. Allergy Immunol. 59(1), 101-108. PubMed, 2021

USES OF BENZYL ALCOHOL

Manufacture	Manufacture of benzyl alcohol (PROC 1, 2, 3, 4, 5, 6, 8a, 8b, 9)
Formulation	Formulation (PROC 1, 2, 3, 4, 5, 8a, 8b, 9, 19) Formulation (PROC 1, 2, 3, 4, 5, 8a, 8b, 9, 13, 15, 19) Formulation in materials – industrial (PROC 1, 2, 3, 4, 5, 8a, 8b, 9, 13) Formulation of preparations – industrial (PROC 1, 2, 3, 4, 5, 8a, 8b, 9, 13) Formulation of preparations – professional (PROC 1, 2, 3, 4, 5, 8a, 8b, 9, 13, 19)
Uses at industrial sites	Paper/board dye, finishing/impregnation (PROC 5, 6, 7, 8b, 10, 13, 14) Building & construction/distributors (PROC 5, 7, 8a, 8b, 9, 10, 13, 14, 15, 19) Building & construction/distributors (PROC 5, 8a, 8b, 9, 10, 13, 14) Polymer preparations - industrial use (PROC 13) Non-metal surface treatment products - industrial use (PROC 5, 8a, 8b, 9, 15) Photo-chemicals (PROC 8a, 8b, 13) Photo-chemicals (PROC 8a, 8b) Coatings, paints, fillers, putties thinners - industrial use (PROC 5, 7, 8a, 8b, 9, 10, 13) Metal surface treatment products (PROC 5, 8a, 8b, 9, 15, 23, 24, 25) Washing & cleaning products - industrial use (PROC 7, 8a, 8b, 9, 10, 13) Intermediates (PROC 1, 2, 3) Intermediates (PROC 1, 2, 3, 8b, 9) Adhesives & sealants - industrial use (PROC 5, 7, 8a, 8b, 9, 10, 12, 13, 14) Cosmetics & personal care products (PROC 13) Adhesives & sealants, coatings and paints, thinners, paint removers, fillers, putties , plasters, modelling clay, finger paints, metal surface and non-metal surface treatment products, ink and toners (PROC 5, 7, 8a, 8b, 9, 10, 12, 13, 14, 23, 24, 25) Adhesives & sealants (PROC 5, 7, 8a, 8b, 9, 10, 12, 13, 14) Ink & toners (PROC 7, 8a, 8b, 9, 10, 13) Textile dyes, finishing/impregnation products (PROC 5, 6, 7, 8a, 8b, 9, 10, 13, 14) Laboratory reagent (PROC 15) Lubricants, greases, release products (PROC 18) Building and construction/distributors (PROC 5, 7, 8a, 8b, 14, 15, 19)
Uses by professional workers	Building & construction/distributors (PROC 5, 8a, 8b, 9, 10, 11, 13, 19) Non-metal surface treatment products (PROC 8a, 8b, 9, 10, 11, 13, 19) Metal surface treatment products (PROC 8a, 8b, 9, 10, 11, 13, 19, 23, 24, 25) Photo-chemicals (PROC 8a, 8b) Coatings, paints, fillers, putties thinners (PROC 5, 8a, 8b, 9, 10, 11, 13, 19) Polymer preparations (PROC 8a, 8b, 9, 10, 11) Ink & toners (PROC 5, 8a, 8b, 10, 11, 13, 19) Adhesives & sealants (PROC 5, 8a, 8b, 9, 10, 11, 13, 19) Paper/board, dye finishing/impregnation (PROC 5, 6, 8a, 8b, 11, 13, 14, 19, 21) Cosmetics & personal care products (PROC 13) Laboratory reagent (PROC 15) Laboratory agent (PROC 8a, 8b, 15) Polishes & wax blends (PROC 8b, 10, 11) Professional Use - indoors - all uses (PROC 5, 6, 8a, 8b, 9, 10, 11, 13, 14, 19, 21, 23, 24, 25) Professional Use - outdoor - all uses (PROC 5, 6, 8a, 8b, 9, 10, 11, 13, 14, 19, 21, 23, 24, 25) Washing & cleaning products (PROC 8a, 8b, 9, 10, 11, 13, 19)
Consumer Uses	PC 1: Adhesives, sealants PC 3: Air care products PC 9a: Coatings and paints, thinners, paint removes PC 9b: Fillers, putties, plasters, modelling clay PC 9c: Finger paints PC 12: Fertilisers

	PC 14: Metal surface treatment products PC 18: Ink and toners PC 19: Intermediate (e.g. Use in cosmetics and personal care products) PC 20: Products such as pH-regulators, flocculants, precipitants, neutralisation agents PC 21: Laboratory chemicals PC 23: Leather tanning, dye, finishing, impregnation and care products PC 24: Lubricants, greases, release products PC 28: Perfumes, fragrances PC 31: Polishes and wax blends PC 32: Polymer preparations and compounds PC 34: Textile dyes, finishing and impregnating products; including bleaches and other processing aids PC 35: Washing and cleaning products (including solvent based products) PC 39: Cosmetics, personal care products PC 0: Other: Building & construction PC 0: Other: Tobacco products PC 0: Other: Tobacco Products and Liquids for Electronic Cigarettes PC 0: Other: Electronic cigarette PC 0: Other: Buildings & construction; distributors PC 0: Other: Building & construction
Article service life	AC 8: Paper articles (consumers) AC 13: Plastic articles AC 5: Fabrics, textiles and apparel (workers) AC 6: Leather articles (workers)

“The German Federal Office of Consumer Protection and Food Safety analysed benzyl alcohol in 4213 consumer products as part of the screening programs between 2000 and 2016. Consumer products in these screening programs belong to consumer goods with body contact and personal care products, household chemicals, cosmetics, packing materials and toys (incl. modelling clay). Benzyl alcohol was detected in cosmetic products and articles, air care products, washing and cleaning products, textile processing aids, and modelling clay. Highest concentrations were detected in cosmetic products. The maximum value measured in other typical consumer products is 0.305% (in an all-purpose cleaner), whereas modelling clay contains 0.717% benzyl alcohol at maximum. These concentrations are covered by the ESs in the CSRs. Modelling clay has also to be considered as a toy product. The database does not cover consumer products entirely and products with higher concentrations are considered possible.

GIFAS (Giftinformations- und Archivierungssystem) provides data on 1536 consumer products belonging to product categories 1, 3, 9a, 34, and 35. Maximum concentration of benzyl alcohol are 55% (PC 1), 27.5% (PC 3), 70% (PC 9a; 100% in paint remover), and up to 100% (PC 34/35; 20% in all-purpose cleaner). These concentrations are not covered by the CSRs. They were not considered in the exposure assessment of the eMSCA but will be deliberated in the subsequent RMOA.”

As taken from BAuA, 2021

“Used as a solvent, preservative, and fragrance ingredient.”

“Professional workers: Adhesives, sealants; Air care products; Biocidal products (e.g. disinfectants, pest control); Coatings, paints, thinners, paint removers; Fillers, putties, plasters, modelling clay; Metal surface treatment products; Non-metal-surface treatment products; Ink and toners; Products such as pH-regulators, flocculants, precipitants, neutralisation agents; Laboratory chemicals; Leather treatment products; Lubricant, greases, release products; Paper and board treatment products; Plant protection products; Perfumes, fragrances; Pharmaceuticals; Photo-chemicals; Polishes and wax blends; Polymer preparations and compounds; Textile dyes and impregnating products; Washing and cleaning products; Cosmetics, personal care products

Consumers: Adhesives, sealants; Air care products; Coatings, paints, thinners, paint removers; Fillers, putties, plasters, modelling clay; Ink and toners; Leather treatment products; Perfumes and fragrances; Polishes and wax blends; Textile dyes and impregnating products; Washing and cleaning products; Cosmetics and personal care products”

“Benzyl alcohol is largely available to consumers for day-by-day use. When used in cosmetic products it may be percutaneously absorbed over more or less the entire body and/or on smaller localised skin areas. Exposure could also occur through eye contact.

As taken from BAuA, 2020

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%
benzyl alcohol	5780379404	99.47	5801115433	99.48
Total ion chromatogram	5811178651	100.00	5831438915	100.00

This ingredient was investigated in a pyrolysis study. This ingredient was investigated in a pyrolysis study. Results are given in Baker and Bishop (2004) J. Anal. Appl. Pyrolysis, 71, pp. 223-311.

Ingredients CAS Number Formulae or structure	Chemical Class	Mol. Wt. (M) Bp or Mp (°C)	Max cig Appln. Level (ppm)	Purity of sample Pyrolysed (%)	Composition of pyrolysate (Compound %)	Max level in smoke (µg)
Benzyl alcohol CAS 100-51- 6	Aryl alcohol	M=108 bp 206	1,700	99	Benzyl alcohol 95.2 Benzaldehyde 4.8	800 40

In a pyrolysis study, 94.2% of benzyl alcohol added to cigarettes was transferred intact to the smoke (Purkis et al. 2011).

2.3. Ingredient(s) from which it originates

Natural Occurring Sources:

Benzyl alcohol occurs primarily in flower oils and tree exudates(1). Several plants containing benzyl alcohol include tea, hyacinth, daffodil, jasmine, rosemary, tangerine, peppermint, and blueberry(2).

Benzyl alcohol is a constituent of jasmine, hyacinth, ylang-ylang oils, and at least 2 dozen additional essential oils(1,3). It is an important part of the odor of gardenia, some rose varieties, narcissus and peony, as well as castoreum, balsams of perus and tolu, and propolis(1). [(1) Mookerjee BD, Wilson RA; Benzyl Alcohol and beta-Phenethyl Alcohol. Kirk-Othmer Encyclopedia of Chemical Technology. (1999-2014). New York, NY: John Wiley & Sons. Online Posting Date: 4 Dec 2000 (2) USDA; Dr. Duke's Phytochemical and Ethnobotanical Databases. Plants with a chosen chemical. Benzyl alcohol. Washington, DC: US Dept Agric, Agric Res Service. Available from, as of Sept 15, 2014: <http://www.ars.grin.gov/duke/> (3) Lewis RJ Sr, ed; Sax's Dangerous Properties of Industrial Materials. 11th ed. Hoboken, NJ: Wiley-Interscience, Wiley & Sons, Inc.V2: 398 (2004)] **PEER REVIEWED**

Food Survey Values:

Benzyl alcohol has been identified as a volatile flavor component of baked potatoes(1), Beaufort (Gruyere) cheese(2), bacon(3), and roasted filberts (nuts)(4). It has been identified as a volatile component of blended nectarines, but not in a headspace analysis of the intact fruit(5). Benzyl alcohol was detected in different forms of cooked sweet corn at concentrations of less than 1 ppb to 8 ppb(6). Benzyl alcohol was identified, not quantified, in the edible portion of Korean chamchwi plants(7). [(1) Coleman EC et al; J Agric Food Chem 29: 42-8 (1981) (2) Dumont JP, Adda J; J Agric Food Chem 26: 364-7 (1978) (3) Ho CT et al; J Agric Food Chem 31: 336-42 (1983) (4) Kinlin TE et al; J Agr Food Chem 20: 1021-8 (1972) (5) Takeoka GR et al; J Agric Food Chem 36: 553-60 (1988) (6) Buttery RG et al; J Agric Food Chem 42: 791-95 (1994) (7) Chung TY et al; J Agric Food Chem 41: 1693-97 (1993)] **PEER REVIEWED**

The concentrations in three commercial fermented soybean (Glycine max) available in Hong Kong markets were 107.6, 138., and 211.7 ug/kg(1). Benzyl alcohol was detected in Australian honey at concentrations of and 0.1-0.6 mg/kg from sources of yellow box (Eucalyptus melliodora); it was not detected in honey based on Australian blue gum (Eucalyptus leucoxylon)(2). The compound is a volatile of ground musty sorghum(3). Benzyl alcohol was identified in Korean red pine (Pinus densiflora) pine sprout tea and pine needle tea(4). [(1) Chung HY; J Agric Food Chem 47: 2690-6 (1999) (3) Darcy BR et al; J Agric Food Chem 45: 1834-43 (1997) (4) Seitz LM et al; J Agric Food Chem 47: 1051-61 (1999) (4) Kim KJ, Chung HJ; J Agric Food Chem 48: 1269-72 (2000)] **PEER REVIEWED**

Fish/Seafood Concentrations:

Benzyl alcohol was detected in salt-fermented fish and shrimp pastes at concentrations of 352-1,230 ng/g(1). Benzyl alcohol was identified, not quantified, in the volatile emissions of cooked clams(2). Benzyl alcohol was found to be a volatile component of Southeast Asian crab meat (Charybdis feriatus) at concentrations of 4.4, 3.8 and 71.7 ug/kg from leg, body and carapace, respectively(3). [(1) Cha YJ, Cadwallar KR; J Food Sci 60: 19-24 (1995) (2) Kubota K et al; J Agric food Chem 39: 1127-30 (1991) (3) Chung HY; J Agric Food Chem 47: 2280-7 (1999)] **PEER REVIEWED**

Plant Concentrations:

Benzyl alcohol was quantitatively detected in the skin and pulp of Queen Anne's pocket melon (Cucumis melo L) at 164.5 and 25.5 ug/kg, respectively(1). [(1) Aubert C, Pitrat M; J Agric Food Chem 54: 8177-82 (2006)] **PEER REVIEWED**

Benzyl alcohol plant concentrations(1).

Genus species	Family	Common name	Part	Quantity (ppm)
Camellia sinensis	Theaceae	Tea	Shoot	1,400

Hyacinthus orientalis	Liliaceae	Hyacinth	Flower	920
Narcissus tazetta	Liliaceae	Daffodil	Flower	360
Jasminum officinale	Oleaceae	Jasmine	Flower	228
Camellia sinensis	Theaceae	Tea	Leaf	160
Hyssopus officinalis	Lamiaceae	Hyssop	Flower	32
Rosmarinus officinalis	Lamiaceae	Rosemary	Plant	32
Hyssopus officinalis	Lamiaceae	Hyssop	Leaf	30
Levisticum officinale	Apiaceae	Lovage	Root	30
Citrus reticulata	Rutaceae	Mandarin	Fruit	2
Origanum minutiflorum	Lamiaceae	Small-flowered Oregano	Shoot	0-2
Mentha x piperita subsp nothosubsp piperita	Lamiaceae	Peppermint	Leaf	
Vaccinium corymbosum	Ericaceae	Blueberry	fruit juice	

[(1) USDA; Dr. Duke's Phytochemical and Ethnobotanical Databases. Plants with a chosen chemical. Benzyl alcohol. Washington, DC: US Dept Agric, Agric Res Service. Available from, as of Sept 15, 2014: <http://www.arsgrin.gov/duke/> **PEER REVIEWED**As taken from HSDB, 2018

The free alcohol is often present in several essential oils and extracts of jasmine, tobacco, tea, neroli, copaiba, Acacia farnesiana Willd., Acacia cavenia Hook. and Arn., Robina pseudacacia, ylang-ylang, Pandanus odoratissimus, Michelia champaca, Prunus laurocerasus, tuberose, orris, castoreum, violet leaves, clove buds and others. Also found in fresh apple, apricot, mandarin peel oil, high bush blueberry, raspberry, strawberry fruit, American cranberry and cooked asparagus.

As taken from Burdock, 2010.

Natural occurrence:

Food	mg/kg
Apple juice	0.002
Other fruits	up to 5.00
Mushrooms (fresh)	0.02-3.7
Asparagus	0.4

Tea (green)	1-30
Tea (black)	1-15
Alcohol	Up to 0.8

As taken from CoE, 2000

3. Status in legislation and other official guidance

Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives BENZYL ALCOHOL	
COE No.:	58
FEMA No.:	2137
JECFA No.:	25
Chemical names:	BENZYL ALCOHOL
Synonyms:	PHENYL CARBINOL; PHENYLMETHANOL; alpha-HYDROXYTOLUENE
Functional class:	FLAVOURING AGENT; CARRIER SOLVENT
Latest evaluation:	2001
ADI:	0-5 mg/kg bw (1996)
Comments:	No safety concern at current levels of intake when used as a flavouring agent. The 1996 group ADI of 0-5 mg/kg bw for benzoic acid, the benzoate salts (calcium, potassium and sodium), benzaldehyde, benzyl acetate, benzyl alcohol and benzyl benzoate, expressed as benzoic acid equivalents, was maintained at the fifty-seventh meeting (2001).
Report:	TRS 909-JECFA 57/73
Specifications:	COMPENDIUM ADDENDUM 12/FNP 52 Add. 12/67 (METALS LIMITS) (2004)
Tox monograph:	FAS 48-JECFA 57/227
Previous status:	2001, COMPENDIUM ADDENDUM 9/FNP 52 Add.9/110. R 1996, TRS 868-JECFA 46/41, COMPENDIUM ADDENDUM 4/FNP 52 Add.4/21 (SOLVENT); 175 (FLAVOUR), FAS 37-JECFA 46/29. 0-5 mg/kg bw (GROUP ADI FOR BENZOIC ACID, THE BENZOATE SALTS (CALCIUM, POTASSIUM AND SODIUM), BENZALDEHYDE, BENZYL ACETATE, BENZYL ALCOHOL AND BENZYL BENZOATE, EXPRESSED AS BENZOIC ACID). FU. R 1979, TRS 648-JECFA 23/15, FNP 12-JECFA 23/11 (COMPENDIUM/193). NOT PREPARED. 0-5 mg/kg bw (THIS GROUP ADI FOR BENZYL ALCOHOL AND BENZYL BENZOATE SHOULD APPLY TO THE BENZYL/BENZOIC MOIETY RELATED TO BENZOIC ACID REPRESENTING TOTAL BENZOATE FROM ALL FOOD ADDITIVE SOURCES). FU. N
Intake:	See BENZOATES

As taken from JECFA, 2005. Evaluation of Benzyl Alcohol

Other Standards Regulations and Guidelines:

Workplace Environmental Exposure Level (WEEL): 8-hr Time-weighted Average (TWA) 10 ppm. 2013 Emergency Response Planning Guidelines (ERPG) & Workplace Exposure Level (WEEL). American Industrial Hygiene Association, Falls Church, VA 2013, p. 43

FIFRA Requirements:

As the federal pesticide law FIFRA directs, EPA is conducting a comprehensive review of older pesticides to consider their health and environmental effects and make decisions about their future use. Under this pesticide reregistration program, EPA examines health and safety data for pesticide active ingredients initially registered before November 1, 1984, and determines whether they are eligible for reregistration. In addition, all pesticides must meet the new safety standard of the Food Quality Protection Act of 1996. Pesticides for which EPA had not issued Registration Standards prior to the effective date of FIFRA '88 were divided into three lists based upon their potential for human exposure and other factors, with List B containing pesticides of greater concern and List D pesticides of less concern. Benzyl alcohol is found on List D. Case No: 4013; Pesticide type: insecticide, fungicide; Case Status: OPP is reviewing data from the pesticide's producers regarding its human health and/or environmental effects, or OPP is determining the pesticide's eligibility for reregistration and developing the RED document.; Active ingredient (AI): benzyl alcohol; AI Status: The active ingredient is no longer contained in any registered products ... "cancelled." United States Environmental Protection Agency/ Prevention, Pesticides and Toxic Substances; Status of Pesticides in Registration, Reregistration, and Special Review. (1998) EPA 738-R-98-002, p. 297

Atmospheric Standards:

This action promulgates standards of performance for equipment leaks of Volatile Organic Compounds (VOC) in the Synthetic Organic Chemical Manufacturing Industry (SOCMI). The intended effect of these standards is to require all newly constructed, modified, and reconstructed SOCMI process units to use the best demonstrated system of continuous emission reduction for equipment leaks of VOC, considering costs, non air quality health and environmental impact and energy requirements. Benzyl alcohol is produced, as an intermediate or a final product, by process units covered under this subpart. 40 CFR 60.489 (USEPA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of October 22, 2014: <http://www.ecfr.gov>

State Drinking Water Guidelines:

(FL) FLORIDA 2,100 ug/L USEPA/Office of Water; Federal-State Toxicology and Risk Analysis Committee (FSTRAC). Summary of State and Federal Drinking Water Standards and Guidelines (11/93) To Present

FDA Requirements:

Diluents in color additive mixtures for drug use exempt from certification. The following diluents may be safely used in color additive mixtures that are exempt from certification and which are to be used for coloring drugs, subject to the condition that each straight color in the mixture has been exempted from certification or, if not so exempted, is from a batch that has previously been certified and has not changed in composition since certification. Such listing of diluents is not to be construed as superseding any of the other requirements of the Federal Food, Drug, and Cosmetic Act with respect to drugs, including new drugs. If a definition and specification for a particular diluent is not set forth in this subpart, the material shall be of a purity consistent with its intended use. ... Externally applied drugs: Benzyl alcohol is included on this list. 21 CFR 73.1001(b) (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of October 22, 2014: <http://www.ecfr.gov>

Benzyl alcohol is a food additive permitted for direct addition to food for human consumption as a synthetic flavoring substance and adjuvant in accordance with the following conditions: a) they are used in the minimum quantity required to produce their intended effect, and otherwise in

accordance with all the principles of good manufacturing practice, and 2) they consist of one or more of the following, used alone or in combination with flavoring substances and adjuvants generally recognized as safe in food, prior-sanctioned for such use, or regulated by an appropriate section in this part. 21 CFR 172.515 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of October 22, 2014: <http://www.ecfr.gov>

Benzyl alcohol is an indirect food additive for use only as a component of adhesives. 21 CFR 175.105 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of June 26, 2008: <http://www.gpoaccess.gov/ecfr>

Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses. A number of active ingredients have been present in OTC drug products for various uses, as described below. However, based on evidence currently available, there are inadequate data to establish general recognition of the safety and effectiveness of these ingredients for the specified uses: benzyl alcohol is included in male genital desensitizer drug products. 21 CFR 310.545 (a)(10)(iii) (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of October 22, 2014: <http://www.ecfr.gov>

Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses. A number of active ingredients have been present in OTC drug products for various uses, as described below. However, based on evidence currently available, there are inadequate data to establish general recognition of the safety and effectiveness of these ingredients for the specified uses: benzyl alcohol is included in poison ivy, poison oak, and poison sumac drug products. 21 CFR 310.545 (a)(18)(vi) (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of October 22, 2014: <http://www.ecfr.gov>

Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses. A number of active ingredients have been present in OTC drug products for various uses, as described below. However, based on evidence currently available, there are inadequate data to establish general recognition of the safety and effectiveness of these ingredients for the specified uses: benzyl alcohol is included in pediculicide drug products. 21 CFR 310.545 (a)(25)(i) (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of October 22, 2014: <http://www.ecfr.gov>

Local anesthetic active ingredients. The active ingredient of the product consists of any of the following when used in the concentration or within the concentration range established for each ingredient: Benzyl alcohol 1 to 4 percent is included on this list. 21 CFR 346.10(b) (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of October 22, 2014: <http://www.ecfr.gov>

As taken from HSDB, 2018

At a meeting of the Expert Panel of the Cosmetics Ingredients Review, it was concluded that benzyl alcohol was safe in the present practices of use and concentration described in their safety assessment (CIR, 2011).

Category A - Permitted for use in food – no upper limits given (CoE, 2000)

There are REACH dossiers on benzyl alcohol (ECHA).

Benzyl alcohol is included on the FDA's inventory of "Substances Added to Food (formerly EAFUS)" as a flavoring agent or adjuvant, and solvent or vehicle, and FDA's Inventory of Food Contact Substances Listed in 21 CFR and is included under 21 CFR sections 172.515 (Synthetic flavoring substances and adjuvants), 175.105 (Adhesives), 175.300 (Resinous and polymeric coatings), 177.1210 (Closures with sealing gaskets for food containers) and 73.1001 (Diluents in color additive mixtures for drug use exempt from certification) (FDA, 2022,2023a,b).

Listed in the US EPA InertFinder Database (2023) as approved for food, non-food and fragrance use pesticide products.

Benzyl alcohol (CAS RN 100-51-6) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory and also in the US EPA 2020 CDR list (Chemical Data Reporting Rule). The CDR regulation requires companies that manufacture (including import) certain chemicals at certain volumes in the U.S. to report to EPA every four years through its CDR.

US EPA Substance Registry Services (SRS) Benzyl alcohol (CAS RN 100-51-6) is classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2023a).

Benzyl alcohol is included in the ECHA Biocidal Active Substances database and is currently under review for use in product type 6 (preservatives for products during storage) (ECHA, 2023b).

IFRA recommendation: Restriction.

Restriction limits in the finished product (%):			
Category 1	0.45 %	Category 7A	0.68 %
Category 2	0.14 %	Category 7B	0.68 %
Category 3	0.34 %	Category 8	0.057 %
Category 4	2.5 %	Category 9	2.2 %
Category 5A	0.64 %	Category 10A	2.2 %
Category 5B	0.17 %	Category 10B	8.50 %
Category 5C	0.34%	Category 11A	0.057 %
Category 5D	0.057 %	Category 11B	0.057 %
Category 6	1.5%	Category 12	No Restriction

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

This is a non-exhaustive indicative list of typical natural presence for Benzyl alcohol and is intended to be used in the absence of own analytical data. If analysis has shown that the level of the restricted ingredient in a natural complex substance is different from what is provided in this Annex I, then the analytically determined level should be used in place of the indicative level.

It should further be noted that natural complex substances themselves can be restricted by an IFRA Standard. For a detailed list of natural contributions, please refer to the Annex I of IFRA Standards, publicly available on the IFRA website (www.ifrafragrance.org)

INTRINSIC PROPERTY DRIVING RISK MANAGEMENT: DERMAL SENSITIZATION AND SYSTEMIC TOXICITY

As taken from IFRA, 2020, 2022

Benzyl alcohol is listed as authorised for use as a flavouring substance in all categories of flavoured foods in the EU under regulation (EU) No. 872/2012 (European Commission (undated). Food flavourings and food additives databases)

Substance	Benzyl Alcohol
CAS #	100-51-6
EC #	202-859-9
Name of Common Ingredients Glossary	BENZYL ALCOHOL
INN/ISO/AN	
Regulation	(EC) 2013/344
Annex/Ref #	III/45
Product Type, body parts	(a)Solvent (b) Fragrance/ aromatic compositions/ their raw materials
Other	For purposes other than inhibiting the development of microorganisms in the product. This purpose has to be apparent from the presentation of the product. (b) The presence of the substance must be indicated in the list of ingredients referred to in Article 19(1)(g) when its concentration exceeds: — 0,001 % in leave-on products — 0,01 % in rinse-off products.
Chemical/IUPAC Name	Benzyl Alcohol
Note	III (45) For use as a preservative, see Annex V, No. 34
Current Version	v.1

Substance	Benzyl alcohol (7)
CAS #	100-51-6
EC #	202-859-9
Name of Common Ingredients Glossary	BENZYL ALCOHOL
Regulation	(EC) 2013/344
Regulated By	86/199/EEC
Annex/Ref #	V/34
Maximum concentration in ready for use preparation	1.0%
Identified INGREDIENTS or substances e.g.	BENZYL ALCOHOL
Note	V (7) For use other than as a preservative, see Annex III, No. 45 (a) (b)
Current Version	v.1

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

“The chemical is listed on the following (Galleria Chemica):-

-EU Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products—Annex III—List of Substances which cosmetic products must not contain except subject to the restrictions laid down;

-New Zealand Cosmetic Products Group Standard—Schedule 5—Table 1: Components cosmetic products must not contain except subject to the restrictions and conditions laid down;

-The Association of Southeast Asian Nations (ASEAN) Cosmetic Directive Annex III—Part 1 List of substances which cosmetic products must not contain except subject to restrictions and conditions laid down;

-EU Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products—Annex V List of preservatives allowed in cosmetic products;

-New Zealand Cosmetic Products Group Standard—Schedule 7: Preservatives cosmetic products may contain with restrictions—Table 1: List of preservatives allowed; and

-ASEAN Cosmetic Directive Annex VI—Part 1—List of preservatives allowed for use in cosmetic products.”

As taken from AICIS, 2016.

Benzyl alcohol is included in the European Union List of Fragrance Allergens Requiring Labelling on Cosmetic and Detergent Products (EMA, 2019).

Benzyl alcohol (FEMA no. 2137) has been designated as GRAS (generally recognized as safe) for use in food by FEMA (Hall RL and Oser BL, 1965).

Benzyl alcohol (CAS RN 100-51-6) is included on the US EPA's Safer Chemical Ingredients List (US EPA, 2023)

Benzyl alcohol is included on the US FDA's list of inactive ingredients for approved drug products. It is permitted for use as ingredients in various products, at the following maximum potencies per unit dose and maximum daily exposures (MDE):

Route	Dosage Form	CAS Number	UNII	Maximum Potency per unit dose	Maximum Daily Exposure (MDE)
AURICULAR (OTIC)	SUSPENSION	100516	LKG8494WBH	0.9 %w/v	
ENDOTRACHEAL	INJECTION	100516	LKG8494WBH	0.9 %w/v	
EPIDURAL	INJECTION	100516	LKG8494WBH	1 %w/v	
INTRA-ARTERIAL	INJECTION	100516	LKG8494WBH	0.9 %w/v	
INTRA-ARTICULAR	INJECTION	100516	LKG8494WBH		40 mg
INTRA-ARTICULAR	INJECTION, SOLUTION	100516	LKG8494WBH		23 mg
INTRA-ARTICULAR	INJECTION, SUSPENSION	100516	LKG8494WBH		40 mg
INTRABURSAL	INJECTION	100516	LKG8494WBH	1 %w/v	
INTRACAVITARY	INJECTION	100516	LKG8494WBH	0.01 ml	
INTRACAVITARY	INJECTION, POWDER, LYOPHILIZED, FOR SOLUTION	100516	LKG8494WBH	8.4 mg	

INTRALESIONAL	INJECTION	100516	LKG8494WBH		18 mg
INTRALESIONAL	INJECTION, SUSPENSION	100516	LKG8494WBH		18 mg
INTRAMUSCULAR	INJECTION	100516	LKG8494WBH		1000 mg
INTRAMUSCULAR	INJECTION, EMULSION	100516	LKG8494WBH		45 mg
INTRAMUSCULAR	INJECTION, POWDER, FOR SOLUTION	100516	LKG8494WBH	144 mg	
INTRAMUSCULAR	INJECTION, SOLUTION	100516	LKG8494WBH		500 mg
INTRAMUSCULAR	INJECTION, SUSPENSION	100516	LKG8494WBH		40 mg
INTRAMUSCULAR	SOLUTION	100516	LKG8494WBH		20 mg
INTRASYNOVIAL	INJECTION	100516	LKG8494WBH	0.92 %w/v	
INTRASYNOVIAL	INJECTION, SUSPENSION	100516	LKG8494WBH	0.92 %w/v	
INTRAVENOUS	INJECTION	100516	LKG8494WBH		1620 mg
INTRAVENOUS	INJECTION, EMULSION	100516	LKG8494WBH		45 mg
INTRAVENOUS	INJECTION, POWDER, FOR SOLUTION	100516	LKG8494WBH	144 mg	
INTRAVENOUS	INJECTION, SOLUTION	100516	LKG8494WBH		400 mg
INTRAVENOUS	LIQUID	100516	LKG8494WBH	1.04 %w/v	
INTRAVENOUS	SOLUTION	100516	LKG8494WBH		242 mg

NASAL	SPRAY	100516	LKG8494WBH	1.8 mg/0.2ml	
NASAL	SPRAY, METERED	100516	LKG8494WBH	5 mg/1ml	
ORAL	CAPSULE	100516	LKG8494WBH		450 mg
ORAL	CAPSULE, EXTENDED RELEASE	100516	LKG8494WBH	1.23 mg	
ORAL	SOLUTION	100516	LKG8494WBH	50 mg/1ml	
ORAL	SUSPENSION	100516	LKG8494WBH		100 mg
ORAL	TABLET	100516	LKG8494WBH	1.06 mg	
ORAL	TABLET, DELAYED RELEASE	100516	LKG8494WBH	2.31 mg	
ORAL	TABLET, FILM COATED, EXTENDED RELEASE	100516	LKG8494WBH	1.25 mg	
RECTAL	GEL	100516	LKG8494WBH	1.55 %w/v	
RECTAL	SUPPOSITORY	100516	LKG8494WBH	129 mg	
SOFT TISSUE	INJECTION	100516	LKG8494WBH		18 mg
SOFT TISSUE	INJECTION, SUSPENSION	100516	LKG8494WBH		40 mg
SUBCONJUNCTIVAL	INJECTION	100516	LKG8494WBH		252 mg
SUBCUTANEOUS	INJECTION	100516	LKG8494WBH		90 mg
SUBCUTANEOUS	INJECTION, EMULSION	100516	LKG8494WBH		45 mg
SUBCUTANEOUS	INJECTION, SOLUTION	100516	LKG8494WBH	0.95 %w/v	
TOPICAL	CREAM	100516	LKG8494WBH		160 mg
TOPICAL	CREAM, AUGMENTED	100516	LKG8494WBH	1 %w/w	

TOPICAL	GEL	100516	LKG8494WBH		185 mg
TOPICAL	LOTION	100516	LKG8494WBH	2 %w/w	
TOPICAL	OINTMENT	100516	LKG8494WBH	2.2 %w/w	
TOPICAL	SHAMPOO	100516	LKG8494WBH	1.25 %w/w	
TOPICAL	SOLUTION	100516	LKG8494WBH	2 %w/w	
TOPICAL	SUSPENSION	100516	LKG8494WBH	1 %w/w	
VAGINAL	CREAM	100516	LKG8494WBH	1 %w/w	
VAGINAL	SUPPOSITORY	100516	LKG8494WBH	NA	

As taken from FDA, 2023c.

Benzyl alcohol is listed by the US EPA Office of Pesticide Programs (2022) and was first registered as an antimicrobial and “conventional chemical” pesticide on 28 July 1986.

“Benzyl alcohol (E 1519) is authorised as a food additive in the European Union (EU) in accordance with Annex III to Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in Commission Regulation (EU) No 231/2012.

Benzyl alcohol was evaluated by JECFA in 1980 (JECFA, 1980) and concluded a group acceptable daily intake (ADI) (for benzoic acid together with benzyl alcohol) of 0–5 mg/kg body weight (bw), subsequently agreed by the Scientific Committee for Food (SCF) in 2002.

Benzyl alcohol is also included in the EU Union list (Commission Implementing Regulation (EU) No 872/2012) of authorised food flavourings substance with FL-no: 02.010, following its evaluation by the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) in 2008 in flavouring group evaluation 54 (FGE.54Rev1). The European Medicines Agency (EMA) has registered the use of benzyl alcohol as an excipient in medical products.”

“Considering the available data set, the Panel established an ADI of 4 mg benzyl alcohol/kg bw per day based on a NOAEL of 400 mg/kg bw per day from the carcinogenicity study in rats, based on no effect in the highest dose tested.”

“The Panel recommend that the European Commission considers introducing the maximum levels of chlorinated organic compounds as impurities in the specifications for purity of E 1519 in EU Regulation 231/2012.”

As taken from EFSA, 2019

Benzyl alcohol (CAS RN 100-51-6) is classified as a natural health product (NHP) for medicinal use under Schedule 1, item 2 (an isolate) of the Natural Health Products Regulations (Health Canada, 2021).

FDA (2022d)“Applying a chemical specific adjustment factor of 2 for interspecies toxicokinetics variation instead of the default factor of 4.0, the Committee established a group ADI of 0–20 mg/kg bw, which applies to benzoic acid, the benzoate salts (calcium, potassium and sodium), benzaldehyde, benzyl acetate, benzyl alcohol and benzyl benzoate, expressed as benzoic acid equivalents. The Committee withdrew the previous group ADI of 0–5 mg/kg bw. The Committee noted that the high dietary exposure estimate, expressed as benzoic acid, of 7.1 mg/kg bw per day for children aged 3–9 years does not exceed the group ADI of 0–20 mg/kg bw.”

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

Benzyl alcohol is normally oxidized rapidly to benzoic acid, conjugated with glycine in the liver, and excreted as hippuric acid. However, this metabolic pathway may not be well developed in premature infants. The benzyl alcohol may therefore have been metabolized to benzoic acid, which could not be conjugated by the immature liver but accumulated, causing metabolic acidosis CDC/MMWR; Neonatal Deaths Associated With Use Of Benzyl Alcohol -- United States. Morbidity and Mortality Weekly Report 31(22);290-1 (June 11, 1982). Available from, as of July 23, 2008: <http://www.cdc.gov/mmwr/preview/mmwrhtml/00001109.htm>

When metabolized, benzyl alcohol is converted to benzoic acid by simple oxidation. The relevant data, therefore, relate to benzoic acid and sodium benzoate. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed AbstractBenzyl alcohol was an intermediate product in the metabolic pathway of benzyl acetate; the subsequent metabolism was identical to that of benzyl alcohol. European Medicines Agency (EMA), The European Agency for the Evaluation of Medicinal Products, Veterinary Medicines Evaluation Unit, Committee for Veterinary Medicinal Products; Benzyl Alcohol, Summary Report (1997). Available from, as of July 8, 2008: <http://www.emea.europa.eu/pdfs/vet/mrls/Benzylalcohol.pdf>

In adults, benzyl alcohol is oxidized to benzoic acid, conjugated in the liver with glycine, and excreted in the urine as hippuric acid. The immature metabolic capacities of infants diminish their ability to metabolize and excrete benzyl alcohol. Preterm babies have a greater ability to metabolize benzyl alcohol to benzoic acid than do term babies, but are unable to convert benzoic acid to hippuric acid, possibly because of glycine deficiency. This results in the accumulation of benzoic acid. Goldfrank, L.R., Goldfrank's Toxicologic Emergencies 8th Ed. 2006., McGraw-Hill, New York, N.Y., p. 831

Benzyl alcohol is readily absorbed from the gastrointestinal tract and rapidly oxidized to benzoic acid, which is conjugated with glycine and excreted as hippuric acid in the urine. ...Human subjects eliminated 75 to 85% of the dose in the urine as hippuric acid within 6 hr after taking 1.5 g of benzyl alcohol orally. Bingham, E.; Cohrssen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. V6 498

/Benzyl alcohol is/ ... oxidized by liver alcohol dehydrogenase. Testa, B. and P. Jenner. Drug Metabolism: Chemical & Biochemical Aspects. New York: Marcel Dekker, Inc., 1976., p. 4

Yields benzaldehyde in rabbits. /from table/. Goodwin, B.L. Handbook of Intermediary Metabolism of Aromatic Compounds. New York: Wiley, 1976., p. B-13

Yields phenol in guinea pigs. /from table/. Goodwin, B.L. Handbook of Intermediary Metabolism of Aromatic Compounds. New York: Wiley, 1976., p. B-13

As taken from HSDB, 2018

“As already stated, the benzyl alcohol is expected to be oxidised to benzoic acid, which will be conjugated with glycine and excreted as hippuric acid (Bridges et al., 1970). This has been demonstrated in a study by LeBel et al. (1988) in which similar intravenous doses (range 0.036 to 0.222 micromole/kg bw) of benzyl alcohol were given via medications to 14 full-term and nine pre-term infants, in order to estimate plasma levels of benzoic and hippuric acids. In the urine of full-term new-borns a larger proportion of benzyl alcohol was found as hippuric acid and a smaller proportion benzoic acid, which is in line with what Bridges et al suggest. However, the mean peak

concentrations of benzoic acid in the plasma of pre-term babies were almost 10 times higher than in full-term new-borns and in the urine of pre-term babies a larger proportion of

benzyl alcohol was found as benzoic acid and a smaller proportion as hippuric acid in contrary to what was found in full-term new-borns. The results suggest that hippuric acid formation is deficient in pre-term newborns (LeBel et al., 1988). Overall, the results suggest that humans metabolise benzyl alcohol to both benzoic acid and hippuric acid but hippuric acid formation is deficient in pre-term new-borns.”

As taken from EFSA (2012). Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific opinion on Flavouring Group Evaluation 20, Revision 4 (FGE.20Rev4): Benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters from chemical groups 23 and 30. Available at <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2012.2994/epdf>

“Benzyl alcohol, a potent anesthetic and bacteriostatic, can be efficiently glucosylated by α -glucosidase from *Saccharomyces cerevisiae* to produce benzyl alcohol α -glucoside with a 75% yield. However, while studying the transglucosylation reaction conditions, it was found out that benzyl alcohol is a non-competitive inhibitor of α -glucosidase's hydrolytic activity ($K_i=18\text{mM}$, toward maltose). Due to its interesting ability to be glycosylated by the enzyme and to inhibit its hydrolytic activity, we proposed a plausible mechanism for the phenolic α -glucosydase inhibitor's binding, since the mechanism of inhibition has not yet been elucidated.” As taken from Pavlovic M et al. 2014. Carbohydr. Res. 387, 14-18. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24531390>

“To compare the pharmacokinetics and pharmacodynamics of propofol with or without 2% benzyl alcohol administered intravenously (IV) as a single induction dose in cats. Prospective experimental study. Six healthy adult cats, three female intact, three male castrated, weighing 4.8 ± 1.8 kg. Cats received 8 mg kg^{-1} IV of propofol (P) or propofol with 2% benzyl alcohol (P28) using a randomized crossover design. Venous blood samples were collected at predetermined time points to 24 hours after drug administration to determine drug plasma concentrations. Physiologic and behavioral variables were also recorded. Propofol and benzyl alcohol concentrations were determined using high pressure liquid chromatography with fluorescence detection. Pharmacokinetic parameters were described using a 2-compartment model. Pharmacokinetic and pharmacodynamic parameters were analyzed using repeated measures anova ($p < 0.05$). Plasma concentrations of benzyl alcohol were below the lower limits of quantification (LLOQ) at all time points for two of the six cats (33%), and by 30 minutes for the remaining four cats.” As taken from Griffenhagen GM et al. 2015. Vet. Anaesth. Analg. 42(5), 472-83. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25327817>

“The aim of the present study was to identify human cytosolic sulfotransferases (SULTs) that are capable of sulfating benzyl alcohol and to examine whether benzyl alcohol sulfation may occur in cultured human cells as well as in human organ homogenates. A systematic analysis revealed that of the 13 known human SULTs, SULT1A1, SULT1A2, SULTA3, and SULT1B1 are capable of mediating the sulfation of benzyl alcohol. The kinetic parameters of SULT1A1 that showed the strongest benzyl alcohol-sulfating activity were determined. HepG2 human hepatoma cells were used to demonstrate the generation and release of sulfated benzyl alcohol under the metabolic settings. Moreover, the cytosol or S9 fractions of human liver, lung, kidney and small intestine were examined to verify the presence of benzyl alcohol sulfating activity in vivo.” As taken from Zhang L et al. 2016. J. Appl. Toxicol. 36(9), 1090-1094. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/26663444>

“Acetaminophen (APAP) hepatotoxicity is a serious public health problem in western countries. Current treatment options for APAP poisoning are limited and novel therapeutic intervention strategies are needed. A recent publication suggested that benzyl alcohol (BA) protects against

APAP hepatotoxicity and could serve as a promising antidote for APAP poisoning. To assess the protective mechanisms of BA, C56Bl/6J mice were treated with 400 mg/kg APAP and/or 270 mg/kg BA. APAP alone caused extensive liver injury at 6 h and 24 h post-APAP. This injury was attenuated by BA co-treatment. Assessment of protein adduct formation demonstrated that BA inhibits APAP metabolic activation. In support of this, in vitro experiments also showed that BA dose-dependently inhibits cytochrome P450 activities. Correlating with the hepatoprotection of BA, APAP-induced oxidant stress and mitochondrial dysfunction were reduced. Similar results were obtained in primary mouse hepatocytes. Interestingly, BA alone caused mitochondrial membrane potential loss and cell toxicity at high doses, and its protective effect could not be reproduced in primary human hepatocytes (PHH). We conclude that BA protects against APAP hepatotoxicity mainly by inhibiting cytochrome P450 enzymes in mice. Considering its toxic effect and the loss of protection in PHH, BA is not a clinically useful treatment option for APAP overdose patient.” As taken from Du K et al. 2015. Food Chem. Toxicol. 86, 253-61. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26522885>

“As benzyl alcohol is quickly metabolized in benzoic acid after administration, it is not present at measurable level in the blood but its oxidation product, benzoic acid, is present and may be used for kinetic. A perturbation of metabolism (anomaly, ADH2*3 allelic variant [30] or immaturity) or elimination of benzyl alcohol may lead to toxicity. Indeed, there is a greater accumulation of benzyl alcohol in serum of pre-term neonates expressed in peak level and in area under curve than in full-term neonates.

Benzoic acid undergoes conjugation by acyl CoA synthetase then by glycine N acyl transferase to form hippuric acid. The newly synthesised hippuric acid is then eliminated in the urine. For an adult, after intravenous administration of a dose of 15 mg/kg, the elimination half-life from plasma is 16 minutes for benzoic acid and 31 minutes for hippuric acid. Eighty per cent of the dose is eliminated in the urine in the form of hippuric acid. Clearance is approximately 600 ml/min. The benzoic acid detoxification process is immature in those pre-term neonates [16] and conjugation of benzoic acid is saturated more quickly. This reaction is furthermore limited by the glycine available. This results in an accumulation of benzoic acid.”

As taken from EMA, 2017

“In humans and animal species, benzyl alcohol is metabolised to benzoic acid which reacts with glycine in the liver and the resulting hippuric acid is excreted quickly in urine.”

As taken from EFSA, 2019

“Findings of kinetic studies on humans and test animals as well as results of in vitro studies demonstrated that B [benzyl alcohol] is rapidly oxidated to the main metabolite benzoic acid via benzaldehyde in the liver; the benzoic acid is then conjugated with glycine to hippuric acid.”

As taken from GESTIS

4.2. Absorption, distribution and excretion

The percutaneous absorption was determined in vivo in rhesus monkeys. Absorption through occluded skin was high (56-80 %) in 24 hr. No correlation was seen between skin penetration and the octanol-water partition coefficient. Under unoccluded conditions skin penetration was reduced (32 %), because of evaporation of the compound. OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.298 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

High levels of benzyl alcohol (5-500 ug/10 mL plasma) were found in uremic patients on hemodialysis; benzyl alcohol was not detected in normal controls. OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate,

Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.294 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

Rabbits given 1 g of benzyl alcohol sc eliminated 300-400 mg of hippuric acid within the following 24 hr. Within 6 hr after oral administration of 0.40 g benzyl alcohol/kg bw, rabbits eliminated 65.7% of dose as hippuric acid in the urine. Clayton, G.D., F.E. Clayton (eds.) Patty's Industrial Hygiene and Toxicology. Volumes 2A, 2B, 2C, 2D, 2E, 2F: Toxicology. 4th ed. New York, NY: John Wiley & Sons Inc., 1993-1994., p. 2704

In humans and animals, benzyl alcohol was readily absorbed from the gastrointestinal tract. Percutaneous absorption was high following topical use. Rhesus monkeys absorbed 56-80% of a topical dose administered under occlusive conditions in 24 hours; absorption was less under unoccluded conditions due to evaporation. Benzyl alcohol rapidly disappeared from the injection site following intramuscular administration in rats; the disappearance half-life was estimated to be less than 10 minutes. ... European Medicines Agency (EMA), The European Agency for the Evaluation of Medicinal Products, Veterinary Medicines Evaluation Unit, Committee for Veterinary Medicinal Products; Benzyl Alcohol, Summary Report (1997). Available from, as of July 8, 2008: <http://www.ema.europa.eu/pdfs/vet/mrls/Benzylalcohol.pdf>

... Vapors can penetrate the intact skin ... Opdyke, D.L.J. (ed.). Monographs on Fragrance Raw Materials. New York: Pergamon Press, 1979., p. 128

The dermal flux for benzyl alcohol across human skin in vitro was reported at 0.073 mg/sq cm/hr, indicating a low rate of dermal uptake. The percentage of the applied dose that penetrated through human skin in vitro in 6 hr was 1.42% for adult skin and 0.73% for full term infant skin. Bingham, E.; Cohrssen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. V6 499

Biological Half-Life:

The plasma half-life of benzyl alcohol administered as 2.5% solution in saline was found to be approximately 1.5 hr in dogs injected iv at doses of 52 and 105 mg/kg. Clayton, G. D. and F. E. Clayton (eds.). Patty's Industrial Hygiene and Toxicology: Volume 2A, 2B, 2C: Toxicology. 3rd ed. New York: John Wiley Sons, 1981-1982., p. 4640

Benzyl alcohol rapidly disappeared from the injection site following intramuscular administration in rats; the disappearance half-life was estimated to be less than 10 minutes. ... European Medicines Agency (EMA), The European Agency for the Evaluation of Medicinal Products, Veterinary Medicines Evaluation Unit, Committee for Veterinary Medicinal Products; Benzyl Alcohol, Summary Report (1997). Available from, as of July 8, 2008: <http://www.ema.europa.eu/pdfs/vet/mrls/Benzylalcohol.pdf>

As taken from HSDB, 2018

"Five minutes after single intraperitoneal doses of 500 - 1100 mg/kg bw of benzyl alcohol administered to CD1 mice, benzyl alcohol was detected in plasma (McCloskey et al., 1986)."

"Conjugation of benzyl alcohol with glutathione has been observed by Van Doorn et al (1981) (van Doorn et al., 1981), who estimated that after an intraperitoneal dose of 1 mmol/kg bw to rats, approximately 0.8 % of the dose was eliminated as mercapturic acid via the urine. From additional studies it was anticipated that the sulphate ester of the alcohol would participate as an intermediate in the ultimate conjugation to glutathione."

As taken from EFSA (2012). Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific opinion on Flavouring Group Evaluation 20, Revision 4 (FGE.20Rev4): Benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters

“The chemical is readily and totally absorbed from the alimentary tract in humans following an oral application of 1.5 g; the chemical is metabolised within six hours and about 75–85 % is excreted in the urine.”

As taken from AICIS, 2016

“Pharmaceutical oil depots are meant to release active substances at a sustained rate. Most of these depots contain benzyl alcohol (BOH) to facilitate the production and administration. Because BOH changes the solubility of components in both the body fluid and the oil formulation, it is relevant to know the change in the BOH concentration in the oil over time. In this study, volunteers were subcutaneously injected with an oil depot that contained 10% BOH, nandrolone decanoate, and cholecalciferol. The aim of this study was to determine the pharmacokinetic profiles of BOH and its metabolites benzoic acid and hippuric acid simultaneously in serum to estimate the BOH release out of the depot. For this, an HPLC bioassay was developed and adequately validated. Hereafter, the bioassay was applied to serum samples obtained at several time points between 0 and 35 days. BOH appeared immediately in serum after injection. The pharmacokinetic profile revealed that all BOH was depleted from the depot within 52 h after injection. Thus, the partition coefficient of active substances between the oil formulation and the body tissue changes rapidly in the first days after injection but will remain constant hereafter.” As taken from Kalicharan R et al. 2016. J. Pharm. Sci. 105(4), 1519-25. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27019966>

Oral and cutaneous absorption rates are 100% and up to 60%, respectively (EMA, 2017).

“As benzyl alcohol is quickly metabolized in benzoic acid after administration, it is not present at measurable level in the blood but its oxidation product, benzoic acid, is present and may be used for kinetic. A perturbation of metabolism (anomaly, ADH2*3 allelic variant [30] or immaturity) or elimination of benzyl alcohol may lead to toxicity. Indeed, there is a greater accumulation of benzyl alcohol in serum of pre-term neonates expressed in peak level and in area under curve than in full-term neonates.

Benzoic acid undergoes conjugation by acyl CoA synthetase then by glycine N acyl transferase to form hippuric acid. The newly synthesised hippuric acid is then eliminated in the urine. For an adult, after intravenous administration of a dose of 15 mg/kg, the elimination half-life from plasma is 16 minutes for benzoic acid and 31 minutes for hippuric acid. Eighty per cent of the dose is eliminated in the urine in the form of hippuric acid. Clearance is approximately 600 ml/min. The benzoic acid detoxification process is immature in those pre-term neonates [16] and conjugation of benzoic acid is saturated more quickly. This reaction is furthermore limited by the glycine available. This results in an accumulation of benzoic acid.

Benzoic acid may be used for managing hyperammonaemia in urea cycle disorders in the neonates. However, the main problem linked to the use of benzoic acid concerns neonates (pre-term and fullterm) who have immature metabolising enzymes leading to accumulation. The risk associated with the accumulation of benzoic acid is an increase in bilirubinaemia following displacement of bilirubin from albumin and which may cause newborn jaundice to develop into kernicterus (non-conjugated bilirubin deposits in the brain tissue).”

“The risk inherent to benzyl alcohol is due to its accumulation in the organism. Elimination of benzyl alcohol is highly variable as both the age (immaturity) and ethnic polymorphism of alcohol deshydrogenase may lead to accumulation of benzyl alcohol and thus toxicity. It is thus difficult to establish an exemption threshold.”

As taken from EMA, 2017

"B [benzyl alcohol] is easily absorbed via the skin. In-vitro studies on human skin yielded flux values of 29 to 275 $\mu\text{g}/\text{cm}^2$ per hour, if physiological acceptor media were used. This corresponds to a total absorption of approx. 58–550 mg with one-hour exposure of both hands and forearms (approx. 2000 cm^2). In animal experiments on rhesus monkeys, 56–80% of the applied dose was absorbed via the (covered) skin within 24 hours."

"After oral intake, B is rapidly and almost completely absorbed via the gastrointestinal tract."

"Hippuric acid is rapidly eliminated with the urine via the kidneys. In a study on humans, 75–85% of an orally absorbed B dose of 1.5 g was eliminated via this route within 6 hours. A smaller portion (up to 20%) of the B absorbed in the body is eliminated with the urine without oxidation after direct conjugation with glucuronic acid."

As taken from GESTIS

"No information is available on the clinical use of spinosad during breastfeeding. Because it is not systemically absorbed after topical application, it should not be present in breastmilk. However, the topical suspension contains benzyl alcohol, which may be systemically absorbed through the skin. High doses of benzyl alcohol are potentially toxic to neonates, but it is unlikely that doses this high reach breastmilk. The manufacturer states that mothers concerned about benzyl alcohol could pump and discard breastmilk for 8 hours, but this is probably unnecessary."

As taken from LactMed, 2021

4.3. Interactions

"To compare the pharmacokinetics and pharmacodynamics of propofol with or without 2% benzyl alcohol administered intravenously (IV) as a single induction dose in cats. Prospective experimental study. Six healthy adult cats, three female intact, three male castrated, weighing 4.8 ± 1.8 kg. Cats received 8 mg kg^{-1} IV of propofol (P) or propofol with 2% benzyl alcohol (P28) using a randomized crossover design. Venous blood samples were collected at predetermined time points to 24 hours after drug administration to determine drug plasma concentrations. Physiologic and behavioral variables were also recorded. Propofol and benzyl alcohol concentrations were determined using high pressure liquid chromatography with fluorescence detection. Pharmacokinetic parameters were described using a 2-compartment model. Pharmacokinetic and pharmacodynamic parameters were analyzed using repeated measures anova ($p < 0.05$). Plasma concentrations of benzyl alcohol were below the lower limits of quantification (LLOQ) at all time points for two of the six cats (33%), and by 30 minutes for the remaining four cats. Propofol pharmacokinetics, with or without 2% benzyl alcohol, were characterized by rapid distribution, a long elimination phase, and a large volume of distribution. No differences were noted between treatments with the exception of clearance from the second compartment (CLD2), which was 23.6 and 38.8 $\text{mL kg}^{-1} \text{ minute}^{-1}$ in the P and P28 treatments, respectively. Physiologic and behavioral variables were not different between treatments with the exception of heart rate at 4 hours post administration. The addition of 2% benzyl alcohol as a preservative minimally altered the pharmacokinetics and pharmacodynamics of propofol 1% emulsion when administered as a single IV bolus in this group of cats. These data support the cautious use of propofol with 2% benzyl alcohol for induction of anesthesia in healthy cats." As taken from Griffenhagen GM et al. 2015. Vet. Anaesth. Analg. 42(5), 472-83. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25327817>

The anabolic steroid nandrolone decanoate has been reported to increase voluntary ethanol intake in Wistar rats. In recent experiments we received opposite results, with decreased voluntary ethanol intake in both high drinking AA and low drinking Wistar rats after nandrolone treatment. The difference between the two studies was that we used pure nandrolone decanoate in oil, whereas in the previous study the nandrolone product Deca-Durabolin containing benzyl alcohol (BA) was used. The aims of the present study were to clarify whether the BA treatment could promote ethanol drinking and to assess the role of the hypothalamic-pituitary-adrenal-gonadal axes

(HPAGA) in the potential BA effect. Male AA and Wistar rats received subcutaneously BA or vehicle oil for 14 days. Hereafter followed a 1-week washout and consecutively a 3-week voluntary alcohol consumption period. The median ($\pm$ median absolute deviation) voluntary ethanol consumption during the drinking period was higher in BA-treated than in control rats (4.94 ± 1.31 g/kg/day vs. 4.17 ± 0.31 g/kg/day, $p = 0.07$ and 1.01 ± 0.26 g/kg/day vs. 0.38 ± 0.27 g/kg/day, $p = 0.05$, for AA and Wistar rats, respectively; combined effect $p < 0.01$). The present results can explain the previous discrepancy between the two nandrolone studies. No significant BA effects on basal and ethanol-mediated serum testosterone and corticosterone levels were observed in blood samples taken at days 1, 8 and 22. However, 2h after ethanol administration significantly ($p = 0.02$) higher frequency of testosterone elevations was detected in high drinking AA rats compared to low drinking Wistars, which supports our previous hypotheses of a role of testosterone elevation in promoting ethanol drinking. Skin irritation and dermatitis were shown exclusively in the BA-treated animals. Altogether, the present results indicate that earlier findings obtained with Deca-Durabolin containing BA need to be re-evaluated. As taken from Etelalahti TJ and Eriksson CJ. 2014. Pharmacol. Biochem. Behav. 124, 81-85. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24871566>

“Acetaminophen (APAP) hepatotoxicity is a serious public health problem in western countries. Current treatment options for APAP poisoning are limited and novel therapeutic intervention strategies are needed. A recent publication suggested that benzyl alcohol (BA) protects against APAP hepatotoxicity and could serve as a promising antidote for APAP poisoning. To assess the protective mechanisms of BA, C56Bl/6J mice were treated with 400 mg/kg APAP and/or 270 mg/kg BA. APAP alone caused extensive liver injury at 6 h and 24 h post-APAP. This injury was attenuated by BA co-treatment. Assessment of protein adduct formation demonstrated that BA inhibits APAP metabolic activation. In support of this, in vitro experiments also showed that BA dose-dependently inhibits cytochrome P450 activities. Correlating with the hepatoprotection of BA, APAP-induced oxidant stress and mitochondrial dysfunction were reduced. Similar results were obtained in primary mouse hepatocytes. Interestingly, BA alone caused mitochondrial membrane potential loss and cell toxicity at high doses, and its protective effect could not be reproduced in primary human hepatocytes (PHH). We conclude that BA protects against APAP hepatotoxicity mainly by inhibiting cytochrome P450 enzymes in mice. Considering its toxic effect and the loss of protection in PHH, BA is not a clinically useful treatment option for APAP overdose patient.” As taken from Du K et al. 2015. Food Chem. Toxicol. 86, 253-61. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26522885>

“Multidose formulations of biotherapeutics, which offer better dosage management and reduced production costs, require the addition of antimicrobial preservatives (APs). APs have been shown, however, to decrease protein stability in solution and cause protein aggregation. In this report, the effect of 4 APs, m-cresol, phenol, phenoxyethanol, and benzyl alcohol on conformational stability, aggregation propensity, and backbone flexibility of an IgG1 mAb, mAb-4, is investigated. Compared with no preservative control, each of the APs decreased the conformational stability of mAb-4 as measured by differential scanning calorimetry and extrinsic fluorescence spectroscopy. The addition of APs resulted in increased monomer loss and aggregate accumulation at 50°C over 28 days, as monitored by size-exclusion chromatography. The extent of conformational destabilization and protein aggregation of mAb-4 induced by APs followed their calculated octanol-water partition coefficients. Increases in backbone flexibility, as measured by hydrogen exchange, of a region located in the CH2 domain of the mAb (heavy chain 237-254) in the presence of APs also correlated with hydrophobicity. Based on these results, the destabilizing effect of APs on mAb-4 correlates with the increased hydrophobicity of the APs and their ability to enhance the local backbone flexibility of an aggregation hot spot within the CH2 domain of the mAb.” As taken from Arora J et al. 2017. J. Pharm. Sci. 106(6), 1508-1518. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28212986>

"Multi-dose formulation of biopharmaceuticals contains an antimicrobial preservative, which may facilitate aggregation of the proteins due to their hydrophobic protein binding. Here, we have investigated the effects of co-solvents on heat-induced protein aggregation in the presence of an antimicrobial preservative. Human immunoglobulin G (IgG) and benzyl alcohol were chosen because of their wide usage as a pharmaceutical protein and a preservative. Trimethylamine N-oxide (TMAO) was found to be the most effective additive in suppressing benzyl alcohol-induced IgG aggregation among the additives tested. Interestingly, TMAO was less effective in increasing the thermal-unfolding temperature of IgG than trehalose, indicating that the observed suppression of IgG aggregation by TMAO in the presence of benzyl alcohol is not simply due to its stabilization effect of the protein structure. The solubility of benzyl alcohol increased with increasing concentrations of TMAO, suggesting that the hydrophobic interaction of TMAO with benzyl alcohol is responsible for the observed aggregation suppression. Thus, TMAO may be a potential aggregation suppressor in multi-dose formulation of biopharmaceuticals." As taken from Yoshizawa S et al. 2018. *Int. J. Biol. Macromol.* 107(Pt. A), 984-989. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28939518>

5. Toxicity

5.1. Single dose toxicity

"Benzyl alcohol is an excipient used in many drugs as a stabilizer. Depending on the amount present in drug formulations there might be confounding findings in the Abnormal Toxicity Test (ATT). The ATT is utilized as a quality control (QC) release test to detect extraneous contaminants according to national pharmacopoeias. Our study assessed the effects of benzyl alcohol as defined in ATT designs. This study - the first thorough evaluation of the confounding effects of benzyl alcohol on the ATT - was conducted in relation to particular health authority questions and was part of the root-cause analyses resulting from some transient behavioral findings observed in the test. Two strains of mice, CD-1 & Kunming, plus Hartley guinea pigs were administered intraperitoneally (ip), subcutaneously (sc), or intravenously (iv) with benzyl alcohol at dose level defined in the ATT design. In both mice and guinea pigs, only after ip administration, minimal behavioral changes were observed transiently within 2-3 min after administration. Therefore, the presence of benzyl alcohol in the product batch may confound the ATT results. This study provides further evidence to question the validity of the ATT for its intended use." As taken from Xie J et al. 2015. *Regul. Toxicol. Pharmacol.* 73, 509-14. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26449397>

"LD50 values to mammals in oral exposure, mg/kg :

3100 orl-rat, Patty 1967

1230 orl-rat, Sax 1986

1580 orl-mus

1040 orl-rbt

LD50 values to mammals in non-oral exposure , mg/kg :

64 ivn-rat, Sax 1986

324 ivn-mus

2000 skn-rbt

LC50 values to mammals in inhalation exposure, mg/m³ :

4420 ihl-rat, 8hr, Sax 1986

LDLo values to mammals in non-oral exposure , mg/kg :

400 ipr-rat, Sax 1986

1700 scu-rat

50 ivn-dog

9 par-dog

10000 skn-cat

60 ivn-cat

400 ipr-gpg

LCLo values to mammals in inhalation exposure, ppm :

1000 ihl-rat, 8hr, Sax 1986

Other information of mammals :

Rat: inhalation: LC0: 200 - 300 ppm, 2 hr

Rat: inhalation: LC33: 200 - 300 ppm, 4 hr

Rat: inhalation: LC100: 200 - 300 ppm, 8 hr

(Patty 1967).“

“Health effects: Moderately toxic. Can cause headaches, vertigo, nausea, vomiting, and diarrhea (Sax 1986).

Aqueous concentrations up to 4 % well tolerated. Produces transient anesthesia of mucous membranes. Ingestion of large volumes results in vomiting, diarrhea, CNS depression. Converted to benzoic and hippuric acids. One human fatality attributed to rectal administration of 45 ml ... Dermal application to humans ... exerts narcotic action. Low toxicity. High exposure can cause decrease in blood pressure, depressant effect on system and death through respiratory paralysis (Sax 1986).”

As taken from SKYE data bank of environmental chemicals.

Oral, rat LD50: 1610 mg/kg bw (OECD SIDS Initial Assessment Report, 2001)

Inhalation rat LC50: >4.178 mg/l/4h (OECD SIDS Initial Assessment Report, 2001))

Dermal, guinea pig LD50: >5 mg/kg bw

Inhalation, rat LC50: >4.178 mg/L/4h

As taken from Bassan A et al. 2012. Reports on toxicokinetics, toxicity and allergenicity data on substances to be evaluated as acceptable previous cargoes for edible fats and oils (NP/EFSA/CONTAM/2011/01) - Batches n. 1, 2 and 3.

Type of Test	Route of Exposure or Administration	Species / Test System	Dose Data	Toxic Effects	Reference
LC50 - Lethal concentration, 50 percent kill	Inhalation	Rodent - mouse	>500 mg/m3	Behavioral somnolence (general depressed activity) - Behavioral ataxia Lungs, Thorax, or Respiration - respiratory depression	VCVGK* "Vrednie chemichescie veshestva, galogen I kislorod sodergashie organicheskije soedinenia". (Hazardous substances. Galogen and oxygen containing substances), Bandman A.L. et al., Chimia, 1994. Volume(issue)/page/year : -,132,1984
LC50 - Lethal concentration, 50 percent kill	Inhalation	Rodent - rat	>500 mg/m3	Behavioral somnolence (general depressed activity) - Behavioral ataxia Lungs, Thorax, or Respiration - respiratory depression	VCVGK* "Vrednie chemichescie veshestva, galogen I kislorod sodergashie organicheskije soedinenia". (Hazardous substances. Galogen and oxygen containing substances), Bandman A.L. et al., Chimia, 1994. Volume(issue)/page/year : -,132,1984
LD50 - Lethal dose, 50 percent kill	Oral	Rodent - mouse	1360 mg/kg	Behavioral somnolence (general depressed activity) - Behavioral ataxia Lungs, Thorax, or Respiration - respiratory depression	VCVGK* "Vrednie chemichescie veshestva, galogen I kislorod sodergashie organicheskije soedinenia". (Hazardous substances. Galogen and oxygen containing substances), Bandman A.L. et al., Chimia, 1994. Volume(issue)/page/year : -,132,1984
LD50 - Lethal dose, 50 percent kill	Oral	Rodent - rat	1660 mg/kg	Behavioral somnolence (general depressed activity) - Behavioral ataxia Lungs, Thorax, or Respiration - respiratory depression	VCVGK* "Vrednie chemichescie veshestva, galogen I kislorod sodergashie organicheskije soedinenia". (Hazardous substances. Galogen and oxygen containing substances), Bandman

				respiratory depression	A.L. et al., Chimia, 1994. Volume(issue)/page/year : -,132,1984
LD50 - Lethal dose, 50 percent kill	Oral	Rodent - guinea pig	2500 mg/kg	Behavioral somnolence (general depressed activity) - Behavioral ataxia Lungs, Thorax, or Respiration - respiratory depression	VCVGK* "Vrednie chemichescie veshestva, galogen I kislorod sodergashie organicheskije soedinenia". (Hazardous substances. Galogen and oxygen containing substances), Bandman A.L. et al., Chimia, 1994. Volume(issue)/page/year : -,132,1984
LD50 - Lethal dose, 50 percent kill	Oral	Rodent - rabbit	1040 mg/kg	Behavioral somnolence (general depressed activity) - Behavioral ataxia Lungs, Thorax, or Respiration - respiratory depression	VCVGK* "Vrednie chemichescie veshestva, galogen I kislorod sodergashie organicheskije soedinenia". (Hazardous substances. Galogen and oxygen containing substances), Bandman A.L. et al., Chimia, 1994. Volume(issue)/page/year : -,132,1984
LD50 - Lethal dose, 50 percent kill	Intraperitoneal	Rodent - rat	650 mg/kg	Behavioral somnolence (general depressed activity) - Behavioral ataxia Lungs, Thorax, or Respiration - respiratory depression	VCVGK* "Vrednie chemichescie veshestva, galogen I kislorod sodergashie organicheskije soedinenia". (Hazardous substances. Galogen and oxygen containing substances), Bandman A.L. et al., Chimia, 1994. Volume(issue)/page/year : -,132,1984
LCLo - Lowest published toxic concentration	Inhalation	Rodent - rat	46 mg/m3	Brain and Coverings - other degenerative changes - Behavioral alteration of classical conditioning	VCVGK* "Vrednie chemichescie veshestva, galogen I kislorod sodergashie organicheskije soedinenia". (Hazardous substances. Galogen and oxygen containing substances), Bandman A.L. et al., Chimia, 1994. Volume(issue)/page/year : -,132,1984
LDLo - Lowest published toxic dose	Intraperitoneal	Rodent - rat	514 mg/kg	Behavioral ataxia	VCVGK* "Vrednie chemichescie veshestva, galogen I kislorod sodergashie

					organicheskie soedinenia". (Hazardous substances. Galogen and oxygen containing substances), Bandman A.L. et al., Chimia, 1994. Volume(issue)/page/year : -,132,1984
LD50 - Lethal dose, 50 percent kill	Administration onto the skin	Rodent - rabbit	2000 mg/kg	Details of toxic effects not reported other than lethal dose value	VCVGK* "Vrednie chemicheskies veshestva, galogen i kislorod sodergashie organicheskie soedinenia". (Hazardous substances. Galogen and oxygen containing substances), Bandman A.L. et al., Chimia, 1994. Volume(issue)/page/year : -,132,1984
LD50 - Lethal dose, 50 percent kill	Oral	Rodent - rat	1.5 mL/kg	Details of toxic effects not reported other than lethal dose value	TPKVAL Toksikologiya Novykh Promyshlennykh Khimicheskikh Veshchestv. Toxicology of New Industrial Chemical Substances. For English translation, see TNICS*. (Izdatel'stvo Meditsina, Moscow, USSR) No.1-1961- Volume(issue)/page/year : 11,135,1969
TDLo - Lowest published toxic dose	Intraperitoneal	Rodent - mouse	250 mg/kg	Behavioral ataxia	RTOPDW Regulatory Toxicology and Pharmacology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1- 1981- Volume(issue)/page/year : 73,509,2015
TDLo - Lowest published toxic dose	Intraperitoneal	Rodent - guinea pig	200 mg/kg	Behavioral ataxia	RTOPDW Regulatory Toxicology and Pharmacology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1- 1981- Volume(issue)/page/year : 73,509,2015
TCLo - Lowest published toxic concentration	Inhalation	Rodent - mouse	0.45 pph/10M	Behavioral antianxiety	PYTOEY Phytomedicine. (Gustav Fischer Verlag, Postfach 720143, D-70577 Stuttgart, Germany) V.1-1994-

					Volume(issue)/page/year : 23,1734,2016
LD50	intravenous	mouse	324mg/kg (324mg/kg)		Archives Internationales de Pharmacodynamie et de Therapie. Vol. 135, Pg. 330, 1962.
LD50	intraarterial	rat	441mg/kg (441mg/kg)	LUNGS, THORAX, OR RESPIRATION: CHRONIC PULMONARY EDEMA LUNGS, THORAX, OR RESPIRATION: DYSPNEA	Toxicology and Applied Pharmacology. Vol. 18, Pg. 60, 1971.
LD50	intraperitoneal	rat	400mg/kg (400mg/kg)		Raw Material Data Handbook, Vol.1: Organic Solvents, 1974. Vol. 1, Pg. 6, 1974.
LD50	intravenous	rat	53mg/kg (53mg/kg)	LUNGS, THORAX, OR RESPIRATION: DYSPNEA	Toxicology and Applied Pharmacology. Vol. 18, Pg. 60, 1971.
LD50	oral	rat	1230mg/kg (1230mg/kg)	BEHAVIORAL: SOMNOLENCE (GENERAL DEPRESSED ACTIVITY) BEHAVIORAL: EXCITEMENT BEHAVIORAL: COMA	Food and Cosmetics Toxicology. Vol. 2, Pg. 327, 1964.
LDLo	subcutaneous	rat	1700mg/kg (1700mg/kg)	SENSE ORGANS AND SPECIAL SENSES: MIOSIS (PUPILLARY CONSTRICTION) : EYE BEHAVIORAL: COMA KIDNEY, URETER, AND BLADDER: OTHER CHANGES	Revue Medicale de la Suisse Romande. Vol. 15, Pg. 561, 1895.

As taken from RTECS, 2019.

/LABORATORY ANIMALS: Acute Exposure/ A polyvinyl chloride (PVC) cup containing 10% w/v benzyl alcohol was fastened (using surgical tape) to the dorsal side of three male nude mice for 24 hours of contact. Following exposure, mice were immediately killed and specimens of the exposed areas and of an adjacent untreated area were taken for microscopic examination. The skin sections were fixed in formalin, dehydrated, and embedded in paraffin. Sections were stained with hematoxylin and eosin and scored using the Ingram & Grasso system. A typical section from benzyl alcohol-treated areas had severe compact hyperkeratosis, acanthosis, spongiosis, intracellular edema, and some areas of ulceration of the epidermis. The collagen bundles in the dermis appeared slightly fragmented and slight cell infiltration of the area was noted. The final score for benzyl alcohol was 22, the modal score for at least three animals. Scores greater than 21 were considered "unacceptably severe damage." The investigators acknowledged that male nude mice were not an ideal model for human skin; however, the study was done to establish the relative dermal tolerance of various penetration enhancers. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/LABORATORY ANIMALS: Acute Exposure/ Undiluted benzyl alcohol /was administered/ intravenously (via the tail vein) to groups of 10 mice (5 of each sex). Three different mice strains were used with the following dose ranges. CD2F1 mice received 0.05 to 0.2 mL/kg, B6D2F1 mice received 0.05 to 0.4 mL/kg, and C57BL/6N mice received 0.025 to 0.1 mL/kg. All mice weighed between 14 and 18 g. The highest dose given did not exceed the LD50. Body weight was determined prior to the start of dosing, and 1 week thereafter. Animals were observed for 14 days and postmortem examinations were performed on day 15. Blood samples were withdrawn from the abdominal aorta and analyzed for hemolysis and precipitation potential. Convulsions, dyspnea, and reduced mobility were noted at the first 24-hour observation in mice treated with all but the lowest dose of benzyl alcohol. Decreased body weight gain or slight decrease in body weight was noted in B6D2F1 and C57BL/6N mice treated with all but the lowest dose. Postmortem alteration included hyperemia and edema in most animals that had died during the observation period (number not reported). Occasional hemorrhagic foci were observed in the spleen of C57BL/6N mice from all dose groups that had survived benzyl alcohol treatment. The blood from benzyl alcohol- treated mice had a potential for hemolysis and precipitation. Undiluted benzyl alcohol was ranked the most toxic of the five vehicles tested, which included dimethyl sulfoxide, polyethylene glycol 400, dimethylformamide, and absolute ethanol. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/LABORATORY ANIMALS: Acute Exposure/ Rats could inhale air saturated with benzyl alcohol vapor for a maximum of 2 hours. Inhalation at a concentration of 1000 ppm for 8 hours caused death of three of six animals within 14 days of exposure. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/LABORATORY ANIMALS: Acute Exposure/ Three groups of six Sherman rats were exposed for 4 hours to a 2000-ppm concentration of benzyl alcohol vapor in normal atmosphere. Nine rats died within 14 days of exposure. The investigators considered the compound to be a moderate hazard. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/LABORATORY ANIMALS: Acute Exposure/ Acute intravenous toxicity of benzyl alcohol was determined in CD2F1 (0.05-0.2 mL/kg bw), B6D2F1 (0.05-0.4 mL/kg) and C57BL/6 mice. The lowest dose was a safe dose and the highest one was the dose causing mortality in no more than half the animals of each group. Clinical signs were convulsion, dyspnea and reduced mortality in all strains for 24 hours. The slight decrease in body weight in the first week following treatment

returned to normal in the second week. OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.303 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

/LABORATORY ANIMALS: Acute Exposure/ ...To test the toxicity of intravitreal injections of benzyl alcohol ... nine New Zealand rabbits were injected with either a control or ... benzyl alcohol calculated to give final injected concentrations of 0.0073%, 0.022%, 0.073%, 0.222%, and 0.733% benzyl alcohol. The 0.022% concentration corresponds to the concentration of benzyl alcohol in human eyes when 0.1 mL of /a / commercial /product/... is used. Baseline examination of the rabbits was performed along with postinjection examinations on days 1, 3, 7, and 14. The eyes were enucleated and examined by light and electron microscopic examinations. ... Eyes injected with benzyl alcohol concentrations of 0.073%, 0.222%, and 0.733% displayed changes in the outer retina including loss of, and shortening of, outer segments and photoreceptors. [Morrison VL et al; Retina 26 (3): 339-44 (2006); Comment in: Retina 26 (9): 1100 (2006)] **PEER REVIEWED** PubMed Abstract

/LABORATORY ANIMALS: Acute Exposure/ ...To assess retinal toxicity of the vehicle of triamcinolone, benzyl alcohol (BA), when injected into the vitreous cavity of rabbits.... 24 pigmented rabbits /were/ assigned into two groups: group 1 (experimental, n = 12) received intravitreal 0.1 ml of BA, and group 2 (control, n = 12) received intravitreal 0.1 mL of balanced salt solution (BSS); all injections were done in the right eyes. Clinical examinations [slit lamp biomicroscopy, indirect ophthalmoscopy, and three intraocular pressure (IOP) measurements] were done on both eyes before injection, at 1 and 3 hr post injection, together with electroretinograms (ERGs) at 3 days, 1, 2, 4, and 6 weeks following injections. Three rabbits from each group were euthanased at 1, 2, 4, or 6 weeks and eyes were sent for light and electron microscopic examination for quantitative morphometric measurements. ... The mean amplitudes of the a and b waves of the BA-injected eyes were 6.42 +/- 9.02 uv and 11.18 +/- 15.18 uv at 3 days, respectively, which were significantly reduced compared with the BSS-injected eyes (30.87 +/- 8.22 uv and 57.90 +/- 13.38 uv, respectively; P < 0.01 t-test) and the non-injected contralateral eyes (36.20 +/- 7.85 uv and 64.10 +/- 9.36 uv, respectively; P < 0.01 t-test). These ERG responses continued to be significantly reduced in the BA-injected eyes (P < 0.01 t-test) throughout the study period. The mean ganglion cell count was significantly reduced (P < 0.005 t-test) in the BA-injected eyes (8.42 +/- 2.4) compared with the BSS- and non-injected eyes (16.42 +/- 3.9 and 16.5 +/- 4.2, respectively). The mean thicknesses of the inner nuclear layer (INL) and outer nuclear layer (ONL) were significantly reduced (P < 0.005 t-test) in the BA-injected eyes (3.78 +/- 0.96 um and 11.77 +/- 1.29 um, respectively) compared with the BSS- (6.1 +/- 0.92 um and 21.82 +/- 0.95 um, respectively) and non-injected eyes (7.05 +/- 1.9 um and 22.49 +/- 1.01 um, respectively). Electron microscopy showed moderate to severe intracellular changes in the ganglion cell layer, INL, ONL, and photoreceptor layer at 6 weeks in BA-injected eyes, with no significant changes in BSS-injected eye. There was no significant rise in the IOP or clinical evidence of increased lens density during the study period in any of the eyes. /It was concluded that/ triamcinolone acetate's vehicle, BA, produced severe ERG and structural damage to the retina when injected intravitreally. [Mackey TA et al; Graefes Arch Clin Exp Ophthalmol 245 (6): 817-24 (2007)] **PEER REVIEWED** PubMed Abstract

/LABORATORY ANIMALS: Acute Exposure/ No fatalities or symptoms were found in rats exposed for 6 hr to a calculated concentration of 61 ppm nor were symptoms produced by exposures obtained by bubbling air through the liquid heated to 100 and 150 deg C. ... Doses of 0.2 mL/kg or more, administered to dogs by stomach tube induced emesis and defecation. This was apparently due to irritation of the gastric mucosa ... Diuresis was more pronounced in the rabbit than in the dog, after administration of benzyl alcohol by various routes. The injection of 5 to 10% benzyl

alcohol in oil of sweet almond in the region of the auditory meatus of cats caused temporary degeneration of the small facial nerves. Clayton, G. D. and F. E. Clayton (eds.). Patty's Industrial Hygiene and Toxicology: Volume 2A, 2B, 2C: Toxicology. 3rd ed. New York: John Wiley Sons, 1981-1982., p. 4639

/LABORATORY ANIMALS: Acute Exposure/ Mice suffered respiratory stimulation, respiratory and muscular paralysis, convulsions, and ... /CNS depression/ following sc injection. ... Observed a decrease in arterial blood pressure of rabbits, cats, and dogs following iv injection of benzyl alcohol, but failed to find such decrease in the case of dogs following oral administration of 0.1-1.0 mL/kg of bw. ... Iv injection of 94% ... into dogs caused dyspnea, diarrhea, ataxia, mydriasis, nystagmus, urination, respiratory arrest, collapse, and cardiac arrest. In some instances deaths were delayed; in these cases, death was due to pulmonary hemorrhage and edema. Clayton, G. D. and F. E. Clayton (eds.). Patty's Industrial Hygiene and Toxicology: Volume 2A, 2B, 2C: Toxicology. 3rd ed. New York: John Wiley Sons, 1981-1982., p. 4639

/LABORATORY ANIMALS: Acute Exposure/ When aqueous humor was replaced in one /rabbit/ eye with pure sterile 0.9% sodium chloride solution and in the other eye was replaced with the same solution plus 2% benzyl alcohol, no toxic effect was produced by the plain saline solution, but the eyes with benzyl alcohol solution rapidly developed evidence of injury of endothelium, with much bluish swelling of the cornea. Also, the irises in the eye with benzyl alcohol solution became hyperemic and had poorly reactive pupils. The corneal edema in rabbits disappeared more rapidly than in the human patients, clearing partially in one week and completely in two weeks. Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 143

/LABORATORY ANIMALS: Acute Exposure/ Benzyl alcohol (1 mL/kg iv) had no effect on respiration, ECG, or blood pressure on anesthetized monkeys and dogs. Lethal iv dose of 0.9% benzyl alcohol in anesthetized dogs was 0.83-1.06 g/kg. [Kimura ET et al; Toxicol Appl Pharmacol 18 (1): 60-8 (1971)] **PEER REVIEWED** PubMed Abstract

/LABORATORY ANIMALS: Acute Exposure/ Administration of 9% benzyl alcohol produced transient respiratory arrest in adult dogs and death in immature dogs, and 7% and 4.5% benzyl alcohol produced clonic seizures in puppies. [Deland FH; Toxicol Appl Pharmacol 25 (2): 153-6 (1973)] **PEER REVIEWED** PubMed Abstract

LD50 Mouse sc 950 mg/kg bw OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.259 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

LD50 Rat sc 1700 mg/kg bw OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.258 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

LD50 Guinea pig ip > 400-800 mg/kg bw OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.258 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

LD50 Rat ip > 400-800 mg/kg bw OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.258 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

LD50 Rat oral 1230-3120 mg/kg bw European Medicines Agency (EMA), The European Agency for the Evaluation of Medicinal Products, Veterinary Medicines Evaluation Unit, Committee for Veterinary Medicinal Products; Benzyl Alcohol, Summary Report (1997). Available from, as of July 8, 2008: <http://www.emea.europa.eu/pdfs/vet/mrls/Benzylalcohol.pdf>

LD50 Mouse iv < 0.5 mL/kg /94% benzyl alcohol/ [Kimura ET et al; Toxicol Appl Pharmacol 18 (1): 60-8 (1971)] **PEER REVIEWED** PubMed Abstract

LD50 Rabbit oral 1940 mg/kg Bingham, E.; Cohrssen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. V6 498

LD50 Guinea pig dermal <5 mL/kg Bingham, E.; Cohrssen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. V6 498

LC50 Rat inhalation 1000 ppm/8 hr Bingham, E.; Cohrssen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. V6 498

LD50 Rat iv 53 mg/kg bw OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.259 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

LD50 Rat iv 314 mg/kg bw OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.259 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

LD50 Mouse CD-1, Male ip 1000mg/kg bw after 4 hours OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.258 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

As taken from HSDB, 2018.

"In adults, a single case of toxicity linked to the administration of benzyl alcohol has been reported in the literature even if the role of benzyl alcohol is uncertain. The dose administered was estimated as 90 mg/kg intravenously."

As taken from EMA, 2017.

"The Panel noted that benzyl alcohol was of low acute toxicity."

As taken from EFSA, 2019

LD50 Rat Oral 1.3 g/kg

LC50 Rat inhalation >4.15 mg/l

As taken from IGS, 2021

"B [benzyl alcohol] shows minor systemic toxicity after dermal absorption. In animal experiments, the dermal LD50 for rabbits was > 2000 mg per kg of body weight; < 5 ml per kg of body weight were reported for guinea pigs."

"Animal experiments yielded oral LD50 values of 1000–3100 mg per kg of body weight for rats, rabbits and guinea pigs. Toxicity symptoms included CNS depression, irritability and coma."

Inhalative exposure must be expected to entail irritations of the respiratory tract and also local anaesthetic effects as those observed in the eyes, the skin and the oral mucosae (see below). 4-hour exposure to 4178 mg/m³ B vapours or aerosols in an animal experiment conducted in

accordance with OECD directive in rats, caused a temporarily reduced respiratory rate as a symptom of sensory irritation. Histological injuries in the respiratory tract or lethal effects did not occur. However, according to data from an older study, deaths occurred in rats after 8-hour exposure to this level (8-hour LC50: 4492 mg/m³ = 1000 ppm; further details were not provided).

As taken from GESTIS

5.2. Repeated dose toxicity

“NSF International has evaluated the noncancer oral toxicity data for benzyl alcohol, and calculated a reference dose (RfD) of 0.5 mg/kg-day. The RfD was calculated based on the NOAEL of 200 mg/kg-day from a two-year gavage study in mice (NTP, 1989). This experimental dose was adjusted from a five-day dosing schedule to a daily dose of 143 mg/kg-day (200 mg/kg-day x 5 days/7 days = 143 mg/kg-day). NSF International applied a composite uncertainty factor of 300 (10 each for intra- and interspecies extrapolation and 3 for database deficiencies).”

As taken from ITER, 2018.

/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ Groups of 20 F344/N rats and B6C3F1 mice (10 of each sex) received 50, 100, 200, 400, or 800 mg/kg benzyl alcohol, 5 days a week for 13 weeks. Experimental conditions were the same as in the 16-day study. The death of five rats was attributed to rupture caused by the gavage procedure. Gavage related deaths were considered to result from the trauma of the gavage procedure combined with the neurotoxic/anesthetic effect of the compound. Aside from these, four male rats and one female of the 800-mg/kg group, as well as one female of the 400-mg/kg group and one male of the 200-mg/kg group died on study. The 800-mg/kg group had signs of neurotoxicity, including staggering, labored breathing, and lethargy after dosing. Blood around the nose and mouth was noted in 5 of 10 males of this group after week 8. Compared to vehicle controls, final mean body weights were 7% and 5% smaller, respectively, in male and female rats of the highest dose group. At histopathologic evaluation, lesions observed in rats of the highest dose group included necrosis of the dentate gyrus of the hippocampus in 7 of 7 males and 9 of 9 females; skeletal muscle necrosis in 5 of 10 males, thymic congestion, hemorrhage, and atrophy in 8 of 10 males, and nephrosis in 6 of 9 males. Renal lesions were similar to those noted in age-related spontaneous renal disease. Nine of 10 deaths (mice) were attributed to the gavage procedure. Final mean body weights of females of the 400- and 800-mg/kg groups were 5% and 8% lower, respectively, than the vehicle control. Staggering was noted during the first and second weeks of dosing in mice of the high-dose group. No compound-related histopathologic alterations were observed. A Sendai virus infection was suspected. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] ****PEER REVIEWED**** PubMed Abstract

/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ Technical grade benzyl alcohol (99% pure) in corn oil at doses of 125, 250, 500, 1000, or 2000 mg/kg was administered to groups of 10 F344/N rats and B6C3F1 mice (5 of each sex). Animals were dosed 5 days a week for 16 days (total of 12 doses). Feed and water were provided ad libitum. On days 8 and 9, both rats and mice of the 125-mg/kg group received doses that were 10-fold too high. All rats that received 2000 mg/kg and two of five males and three of five females that received 1000 mg/kg benzyl alcohol died before the end of the study. Rats of the two highest dose groups had blood around the nose and mouth, subcutaneous hemorrhages, and blood in the urinary and gastrointestinal tracts. Final body weight of male rats of the 1000 mg/kg group was 18% less than that of vehicle controls. Lethargy was observed in rats of the two highest dose groups; rough coats were noted in males of the 500- and 1000-mg/kg groups and in females of the 250- and 500-mg/kg groups. No compound-related histopathologic changes were noted. All mice that received 2000 mg/kg and one of five males and two of five females that received 1000 mg/kg benzyl alcohol died before the end of the study. Lethargy and rough coats were noted in males that received $\geq$ 500 mg/kg and in females that

received $\geq$ 1000 mg/kg. Blood in the urinary bladder was noted at necropsy in mice of the two highest dose groups. No compound related histopathologic changes were noted. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ Benzyl alcohol was administered by gavage daily and for 8 days to mice at doses of 325, 645, 1300, and 2595 mg/kg/day. Decreased muscle coordination, a "hunched" appearance, depression, and fur changes were reported in mice given 645 mg/kg, but not in those receiving 325 mg/kg or below. At 1300 mg/kg, animals additionally suffered breathing difficulties, discharge from the eyes, and various CNS effects, and death occurred on day 1 in all mice given 2595 mg/kg. [OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.274 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ Fischer 344 rats were given oral doses of 50, 100, 200, 400, and 800 mg/kg for 13 weeks. The high dose produced clinical signs indicative of neurotoxicity including staggering, respiratory difficulty, and lethargy. Reduction in weight gain was noted in males at 800 mg/kg and females at equal to or greater than 200 mg/kg. The high dose animals also showed hemorrhages around the mouth and nose, and histological lesions in the brain, thymus, skeletal muscle, and kidney. Bingham, E.; Cohrssen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. V6 498

/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ B6C3F1 mice were given oral doses of 50, 100, 200, 400, and 800 mg/kg for 13 weeks. The high dose appeared to produce clinical signs of neurotoxicity. Reduction in weight gain was noted in males at equal to or greater than 400 mg/kg and females at equal to or greater than 200 mg/kg. No treatment related histopathological effects were noted. Bingham, E.; Cohrssen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. V6 498

/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ Benzyl alcohol applied daily to guinea pigs with experimental trichophytosis on surface of scarred infected skin produced paralysis in hind limbs after several days. [Wollmann H et al; Pharmazie 22 (8): 455 (1967)] **PEER REVIEWED** PubMed Abstract

/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ The blood sugar of fasting animals was increased somewhat by prolonged admin of benzyl alcohol. Clayton, G. D. and F. E. Clayton (eds.). Patty's Industrial Hygiene and Toxicology: Volume 2A, 2B, 2C: Toxicology. 3rd ed. New York: John Wiley Sons, 1981-1982., p. 4640

16 Day Rat: Toxicology and carcinogenesis studies of technical-grade benzyl alcohol (99% pure), a textile dye additive, solvent, and food flavoring agent, were conducted by administering the chemical by gavage in corn oil vehicle to groups of F344/N rats ... of each sex for 16 days. In 16-day studies, all five male and five female rats ... dosed with 2,000 mg/kg benzyl alcohol died. Two of five male and 3/5 female rats ... dosed with 1,000 mg/kg died. Rats ... of each sex in the two highest dose groups were lethargic after dosing. Other toxic responses to benzyl alcohol in these dose groups included blood around the mouth and nose, subcutaneous hemorrhages, and blood in the urinary and gastrointestinal tracts of rats. ... Animals administered lower doses of benzyl alcohol (125, 250, or 500 mg/kg) had no compound-related histologic lesions. [DHHS/NTP; Toxicology and Carcinogenesis Studies of Benzyl Alcohol in F344/N Rats and B6C3F1 Mice (Gavage Studies) (1989) Technical Rpt Series No. 343 NIH Pub No. 89-2599. Available from, as of November 21, 2014: <http://ntp-server.niehs.nih.gov/>

16 Day Mouse: Toxicology and carcinogenesis studies of technical-grade benzyl alcohol (99% pure), a textile dye additive, solvent, and food flavoring agent, were conducted by administering the chemical by gavage in corn oil vehicle to groups of ... B6C3F1 mice of each sex for 16 days. In 16-day studies, all five male and five female ... mice dosed with 2,000 mg/kg benzyl alcohol died. ... One of the 5 male and 2/5 female mice dosed with 1,000 mg/kg died. ... Mice of each sex in the two highest dose groups were lethargic after dosing. Other toxic responses to benzyl alcohol in these dose groups included blood around the mouth and nose, subcutaneous hemorrhages, ... and blood in the urinary bladder of mice. Animals administered lower doses of benzyl alcohol (125, 250, or 500 mg/kg) had no compound-related histologic lesions. [DHHS/NTP; Toxicology and Carcinogenesis Studies of Benzyl Alcohol in F344/N Rats and B6C3F1 Mice (Gavage Studies) (1989) Technical Rpt Series No. 343 NIH Pub No. 89-2599. Available from, as of November 21, 2014: <http://ntp-server.niehs.nih.gov/>

13 Weeks Rat: Toxicology and carcinogenesis studies of technical-grade benzyl alcohol (99% pure), a textile dye additive, solvent, and food flavoring agent, were conducted by administering the chemical by gavage in corn oil vehicle to groups of F344/N rats ... of each sex for ... 13 weeks. Doses selected for the 13-week studies were 0, 50, 100, 200, 400, and 800 mg/kg for rats. ... Eight of 10 male rats dosed with 800 mg/kg died during weeks 7 and 8; four of these deaths were described as gavage related. Rats dosed with 800 mg/kg exhibited clinical signs indicative of neurotoxicity including staggering, respiratory difficulty, and lethargy. Hemorrhages occurred around the mouth and nose, and there were histologic lesions in the brain, thymus, skeletal muscle, and kidney. ... Some of the deaths in the rats ... may have been caused by a combination of the gavage procedure and chemical toxicity, since there was evidence that benzyl alcohol induced neurotoxic effects. There were reductions in relative weight gain in male rats dosed with 800 mg/kg benzyl alcohol and in female rats dosed with 200 mg/kg or more. ... No notable changes in body weight gain or compound-related histopathologic lesions were observed in rats ... from the lower dose groups. DHHS/NTP; Toxicology and Carcinogenesis Studies of Benzyl Alcohol in F344/N Rats and B6C3F1 Mice (Gavage Studies) (1989) Technical Rpt Series No. 343 NIH Pub No. 89-2599. Available from, as of November 21, 2014: <http://ntp-server.niehs.nih.gov/>

13 Weeks Mouse: Toxicology and carcinogenesis studies of technical-grade benzyl alcohol (99% pure), a textile dye additive, solvent, and food flavoring agent, were conducted by administering the chemical by gavage in corn oil vehicle to groups of ... B6C3F1 mice of each sex for ... 13 weeks. Doses selected for the 13-week studies were 0, 50, 100, 200, 400, and 800 mg/kg for ... mice. ... In mice, deaths were scattered among all dose levels, but none occurred in vehicle controls. Four male and six female mice died after being dosed; all deaths but one were described as gavage related. Staggering after dosing also occurred during the first 2 weeks of the studies in mice dosed with 800 mg/kg. Some of the deaths in the ... mice may have been caused by a combination of the gavage procedure and chemical toxicity, since there was evidence that benzyl alcohol induced neurotoxic effects. There were reductions in relative weight gain in ... male mice dosed with 400 or 800 mg/kg, and in female mice dosed with 200 mg/kg or more. No notable changes in body weight gain or compound-related histopathologic lesions were observed in ... mice from the lower dose groups. [DHHS/NTP; Toxicology and Carcinogenesis Studies of Benzyl Alcohol in F344/N Rats and B6C3F1 Mice (Gavage Studies) (1989) Technical Rpt Series No. 343 NIH Pub No. 89-2599. Available from, as of November 21, 2014: <http://ntp-server.niehs.nih.gov/>

As taken from HSDB, 2018

Flavouring agent (No.)	Species, sex	No. of test groups/ no. per group	Route	Length (days)	NOEL (mg/kg bw per day)	Reference
Benzyl alcohol	Rat, M,F	10/5	Gavage	16	130	National Toxicology

(25)	Mouse, M,F	10/5	Gavage	16	250	Program (1989)

As taken from WHO Food Additives Series 48

Species: Sex, No. Group	Route	Dose Levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
Rat; M, F 20	Gavage	0, 50, 100, 200, 400, 800 mg/kg bw/day	13 wks	100	NTP, 1989a	Fully described NTP study. Reduced relative weight gain in females at 200 mg/kg bw/day and more, and at 800 mg/kg bw/day in males.
Rat; M, F 100	Gavage	0, 200, 400 mg/kg bw/day	103 wks	ND	NTP	Fully described NTP study. Survival in both dose group of females was 50 % that of controls. This was, as concluded by NTP, primarily due to an increased number of gavage-related deaths. NTP conclusion on carcinogenicity: No evidence of carcinogenic activity.
Mouse; M, F 20	Gavage	0, 50, 100, 200, 400, 800 mg/kg bw/day	13 wks	100	NTP	Fully described NTP study. Reduced relative weight gain in females at 200 mg/kg bw/day and more, and at 400 and 800 mg/kg bw/day in males.
Mouse; M, F 100	Gavage	0, 100, 200 mg/kg bw/day	103 wks	200	NTP	Fully described NTP study. NTP conclusion on carcinogenicity: No evidence of carcinogenic activity.

As taken from EFSA (2012). Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific opinion on Flavouring Group Evaluation 20, Revision 4

(FGE.20Rev4): Benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters from chemical groups 23 and 30. Available at <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2012.2994/epdf>

Type of Test	Route of Exposure or Administration	Species / Test System	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Rodent - rat	2100 mg/kg/2 1D (intermittent)	Nutritional and Gross Metabolic - weight loss or decreased weight gain Related to Chronic Data - death	GISAAA Gigiena i Sanitariya. For English translation, see HYSAAV. (V/O Mezhdunarodnaya Kniga, 113095 Moscow, USSR) V.1- 1936- Volume(issue)/page/year: 50(7),81,1985
TDLo - Lowest published toxic dose	Oral	Rodent - rat	13 gm/kg/1 3W (intermittent)	Brain and Coverings - other degenerative changes Related to Chronic Data - death	NTPTR* National Toxicology Program Technical Report Series. (Research Triangle Park, NC 27709) No.206- Volume(issue)/page/year: NTP-TR-343,1989
TDLo - Lowest published toxic dose	Oral	Rodent - rat	24 mL/kg/1 2D (intermittent)	Liver - other changes Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels - dehydrogenases	CBPCEE Comparative Biochemistry and Physiology, C: Pharmacology, Toxicology and Endocrinology. (Elsevier Science, 660 White Plains Rd., Tarrytown, NY 10591) V.74- 1983- Volume(issue)/page/year: 99,445,1991
TDLo - Lowest published toxic dose	Oral	Rodent - mouse	12 gm/kg/1 6D (intermittent)	Related to Chronic Data - death	NTPTR* National Toxicology Program Technical Report Series. (Research Triangle Park, NC 27709) No.206- Volume(issue)/page/year: NTP-TR-343,1989
TCLo - Lowest published toxic concentration	Inhalation	Human	10 pph/45 D (intermittent)	Behavioral - somnolence (general depressed activity) Behavioral - headache Gastrointestinal - nausea or vomiting	VCVGK* "Vrednie chemicheskije veshestva, galogen i kislorod sodergashie organicheskie soedinenia". (Hazardous substances. Galogen and oxygen containing substances), Bandman A.L. et al., Chimia, 1994. Volume(issue)/page/year: -,132,1994
TCLo - Lowest published	Inhalation	Mammal - specie	42 mg/m3/ 122D	Cardiac - other changes Liver - other	VCVGK* "Vrednie chemicheskije veshestva, galogen i kislorod sodergashie organicheskie soedinenia". (Hazardous

d toxic concentration		s unspecified	(intermittent)	changes Kidney/Ureter/Bladder - other changes	substances. Galogen and oxygen containing substances), Bandman A.L. et al., Chimia, 1994. Volume(issue)/page/year: -,132,1994
TDLo - Lowest published toxic dose	Oral	Rodent - rat	103000 mg/kg/103W (continuous)	Endocrine - tumors Tumorigenic - active as anti-cancer agent	NTIS** National Technical Information Service. (Springfield, VA 22161) Formerly U.S. Clearinghouse for Scientific & Technical Information. Volume(issue)/page/year: PB90-110206
TDLo - Lowest published toxic dose	Oral	Rodent - rat	25200 mg/kg/6W (intermittent)	Nutritional and Gross Metabolic - metabolic acidosis	TOXID9 Toxicologist. (Soc. of Toxicology, Inc., 475 Wolf Ledge Parkway, Akron, OH 44311) V.1- 1981- Volume(issue)/page/year: 44,55,2005
TDLo - Lowest published toxic dose	Oral	Rodent - rat	52000 mg/kg/13W (intermittent)	Brain and Coverings - other degenerative changes Kidney/Ureter/Bladder - changes in tubules (including acute renal failure, acute tubular necrosis) Related to Chronic Data - death	HPV141 U.S. Environmental Protection Agency; High Production Volume (HPV) Challenge; Benzyl Derivatives.pdf http://www.epa.gov/HPV/pubs/summaries/benzylde/c13450tc.htm Volume(issue)/page/year: -, -,2001
TDLo - Lowest published toxic dose	Oral	Rodent - rat	206000 mg/kg/103W (intermittent)	Related to Chronic Data - death	HPV141 U.S. Environmental Protection Agency; High Production Volume (HPV) Challenge; Benzyl Derivatives.pdf http://www.epa.gov/HPV/pubs/summaries/benzylde/c13450tc.htm Volume(issue)/page/year: -, -,2001
TDLo - Lowest published toxic dose	Oral	Rodent - rat	10000 mg/kg/16D (intermittent)	Related to Chronic Data - death	HPV141 U.S. Environmental Protection Agency; High Production Volume (HPV) Challenge; Benzyl Derivatives.pdf http://www.epa.gov/HPV/pubs/summaries/benzylde/c13450tc.htm Volume(issue)/page/year: -, -,2001
TDLo - Lowest published toxic dose	Oral	Rodent - mouse	10000 mg/kg/16D (intermittent)	Behavioral - somnolence (general depressed activity) Related to Chronic Data - death	HPV141 U.S. Environmental Protection Agency; High Production Volume (HPV) Challenge; Benzyl Derivatives.pdf http://www.epa.gov/HPV/pubs/summaries/benzylde/c13450tc.htm Volume(issue)/page/year: -, -,2001
TDLo - Lowest published	Oral	Rodent - mouse	52000 mg/kg/13W	Behavioral - changes in motor activity	HPV141 U.S. Environmental Protection Agency; High Production Volume (HPV) Challenge; Benzyl Derivatives.pdf

d toxic dose			(intermittent)	(specific assay)	http://www.epa.gov/HPV/pubs/summaries/benzylde/c13450tc.htm Volume(issue)/page/year: -, -, 2001
TDLo - Lowest published toxic dose	Oral	Rodent - mouse	6000 mg/kg/8 D (intermittent)	Behavioral - ataxia Lungs, Thorax, or Respiration - dyspnea Related to Chronic Data - death	HPV141 U.S. Environmental Protection Agency; High Production Volume (HPV) Challenge; Benzyl Derivatives.pdf http://www.epa.gov/HPV/pubs/summaries/benzylde/c13450tc.htm Volume(issue)/page/year: -, -, 2001

As taken from RTECS, 2019.

Based on two years feeding studies (by gavage), a NOAEL of > 400 mg/kg b.w. was set for rats and of > 200 mg/kg b.w. for mice. At higher doses lethargy, respiratory problems, staggering, hemorrhages around mouth and nose, reduction in weight gain and histological lesions in brain, thymus, kidney and skeletal muscle were observed. Benzyl alcohol has been administered by gavage to mice five days a week for 16 days. At doses of 1,000 and 2,000 mg/kg blood in the urinary bladder was observed; at 800 mg/kg animals showed staggering. (US NTP Technical Report No. TR 343, 1989. Ks: 2). A study has been conducted to evaluate the effects of the administration of benzyl alcohol to rat pups by gavage for 6 weeks. After 3 weeks, at the dose of 600 mg/kg per day, a decrease in body weight gain, severe respiratory problems and excessive salivation have been observed. No histopathological changes have been observed. Based on weight results, a NOAEL of 300 mg/kg per day was established (Foulon et al., 2005. Ks: 4).

As taken from Bassan A et al. 2012. Reports on toxicokinetics, toxicity and allergenicity data on substances to be evaluated as acceptable previous cargoes for edible fats and oils (NP/EFSA/CONTAM/2011/01) - Batches n. 1, 2 and 3. Available at <http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2012.EN-274/epdf>

Record for benzenemethanol:

Species Common Name	Exposure Type	Test Location	Conc 1 Type (Author)	Conc 1 (Author)	Conc 1 Units (Author)	Effect	Effect Measurement	Endpoint	Exposure Duration Mean (Days)	Response Site	Observed Duration (Days)
House Mouse	Gavage	Lab	Formulation	750	mg/kg bdwt/d	Growth	Weight gain	LOEL	18	Whole organism	18
House Mouse	Gavage	Lab	Formulation	750	mg/kg bdwt/d	Growth	Weight gain	LOEL	18	Whole organism	18
House Mouse	Gavage	Lab	Formulation	750	mg/kg bdwt/d	Growth	Weight	LOEL	15	Whole organism	15
House Mouse	Gavage	Lab	Formulation	750	mg/kg g	Mortality	Survival	NOEL	18	Not reported	18

Mouse			tion		bdwt/d	lity				d	
House Mouse	Gavage	Lab	Formula tion	750	mg/kg bdwt/d	Mortality	Mortality	NR	18	Not reported	18

As taken from the US EPA ECOTOX database, 2021

The US EPA has evaluated the noncancer toxicity of benzyl alcohol and has set a reference dose of 0.1 mg/kg-day (US EPA, 2019b).

"Ingredient chemicals like fragrances may cause adverse health effects. Frequent health risk assessments and stringent management of consumer products are of paramount importance to reduce these serious occurrences. In this study, the respiratory and dermal health effects were assessed in relation to air fresheners. Twenty six fragrance ingredients, thirty four biocidal ingredients, and sixteen hazardous ingredients were analyzed and assessed according to their risk to human health on five groups by application type in eighty two air fresheners. For hazard characterization of ingredients, toxicological information on the intrinsic properties of the ingredients was collected, and reference values were determined as chronic NOAEL. Exposure assessment was performed in two steps. The 95th exposure factor values were used to estimate exposure to assume the worst-case scenario and the maximum concentration determined by the product purpose and application type was used type in tiered 1 assessment. The values input into the exposure algorithms were developed via the exposure route. In the tiered 2 assessment, the 75th exposure factor values were used to estimate the assumed reasonable exposure to ingredients. Six ingredients for the inhalation and twelve ingredients for the dermal route were conducted for tiered 2 assessment. This study showed that the assessed ingredients have no health risks at their maximum concentrations in air fresheners. The approach should be used to establish improved guidelines for specific ingredients that may pose inhalation and dermal hazard." As taken from Kim JH et al. 2018. Regul. Toxicol. Pharmacol. 98, 31-40. PubMed, 2019 available at: <https://www.ncbi.nlm.nih.gov/pubmed/29857116>

Quantitative Risk Type	Quantitative Risk Value	Product Use	Safety Evaluation Owner	POD Method	POD Value	POD Owner
Not calculated	Not calculated	Not specified	COSMOS TTC (NON-CANCER)	NOAEL	66.7	COSMOS TTC (NON-CANCER)

Adjustment factors used in calculations:

Adjustment factor: Study: Dose Duration: 3.0 (3)

Critical study: RAT (Reproductive/Developmental Toxicity) Oral - dietary exposure for 1 GEN

NOEL/LEL Owner	Original NOEL	Original LEL	Critical Sites	Critical Effects
PAFA	200.0 mg/kg bw/day	400.0 mg/kg bw/day		• BODY WEIGHT CHANGES

Safety Evaluation Comments: no comments available.

Source Document: no source document available

POD Method	POD Value	POD Owner
NOEL	100.0	MUNRO

Lowest-observed effect

Owner	Type	Value	Sites	Effects
MUNRO	LOEL	200.0 mg/kg bw/day	UNSPECIFIED - MULTIPLE EFFECTS	UNSPECIFIED - MULTIPLE EFFECTS

No-observed effect: MUNRO: NOEL: 100.0 mg/kg bw/day

Adjustment factors: >Critical study: Carcinogenicity > Chronic/Carcinogenicity (Mouse, Oral - Gavage/Intubation exposure) for 720 day

POD Method	POD Value	POD Owner
HNEL	Not Calculated	PAFA

Lowest-observed effect

Owner	Type	Value	Sites	Effects
PAFA	LOAEL	800.0 mg/kg bw/day	- BODY WEIGHT CHANGES - CLINICAL SIGNS - CONDITION; RESPIRATORY - BRAIN - PATHOLOGY - MORTALITY	- BODY WEIGHT CHANGES - CLINICAL SIGNS - CONDITION; RESPIRATORY - BRAIN - PATHOLOGY - MORTALITY

No-observed effect: PAFA: HNEL: 400.0 mg/kg bw/day

Adjustment factors: >Critical study: Target Organ Toxicity > Subchronic Toxicity (Rat, Oral - Gavage/Intubation exposure) for 13 week

As taken from the COSMOS database available at <https://ng.cosmosdb.eu/https://cosmosdb.eu/cosmosdb.v2/dbsearch/full>

“Read-across material benzyl alcohol (CAS # 100-51-6) has numerous repeated dose toxicity studies. Gavage 13- week subchronic toxicity studies were conducted with benzyl alcohol in rats and mice by the US National Toxicology Program (NTP). The NOAEL was determined to be 100 mg/kg/day, based on reduced bodyweight gain (NTP, 1989; data also available in National Toxicology Program, 1980a).”

As taken from Api AM et al. 2020. Food Chem. Toxicol. 141(S1), 111338. Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/536-60-7_0.pdf

“With 13-week (5 days per week via gavage) oral administration of B [benzyl alcohol] to rats, 800 mg per kg of body weight per day led to lethargy, breathing disorders and histopathological changes in the brain, the thymus, the skeletal muscles and the kidneys. Following 2-year oral administration, no substance-related changes in body and organ weights or histopathological organ changes occurred up to the highest investigated dose of 400 mg per kg of body weight per day in rats, and 200 mg per kg of body weight per day in mice.”

As taken from GESTIS

Name	Updated on	Route of administration	Threshold	Information for the Package Leaflet	Comments

Benzyl alcohol	09/10/2017	All	Zero	This medicine contains x mg benzyl alcohol in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>. Benzyl alcohol may cause allergic reactions.	
Benzyl alcohol	09/10/2017	Oral, parenteral	Zero	Benzyl alcohol has been linked with the risk of severe side effects including breathing problems (called "gasping syndrome") in young children. Do not give to your newborn baby (up to 4 weeks old), unless recommended by your doctor.	Intravenous administration of benzyl alcohol has been associated with serious adverse events and death in neonates ("gasping syndrome"). The minimum amount of benzyl alcohol at which toxicity may occur is not known. Warning in section 4.4 in the SmPC should be given if used in neonates.
Benzyl alcohol	09/10/2017	Oral, parenteral	Zero	Do not use for more than a week in young children (less than 3 years old), unless advised by your doctor or pharmacist.	Increased risk due to accumulation in young children.
Benzyl alcohol	09/10/2017	Oral, parenteral	Zero	Ask your doctor or pharmacist for advice if you are pregnant or breast-feeding. This is because large amounts of benzyl alcohol can build-up in your body and may cause side effects (called "metabolic acidosis").	
Benzyl alcohol	09/10/2017	Oral, parenteral	Zero	Ask your doctor or pharmacist for advice if you have a liver or kidney disease. This is because large amounts of benzyl alcohol can build-up in your body	High volumes should be used with caution and only if necessary, especially in subjects with liver or

				and may cause side effects (called "metabolic acidosis").	kidney impairment because of the risk of accumulation and toxicity (metabolic acidosis).
Benzyl alcohol	09/10/2017	Topical	Zero	Benzyl alcohol may cause mild local irritation.	

EMA (2022)

5.3. Reproduction toxicity

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ In a study which assayed the teratogenic activity of ethinyloestradiol sulfonate in Wistar rats, a vehicle control group that was treated with benzyl alcohol/peanut oil was maintained. On GDs 10, 13, 6 to 10, or 10 to 14, rats (number not stated) received intraperitoneal (ip) injections of either the test material or an unspecified amount of vehicle. Fetuses were removed on day 21 and examined. No teratogenic effect was noted. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] ****PEER REVIEWED**** [PubMed Abstract](http://www.ncbi.nlm.nih.gov/pubmed/11766131?dopt=Abstract)

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ A group of 50 pregnant SPF CD-1 albino mice was dosed with 550 mg benzyl alcohol/kg/day on GDs 6 to 15 by gavage. The benzyl alcohol was dissolved in corn oil; a vehicle-control group was maintained. Maternal status (survival, body weight changes), gestation index (length of gestation), reproductive index, postnatal survival, average litter weight, and average pup weight were comparable between treated and control animals. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] ****PEER REVIEWED**** [PubMed Abstract](http://www.ncbi.nlm.nih.gov/pubmed/11766131?dopt=Abstract)

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ In screening a new developmental toxicity assay, 50 pregnant CD-1 mice were gavaged on GDs 6 to 13 with benzyl alcohol at a rate of 750 mg/kg/day. The dose selected was the LD(10) value determined in preliminary dose-finding studies. Mice were allowed to deliver. Litter size, birth weight, and neonatal growth and survival to postnatal day 3 were measured. Nineteen (38%) of the dams of the benzyl alcohol group died prior to delivery; the corresponding vehicle control group (which received water) had no maternal death. (Mice that died were not necropsied.) Maternal weight was significantly less changed in the benzyl alcohol group (6.2 +/- 3.6 g) as compared to controls (7.9 +/- 2.3 g). Viability in the benzyl alcohol group was 21 of 22 litters (controls had 29/29 viability) with an average of 10.0 liveborns per litter. Birth weight (1.6 g/pup) and 3-day weight gain (0.5 g/pup) for pups of the Benzyl Alcohol treatment group were significantly less (p < .05) than the corresponding values in controls (1.7 and 0.7 g/pup, respectively). The reduced birth weight was classified as "some evidence of developmental toxicity." The researchers noted the 10% false-negative rate for toluene, a "presumptive teratogen." [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] ****PEER REVIEWED**** [PubMed Abstract](http://www.ncbi.nlm.nih.gov/pubmed/11766131?dopt=Abstract)

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ A group of 50 specific pathogen-free (SPF) CD-1 mice received 750 mg/kg/day benzyl alcohol (in distilled water) by gavage on GDs 7 to 14. (Earlier toxicity studies had determined the maximum tolerated dose was between 645 and 1300 mg/kg/day, and the 750-mg/kg/day dose was selected for the reproduction study.) The concurrent vehicle control had 50 mice. Mice were individually caged and feed and water were available ad libitum. Clinical observations were made daily. Maternal body weights were

recorded prior to dosing, on day 18, and on postnatal day 3; the weight on day 7 determined the dose volume administered over the entire treatment period. Mice were allowed to deliver their litters and nurse the pups for 3 days. There were 18 compound-related deaths during the dosing period, and one on GD 15. Mice that died were discarded without necropsy. No procedure-related deaths (i.e., gavage error) were recorded. Body tremors, hunching, subdued behavior, prostration, ataxia, swelling, and/or cyanosis of the abdomen and piloerection were noted in mice that died during the study as well as those that produced litters. No significant differences in reproductive and gestation indices, or in mean gestation length were noted between treated and control mice. A significantly lower day 18 mean body weight and a marginally reduced maternal weight on postpartum day 3 were noted in dosed dams. Decreased mean litter mean pup weight was noted on postpartum days 1 ($p < .01$) and 3 ($p < .001$). On postpartum days 1 to 3, a decreased mean litter weight change ($p < .05$) and decreased mean litter mean pup weight ($p < .001$) were noted. No significant differences were noted between treated and control pups in group litter viability. The investigators considered benzyl alcohol a suspect reproductive hazard and was 58 mg/kg/day. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ Fifty female mice were given benzyl alcohol at 550 mg/kg bw per day by gavage on days 6-15 of gestation; a further 50 mice received the corn oil vehicle. All dams were allowed to deliver naturally, and pups and dams were observed until day 3 post partum, when the experiment was terminated. Body weight, clinical observations, and mortality were recorded daily throughout treatment and up to day 3 post partum. Mortality was not significantly increased in animals given benzyl alcohol over that in the control group. One treated mouse showing languid behavior, labored breathing, and a rough coat died, but no other deaths or clinical signs were reported. Maternal body weight and body-weight gain during treatment and up to day 3 post partum were virtually identical for treated and control animals. All other parameters examined, including gestation index, average number of live pups per litter, and postnatal survival and pup body weight on days 0 and 3 post partum, were not significantly different from the control values. The authors concluded that, at the predicted LD₁₀, benzyl alcohol had no significant effects on the development of CD-1 mice. NOAEL = 550 mg/kg bw per day OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.27 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ Benzyl alcohol was evaluated for developmental toxicity in a proposed new short term in vivo animal bioassay. In this assay, pregnant mice were dosed with the test agent in mid-pregnancy and allowed to go to term. Observations were then made on litter size as well as the birth weight, neonatal growth, and survival of pups as indicators of developmental toxicity. Fifty pregnant CD-1 mice were given 750 mg/kg/day benzyl alcohol in water by gavage on days 6-13 of gestation and were allowed to deliver. A decrease in the birth weight and weight gain in the pups was observed, but was not toxic to the mothers and had no effect on pup viability. [Hardin BD et al; Teratog Carcinog Mutagen 7: 29-48 (1987)] **PEER REVIEWED** PubMed Abstract

/LABORATORY ANIMALS: Developmental or Reproductive Toxicity/ ... 0.01 or 0.02 mL of benzyl alcohol /injected/ into yolk sac of the chick from before incubation up to the 7th day. Meningoceles and skeletal defects were produced. Shepard, T.H. Catalog of Teratogenic Agents. 5th ed. Baltimore, MD: The Johns Hopkins University Press, 1986., p. 71

/LABORATORY ANIMALS: Acute Exposure/ The length of the estrus cycle was reduced when 0.52-2.1 d (1-4 mg/kg bw) benzyl alcohol was injected into the uterus of each of 48 cows. OECD;

SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.300 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

As taken from HSDB, 2018

"The margin of exposure for Benzyl alcohol is adequate for the developmental and reproductive toxicity endpoints at the current level of use... The developmental toxicity data on benzyl alcohol are sufficient for the developmental toxicity endpoint. A gavage post-natal screening study conducted in mice determined the developmental NOAEL to be 550 mg/kg/day, the only dosage tested (RIFM, 1986a). In a separate gavage post-natal screening study conducted in mice at 750 mg/kg/day, reduced pup body weights were noted (Hardin et al., 1987; data also available in RIFM, 1983). This effect occurred in the presence of significant maternal toxicity. Therefore, the MOE for developmental toxicity is equal to the NOAEL in mg/kg/day divided by the total systemic exposure, 550/0.036 or 15278.

The reproductive toxicity data on benzyl alcohol are insufficient for the reproductive toxicity endpoint. In gavage mouse post-natal screening studies, no maternal toxicity was observed at 550 mg/kg/day (RIFM, 1986a), while significant maternal toxicity (mortality and adverse clinical signs) was observed when the dosage was increased to 750 mg/kg/day (Hardin et al., 1987; data also available in RIFM, 1983). There are no male reproductive toxicity data on benzyl alcohol. Benzyl acetate (CAS # 140-11-4; see Section V) is rapidly hydrolyzed to benzyl alcohol, which is then oxidized to benzoic acid (CAS # 65-85-0; see Section V) (numerous references, metabolic scheme detailed in Chidgey et al., 1986 and Yuan et al., 1995; see Section 6). Precursor benzyl acetate has 13-week dietary subchronic toxicity studies in rats and mice in which sperm morphology and vaginal cytology examinations were evaluated (Morrissey et al., 1988; data also available in NTP, 1993). There were no effects on sperm parameters in mice or rats up to the high dosage of 7900 or 3900 mg/kg/day, respectively. There were no effects on estrous cycling in female rats up to the high dosage of 4500 mg/kg/day. Lengthening of the estrous cycle occurred in high-dose female mice (9400 mg/kg/day), which the authors concluded was related to decreases in body weight. For the benzoic acid metabolite, a dietary chronic toxicity and 4-generation reproductive toxicity study conducted in rats determined the NOAEL for reproductive toxicity to be 1%, or 500 mg/kg/day, the highest dosage tested (Kieckebusch and Lang, 1960). These data indicate no specific concern for reproductive toxicity. The most conservative NOAEL was selected for this safety assessment. Therefore, the MOE for reproductive toxicity is equal to the benzoic acid NOAEL in mg/kg/day divided by the total systemic exposure, 500/0.036 or 13889."

As taken from Api AM et al., (2015).

"The chemical does not show specific reproductive or developmental toxicity. Any reproductive and developmental effects were only observed secondary to maternal toxicity."

As taken from AICIS, 2016.

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Sex/Duration	Toxic Effects	Reference

TDLo - Lowest published toxic dose	Oral	Rodent mouse	6 gm/kg	female 6-13 day(s) after conception	Reproductive - Effects on Newborn - growth statistics (e.g.%, reduced weight gain)	TCMUD8 Teratogenesis, Carcinogenesis, and Mutagenesis. (Alan R. Liss, Inc., 41 E. 11th St., New York, NY 10003) V.1- 1980- Volume(issue)/page/year: 7,29,1987

As taken from RTECS, 2019.

"In rats, no action on the growth, fertility, lactation or survival has been observed during a multigeneration reproductive study (because of the consumption of glycine occurring during elimination of benzoic acid and the possibility that glycine is essential for growth, a detrimental effect on growth could be envisaged). Toxicity on foetal development has only been observed at doses that are toxic to the mother. Three reproductive studies and 3 developmental toxicity studies exist, which lead to the conclusion that the no observable adverse effect level (NOAEL) after ingestion is in the order of 500 mg/kg/day."

As taken from EMA, 2017

"The Panel noted that reproductive studies were not available and that two developmental screening studies in mice were of limited design."

As taken from EFSA, 2019

Record for benzenemethanol:

Species Common Name	Exposure Type	Test Location	Conc 1 Type (Author)	Conc 1 (Author)	Conc 1 Units (Author)	Effect	Effect Measurement	Endpoint	Exposure Duration Mean (Days)	Response Site	Observed Duration (Days)
House Mouse	Gavage	Lab	Formulation	750	mg/kg bw/d	Reproduction	Progeny counts/numbers	NOEL	15	Not reported	15
House Mouse	Gavage	Lab	Formulation	750	mg/kg bw/d	Reproduction	Viability	NOEL	15	Not reported	15

As taken from the US EPA ECOTOX database, 2021

Read-across material benzyl alcohol (CAS # 100-51-6) has several gavage mouse postnatal screening studies; no maternal toxicity was observed at 550 mg/kg/day (Hardin, 1986), while significant maternal toxicity (mortality and adverse clinical signs) was observed when the dosage was increased to 750 mg/kg/day (Hardin et al., 1987; data also available in National Institute for Occupational Safety and Health, 1983). There are no male reproductive data on benzyl alcohol or any other read-across material."

The reproductive toxicity endpoint was evaluated using the TTC for a Cramer Class I material, and the exposure is below the TTC (0.03 mg/kg/day).

As taken from Api AM et al. 2020. Food Chem. Toxicol. 141(S1), 111338. Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/536-60-7_0.pdf

5.4. Mutagenicity

/GENOTOXICITY/ Benzyl alcohol was not mutagenic when tested by the preincubational protocol in the presence or absence of exogenous metabolic activation in Salmonella typhimurium strains TA98, TA100, TA1535, or TA1537. In the mouse L5178Y/TK+/- lymphoma assay, benzyl alcohol induced an increase in trifluorothymidine (Tft)-resistant cells in the absence, but not in the presence, of S9; the effect was associated with toxicity. In cytogenetic assays with Chinese hamster ovary (CHO) cells, treatment with benzyl alcohol produced an increase in sister chromatid exchanges (SCEs) which was judged to be equivocal both with and without S9; a significant increase in chromosomal aberrations was observed after exposure to benzyl alcohol in the presence, but not the absence, of S9. DHHS/NTP; Toxicology and Carcinogenesis Studies of Benzyl Alcohol in F344/N Rats and B6C3F1 Mice (Gavage Studies) (1989) Technical Rpt Series No. 343 NIH Pub No. 89-2599. Available from, as of November 21, 2014: <http://ntp-server.niehs.nih.gov/>

/GENOTOXICITY/ Benzyl alcohol was negative in the Ames test with and without metabolic activation, sex-linked recessive lethal (flies), and replicative DNA synthesis (male rats) assays. Results of a mouse lymphoma forward mutation assay in the absence of S9 activation /were considered/ to be "questionable," whereas a positive response /was reported/ at concentrations associated with toxicity. Both studies were negative with S9 activation. Benzyl alcohol, with S9 activation, was positive in the chromosome aberration test in Chinese hamster ovary (CHO) cells. Equivocal results were noted in the sister chromatid exchange (SCE) assay. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/GENOTOXICITY/ A Drosophila melanogaster SRL assay with benzylalcohol 5000 ppm (feed) and 8000 ppm (injection) was negative. OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.21 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

/GENOTOXICITY/ /In a/ recombination assay with Bacillus subtilus H17 and M45, /benzyl alcohol/ was positive. OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.19 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

/GENOTOXICITY/ In a cytogenic assay using CHO cells /benzyl alcohol/ was negative without metabolic activation and positive with metabolic activation. OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.19 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

/GENOTOXICITY/ Benzyl alcohol was not mutagenic /in the/ Escherichia coli reverse mutation assay ... with and without metabolic activation. OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.19 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

/GENOTOXICITY/ ... /Benzyl alcohol was/ evaluated for genotoxicity in the wing somatic mutation and recombination test (SMART) of Drosophila melanogaster. Third-instar larvae trans-heterozygous for two genetic markers mwh and flr, were treated at different concentrations (0.1,

0.5, 1, 10, 25 and 50 mM) of the test compounds. Wings of the emerging adult flies were scored for the presence of spots of mutant cells, which can result from either somatic mutation or mitotic recombination. Also lethal doses of benzyl derivatives used as flavor ingredients were determined in the experiments. For the evaluation of genotoxic effects, the frequencies of spots per wing in the treated series were compared to the control group, which is distilled water. [Demir E et al; Food Chem Toxicol 46 (3): 1034-41 (2008)] **PEER REVIEWED** PubMed Abstract

/GENOTOXICITY/ Benzyl alcohol was found to be negative when tested for mutagenicity in the Salmonella/microsome preincubation assay using the standard protocol approved by the National Toxicology Program (NTP). Benzyl alcohol was tested at doses of 0.1, 0.333, 1.0, 3.333, 5.0, and 6.666 mg/plate in as many as 5 Salmonella typhimurium strains (TA1535, TA1537, TA97, TA98, and TA100) in the presence and absence of rat or hamster liver S-9. The highest ineffective dose tested without toxicity in any S. typhimurium strains was 5.0 mg/plate. Slight inhibition of the background bacterial lawn occurred in cultures at 6.666 mg/plate but no significant change was seen in the results. Mortelmans K et al; Environ Mutagen 8: 1-119 (1986)

'In Vivo' Genotoxicity:

/GENOTOXICITY/ The A31-1-13 clone of BALB/c-3T3 cells was used to evaluate the transforming potential of numerous chemicals including benzyl alcohol. Each transformation assay contained a standard clonal survival assay, a co-culture clonal survival assay, and a transformation assay. ... Benzyl Alcohol can be oxidized by air and may have been altered during the treatment period. ... Benzyl Alcohol was noncytotoxic to BALB/c-3T3 cells and ... the statistical sensitivities for trial 1 and 2 were 2 and 38/110, respectively. Benzyl Alcohol was evaluated as active in this assay with actual and estimated rank t-statistics both 1.95. ... Benzyl alcohol was grouped as a noncytotoxic, nonmutagenic, noncarcinogenic chemical. OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.277 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

/GENOTOXICITY/ A Mouse Micronucleus assay using 50, 100, 200 mg/kg benzyl alcohol by ip injection was negative at all doses tested. [OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.21 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

/GENOTOXICITY/ A Replicative DNA Synthesis assay using male B6C3F1 male mice given a single dose of 0, 400 or 800 mg/kg bw benzyl alcohol by gavage was negative at all doses tested. OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.21 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

/GENOTOXICITY/ A Replicative DNA Synthesis assay using male Fischer 344 rats given a single dose of 0, 300, or 600 mg/kg bw benzyl alcohol by gavage was negative at all doses tested. OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.21 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

As taken from HSDB, 2018

Test System:	AMES SALMONELLA TYPHIMURIUM

Strain Indicator:	TA98
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Method:	PREINCUBATION
Dose:	100-6666 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-343 Y89]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA100
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Method:	PREINCUBATION
Dose:	100-6666 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-343 Y89]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1535

Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Method:	PREINCUBATION
Dose:	100-6666 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-343 Y89]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1537
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Method:	PREINCUBATION
Dose:	100-6666 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-343 Y89]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA98
Metabolic Activation:	HAMSTER, LIVER, S-9, AROCLOR 1254

Method:	PREINCUBATION
Dose:	100-6666 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-343 Y89]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA100
Metabolic Activation:	HAMSTER, LIVER, S-9, AROCLOR 1254
Method:	PREINCUBATION
Dose:	100-6666 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-343 Y89]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1535
Metabolic Activation:	HAMSTER, LIVER, S-9, AROCLOR 1254
Method:	PREINCUBATION

Dose:	100-6666 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-343 Y89]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1537
Metabolic Activation:	HAMSTER, LIVER, S-9, AROCLOR 1254
Method:	PREINCUBATION
Dose:	100-6666 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-343 Y89]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA98
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	100-6666 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)

Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-343 Y89]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA100
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	100-6666 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-343 Y89]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1535
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	100-6666 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE

Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-343 Y89]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA1537
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	100-6666 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-343 Y89]
Test System:	MOUSE LYMPHOMA
Strain Indicator:	L5178Y (TK+/TK-)
Metabolic Activation:	NONE
Method:	SUSPENSION/PLATE
Dose:	156.25-5000 UG/ML (TEST MATERIAL SOLVENT: DMSO)
Results:	POSITIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-343 Y89]

Test System:	MOUSE LYMPHOMA
Strain Indicator:	L5178Y (TK+/TK-)
Metabolic Activation:	NONE
Method:	SUSPENSION/PLATE
Dose:	250-3500 UG/ML (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-343 Y89]
Test System:	MOUSE LYMPHOMA
Strain Indicator:	L5178Y (TK+/TK-)
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Method:	SUSPENSION/PLATE
Dose:	250-3500 UG/ML (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-343 Y89]
Test System:	AMES SALMONELLA TYPHIMURIUM

Strain Indicator:	TA100
Metabolic Activation:	NONE
Method:	STANDARD PLATE
Dose:	100-1000 UG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[BALL,JC, FOXALL-VANAKEN,S AND JENSEN,TE; MUTAGENICITY STUDIES OF P-SUBSTITUTED BENZYL DERIVATIVES IN THE AMES SALMONELLA PLATE-INCORPORATION ASSAY; MUTAT. RES. 138(2-3):145-151, 1984]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA97
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	0.01-1 MG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[FUJITA,H, SUMI,C AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. VII;KENKYU NENPO - TOKYO-TORITSU EISEI KENKYUSHO 43:219-227, 1992]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA97

Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Method:	PREINCUBATION
Dose:	0.01-1 MG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[FUJITA,H, SUMI,C AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. VII;KENKYU NENPO - TOKYO-TORITSU EISEI KENKYUSHO 43:219-227, 1992]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA102
Metabolic Activation:	NONE
Method:	PREINCUBATION
Dose:	0.01-1 MG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[FUJITA,H, SUMI,C AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. VII;KENKYU NENPO - TOKYO-TORITSU EISEI KENKYUSHO 43:219-227, 1992]
Test System:	AMES SALMONELLA TYPHIMURIUM
Strain Indicator:	TA102
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254

Method:	PREINCUBATION
Dose:	0.01-1 MG/PLATE (TEST MATERIAL SOLVENT: DMSO)
Results:	NEGATIVE
Reference:	[FUJITA,H, SUMI,C AND SASAKI,M; MUTAGENICITY TEST OF FOOD ADDITIVES WITH SALMONELLA TYPHIMURIUM TA97 AND TA102. VII;KENKYU NENPO - TOKYO-TORITSU EISEI KENKYUSHO 43:219-227, 1992]
Test System:	CHO CELLS
End Point:	IN VITRO CHROMOSOMAL ABERRATIONS
Metabolic Activation:	NONE
Dose:	160; 500; 1600; 5000 UG/ML (TEST MATERIAL SOLVENT: DMSO)
Dose Regimen:	8 HR TREATMENT, 4.5 HR RECOVERY WITH COLCEMID ADDED IN FINAL 2-2.5 HR
Results:	NEGATIVE
Reference:	[ANDERSON,BE, ZEIGER,E, SHELBY,MD, RESNICK,MA, GULATI,DK, IVETT,JL AND LOVEDAY,KS; CHROMOSOME ABERRATION AND SISTER CHROMATID EXCHANGE TEST RESULTS WITH 42 CHEMICALS; ENVIRON.MOL. MUTAGEN. 16(SUPPL. 18):55-137, 1990]
Test System:	CHO CELLS
End Point:	IN VITRO CHROMOSOMAL ABERRATIONS
Metabolic Activation:	NONE
Dose:	2000; 3000; 4000 UG/ML (TEST MATERIAL SOLVENT: DMSO)

Dose Regimen:	8 HR TREATMENT, 4 HR RECOVERY WITH COLCEMID ADDED IN FINAL 2-2.5 HR
Results:	POSITIVE
Reference:	[ANDERSON,BE, ZEIGER,E, SHELBY,MD, RESNICK,MA, GULATI,DK, IVETT,JL AND LOVEDAY,KS; CHROMOSOME ABERRATION AND SISTER CHROMATID EXCHANGE TEST RESULTS WITH 42 CHEMICALS; ENVIRON.MOL. MUTAGEN. 16(SUPPL. 18):55-137, 1990]
Test System:	CHO CELLS
End Point:	IN VITRO CHROMOSOMAL ABERRATIONS
Metabolic Activation:	NONE
Dose:	250; 500; 1600 UG/ML (TEST MATERIAL SOLVENT: DMSO)
Dose Regimen:	8 HR TREATMENT, 4 HR RECOVERY WITH COLCEMID ADDED IN FINAL 2-2.5 HR
Results:	NEGATIVE
Reference:	[ANDERSON,BE, ZEIGER,E, SHELBY,MD, RESNICK,MA, GULATI,DK, IVETT,JL AND LOVEDAY,KS; CHROMOSOME ABERRATION AND SISTER CHROMATID EXCHANGE TEST RESULTS WITH 42 CHEMICALS; ENVIRON.MOL. MUTAGEN. 16(SUPPL. 18):55-137, 1990]
Test System:	CHO CELLS
End Point:	IN VITRO CHROMOSOMAL ABERRATIONS
Metabolic Activation:	NONE
Dose:	500; 1600; 3000 UG/ML (TEST MATERIAL SOLVENT: DMSO)

Dose Regimen:	8 HR TREATMENT, 9 HR RECOVERY WITH COLCEMID ADDED IN FINAL 2-2.5 HR
Results:	NEGATIVE
Reference:	[ANDERSON,BE, ZEIGER,E, SHELBY,MD, RESNICK,MA, GULATI,DK, IVETT,JL AND LOVEDAY,KS; CHROMOSOME ABERRATION AND SISTER CHROMATID EXCHANGE TEST RESULTS WITH 42 CHEMICALS; ENVIRON.MOL. MUTAGEN. 16(SUPPL. 18):55-137, 1990]
Test System:	CHO CELLS
End Point:	IN VITRO CHROMOSOMAL ABERRATIONS
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Dose:	50; 160; 500; 1600; 5000 UG/ML (TEST MATERIAL SOLVENT: DMSO)
Dose Regimen:	2 HR TREATMENT, 10 HR RECOVERY WITH COLCEMID ADDED IN FINAL 2 HR
Results:	NEGATIVE
Reference:	[ANDERSON,BE, ZEIGER,E, SHELBY,MD, RESNICK,MA, GULATI,DK, IVETT,JL AND LOVEDAY,KS; CHROMOSOME ABERRATION AND SISTER CHROMATID EXCHANGE TEST RESULTS WITH 42 CHEMICALS; ENVIRON.MOL. MUTAGEN. 16(SUPPL. 18):55-137, 1990]
Test System:	CHO CELLS
End Point:	IN VITRO CHROMOSOMAL ABERRATIONS
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Dose:	500; 1600; 3000; 4000 UG/ML (TEST MATERIAL SOLVENT: DMSO)

Dose Regimen:	4 HR TREATMENT, 11 HR RECOVERY WITH COLCEMID ADDED IN FINAL 2 HR
Results:	POSITIVE
Reference:	[ANDERSON,BE, ZEIGER,E, SHELBY,MD, RESNICK,MA, GULATI,DK, IVETT,JL AND LOVEDAY,KS; CHROMOSOME ABERRATION AND SISTER CHROMATID EXCHANGE TEST RESULTS WITH 42 CHEMICALS; ENVIRON.MOL. MUTAGEN. 16(SUPPL. 18):55-137, 1990]
Test System:	CHO CELLS
End Point:	IN VITRO CHROMOSOMAL ABERRATIONS
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Dose:	1600; 3000; 4000 UG/ML (TEST MATERIAL SOLVENT: DMSO)
Dose Regimen:	2 HR TREATMENT, 10.3 HR RECOVERY WITH COLCEMID ADDED IN FINAL 2 HR
Results:	POSITIVE
Reference:	[ANDERSON,BE, ZEIGER,E, SHELBY,MD, RESNICK,MA, GULATI,DK, IVETT,JL AND LOVEDAY,KS; CHROMOSOME ABERRATION AND SISTER CHROMATID EXCHANGE TEST RESULTS WITH 42 CHEMICALS; ENVIRON.MOL. MUTAGEN. 16(SUPPL. 18):55-137, 1990]
Test System:	CHO CELLS
End Point:	IN VITRO CHROMOSOMAL ABERRATIONS
Metabolic Activation:	RAT, LIVER, S-9, AROCLOR 1254
Dose:	1600; 3000; 4000 UG/ML (TEST MATERIAL SOLVENT: DMSO)

Dose Regimen:	2 HR TREATMENT, 16 HR RECOVERY WITH COLCEMID ADDED IN FINAL 2 HR
Results:	POSITIVE
Reference:	[ANDERSON,BE, ZEIGER,E, SHELBY,MD, RESNICK,MA, GULATI,DK, IVETT,JL AND LOVEDAY,KS; CHROMOSOME ABERRATION AND SISTER CHROMATID EXCHANGE TEST RESULTS WITH 42 CHEMICALS; ENVIRON.MOL. MUTAGEN. 16(SUPPL. 18):55-137, 1990]
Test System:	BONE MARROW POLYCHROMATIC ERYTHROCYTES
End Point:	IN VIVO MICRONUCLEUS
Species:	MOUSE
Strain/Sex:	DDY/MALE
Route:	IP
Dose:	100 MG/KG (TEST MATERIAL SOLVENT: SALINE)
Dose Regimen:	1/D FOR 4D; 24 HR HARVEST
Results:	NEGATIVE
Reference:	[HAYASHI,M, KISHI,M, SOFUNI,T AND ISHIDATE MJR; MICRONUCLEUS TESTS IN MICE ON 39 FOOD ADDITIVES AND EIGHT MISCELLANEOUS CHEMICALS, FOOD CHEM. TOXICOL. 26(6):487-500, 1988]
Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA98
Metabolic	None

Activation:	
Method:	Direct plate incorporation
Dose:	100-5,000 ug/plate (Test material solvent: DMSO)
Results:	Negative
Reference:	[FALL,M, HADDOUK,H, MORIN,J-P AND FORSTER,R; MUTAGENICITY OF BENZYL CHLORIDE IN THE SALMONELLA/MICROSOME MUTAGENESIS ADSSAY DEPENDS ON EXPOSURE CONDITIONS; MUTAT. RES. GEN. TOXICOL. ENVIRON. MUTAGEN. 633(1):13-20, 2007]
Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA98
Metabolic Activation:	Rat, liver, S-9, Aroclor 1254
Method:	Direct plate incorporation
Dose:	100-5,000 ug/plate (Test material solvent: DMSO)
Results:	Negative
Reference:	[FALL,M, HADDOUK,H, MORIN,J-P AND FORSTER,R; MUTAGENICITY OF BENZYL CHLORIDE IN THE SALMONELLA/MICROSOME MUTAGENESIS ADSSAY DEPENDS ON EXPOSURE CONDITIONS; MUTAT. RES. GEN. TOXICOL. ENVIRON. MUTAGEN. 633(1):13-20, 2007]
Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA100
Metabolic Activation:	None

Method:	Direct plate incorporation
Dose:	100-5,000 ug/plate (Test material solvent: DMSO)
Results:	Negative
Reference:	[FALL,M, HADDOUK,H, MORIN,J-P AND FORSTER,R; MUTAGENICITY OF BENZYL CHLORIDE IN THE SALMONELLA/MICROSOME MUTAGENESIS ADSSAY DEPENDS ON EXPOSURE CONDITIONS; MUTAT. RES. GEN. TOXICOL. ENVIRON. MUTAGEN. 633(1):13-20, 2007]
Test System:	Ames Salmonella typhimurium
Strain Indicator:	TA100
Metabolic Activation:	Rat, liver, S-9, Aroclor 1254
Method:	Direct plate incorporation
Dose:	100-5,000 ug/plate (Test material solvent: DMSO)
Results:	Negative
Reference:	[FALL,M, HADDOUK,H, MORIN,J-P AND FORSTER,R; MUTAGENICITY OF BENZYL CHLORIDE IN THE SALMONELLA/MICROSOME MUTAGENESIS ADSSAY DEPENDS ON EXPOSURE CONDITIONS; MUTAT. RES. GEN. TOXICOL. ENVIRON. MUTAGEN. 633(1):13-20, 2007]

As taken from CCRIS, 2008

Benzyl alcohol was negative in the rec assay using E.coli polA (W3110 vs P3478) (Leifer et al. 1981).

“In this study, different concentrations of four benzyl derivatives (benzyl alcohol, benzyl acetate, benzoic acid and benzaldehyde) used as flavour ingredients were investigated for genotoxicity in in vitro. By taking blood from two healthy people comet assay was carried on to investigate the potential health damages of benzyl derivatives. For the evaluation of genotoxic effects, the tail moment and % tail DNA in the treated chemicals were compared to the solvent control, which is distilled water. The alkaline comet assay showed significantly increased tail moment and % tail DNA at 25 and 50 mM concentrations of benzyl alcohol.....” (Demir et al. 2010).

“Current in vitro mammalian cell genotoxicity assays show a high rate of positive results, many of which are misleading when compared with in vivo genotoxicity or rodent carcinogenicity data. P53-deficiency in many of the rodent cell lines may be a key factor in this poor predictivity. As part of an European Cosmetics Industry Association initiative for improvement of in vitro mammalian cell assays, we have compared several rodent cell lines (V79, CHL, CHO) with p53-competent human peripheral blood lymphocytes (HuLy), TK6 human lymphoblastoid cells, and the human liver cell line, HepG2. We have compared in vitro micronucleus (MN) induction following treatment with 19 compounds that were accepted as producing misleading or "false" positive results in in vitro mammalian cell assays [6]. Of these, ... benzyl alcohol, ...was not toxic and did not induce any MN at concentrations up to 10mM. These data suggest that a reduction in the frequency of misleading positive results can be achieved by careful selection of the mammalian cell type for genotoxicity testing” (Fowler et al. 2012).

Benzyl alcohol has been reported to produce negative effects in the following genotoxicity tests:

- Ames tests on *S. typhimurium*, at concentrations up to 50,000 $\mu\text{g}/\text{plate}$
- Mutation assay in *Escherichia coli* WP2 *uvrA*, at concentrations of 1000 to 8000 $\mu\text{g}/\text{plate}$
- Cytotoxicity and DNA damage assays in human alveolar tumour cells, at a concentration of 0.5 mM
- In vivo SLRL test in *Drosophila melanogaster* (injection), at a concentration of 8000 ppm
- In vivo micronucleus test in mouse bone marrow cells (IP injection), at a concentration of 200 mg/kg bw
- In vivo replicative DNA synthesis test in mouse hepatocytes (oral gavage) at a concentration of 800 mg/kg
- In vivo replicative DNA synthesis test in rat hepatocytes (oral or SC injection) at a concentration of 600 mg/kg

Benzyl alcohol has been reported to produce positive effects in the following genotoxicity tests:

- Rec assay in *B. subtilis*, at concentrations of 10 $\mu\text{g}/\text{disc}$ or 20 $\mu\text{g}/\text{disc}$
- Sister chromatid exchange assay in Chinese hamster ovary cells, at concentrations of 16 to 5000 $\mu\text{g}/\text{ml}$

Benzyl alcohol has been reported to produce equivocal effects in the following genotoxicity tests:

- Chromosomal aberration test in Chinese hamster ovary cells, at concentrations of 50 to 5000 $\mu\text{g}/\text{ml}$
- Mammalian cell gene mutation test in mouse lymphoma L5178Y cells, at concentrations of 150 to 5000 $\mu\text{g}/\text{ml}$

As taken from EFSA (2012). Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific opinion on Flavouring Group Evaluation 20, Revision 4 (FGE.20Rev4): Benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters from chemical groups 23 and 30. Available at <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2012.2994/epdf>

“At cytotoxic concentration benzyl alcohol induces DNA double strand breaks (experiment on rat hepatocyte cells) (SCF, 2002. Ks: 2).”

As taken from Bassan A et al. 2012 Reports on toxicokinetics, toxicity and allergenicity data on substances to be evaluated as acceptable previous cargoes for edible fats and oils (NP/EFSA/CONTAM/2011/01) - Batches n. 1, 2 and 3. Available at <http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2012.EN-274/epdf>

This study presents the first application of an in vivo alkaline comet assay using hemocytes in the hemolymph of *Drosophila melanogaster* larvae for the genotoxic evaluation of benzyl derivatives. Haemocytes are playing a function similar to mammalian blood. In this study, different concentrations. (5, 10, 25 and 50 mM) of benzyl alcohol, benzyl acetate, benzaldehyde and benzoic acid used as flavor ingredients were investigated for genotoxicity as in vivo. Haemolymph was extracted from the larvae, and hemocytes were isolated by centrifugation and used in the comet assay. In the comet assay with hemocytes for benzyl derivatives observed significantly and dose-dependently induced DNA damage formation. The highest concentration of each tested benzyl derivative was significantly induced DNA damage. Their genotoxic potencies according to the induced % DNA tail in the highest concentration are as follows: benzyl acetate (35.51 ± 0.31), benzyl alcohol (34.87 ± 2.01), benzaldehyde (33.27 ± 0.95) and benzoic acid (28.21 ± 0.31). These results confirm the benefit of the comet assay with hemocytes as an in vivo model and support the possibility that there is a genotoxic risk associated with benzyl derivatives exposure (Esref Demir & Bülent Kaya, 2013).

“The mutagenic activity of benzyl alcohol was assessed in an Ames study conducted in compliance with GLP regulations by the NTP in accordance with OECD TG 471 using the preincubation method. *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 were treated with benzyl alcohol at concentrations of 0, 100, 333, 1000, 1333, 3000 and 6666 µg/plate in the presence and absence of metabolically active microsomal mix (S-9 mix). There were no significant increases observed in the number of revertant colonies in the strains at any concentration (Zeiger et al., 1990). Under the conditions of the study, benzyl alcohol was considered not mutagenic in the Ames test. This was confirmed in an in vitro mammalian gene mutation test using mouse lymphoma L5178Y cells. Benzyl alcohol up to concentrations of 5000 µg/ml was unable to induce a dose related, statistically significant increase in mutation frequency and was considered not mutagenic in the in vitro mammalian gene mutation test.

The clastogenic activity of benzyl alcohol was assessed in an in vivo micronucleus assay conducted equivalent to OECD TG 474. Groups of male ddY mice were administered benzyl alcohol in saline via either a single intraperitoneal injection, at the concentrations of 0, 50, 100 and 200 mg/kg body weight or multiple injections every 24 h for four days at concentrations of 0 and 100 mg/kg body weight. Animals were euthanized 24 h after last administration, bone marrow was extracted and smears prepared. No increase in the number of micronucleated polychromatic erythrocytes was observed (Hayashi et al., 1988). Under the conditions of the study, benzyl alcohol was considered unable to induce micronuclei in the in vivo micronucleus test.

Based on the available data, benzyl alcohol does not present a concern for genotoxic potential.”

As taken from Api AM et al., (2020).

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Reference
DNA repair		Bacteria - Bacillus subtilis	21 mg/disc	OIGZDE Osaka-shi Igakkai Zasshi. Journal of Osaka City Medical Association. (Osaka-shi Igakkai, c/o Osaka-shiritsu Daigaku Igakubu, 1-4-54 Asahi-cho, Abeno-ku, Osaka, 545, Japan) V.24- 1975- Volume(issue)/page/year: 34,267,1985
DNA damage		Rodent - rat Liver	10 mmol/L	EMMUEG Environmental and Molecular Mutagenesis. (Alan R. Liss, Inc., 41 E. 11th St., New York, NY 10003) V.10- 1987- Volume(issue)/page/year: 24,181,1994
Mutation in microorganisms		Rodent - mouse Lymphocyte	250 mg/L	NTPTR* National Toxicology Program Technical Report Series. (Research Triangle Park, NC 27709) No.206- Volume(issue)/page/year: NTP-TR-343,1989
Cytogenetic analysis		Rodent - hamster Ovary	4 ug/L	NTPTR* National Toxicology Program Technical Report Series. (Research Triangle Park, NC 27709) No.206- Volume(issue)/page/year: NTP-TR-343,1989
Mutation test systems - not otherwise specified	Oral	Insect - Drosophila melanogaster	50 mmol/L	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 46,1034,2008
DNA damage		Human Liver	0.1 mmol//48H	MUREAV Mutation Research. (Elsevier Science Pub. B.V., POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1964- Volume(issue)/page/year: 696,21,2010
DNA damage		Human Lymphocyte	25 mmol/L	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park,

				Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 48,1239,2010

As taken from RTECS, 2019.

“The chemical was reported to be negative in several Ames tests, with and without metabolic activation, and also negative in an in vitro mammalian chromosome aberration test using Chinese hamster lung (CHL) cells without metabolic activation.

The chemical produced a significant increase in chromosomal aberrations with Chinese hamster ovary (CHO) cells in the presence, but not the absence, of S9. The chemical also produced an increase in sister chromatid exchanges (SCEs) in CHO cells in a sister chromatid exchange assay in mammalian cells, which was judged to be equivocal both with and without metabolic activation. While the chemical produced a positive response in mammalian cells in the mouse lymphoma thymidine kinase forward mutation assay without metabolic activation, negative results were reported for tests with metabolic activation.

The chemical was found to be negative in the in vivo micronucleus assay on mouse bone marrow cells, in an assay on replicative DNA synthesis in rats, and in the sex-linked recessive lethal assay in *Drosophila melanogaster*.

The weight of the evidence of the in vitro and in vivo genotoxicity data indicates that the chemical does not have mutagenic or clastogenic potential”.

As taken from AICIS, 2016.

“In 2008 we published recommendations on chemicals that would be appropriate to evaluate the sensitivity and specificity of new/modified mammalian cell genotoxicity tests, in particular to avoid misleading positive results. In light of new data it is appropriate to update these lists of chemicals. An expert panel was convened and has revised the recommended chemicals to fit the following different sets of characteristics: • Group 1: chemicals that should be detected as positive in in vitro mammalian cell genotoxicity tests. Chemicals in this group are all in vivo genotoxins at one or more endpoints, either due to DNA-reactive or non DNA-reactive mechanisms. Many are known carcinogens with a mutagenic mode of action, but a sub-class of probable aneugens has been introduced. • Group 2: chemicals that should give negative results in in vitro mammalian cell genotoxicity tests. Chemicals in this group are usually negative in vivo and non-DNA-reactive. They are either non-carcinogenic or rodent carcinogens with a non-mutagenic mode of action. • Group 3: chemicals that should give negative results in in vitro mammalian cell genotoxicity tests, but have been reported to induce gene mutations in mouse lymphoma cells, chromosomal aberrations or micronuclei, often at high concentrations or at high levels of cytotoxicity. Chemicals in this group are generally negative in vivo and negative in the Ames test. They are either non-carcinogenic or rodent carcinogens with an accepted non-mutagenic mode of action. This group contains comments as to any conditions that can be identified under which misleading positive results are likely to occur. This paper, therefore, updates these three recommended lists of chemicals and describes how these should be used for any test evaluation program.” As taken from Kirkland D et al. 2016. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* 795, 7-30. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/26774663>

“Benzyl alcohol was tested with negative results in the base set of genotoxicity tests and the Panel concluded that benzyl alcohol does not raise safety concern with respect to genotoxicity.”

As taken from EFSA, 2019

Record for benzenemethanol:

Species Common Name	Exposure Type	Test Location	Conc 1 Type (Author)	Conc 1 (Author)	Conc 1 Units (Author)	Effect	Effect Measurement	Endpoint	Exposure Duration Mean (Days)	Response Site	Observed Duration (Days)
House Mouse	Gavage	Lab	Formulation	400-800	mg/kg bdwt	Genetics	DNA synthesis rate	NR	2	Liver	1-2
Norway Rat	Terrestrial Not Reported	Lab	Formulation	300-600	mg/kg bdwt	Genetics	DNA synthesis rate	NR	1-2	Liver	1-2

As taken from the US EPA ECOTOX database, 2021

“Amongst all toxicological endpoints, carcinogenicity might pose the greatest concern. Genetic damage has been considered an important underlying mechanism for the carcinogenicity of chemical substances. The demand for in vitro genotoxic tests as alternative approaches is growing rapidly with the implementation of new regulations for compounds. However, currently available in vitro genotoxicity tests are often limited by relatively high false positive rates. Moreover, few studies have explored carcinogenicity potential by in vitro genotoxicity testing due to the shortage of suitable toxicological biomarkers to link gene damage with cancer risk. γ -H2AX is a recently acknowledged attractive endpoint (biomarker) for evaluating DNA damage and can simultaneously reflect the DNA damage response and repair of cells. We previously reported an ultrasensitive and reliable method, namely stable-isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS), for detecting cellular γ -H2AX and evaluating genotoxic chemicals. More importantly, our method can dynamically monitor the specific processes of genotoxic compounds affecting DNA damage and repair reflected by the amount of γ -H2AX. To clarify the possibility of using this method to assess the potential carcinogenicity of genotoxic chemicals, we applied it to a set of 69 model compounds recommended by the European Center for the Validation of Alternative Methods (ECVAM), with already-characterized genotoxic potential. Compared to conventional in vitro genotoxicity assays, including the Ames test, the γ -H2AX assay by MS has high accuracy (94-96%) due to high sensitivity and specificity (88% and 100%, respectively). The dynamic profiles of model compounds after exposure in HepG2 cells were explored, and a mathematical approach was employed to simulate and quantitatively model the DNA repair kinetics of genotoxic carcinogens (GCs) based on γ -H2AX time-effect curves up to 8 h. Two crucial parameters, i.e., k (rate of γ -H2AX decay) and t_{50} (time required for γ -H2AX from maximum decrease to half) estimated by the least squares method, were achieved. An open web server to help researchers calculate these two key parameters and profile simulated curves of the tested compound is available online (<http://ccb1.bmi.ac.cn:81/shiny-server/sample-apps/prediction1/>). We detected a positive association between carcinogenic levels and k and t_{50} values of γ -H2AX in tested GCs, validating the potential of using this MS-based γ -H2AX in vitro assay to help preliminarily evaluate carcinogenicity and assess genotoxicity. This approach may be used alone or integrated into an existing battery of in vitro genetic toxicity tests.”

Qu M et al. (2021) Dynamically monitoring cellular γ -H2AX reveals the potential of carcinogenicity evaluation for genotoxic compounds.

5.5. Cytotoxicity

Cytotoxicity	Human alveolar tumour cells	0.5 mmol/L	Negative

As taken from WHO Food Additives Series 48 available at <http://www.inchem.org/documents/jecfa/jecmono/v48je14.htm>

/ALTERNATIVE and IN VITRO TESTS/ The aim of the study was to investigate the toxicity of benzyl alcohol (BA), the preservative in commercial triamcinolone acetonide (TA) suspensions, on retinal pigment epithelial (RPE) cells. Cultured RPE cells from a human cell line (ARPE-19) and from rabbits were exposed to the balanced salt solution (control) or BA (0.0225, 0.225, 0.9, 3 or 9 mg/mL) for 5, 30, 60, or 120 min. ... The proportions of dead cells were quantitatively measured by the trypan blue exclusion assay, and those of functional cells were assessed by a mitochondrial dehydrogenase assay. The mechanism of cytotoxicity was determined by the acridine orange/ethidium bromide staining and DNA laddering technique. Furthermore, ultrastructural changes were observed by transmission electron microscopy. The results showed that RPE cell damage was dose- and time-dependent. BA 0.225 mg/mL, the clinically relevant concentration in TA following intravitreal injection, caused ultrastructural damage and impaired human RPE cell function at 2 hr; but BA 0.0225mg/mL did not. BA 9.0 mg/mL, the concentration in commercial TA suspensions, was toxic within 5 min on each assay for both human and rabbit RPE cells. The major mechanism of cell death was necrosis. [Chang YS et al; Exp Eye Res 86 (6): 942-50 (2008)]
****PEER REVIEWED**** <http://www.ncbi.nlm.nih.gov/pubmed/18420195?dopt=Abstract>
 target=new>PubMed Abstract

As taken from HSDB, 2018

“Acetaminophen (APAP) hepatotoxicity is a serious public health problem in western countries. Current treatment options for APAP poisoning are limited and novel therapeutic intervention strategies are needed. A recent publication suggested that benzyl alcohol (BA) protects against APAP hepatotoxicity and could serve as a promising antidote for APAP poisoning. To assess the protective mechanisms of BA, C56Bl/6J mice were treated with 400 mg/kg APAP and/or 270 mg/kg BA. APAP alone caused extensive liver injury at 6 h and 24 h post-APAP. This injury was attenuated by BA co-treatment. Assessment of protein adduct formation demonstrated that BA inhibits APAP metabolic activation. In support of this, in vitro experiments also showed that BA dose-dependently inhibits cytochrome P450 activities. Correlating with the hepatoprotection of BA, APAP-induced oxidant stress and mitochondrial dysfunction were reduced. Similar results were obtained in primary mouse hepatocytes. Interestingly, BA alone caused mitochondrial membrane potential loss and cell toxicity at high doses, and its protective effect could not be reproduced in primary human hepatocytes (PHH). We conclude that BA protects against APAP hepatotoxicity mainly by inhibiting cytochrome P450 enzymes in mice. Considering its toxic effect and the loss of protection in PHH, BA is not a clinically useful treatment option for APAP overdose patient.” As taken from Du K et al. 2015. Food Chem. Toxicol. 86, 253-61. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26522885>

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
IC50 - Inhibitor Concentration 50	In vitro	Human - skin	>2000 umol/L/48H	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT,	TXAPA9 Toxicology and Applied Pharmacology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1- 1959- Volume(issue)/page/year:

				XTT, MTS, WSTs assays etc.	245,281,2010
ICLo - Inhibitor Concentration Low	In vitro	Rabbit ocular -	5 pph/5M	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc.	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 25,1425,2011
IC50 - Inhibitor Concentration 50	In vitro	Human ocular -	20674 mg/L/1H	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc.	TOLED5 Toxicology Letters. (Elsevier Science Pub. B.V., POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1977- Volume(issue)/page/year: 212,198,2012
IC30 - Inhibitor Concentration 30	In vitro	Human lymphocyte -	>200 mg/L/45H	In Vitro Toxicity Studies - cell viability (dye exclusion): trypan blue assay etc.	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 29,901,2015
IC50 - Inhibitor Concentration 50	In vitro	Human - skin	6.86 pph/24H	In Vitro Toxicity Studies - cell viability (mitochondrial reductase assays): MTT, XTT, MTS, WSTs assays etc	TOLED5 Toxicology Letters. (Elsevier Science Pub. B.V., POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1977- Volume(issue)/page/year: 271,1,2017

As taken from RTECS, 2019.

“Benzyl alcohol is mainly used as a preservative. Benzoic acid is a bacteriostatic antiseptic that is only active in an acidic environment (pH 2.5 to 4.5).

The minimal inhibitory concentrations (MIC) are as follows:

Micro-organism	Minimal inhibitory concentration (µg/ml)
Aspergillus niger	5000
Candida albicans	2500
Escherichia coli	2000
Pseudomonas aeruginosa	2000
Staphylococcus aureus	25

As taken from EMA, 2017

“In our continuing effort to identify bioactive secondary metabolites from natural sources, the antiproliferative activity of 23 compounds, previously isolated from *Penicillium concentricum*, was assessed using the sulforhodamine B assay. The cytotoxic effect was determined against HeLa cervical, HT-29 colon, MDA-MB-321 breast, PC-3, and DU-145 prostate cancer cell lines. Compounds were also tested in the mitochondrial transmembrane potential (MTP) and nuclear factor kappa B (NF-κB) target-based assays. The results showed that 2-bromogentisyl alcohol (2) and 3-hydroxy-benzenemethanol (8) exhibited the highest cytotoxic activity against different cancer cell lines. Epoxydon (14) showed selectivity against DU-145 prostate cancer cells [inhibitory concentration 50 (IC₅₀)=1.2 µmol/l]. Compounds 2, 8, 14, 18, 21 also induced damage of MTP (IC₅₀=0.1, 0.2, 7.0, 9.6, and 1.8 µmol/l, respectively). In the NF-κB assay, only compound 8 exhibited potent inhibition (IC₅₀=0.3 µmol/l). Compounds 2 and 14 showed cytotoxic activity and induction of damage in mitochondrial membrane potential while compound 8 inhibited NF-κB and MTP damage. Additionally, compound 14 with selectivity against DU-145 prostate cancer cells induced cell cycle arrested in G2/M phase. Thus, compounds 2, 8, and 14 could be useful leads in the development of new anticancer agents from natural sources.” As taken from Anaya-Eugenio GD et al. 2019. *Anticancer Drugs* 30(4), 323-329. PubMed, 2091 available at: <https://www.ncbi.nlm.nih.gov/pubmed/30688672>

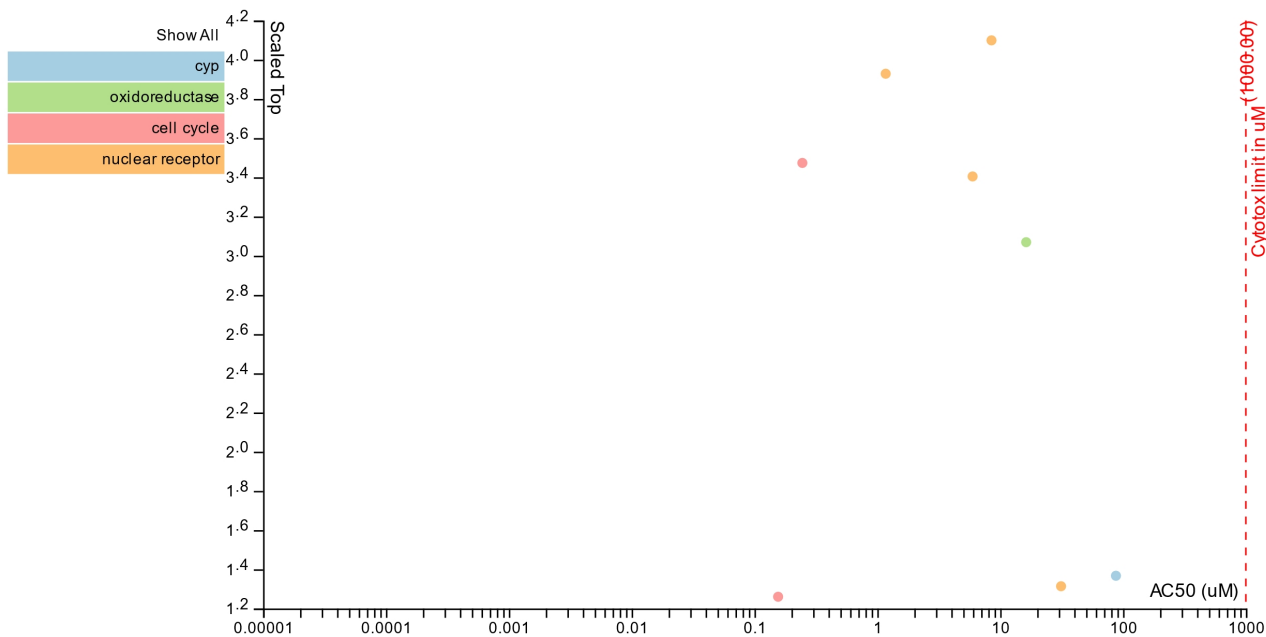
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated benzyl alcohol in a series of high-throughput assays, which are publicly available on the US EPA's CompTox Dashboard (section BIOACTIVITY / sub-section TOXCAST:SUMMARY), available at the following URL: <https://comptox.epa.gov/dashboard>

US EPA provides the following data use considerations for ToxCast data: “The activity of a chemical in a specific assay does not necessarily mean that it will cause toxicity or an adverse health outcome. There are many factors that determine whether a chemical will cause a specific adverse health outcome. Careful review is required to determine the use of the data in a particular decision contexts. Interpretation of ToxCast data is expected to change over time as both the science and analytical methods improve.”

A summary of the ToxCast assay data on benzyl alcohol is provided below in Figure 1. Figure 1 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on US EPA's CompTox Dashboard.

Figure 1



Record for benzenemethanol:

Species Scientific Name	Species Common Name	Exposure Duration Mean (Days)	Test Location	Exposure Type	Endpoint	Effect	Effect Measurement	Response Site	Conc 1 (Author)	Conc 1 Units (Author)	Observed Duration (Days)
Rattus norvegicus	Norway Rat	1	Lab	Not reported	LOEL	Cell(s)	Viability	Liver	600	mg/kg bdwt	1
Rattus norvegicus	Norway Rat	1	Lab	Not reported	NOEL	Cell(s)	Viability	Liver	300	mg/kg bdwt	1
Mus musculus	House Mouse	2	Lab	Gavage	NOEL	Cell(s)	Viability	Liver	800	mg/kg bdwt	2
Rattus norvegicus	Norway Rat	2	Lab	Terrestrial Not Reported	LOEL	Cell(s)	Viability	Liver	300	mg/kg bdwt	2
Rattus norvegicus	Norway Rat	1.625	Lab	Terrestrial Not Reported	NR	Cell(s)	Viability	Liver	300-600	mg/kg bdwt	1.625

As taken from the US EPA ECOTOX database, 2021

"The development of new orally inhaled drug products requires their demonstration of safety, which must be proven in animal experiments. New in vitro methods may replace, or at least reduce, these animal experiments, provided they are able to correctly predict safety or possible toxicity in humans. However, the challenge is to link in vitro data obtained in human cells to human in vivo data. We here present a new approach to the safety assessment of excipients (SAFE) for pulmonary drug delivery. The SAFE model is based on a dose response curve of 23 excipients tested on the human pulmonary epithelial cell lines A549 and Calu-3. The resulting in vitro IC50 values were correlated with the FDA-approved concentrations in pharmaceutical products for either pulmonary (if available) or parenteral administration. Setting a threshold of 0.1% (1 mg/mL) for either value yielded four safety classes and allowed to link IC50 data as measured in human cell cultures in vitro with the concentrations of the same compounds in FDA-approved drug products. The necessary in vitro data for novel excipients can be easily generated, and the SAFE approach allows putting them into context for eventual use in human pulmonary drug products. Excipients that are most likely not safe for use in humans can be excluded early on from further pharmaceutical development. The SAFE approach thus helps to avoid unnecessary animal experiments. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International license (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution and reproduction in any medium, provided the original work is appropriately cited." As taken from Metz JK et al. 2020. ALTEX 37(2), 275-286. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/32052853/>

5.6. Carcinogenicity

Species:	RAT
Strain/Sex:	F344/M
Route:	GAVAGE
Dose:	0; 200; 400 MG/KG IN CORN OIL 5D/WK FOR 103 WK (STUDY DURATION: 105 WK)
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-343 Y89]
Species:	RAT
Strain/Sex:	F344/F
Route:	GAVAGE

Dose:	0; 200; 400 MG/KG IN CORN OIL 5D/WK FOR 103 WK (STUDY DURATION: 105 WK)
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-343 Y89]
Species:	MOUSE
Strain/Sex:	B6C3F1/F
Route:	GAVAGE
Dose:	0; 100; 200 MG/KG IN CORN OIL 5D/WK FOR 103 WK (STUDY DURATION: 106 WK)
Results:	NEGATIVE
Reference:	[NCI/NTP CARCINOGENESIS TECHNICAL REPORT SERIES; NATIONAL CANCER INSTITUTE/NATIONAL TOXICOLOGY PROGRAM; U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, TR-343 Y89]

As taken from CCRIS, 2008

/LABORATORY ANIMALS: Chronic Exposure or Carcinogenicity/ A nonoxidative hair dye containing 2.0% benzyl alcohol and 0.016% benzoic acid was painted onto the skin /of groups 8120 Eppléy Swiss mice (60/sex) at a dose of 0.05 mL/application, three times weekly for 20 months. Sites were shaved of hair 24 hours before each application and a new bottle of dye was used each week. Two groups of control animals were shaved but not treated. Nine months into the study, 10 mice/sex/group were killed. Body weights and survival differed little between treatment and control groups. Varying degrees of chronic dermal inflammation were noted in all groups, including the controls. A significant ($p < .01$) increase in malignant lymphomas was noted in treated females (23/60). However, the researchers noted that one concurrent control group had a very low incidence (7/60 or 12%) for that tumor type. The rate was 22% for the other control group and had averaged 33% for three control groups in previous studies. Thus, the findings were not considered treatment related. The incidence of pulmonary adenomas and hepatic hemangiomas, which are common to this mouse strain, were similar between treated and control groups. No unusual neoplasms were observed. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED**
[PubMed Abstract](http://www.ncbi.nlm.nih.gov/pubmed/11766131?dopt=Abstract)

/LABORATORY ANIMALS: Chronic Exposure or Carcinogenicity/ Groups of 100 F344/N rats (50 each sex) were dosed with 200 or 400 mg/kg benzyl alcohol in corn oil, 5 days per week for 103 weeks. Groups of 100 B6C3F1 mice were dosed with 100 or 200 mg/kg benzyl alcohol following the same schedule. During week 80, mice were mistakenly dosed for four days with 375 (low-dose group) and 750 mg/kg (high-dose group) of alpha-methylbenzyl alcohol. No adverse effects were

apparent. Mean body weights were comparable among dosed and vehicle control rats throughout the study. A number of accidental deaths was due to gavage procedures in female rats of both dose groups (17 deaths, low-dose; 13 deaths, high-dose) and in males of the 400 mg/kg group (14 deaths). Survival of female rats of the low- and high-dose groups was significantly lower than that of vehicle controls after weeks 71 and 50, respectively. At the end of the study, 17 female rats survived from each of the dose groups, compared to 35 female vehicle-controls; 27 low-dose males and 24 high-dose males survived, compared to 28 male vehicle controls. Clinical signs characteristic of sialodacryoadenitis (cervical swelling, pink eyes, red exudate around eyes) were observed in dosed and vehicle-control rats. The diagnosis was confirmed by serum analysis. Epithelial hyperplasia of the nonglandular stomach was noted in four high-dose males. A squamous cell papilloma was noted in 1 of 19 low-dose and 1 of 50 high-dose males. (It was not stated why only 19 low-dose male rats were examined.) No other compound-related clinical signs were observed. Mean body weight was comparable among dosed and vehicle control mice throughout the study. Survival of female vehicle controls was significantly lower than that of the high-dose group after week 74 (female: vehicle control, 26/50; low dose, 32/50; high dose, 36/50). Corpora amylacea (foci of mineralization in the thalamus) was observed at an increased incidence in highdose mice (male: vehicle control, 15/49; low dose, 21/48; high dose, 22/50; female: 14/50; 15/48; 25/50), but was noted to be a common and spontaneously occurring lesion [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] ****PEER REVIEWED**** [PubMed Abstract](http://www.ncbi.nlm.nih.gov/pubmed/11766131?dopt=Abstract)

/LABORATORY ANIMALS: Chronic Exposure or Carcinogenicity/ Under the conditions of these 2-year gavage studies, there was no evidence of carcinogenic activity of benzyl alcohol for male or female F344/N rats dosed with 200 or 400 mg/kg. Survival in both dose groups of female rats was 50% that of vehicle controls, primarily due to an increased number of gavage-related deaths. There was no evidence of carcinogenic activity of benzyl alcohol for male or female B6C3F1 mice dosed with 100 or 200 mg/kg for 2 years.[DHHS/NTP; Toxicology and Carcinogenesis Studies of Benzyl Alcohol in F344/N Rats and B6C3F1 Mice (Gavage Studies) (1989) Technical Rpt Series No. 343 NIH Pub No. 89-2599. Available from, as of November 21, 2014: <http://ntp-server.niehs.nih.gov/>

2 Years Rat: Toxicology and carcinogenesis studies of technical-grade benzyl alcohol (99% pure), a textile dye additive, solvent, and food flavoring agent, were conducted by administering the chemical by gavage in corn oil vehicle to groups of F344/N rats ... of each sex for ... 2 years. Fifty animals of each ... sex were administered benzyl alcohol in corn oil by gavage 5 days per week for 103 weeks at doses of 0, 200, and 400 mg/kg. Administration of benzyl alcohol did not affect survival in male rats (final survival rates: vehicle control, 28/50; low dose, 27/50; high dose, 24/50) but reduced survival of dosed female rats by half (36/50; 18/50; 17/50). Many of the early deaths were considered related to the gavage procedure. ... No effect of benzyl alcohol on body weight gain in rats ... was observed. In the third month of the studies, clinical signs of sialodacryoadenitis virus infection were observed in rats. A positive serologic reaction for rat coronavirus was observed in sentinel animals at 6 months and again at 18 months. No apparent compound-related nonneoplastic responses were observed. Dose-related negative trends in the incidences of anterior pituitary gland neoplasms were seen in female rats (vehicle control, 29/50; low dose, 17/47; high dose, 9/49). ... DHHS/NTP; Toxicology and Carcinogenesis Studies of Benzyl Alcohol in F344/N Rats and B6C3F1 Mice (Gavage Studies) (1989) Technical Rpt Series No. 343 NIH Pub No. 89-2599. Available from, as of November 21, 2014: <http://ntp-server.niehs.nih.gov/>

2 Years Mouse: Toxicology and carcinogenesis studies of technical-grade benzyl alcohol (99% pure), a textile dye additive, solvent, and food flavoring agent, were conducted by administering the chemical by gavage in corn oil vehicle to groups of ... B6C3F1 mice of each sex for ... 2 years. Fifty animals of each ... sex were administered benzyl alcohol in corn oil by gavage 5 days per week for 103 weeks at doses of 0, 100, and 200 mg/kg. ... Survival in mice was not affected by benzyl alcohol administration (male: 34/50; 33/50; 35/50; female: 26/50; 32/50; 36/50). No effect of benzyl alcohol on body weight gain in ... mice was observed. ... No apparent compound-related

nonneoplastic responses were observed. Dose-related negative trends in the incidences ... of harderian gland adenomas in male mice (8/50; 3/50; 2/50). Adenomas of the adrenal cortex occurred at an increased incidence in high dose male mice (0/48; 0/44; 3/48), but this slight increase was not considered to be related to chemical exposure. DHHS/NTP; Toxicology and Carcinogenesis Studies of Benzyl Alcohol in F344/N Rats and B6C3F1 Mice (Gavage Studies) (1989) Technical Rpt Series No. 343 NIH Pub No. 89-2599. Available from, as of November 21, 2014: <http://ntp-server.niehs.nih.gov/>

As taken from HSDB, 2018

"NSF International has evaluated carcinogenicity data for benzyl alcohol. NSF International categorized benzyl alcohol as not likely to be carcinogenic to humans using the U.S. EPA (1999) draft revised guidelines for carcinogen risk assessment. NSF International did not determine a cancer risk level for benzyl alcohol."

As taken from ITER, 2018.

Rats and Mice: Cancer Test Summary

Rat Target Sites		Mouse Target Sites		TD ₅₀ (mg/kg/day)	
Male	Female	Male	Female	Rat	Mouse
no positive	no positive	no positive	no positive	no positive	no positive

As taken from CPDB, 2007

"Benzyl alcohol is not carcinogenic in mice and rats up to 200 and 400 mg/kg bw per day, respectively, which were highest doses tested. The no observable adverse effect level (NOAEL) in the carcinogenicity study in rats was 400 mg/kg bw per day."

As taken from EFSA, 2019

5.7. Irritation/immunotoxicity

"Direct contact: skin and eyes; general sensation: slight hazard on ingestion. Skin irritation grade 4 - capillary injection diluted. Eye irritation grade 8 - severe burns 0.5 ml 10 % solution.

Causes local irritation of skin and mucous membranes, headache, vertigo, nausea, vomiting and diarrhea. Faint aromatic odor; sharp burning taste (Sax1986). May cause irritation if present in recreational waters (Sax 1986). Pure alcohol is irritating and corrosive. Aqueous concentrations up to 4 % well tolerated. Produces transient anesthesia of mucous membranes ... Dermal application to humans of 0.1 g of 32 % concentration in acetone was mildly irritating.

Skin and eye irritation: skn, rbt, 10 mg, 24 hr, mild; eye, rbt, 0.750 mg, severe; skn, pig, 100 %, moderate (Sax 1986)."

As taken from SKYE data bank of environmental chemicals.

Skin, Eye and Respiratory Irritations:

A moderate skin and severe eye irritant. Lewis, R.J. Sr. (ed) Sax's Dangerous Properties of Industrial Materials. 11th Edition. Wiley-Interscience, Wiley & Sons, Inc. Hoboken, NJ. 2004., p. 399

It is slightly irritating to the skin International Labour Office. Encyclopedia of Occupational Health and Safety. Vols. I&II. Geneva, Switzerland: International Labour Office, 1983., p. 111

Vapor: Irritating to eyes, nose and throat. Liquid: Irritating to skin & eyes. U.S. Coast Guard, Department of Transportation. CHRIS - Hazardous Chemical Data. Volume II. Washington, D.C.: U.S. Government Printing Office, 1984-5.

/HUMAN EXPOSURE STUDIES/ Results /were reported/ of a cosmetic intolerance assay that patch tested 5202 patients with possible allergic contact dermatitis (537 of the patients had a history of "intolerance," allergy, or irritation to cosmetics). Patch test conditions were not specified. A reaction was noted in 48 (0.92% incidence) to benzyl alcohol. Reactions were noted in 2 of the 155 patients with cosmetic allergy. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/HUMAN EXPOSURE STUDIES/ Four positive patch tests to 6.5% benzyl alcohol /were reported/ among 242 patients with histories of contact allergy of varying origin. An index of simultaneous reactivity in which the number of reactions to other perfume ingredients was divided by the number of positive reactions to benzyl alcohol had a value of 0.50 (one individual responded to eugenol and another to isoeugenol). [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/HUMAN EXPOSURE STUDIES/ Patch test results /were compiled/ from 12 dermatologists over a 6-year period. Patches had been applied to the upper back for 48 hours of contact, and sites were evaluated at 48 and 72 hours. Three cutaneous reactions to 5% benzyl alcohol in petrolatum were noted among 713 cosmetic dermatitis patients. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/HUMAN EXPOSURE STUDIES/ The Research Institute for Fragrance Materials, Inc. (RIFM) report on benzyl alcohol cited an unpublished Kligman Maximization study that tested 10% benzyl alcohol in petrolatum using 25 male volunteers (skin types: 10 were Caucasian and 15 were Black). Benzyl alcohol (and three other test materials) was applied under occlusive patches to the forearm of panelists. A total of five 48-hour exposures occurred during induction and each was preceded by a 24-hour occlusive pretreatment of the sites with 5% aqueous sodium lauryl sulfate (SLS). Following a 10-day nontreatment period, panelists were challenged on the scapular back with a 48 hour patch. Challenge sites were pretreated for 1 hour with 10% SLS. Challenge sites were examined at 48 and 72 hours. No reactions were observed. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/HUMAN EXPOSURE STUDIES/ A repeat-insult patch test (RIPT) was conducted using a nonexclusive group of 110 panelists. Two mascara formulations each containing 0.65% benzyl alcohol were tested. During a 3-week induction period nine occlusive 24-hour patches (containing 0.15 g of test material) were applied to the same site on either the upper arm or back. Sites were evaluated 24 hours after patch removal (i.e., prior to application of subsequent patch). Following a 12- to 20-day nontreatment period, a challenge patch was applied to both the original site and a previously unexposed site. Challenge sites were evaluated at 24 and 48 hours after patch removal. No reactions were noted during induction or at challenge to either formulation. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/HUMAN EXPOSURE STUDIES/ ... During 4 periods of 6 months, from 1 January 2003 to 31 December 2004, 26 fragrances were patch tested additionally to the standard series in a total of 21,325 patients; the number of patients tested with each of the fragrances ranged from 1658 to 4238. ... The following frequencies of sensitization (rates in %, standardized for sex and age) were observed: tree moss (2.4%), HMPCC (2.3), oak moss (2.0), hydroxycitronellal (1.3), isoeugenol (1.1), cinnamic aldehyde (1.0), farnesol (0.9), cinnamic alcohol (0.6), citral (0.6), citronellol (0.5), geraniol (0.4), eugenol (0.4), coumarin (0.4), lilial (0.3), amyl-cinnamic alcohol (0.3), benzyl cinnamate (0.3), benzyl alcohol (0.3), linalool (0.2), methylheptin carbonate (0.2), amyl-cinnamic

aldehyde (0.1), hexyl-cinnamic aldehyde (0.1), limonene (0.1), benzyl salicylate (0.1), gamma-methylionon (0.1), benzyl benzoate (0.0), anisyl alcohol (0.0). ... [Schnuch A et al; Contact Dermatitis 57 (1): 1-10 (2007)] **PEER REVIEWED** PubMed Abstract

/HUMAN EXPOSURE STUDIES/ A blinded, controlled study in healthy volunteers who were treated with 3 mL of saline with or without benzyl alcohol (9 mg/mL) found that the benzyl alcohol group had bronchitis with erythema, tracheobronchial mucosal edema, metaplasia, denudation of cilia, and mucosal lymphocytic infiltration on bronchoscopy. Dart, R.C. (ed). Medical Toxicology. Third Edition, Lippincott Williams & Wilkins. Philadelphia, PA. 2004., p. 1232

/SIGNS AND SYMPTOMS/ Hypersensitivity reactions may occur after parenteral or dermal exposure to benzyl alcohol. Acute reactions include urticaria, erythema, palpable edema, fatigue, nausea, diffuse angioedema, maculopapular rash, and fever. A delayed hypersensitivity reaction characterized by erythema, edema, and vesiculation may appear in 2 to 3 days after an immediate reaction to a single benzyl alcohol challenge in the same patient. Dart, R.C. (ed). Medical Toxicology. Third Edition, Lippincott Williams & Wilkins. Philadelphia, PA. 2004., p. 1232

/CASE REPORTS/ Two patients with contact dermatitis were found to be sensitized by benzyl alcohol: 1 percent in petrolatum. OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.269 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

/SIGNS AND SYMPTOMS/ Benzyl alcohol and especially benzoic acid and sodium benzoate are among various compounds (such as some food additives) recognized in the published literature to induce nonimmunologic contact reactions in certain populations. These agents "produce the reaction without any previous sensitization in most or almost all exposed persons." The hypersensitivity has been indicated by flexural dermatitis, rhinitis, and/or asthma. However, cutaneous changes such as urticaria, angioneurotic edema, and contact urticaria were the more common manifestations. The terms nonimmunologic contact urticaria or nonimmunologic immediate contact reactions were used to describe the occurrence. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/LABORATORY ANIMALS: Acute Exposure/ In a cumulative irritation study, three male albino guinea pigs received a daily open application of 10% benzyl alcohol in squalane (0.3 mL) on the back for 3 successive days. Sites were evaluated for erythema and edema 24 hours after each application and scored on a scale of 0 to 4. Benzyl alcohol in squalane received a cumulative score of 0.4, falling in the <=2.0 range of "none to weak irritant". [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/LABORATORY ANIMALS: Acute Exposure/ In a primary irritation study 10% benzyl alcohol in squalane was applied (0.3 mL) in a 24-hour occlusive patch to the back of eight male albino rabbits. The sites had been clipped free of hair and were abraded in four rabbits. Sites were evaluated according to the Draize scoring system at the time of patch removal and 72 hours later. No irritation was observed; there was a score of zero on a scale of 0 to 8. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/LABORATORY ANIMALS: Acute Exposure/ Benzyl alcohol was found to be not sensitizing in the guinea pig Draize Test, guinea pig maximization test, but was shown to be sensitizing in the guinea

pig by the Freund's Complete Adjuvant Test and the Open Epicutaneous Test. /From table/. OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.264 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

/LABORATORY ANIMALS: Acute Exposure/ Benzyl alcohol was not irritating to rabbit skin in a 4 hour exposure experiment but was moderately irritating following 24 hours exposure. It was a moderate to severe eye irritant. European Medicines Agency (EMA), The European Agency for the Evaluation of Medicinal Products, Veterinary Medicines Evaluation Unit, Committee for Veterinary Medicinal Products; Benzyl Alcohol, Summary Report (1997). Available from, as of July 8, 2008: <http://www.emea.europa.eu/pdfs/vet/mrls/Benzylalcohol.pdf>

/LABORATORY ANIMALS: Acute Exposure/ Undiluted benzyl alcohol was moderately irritating when applied to the depilated skin of guinea pigs for 24 hr. It was moderately irritating when applied to rabbit skin. Benzyl alcohol was severely irritating to the eyes of rabbits. Bingham, E.; Cohrsen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. V6 498

/SIGNS AND SYMPTOMS/ Benzyl alcohol is an aromatic organic alcohol that is a preservative, a solvent, and a local anesthetic. It rarely causes allergic contact dermatitis and is patch-tested as a 5% concentration in petrolatum. Marks, J.G. Jr., DeLeo V.A., Contact and Occupational Dermatology. St. Louis, MO: Mosby Year Book 1992., p. 128

/SIGNS AND SYMPTOMS/ Common side effects of /Benzyl Alcohol Lotion, 5%/ include irritations of the skin, scalp, and eyes, and numbness at the site of application. As with all medications, it is important to use benzyl alcohol, 5%, as labeled to maximize benefits and minimize risks. The product should be applied only to the scalp or the hair attached to the scalp. It is not approved for use in children younger than six months. Use in premature infants could lead to serious respiratory, heart- or brain-related adverse events such as seizure, coma, or death. FDA; FDA Approves Benzyl Alcohol Lotion for the Treatment of Head Lice, FDA News (April 9, 2009) Available from, as of April 20, 2009: <http://www.fda.gov/bbs/topics/NEWS/2009/NEW01993.html>

/CASE REPORTS/ A patient with allergic contact dermatitis caused by benzyl alcohol in a hearing aid impression material and in topical medications is described. In addition, the patient had topical and probably systemic corticosteroid allergy. Benzyl alcohol allergy is reviewed. Allergic contact dermatitis is the most commonly reported allergic reaction to benzyl alcohol. There is also 1 report of contact urticaria. ... Reported allergic reactions to injected benzyl alcohol include generalized urticarial reactions, 1 generalized maculopapular reaction and 1 delayed localized reaction. [Shaw DW; Am J Contact Dermat 10 (4): 228-32 (1999)] **PEER REVIEWED** PubMed Abstract

As taken from HSDB, 2018

"A condition described as relapsing tinea affecting the anogenital region and adjacent thigh area was observed in a man after treatment with antimycotic lotions and ointments. The condition was resolved by treatment with zinc oxide and topical corticosteroids and after stopping use of perfumed soap and foam-bath preparations. Positive reactions to a number of therapeutic ointments were seen in patch testing, and benzyl alcohol was subsequently identified as the sensitizing agent" As taken from WHO Food Additives Series 37 available at <http://www.inchem.org/documents/jecfa/jecmono/v37je05.htm>

In a primary irritation study 10% benzyl alcohol applied in a 24-hour occlusive patch to the back of eight male albino rabbits did not cause irritation. Undiluted benzyl alcohol was moderately irritating when applied to the depilated skin of guinea pigs for 24 hr.

As taken from HSDB, 2018

In a mouse local lymph-node assay (LLNA), an EC3 value was not established for benzyl alcohol (EC3 >50%) as higher concentrations needed to be tested (data reported to the EU Scientific Committee on Consumer Safety (SCCS) by RIFM, 2009).

The EC3 value derived from a mouse local lymph-node assay (LLNA) for benzyl alcohol (>12,500 µg/cm²) tends to over-estimate the no-effect-level for sensitization in humans, the weight-of-evidence no-expected-sensitization-induction-level (NESIL) being 5,800 µg/cm² (Lalko et al. 2009).

Skin irritation:

Species	Protocol	Result	Reference
Rabbit	OECD Guide-line 404	not irritating	Bayer AG data, Report No. 19232, 1990
Rabbit	Other	slightly irritating	Smyth, H. F. et al., 1951; reported in US NTP: TR 343, 1989.

Eye irritation:

Species	Protocol	Result	Reference
Rabbit	OECD Guide-line 405	moderately irritating	Bayer AG data, Report No. 19232, 1990
Rabbit	Other: limited data	highly irritating	Smyth, H. F. et al., 1951; reported in US NTP: TR 343, 1989.

Sensitization:

Species	Protocol	Result	Reference
Guinea pig	Draize test	not sensitizing	Klecak, G. et al., 1977
Guinea pig	Maximization test	not sensitizing	Klecak, G. et al., 1977
Guinea pig	Freund's complete adjuvant test	not sensitizing	Klecak, G. et al., 1977
Guinea pig	Open epicutaneous test	sensitizing	Klecak, G. et al., 1977
Guinea pig	Patch test	sensitizing	Malten, K. E. et al., 1984; Mitchell, J. C. et al., 1982; Nethercott, J. R., 1982
Human		sensitizing	

As taken from OECD SIDS Initial Assessment Report, 2001.

LLNA weighted mean EC3 values (n _{ij} g/cm ²) [no. studies]	Potency Classification ²	Human Data			WoE NESIL ³ (µg/cm ²)
		NOEL – HRIPT (induction) (µg/cm ²)	NOEL – MAX (induction) (µg/cm ²)	LOEL ¹ (induction) (µg/cm ²)	
> 12500 [1]	Weak	5906	6897	8858	5900

NOEL = No observed effect level; HRIPT = Human Repeat Insult Patch Test; MAT = Human Maximization Test; LOEL = lowest observed effect level; NA = Not Available

¹Data derived from HRIPT or HMT

²Gerberick et al., 2001

³WoE NESIL limited to two significant figures

As taken from IFRA (2007). Standard (42nd amendment) for Benzyl alcohol, available at <https://ifrafragrance.org/safe-use/standards-guidance>

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Reaction Severity	Reference
Standard Draize test	Administration onto the skin	Human - man	16 mg/48H	Mild	CTOIDG Cosmetics and Toiletries. (Allured Pub. Corp., POB 318, Wheaton, IL 60189) V.91- 1976- Volume(issue)/page/year: 94(8),41,1979
Standard Draize test	Administration onto the skin	Rodent - rabbit	100 mg/24H	Moderate	CTOIDG Cosmetics and Toiletries. (Allured Pub. Corp., POB 318, Wheaton, IL 60189) V.91- 1976- Volume(issue)/page/year: 94(8),41,1979
Standard Draize test	Administration onto the skin	Mammal - pig	100%	Moderate	FCTXAV Food and Cosmetics Toxicology. (London, UK) V.1-19, 1963-81. For publisher information, see FCTOD7. Volume(issue)/page/year:

					11,1011,1973
Standard Draize test	Administration onto the skin	Human	1%/2D		CODED Contact Dermatitis. Environmental and Occupational Dermatitis. (Munksgaard International Pub., c/o Publications Expediting Inc., 200 Meacham Ave., Elmont, NY 11003) V.1- 1975- Volume(issue)/page/year: 61,217,2009

As taken from RTECS, 2019.

In a study with 666 hairdressers and 1444 clients, patch testing with benzyl alcohol (1%) produced one positive response in each treatment group. (Uter W et al. 2014).

Patch testing of 1951 eczema patients with benzyl alcohol (10%) induced 4 positive responses (0.21%) (Mann J et al. 2014).

“BACKGROUND: Irritant contact dermatitis (ICD) is a common diagnosis in patients with occupational contact dermatitis (OCD). Studies are lacking on the usefulness of material safety data sheets (MSDSs) in making the diagnosis of ICD. OBJECTIVE: To characterize irritant exposures leading to the diagnosis of occupational ICD (OICD), and to evaluate the occurrence of concomitant exposures to contact allergens. METHODS: We included 316 patients with suspected occupational hand dermatitis, referred to the Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark during January 2010-August 2011, in a programme consisting of a clinical examination, exposure assessment, and extensive patch/prick testing. RESULTS: OCD was diagnosed in 228 patients. Of these patients, 118 were diagnosed with OICD. The main irritant exposures identified were wet work (n = 64), gloves (n = 45), mechanical traumas (n = 19), and oils (n = 15). Exposure to specific irritant chemicals was found in 9 patients, and was identified from MSDSs/ingredients labelling in 8 of these patients. Review of MSDSs and ingredients labelling showed that 41 patients were exposed to 41 moderate to potent contact allergens, and 18 patients were exposed to 25 weak workplace contact allergens. CONCLUSION: In the present study, the systematic exposure assessment did not reveal any new irritants. MSDSs have a limited role in the investigation of ICD.” As taken from Friis UF et al. 2014. Contact Dermatitis 71, 364-70. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/25302958>

“Although fragrance mix I (FM I) has been widely used to detect fragrance allergy, it can only detect up to 85% of fragrance-allergic patients. In the recent study by Mann et al., only 57.6% of patients tested with an extended series of fragrance ingredients (the 26 substances required to be labelled in the EU) also reacted to a fragrance marker in the baseline series. In 2005, we reported that the prevalence of contact allergy to FM I in patients with eczema was 15.9%. Given that more and more Chinese are using fragrance-containing cosmetics, and are also exposed to traditional Chinese medicine (TCM), which may also contain fragrances, it is interesting to study fragrance allergy beyond FM I in China.” As taken from Liu J and Li L-F. 2015. Contact Dermatitis 73, 252-253.

“Based on the available UV spectra, benzyl alcohol does not present a concern for phototoxicity or photoallergenicity. The molar absorption coefficient for λ max between 290 and 700 nm is well below the benchmark ($1000 \text{ L mol}^{-1} \text{ cm}^{-1}$) of concern for phototoxicity and photoallergenicity (Henry et al., 2009).”

As taken from Api AM et al., (2015).

“In in chemico experimental studies, little to no reactivity to cysteinebased peptides has been reported with read-across material benzyl alcohol (CAS # 100-51-6; see Section V) (Natsch et al.,

2007; Natsch and Gfeller, 2008). Read-across material benzyl alcohol has been reported to be both positive and negative in guinea pig tests. Additionally, in a local lymph node assay, read-across material benzyl alcohol was reported to have an EC3 value > 50% (12500 µg/cm²) (RIFM, 2005a). In the human repeat insult patch test, read-across material benzyl alcohol had a NESIL of 5906 µg/cm² and a LOEL of 8858 µg/cm² in ethanol contacting vehicles (RIFM, 2005b; RIFM, 2004). In the HMAX, a NOEL of 6897 µg/cm² has been reported with read-across material benzyl alcohol (Epstein 1979; #1697; RIFM, 1970) and 2759 µg/cm² with p-isopropylbenzyl alcohol (RIFM, 1973). Based on the material-specific data and read-across to benzyl alcohol (CAS # 100-51-6; see Section V), p-isopropylbenzyl alcohol is considered to be a weak skin sensitizer with a defined NESIL of 5900 µg/cm² (Table 1). Section IX provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2008; IDEA [International Dialogue for A.M. Api, et al. Food and Chemical Toxicology 141 (2020) 111338 5 the Evaluation of Allergens] project Final Report on the QRA2: Skin Sensitization Quantitative Risk Assessment for Fragrance Ingredients, September 30, 2016, <http://www.ideaproject.info/uploads/Modules/Documents/qra2-dossier-final-september-2016.pdf>) and a reference dose of 1.0 mg/kg/day.”

As taken from Api AM et al. 2020. Food Chem. Toxicol. 141(S1), 111338. Available at http://fragrancematerialsafetyresource.elsevier.com/sites/default/files/536-60-7_0.pdf

“Several sensitization tests in different animal systems yielded both positive and negative results. Thus no firm conclusion on the sensitizing potential of benzyl alcohol in animals could be reached. Further testing with volunteers indicates rather low sensitizing potential for benzyl alcohol in humans. A weight of evidence approach should be used for overall evaluation of the available studies in both animals and humans.”

As taken from ECHA, 2015

INCI Name	Allergy potential	LLNA EC3-value (ECHA RSD or RIVM)	Level of induction/elicitation	Reference
Benzyl alcohol (100-51-6)	Established contact allergen in humans. Listed as “+” in SCCS opinion, i.e. up to 10 positive test reactions reported.	> 50% (RIVM; SCCS) No data in ECHA RSD (sensitising)	Induction: No data Elicitation: No data SCCS concludes that in the absence of adequate substance specific data, it is possible to use a general threshold of 0.8 µg/cm ² (based on a concentration of 100 ppm).	SCCS/1459/11

As taken from Danish EPA, 2016

“No standardized in vitro methods to assess potency of skin sensitizers are available. Recently, we standardized a procedure which combines the epidermal equivalent potency assay with assessment of IL-18 to provide a single test for identification and classification of skin sensitizers. This current study aimed to extend tested chemicals, and to provide a simple in vitro method for estimation of the expected sensitization induction level interpolating in vitro EC50 and IL-18 SI2 values to predict LLNA EC3 and/or human NOEL from standards curves generated using reference contact allergens. Reconstituted human epidermis was challenged with 14 chemicals not previously tested benzoquinone, chlorpromazine, chloramine T, benzyl salicylate, diethyl maleate, dihydroeugenol, 2,4-dichloronitrobenzene, benzyl cinnamate, imidazolidinyl urea, and limonene as contact sensitizers while benzyl alcohol, isopropanol, dimethyl isophthalate and 4-aminobenzoic acid as non-sensitizers in the LLNA. Where for benzyl salicylate and benzyl cinnamate no

sensitization was observed in human predictive studies, positive responses to benzyl alcohol and dimethyl isophthalate were reported. The proposed method correlates better with human data, correctly predicting substances incorrectly classified by LLNA. With the exception of benzoquinone (interference with both MTT and IL-18 ELISA), and chloramine T (underestimated in the interpolation), a good estimation of LLNA EC3 and in vivo available human NOEL values was obtained.” As taken from Galbiati V et al. 2017. *Toxicol. Lett.* 271, 1-11. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28189648>

BA is considered to be an extremely rare sensitizer (2). We report a patient who was first suspected of having corticosteroid allergy but turned out to be sensitized to BA. Kubin ME and Riekkari R (2016). Benzyl alcohol allergy mimicking corticosteroid allergy. *Contact Dermatitis* 75(1), 58-59. PubMed, 2017 available at: <https://www.ncbi.nlm.nih.gov/pubmed/27264293>

“BACKGROUND: The development of non-animal alternatives for skin sensitization potency prediction is dependent upon the availability of a sufficient dataset whose human potency is well characterized. Previously, establishment of basic categorization criteria for 6 defined potency categories, allowed 131 substances to be allocated into them entirely on the basis of human information. OBJECTIVES: To supplement the original dataset with an extended range of fragrance substances. METHODS: A more fully described version of the original criteria was used to assess 89 fragrance chemicals, allowing their allocation into one of the 6 potency categories. RESULTS: None of the fragrance substances were assigned to the most potent group, category 1, whereas 11 were category 2, 22 were category 3, 37 were category 4, and 19 were category 5. Although none were identified as non-sensitizing, note that substances in category 5 also do not pass the threshold for regulatory classification. CONCLUSIONS: The combined datasets of >200 substances placed into potency categories solely on the basis of human data provides an essential resource for the elaboration and evaluation of predictive non-animal methods.” As taken from Api AM et al. 2017. *Dermatitis* 28(5), 299-307. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28691948>

“Twenty-four pure fragrance ingredients have been identified as potential concern for skin sensitization. Several of these compounds are chemically unstable and convert into reactive species upon exposure to air or light. In the present work, a systematic investigation of the correlation between chemical stability and reactivity has been undertaken. The compounds were subjected to forced photodegradation for three months and the chemical changes were studied with GC-MS. At the end of the stability study, two-thirds of the samples were found to be unstable. The generation of chemically reactive species was investigated using the in chemico HTS-DCYA assay. Eleven and fourteen compounds were chemically reactive before and after three months, respectively. A significant increase in reactivity upon degradation was found for isoeugenol, linalool, limonene, lylal, citronellol and geraniol; in the same conditions, the reactivity of hydroxycitronellal decreased. The non-reactive compounds α -isomethyl ionone, benzyl alcohol, amyl cinnamal and farnesol became reactive after photo-oxidative degradation. Overall, forced degradation resulted in four non-reactive fragrance compounds to display in chemico thiol reactivity, while ten out of 24 compounds remained inactive. Chemical degradation does not necessarily occur with generation of reactive species. Non-chemical activation may be involved for the 10 stable unreactive compounds.” As taken from Avonto C et al. 2018. *Toxicol. In Vitro* 46, 237-245. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28927722>

“Benzyl alcohol may cause mild local irritation.” “In addition to allergic reactions in sensitised patients, non-sensitised patients may become sensitised.”

As taken from EMA, 2019

“Some allergic reactions have been reported in humans. Benzyl alcohol may lead to local reactions (contact dermatitis) and general reactions, probably linked to a direct histamine release. Both Benzyl alcohol and Benzoic acid are mucous membrane irritant; it causes serious irritation following application to the skin of mice.”

As taken from EMA, 2017

“BACKGROUND: Fragrance chemicals constitute the second most frequent cause of contact allergy in Spain. There are no data available concerning the individual fragrances that are most frequently involved. OBJECTIVES: To describe the diagnostic contribution provided by specific fragrance series to the results obtained with baseline series fragrance markers by correlating the results of both series. MATERIALS AND METHODS: We performed a 5-year retrospective study of fragrance marker-positive patients tested with specific fragrance series in 23 Spanish centres. We collected the demographic and clinical characteristics, and compared the results of patch tests obtained from different suppliers. RESULTS: Of 19 588 patients patch tested with the Spanish baseline series, 1590 (8.1%) reacted positively to a fragrance marker. Of these, 1013 (63.7%) were patch tested with a fragrance series, and 664 patients reacted positively to at least one individual fragrance other than hydroxyisohexyl 3-cyclohexene carboxaldehyde. Geraniol was the most frequent allergen. Positive reactions to substances not included in fragrance mix (FM) I or FM II were found in 230 patients. Of the 436 FM I-positive patients and the 419 FM II-positive patients, 184 (42%) and 64 (39.1%), respectively, had no positive reactions to fragrance series. In the case of FM I, negative results were more common when individual fragrances were patch tested at low concentrations. CONCLUSIONS: We recommend patch testing all patients positive for any fragrance marker with a specific fragrance series. The correlation between the results of baseline series and fragrance series could be improved by increasing the concentrations of individual fragrances.” As taken from Silvestre JF et al. 2019. Contact Dermatitis 80(2), 94-100. PubMed, 2019 available at: <https://www.ncbi.nlm.nih.gov/pubmed/30430587>

“Cosmetics Europe, the European Trade Association for the cosmetics and personal care industry, is conducting a multi-phase program to develop regulatory accepted, animal-free testing strategies enabling the cosmetics industry to conduct safety assessments. Based on a systematic evaluation of test methods for skin sensitization, five non-animal test methods (DPRA (Direct Peptide Reactivity Assay), KeratinoSens™, h-CLAT (human cell line activation test), U-SENS™, SENS-IS) were selected for inclusion in a comprehensive database of 128 substances. Existing data were compiled and completed with newly generated data, the latter amounting to one-third of all data. The database was complemented with human and local lymph node assay (LLNA) reference data, physicochemical properties and use categories, and thoroughly curated. Focused on the availability of human data, the substance selection nevertheless resulted in a high diversity of chemistries in terms of physico-chemical property ranges and use categories. Predictivities of skin sensitization potential and potency, where applicable, were calculated for the LLNA as compared to human data and for the individual test methods compared to both human and LLNA reference data. In addition, various aspects of applicability of the test methods were analyzed. Due to its high level of curation, comprehensiveness, and completeness, we propose our database as a point of reference for the evaluation and development of testing strategies, as done for example in the associated work of Kleinstreuer et al. We encourage the community to use it to meet the challenge of conducting skin sensitization safety assessment without generating new animal data.” As taken from Hoffmann S et al. 2018. Crit. Rev. Toxicol. 48(5), 344-358. PubMed, 2019 available at: <https://www.ncbi.nlm.nih.gov/pubmed/29474128>

“BACKGROUND: There is limited information regarding contact dermatitis (CD) associated with skin cleansers (SCs). OBJECTIVE: The aim of the study was to evaluate the prevalence of allergic patch test (APT) reactions and irritant CD (ICD) associated with SCs. METHODS: A retrospective cross-sectional analysis was performed using North American Contact Dermatitis Group data, 2000-2014. RESULTS: Of 32,945 tested patients, 1069 (3.24%) had either APT reaction or ICD associated with SCs. Of these, 692 (64.7%) had APT reaction only, 350 (32.7%) had ICD only, and 27 (2.5%) had both. Individuals with APT reaction and/or ICD were more likely to have occupationally related skin disease (relative risk [RR] = 3.8 [95% confidence interval {CI} = 3.3-4.5] for APT reaction and 10.0 [95% CI = 8.2-12.2] for ICD, respectively, P < 0.0001). As compared with those without APT reaction to SC, individuals with APT reaction had significantly higher frequencies

of hand (RR = 2.4 [95% CI = 2.1-2.7]) and arm dermatitis (RR = 1.3 [95% CI = 1.1-1.6], $P \leq 0.001$). Irritant CD was strongly associated with hand dermatitis (RR = 6.2 [95% CI = 5.2-7.3], $P < 0.0001$). More than 50 allergens were associated with SCs including quaternium-15 (11.2%), cocamidopropyl betaine (9.5%), methylchloroisothiazolinone/methylisothiazolinone (8.4%), coconut diethanolamide (7.9%), fragrance mix I (7.7%), Myroxylon pereirae (5.9%), 4-chloro-3,5-xyleneol (5.8%), amidoamine (5.5%), and formaldehyde (4.4%).” As taken from Warshaw EM et al. 2018. Dermatitis 29(1), 32-42. PubMed, 2019 available at: <https://www.ncbi.nlm.nih.gov/pubmed/29256904>

“Cutaneous allergy occurs primarily as a result of using cosmetic, household, and laundry products available on the market that contain fragrances. The aim of this study was to develop a rapid and specific high-performance liquid chromatography with ultraviolet detection (HPLC-UV) method for quantification of 25 fragrance allergens (amyl cinnamyl alcohol, benzyl alcohol, benzyl benzoate, benzyl cinnamate, benzyl salicylate, citronellol, cinnamyl alcohol, citral, coumarin, eugenol, farnesol, geraniol, hydroxycitronellal, HICC (4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carbaldehyde), isoeugenol, isoeugenyl acetate, lilial (butyl phenyl methyl propional), limonene, linalool, methyl 2-octynoate, etc.). In addition, an exposure-based quantitative risk assessment (QRA) was performed to determine safe levels of fragrance ingredients in 107 perfumes. In 76 women's and 31 men's fragrances, 25 allergens were identified at concentrations ranging from undetectable (N.D.) to 8,997.68 mg/kg, and from N.D. to 17,352.34 mg/kg, respectively. An exposure-based sensitization QRA revealed that the ratios of acceptable exposure level (AEL) to consumer exposure level (CEL) of fragrance ingredients were greater than 1, suggesting an absence of skin sensitizing potential. However, the maximum level used in the exposure scenario was determined by the product purpose and application type, and AEL/CEL ratios of lilial, HICC, citral, isoeugenol, and methyl 2-octynoate analyzed in women's perfume were 0.53, 0.67 0.19, 0.13, and 0.57, respectively. As the ratios of AEL:CEL of these fragrance ingredients were below 1, the utilization of these potential skin sensitizers is not considered safe. Our findings indicate that the sensitization risk of allergens with AEL:CEL ratios below 1 detected in fragrances needs to be reduced to the appropriate human safety level for risk management.” As taken from Soo Lim D et al. 2018. J. Toxicol. Environ. Health A 81(22), 1173-1185. PubMed, 2019 available at: <https://www.ncbi.nlm.nih.gov/pubmed/30415634>

List of reference chemicals for determination of reproducibility (12 chemicals for WLR, 20 chemicals for BLR) and predictive capacity (20 chemicals) in a proposed similar or modified protein reactivity assay

12 Test chemicals for Within-Laboratory Reproducibility and Between-Laboratory Reproducibility

Test chemical	Cas No.	Physical state	Molecular weight	Mechanism	LLNA EC3 (%)	In vivo prediction ¹	DRPA prediction	ADRA prediction ²
Benzyl alcohol	100-51-6	Liquid	108.14	Non-reactive	-	Non-sensitizer	Pos/Neg ³ (non-concordant results)	Neg

¹Predictions of in vivo hazard (potency) are based on LLNA data. In vivo potency is derived using criteria proposed by ECETOC

²Result of ADRA (Amino acid Derivative Reactivity Assay) validation study. ³Result of DPRA (Direct Peptide Reactivity Assay) validation study.

Reference range of depletion from DPRA and ADRA.

12 Test chemicals for Within-Laboratory Reproducibility and Between-Laboratory Reproducibility

Test Chemicals	DPRA prediction ^{1,2}						ADRA prediction ³					
	Cys-peptide		Lys-peptide		Mean depletion (%)	N	NAC		NAL		Mean depletion (%)	N
	Depletion (%)	N	Depletion (%)	N	Depletion (%)	N	Depletion (%)	N				
Benzyl alcohol	2-3	4	<26	4	1-14	4	<5	5	<5	5	<3	5

¹Predictions based on published data (9) (11) (12) (13).

²Result of DPRA validation study (4). ³Result of ADRA validation study (5). N: the number of sources for reference ranges

As taken from OECD, 2019.

"Amino acid derivative reactivity assay (ADRA) has previously been developed as an alternative method to direct peptide reactivity assay (DPRA) to evaluate key event 1 in skin sensitization mechanisms. However, when using alternative methods for skin sensitization, integrated approaches to testing and assessment (IATA) that combine the results of multiple tests evaluating different key events are generally required. To verify whether ADRA can be used in IATA, we replaced DPRA with ADRA in five IATA methods combining DPRA, KeratinoSens, and h-CLAT: (i) the "2 out of 3" approach, (ii) the "3 out of 3" approach, (iii) sequential testing strategy (STS), (iv) integrated testing strategy by scoring approach (ITS-SA), and (v) the "ITS by two methods approach" (ITS-2MA). The prediction accuracy of the "2 out of 3" approach using ADRA (1 mM) and ADRA (0.5 mg/mL) was 90.0% and 91.1%, respectively, for human data, and was very similar to that obtained using DPRA (91.1%). The "3 out of 3" approach also showed good predictability (83.2%) using either ADRA (1 mM) or ADRA (0.5 mg/mL) compared to DPRA. Regarding the accuracy of the prediction of sensitization intensity for the human data by the third classification, prediction accuracy using ADRA was almost the same as STS, ITS-SA, or ITS-2MA using DPRA. As a result, this study showed that ADRA can be used as a test method for key event 1 in the evaluation of skin sensitization by combining multiple alternative methods." As taken from Yamamoto Y et al. 2019. J. Toxicol. Sci. 44(9), 585-600. PubMed, 2020 available at <https://pubmed.ncbi.nlm.nih.gov/31474740/>

Record for benzenemethanol:

Species Common Name	Exposure Type	Test Location	Conc 1 Type (Author)	Conc 1 (Author)	Conc 1 Units (Author)	Effect	Effect Measurement	Endpoint	Exposure Duration Mean (Days)	Response Site	Observed Duration (Days)
House Mouse	Subcutaneous	Lab	Active ingredient	25	ug/ml	Immunological	Immunoglobulin G	LOEL	19	Blood	<=19
House	Subcutaneous	Lab	Active ingredient	25	ug/ml	Immunol	Immunogl	LOE	19	Blood	<=19

Mouse	Mouse		Irritant		1	Immunological	Immunoglobulin G	L			
House Mouse	Subcutaneous	Lab	Active ingredient	25	µg/ml	Immunological	immunoglobulin E	NOEL	19	Blood	≤19
House Mouse	Subcutaneous	Lab	Active ingredient	25	µg/ml	Immunological	immunoglobulin E	NOEL	19	Blood	≤19

As taken from the US EPA ECOTOX database, 2021

“Undiluted B [benzyl alcohol] is rated as moderately irritant to the mucosae. B caused conjunctivitis as well as mild chemosis, irritation of the iris and corneal opacity in rabbit eyes; all effects were completely reversible within 21 days. In other, older studies, undiluted B caused severe eye irritation, whilst a 4% solution triggered no eye irritation.

In animal experiments, irritant effects on the eyes (lacrimation) also occurred upon exposure to B in the air (450 mg/m³, presumably as vapour and in aerosol form).

Skin irritation was also caused in test subjects by formulations with low B content. For instance, in an open epicutaneous test, 5% B in Vaseline caused urticaria in 7 of 32 probands, and 0.05% B in ethanol or ointment base caused skin irritations in 18 of 614 test subjects after up to 48 hours of occlusive application. In some cases, it was difficult to distinguish between these effects and allergic skin reactions (see below). As with the eyes, 1% solutions caused anaesthesia.

In an animal experiment, undiluted B caused no irritations in rabbit skin after four-hour semi-occlusive exposure to 0.5 ml in a study conducted in accordance with OECD directive. In other, older studies (not conducted in accordance with OECD guideline), no, mild or moderate effects were observed in rabbits and guinea pigs; in a study on mice, 10% aqueous solution also triggered severe skin irritant effects.

Despite its widespread distribution and use, benzyl alcohol only triggers contact sensitisations and allergic reactions in very rare cases. B is only rated as a very weak contact allergen.

There are only a few reports on individual cases pertaining to occupational skin sensitisations. There are rather reports on presumably allergic reactions to B as component of topically applied therapeutic products, and less frequently as a component of cosmetics. However, in clinical epidemiological studies involving epicutaneous tests with relatively high B concentrations (up to 10%), up to 2.5% or 1.6% of the test subjects showed positive reactions in collectives of up to 4246 and 2028 patients, respectively, when tested with 5 or 10 % B. Even higher rates of about 5% were reported in subjects with pre-existing skin lesions. A 1% preparation (mostly in Vaseline), in contrast, caused significantly lower reaction rates. In a series of studies conducted in German clinics between 1996 and 2007, 223 of 65,398 patients (0.3%) showed positive reactions to 1% B in Vaseline in epicutaneous tests. Further studies at these clinics yielded practically identical reaction rates of about 0.3% for test subjects with and without atopic diathesis (genetic predisposition to allergies). However, a positive reaction was strongly associated with an age > 40 years. [7619]

In epicutaneous tests on patients showing atopic reactions (to unknown substances or with suspected allergy to B), reactions to structurally related substances (benzyl cinnamate, benzaldehyde, benzylparaben and Peru balsam) were observed in addition to reactions to B.

An LLNA (Local Lymph Node Assay) performed in accordance with OECD directive on mice with B solutions of up to 50% in ethanol/diethyl phthalate (1:3) did not result in threefold increases of the lymphocyte proliferation and did thus not provide indications of a skin-sensitising potential. In older

studies, a maximisation test and a Draize test on guinea pigs provided no indications of a sensitising effect, whilst an open epicutaneous test and a test with Freund's complete adjuvant yielded positive results.

Despite the frequent use of B, there are no data on sensitising effects on the respiratory tract. [7619] In view of the skin findings, an allergic reaction on the mucosae of the respiratory tract cannot be ruled out."

"In animal experiments rats showed microscopically detectable minimal to mild changes in the respiratory tract after 4-week inhalative exposure (6 hours per day, 5 days per week, "nose only") to 1072 mg/m³ B (as vapour and aerosol, MMAD 3.3 µm): Hyperplasia and inflammation in the squamous epithelium of the nose, hyperplasia of the lymph nodes and type II pneumocytes as well as increase in eosinophilic and mononuclear infiltrates in the lungs as signs of inflammation. These changes were assessed at least as the beginning of an inflammatory effect; therefore, the concentration of 1072 mg/m³ was considered as LOAEC. Due to these findings, the irritant effect in the respiratory tract is considered a critical effect in occupational exposures to B."

As taken from GESTIS

"The purpose of this study was to evaluate the sensitization potential of 82 compounds classified as volatile and/or semi-volatile organic compounds using the direct peptide reactivity assay (DPRA), given that these chemical compounds have been detected frequently and at high concentrations in a national survey of Japanese indoor air pollution and other studies. The skin sensitization potential of 81 of these compounds was evaluable in our study; one compound co-eluted with cysteine peptide and was therefore not evaluable. Twenty-five of the evaluated compounds were classified as positive. Although all glycols and plasticizers detected frequently and at high concentrations in a national survey of Japanese indoor air pollution were negative, hexanal and nonanal, which are found in fragrances and building materials, tested positive. Monoethanolamine and 1,3-butanediol, which cause clinical contact dermatitis, and several compounds reported to have weak sensitization potential in animal studies, were classified as negative. Thus, it was considered that compounds with weak sensitization potential were evaluated as negative in the DPRA. Although the sensitization potential of the formaldehyde-releasing preservative bronopol has been attributed to the release of formaldehyde (a well-known contact allergen) by its degradation, its degradation products-bromonitromethane and 2-bromoethanol-were classified as positive, indicating that these degradation products also exhibit sensitization potential. The compounds that tested positive in this study should be comprehensively assessed through multiple toxicity and epidemiological studies." As taken from Kawakami T et al. 2020. J. Toxicol. Sci. 45(11), 725-735. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33132246/>

"Background: Preservatives are often necessary components of commercial products. Large-scale North American studies on preservative allergy are limited. Objective: To evaluate demographics, positive patch test reactions (PPTRs), clinical relevance, and trends for preservatives tested by the North American Contact Dermatitis Group. Methods: We conducted a retrospective cross-sectional analysis of North American Contact Dermatitis Group patch testing results of preservatives from 1994 through 2016. Results: A total of 50,799 patients were tested; 11,338 (22.3%) had a PPTR to at least 1 preservative. The most frequent reactions were to methylisothiazolinone 0.2% aqueous (aq) (12.2%), formaldehyde 2% aq (7.8%), formaldehyde 1% aq (7.8%), quaternium-15 2% petrolatum (pet) (7.7%), and methyldibromo glutaronitrile/phenoxyethanol 2% pet (5.1%). Paraben mix 12% pet (1%), iodopropynyl butylcarbamate 0.1% pet (0.4%), benzyl alcohol 1% pet (0.3%), and phenoxyethanol 1% pet (0.2%) had the lowest PPTRs. Linear regression analysis of preservatives tested showed that only methylchloroisothiazolinone/methylisothiazolinone 0.01% aq (parameter estimate, 0.42; 95% CI, 0.17-0.66; P < .005) had a significant increase in PPTRs over time. Limitations: Collected variables are dependent on clinical judgment. Results may be prone to referral selection bias. Conclusions: This large North American study provides insight on preservative PPTRs and trends from 1994 through 2016." As taken from Atwater AR et al. 2021. J.

Am. Acad. Dermatol. 84(4), 965-976. PubMed, 2021 available at <https://pubmed.ncbi.nlm.nih.gov/33579596/>

“Importance

When fragrance- and botanical-related (F/BR) allergy is identified, concomitant reaction (CR) frequencies can help direct avoidance recommendations.

Objective

This study aimed to determine the CR rates for F/BR allergens.

Design

A retrospective cross-sectional analysis of the North American Contact Dermatitis Group data 2007–2016 was conducted. Frequencies of demographics, positive reactions, strength of reactions, trends, and CR rates were calculated.

Results

A total of 5504 (22.7%) of 24,246 patients had F/BR allergic reactions. The F/BR-sensitive patients were more likely to be female, older than age 40 years, and White and have face, leg, or anal/genital dermatitis. Top allergens included fragrance mix I (FMI, 10.6%), Myroxylon pereirae (balsam of Peru [BOP], 8%), and fragrance mix II (FMII, 4.9%). There were increasing trends for FMI, FMII, cinnamic aldehyde, and Compositae mix and decreasing trends for BOP and propolis. When patients were positive to any F/BR allergen, they were likely to be positive to FMI, FMII, and BOP. Concomitant reactions were bidirectional between multiple fragrance allergens and propolis, colophony, and Compositae mix.

Conclusions

Concomitant reactions were identified between fragrances, between fragrances and BR allergens, and between BR allergens and fragrances. If CRs of greater than 10% suggest cross-reactivity, then all patients with fragrance sensitivity should avoid BR allergens and vice versa.”

Atwater AR et al. (2021) Fragrance- and Botanical-Related Allergy and Associated Concomitant Reactions: A Retrospective Analysis of the North American Contact Dermatitis Group Data 2007–2016.

“Background: There is considerable variability across European patch test centres as to which allergens are included in local and national cosmetics series.

Objectives: To propose a standardized, evidence-based cosmetic series for Europe based on up-to-date analysis of relevant contact allergens.

Methods: We collated data from the European Surveillance System on Contact Allergies (ESSCA) from 2009 to 2018 to determine which cosmetic allergens produce a high yield of contact allergy. Contact allergens with a prevalence of >0.3% that were considered relevant were included. Rare contact allergens were excluded if deemed no longer relevant or added to a supplemental cosmetic series for further analysis.

Results: Sensitization prevalences of 39 cosmetic contact allergens were tabulated. Thirty of these allergens yielded >0.3% positive reactions and are therefore included in our proposed European cosmetic series. Six were considered no longer relevant and therefore excluded. Three were included in a supplementary European cosmetic series. An additional nine allergens were included in either the core or supplemental European cosmetic series following literature review.

Conclusion: We have derived a potential European cosmetic series based upon the above methods. This will require ongoing investigation based upon the changing exposure profiles of cosmetic allergens as well as new and evolving substances.”

Horton E (2021) Developing a cosmetic series: Results from the ESSCA network, 2009-2018.

“Background: Benzyl alcohol is a widely used preservative, solvent and fragrance material. According to published data, it is a rare sensitizer in humans.

Objectives: To identify characteristics and sensitization patterns of patients with positive patch test reactions to benzyl alcohol and to check the reliability of the patch test preparation benzyl alcohol 1% pet.

Patients and methods: Retrospective analysis of data from the Information Network of Departments of Dermatology (IVDK), 2010-2019.

Results: Of 70 867 patients patch tested with benzyl alcohol 1% pet., 146 (0.21%) showed a positive reaction, most of them (89%) only weakly positive. The number of doubtful and irritant reactions significantly exceeded the number of positive reactions. Reproducibility of positive test reactions was low. Among benzyl alcohol-positive patients, compared to benzyl alcohol-negative patients, there were significantly more patients with leg dermatitis (17.8% vs. 8.6%), more patients aged 40 years or more (81.5% vs. 70.5%) and more patients who were tested because of a suspected intolerance reaction to topical medications (34.9% vs. 16.6%). Concomitant positive reactions were mainly seen to fragrances, preservatives and ointment bases.

Conclusions: Sensitization to benzyl alcohol occurs very rarely, mainly in patients with stasis dermatitis. In view of our results, benzyl alcohol cannot be regarded as a significant contact allergen, and therefore marking it as skin sensitizer 1B and labelling it with H 317 is not helpful."

Geier J et al. (2022) Is benzyl alcohol a significant contact sensitizer?

"Background: Reliable human potency data are necessary for conducting quantitative risk assessments, as well as development and validation of new nonanimal methods for skin sensitization assessments. Previously, human skin sensitization potency of fragrance materials was derived primarily from human data or the local lymph node assay.

Objectives: This study aimed to define skin sensitization potency of fragrance materials via weight of evidence approach, incorporating all available human, animal, in vitro, in chemico, and in silico data.

Methods: All available data on 106 fragrance materials were considered to assign each material into 1 of the 6 defined potency categories (extreme, strong, moderate, weak, very weak, and nonsensitizer).

Results: None of the 106 materials were considered an extreme sensitizer, whereas a total of 6, 23, 41, and 26 materials were categorized as strong, moderate, weak, and very weak sensitizers, respectively. Ten materials lacked evidence for the induction of skin sensitization.

Conclusions: Skin sensitization potency categorization of the 106 fragrance materials based on the described weight of evidence approach can serve as a useful resource in evaluation of nonanimal methods, as well as in risk assessment.

CAS Number	Chemical Name	Category Call Based on WoE	Human NOEL*, µg/cm2	Human LOEL†, µg/cm2	LLNA, EC3, µg/cm2	DPR A, Peptide Reactivity	KeratinoSens	h-CLAT	Protein Binding Alerts for Skin Sensitization, Toolbox 4.2	Parent Prediction, TIMES-SS	Metabolite Prediction, TIMES-SS
100-51-6	Benzyl alcohol	Weak	5905	8858	NC (12,500)	Minimal	Positive/Negative	Positive	No alert found	Nonsensitizer	Nonsensitizer

Benzyl alcohol (CAS 100-51-6) was categorized as a weak sensitizer, mainly based on a human NOEL of 5900 µg/cm2 and a similar LOEL of 8858 µg/cm2. The negative LLNA data and the lack of protein binding alerts suggest that benzyl alcohol is not a strong sensitizer. There are positive diagnostic patch test data in the literature. For instance, in a patch test study by Schnuch et al, 1% benzyl alcohol led to skin reactions in 0.3% in 2166 patients. In another study by Hausen, patch testing 102 patients with 5% benzyl alcohol led to skin reactions in 7.8% of the tested patients. However, considering the high volume of use as a fragrance ingredient in consumer products (International Fragrance Association, 2015 Volume of Use Survey), combined with the fact that benzyl alcohol is used ubiquitously in consumer products that come in close contact with the skin

such as facial scrub and face wash (Creme-RIFM Aggregate Exposure Model, V3.1.3), benzyl alcohol is a weak sensitizer."

Na, M., et al. (2022). Weight of Evidence Approach for Skin Sensitization Potency Categorization of Fragrance Ingredients. *Dermatitis : contact, atopic, occupational, drug*, 33(2), 161–175. <https://doi.org/10.1097/DER.0000000000000854>

5.8. All other relevant types of toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Phenyl carbinol 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 Phenyl carbinol 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	117	JTI KB Study Report(s)
In vitro cytotoxicity	117	JTI KB Study Report(s)

/ALTERNATIVE and IN VITRO TESTS/ Hemolysis of red blood cells washed with normal saline containing benzyl alcohol was studied. It was confirmed that benzyl alcohol was responsible for hemolysis, when the volume of saline exceeds the volume of blood. McOrmond P et al; *Drug Intell Clin Pharm* 14 (Jul-Aug): 549 (1980)

/ALTERNATIVE and IN VITRO TESTS/ Local anesthetics and alcohols inhibited mitochondrial electron transport at several points along the chain. N-butanol and benzyl alcohol inhibited each of segments of rat liver and beef heart mitochondrial electron transport chain assayed; these include cytochrome c oxidase, durohydroquinone oxidase, succinate oxidase and dehydrogenase, NADH oxidase, succinate-cytochrome c oxidoreductase, and others. [Chazotte B, Vanderkooi G; *Biochim Biophys Acta* 636 (2): 153-61 (1981)] **PEER REVIEWED** PubMed Abstract

/CASE REPORTS/ ... Intraocular use of sodium chloride solution preserved with 2% benzyl alcohol during cataract surgery and peripheral iridectomy caused severe striated keratopathy, progressing to chronic edema of cornea, with vesicles, bullae, and dirty pigmented appearance of endothelium. ... Iris was also affected. Grant, W.M. *Toxicology of the Eye*. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 143

/SIGNS AND SYMPTOMS/ A "gasping" syndrome, which included hypotension, bradycardia, gasping respiration, hypotonia, progressive metabolic acidosis, seizures, cardiovascular collapse, and death, was first described in low-birth-weight neonates in intensive care units. All the infants had received either bacteriostatic water or sodium chloride solution containing 0.9% benzyl alcohol to flush intravenous catheters or in parenteral medications reconstituted with bacteriostatic water or saline. The syndrome occurred in infants who had received greater than 99 mg/kg of benzyl alcohol (range, 99-234 mg/kg). Goldfrank, L.R., *Goldfrank's Toxicologic Emergencies* 8th Ed. 2006., McGraw-Hill, New York, N.Y., p. 831

/SIGNS AND SYMPTOMS/ In 1981 and 1982 several neonatal deaths were ascribed to benzyl alcohol present as a preservative in isotonic saline (9 mg/mL) that had been used to flush catheters. The syndrome consisted of metabolic acidosis, central neural depression, respiratory distress progressing to gasping respiration, hypotension, renal failure, and sometimes seizures and intracranial hemorrhages. In alerting pediatricians of the findings, the FDA reported an estimated daily intake of 99 to 404 mg/kg, which was 20 to 90 times the 4.5-mg/kg dose considered safe for healthy adults. Although the infants involved had "serious underlying disease," biochemical evidence of benzyl alcohol toxicity was found. Blood and urine specimens contained high concentrations of benzyl alcohol, benzoic acid, and hippuric acid. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/SIGNS AND SYMPTOMS/ Benzyl alcohol, which is used as a preservative in intravascular flush solutions, caused neurological deterioration and deaths in very low birth weight infants. Preterm infants who received large volumes of fluids containing 0.9% benzyl alcohol via catheter developed "gasping baby syndrome." Estimated intakes of 99 to 405 mg benzyl alcohol/kg body weight for 2 to 28 days caused effects including severe metabolic acidosis, gasping, neurological deterioration, blood abnormalities, skin breakdown, liver and kidney failure, lowered blood pressure, heart failure, and death. No effects were seen for intakes of 27 to 99 mg/kg body weight over similar periods, although there is one report of 32 to 105 mg/kg body weight for 7 days causing breathing difficulty. Bingham, E.; Cohrssen, B.; Powell, C.H.; Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. V6 500

/LABORATORY ANIMALS: Acute Exposure/ No fatalities or symptoms were found in rats exposed for 6 hr to a calculated concentration of 61 ppm nor were symptoms produced by exposures obtained by bubbling air through the liquid heated to 100 and 150 deg C. ... Doses of 0.2 mL/kg or more, administered to dogs by stomach tube induced emesis and defecation. This was apparently due to irritation of the gastric mucosa ... Diuresis was more pronounced in the rabbit than in the dog, after administration of benzyl alcohol by various routes. The injection of 5 to 10% benzyl alcohol in oil of sweet almond in the region of the auditory meatus of cats caused temporary degeneration of the small facial nerves. [Clayton, G. D. and F. E. Clayton (eds.). Patty's Industrial Hygiene and Toxicology: Volume 2A, 2B, 2C: Toxicology. 3rd ed. New York: John Wiley Sons, 1981-1982., p. 4639

As taken from HSDB, 2018

"PURPOSE: Benzyl alcohol (BA) is the preservative in triamcinolone acetonide (TA) suspensions, which are used in treating vitreoretinal diseases and during surgery. This paper investigates the molecular mechanisms and signaling pathways underlying BA toxicity in human retinal pigment epithelial (RPE) cells. METHODS: Cultured human RPE cells from the ARPE-19 cell line were exposed to culture medium alone (control) or with BA (0.0225, 0.225, 0.9, 3, or 9 mg/mL) for up to 6 hours. BA toxicity was assessed by TUNEL assay, propidium iodide/annexin V-FITC staining and flow cytometry, caspase activation assay, caspase and apoptosis inhibition assays, mitochondrial transmembrane potential by rhodamine staining and flow cytometry, reactive oxygen species by chemiluminescence, and apoptosis-inducing factor staining. RESULTS: BA caused RPE cell death not only by necrosis but also by apoptosis, evidenced by exposure to 9 mg/mL BA for 6 hours leading to 19.0% early apoptotic cells and 64.2% apoptotic necrotic cells. Apoptotic signaling involved the immediate production of reactive oxygen species, activation of caspase-8, impairment of the mitochondrial transmembrane potential, and further activation of caspase-9 and -3. In addition, BA induced translocation of apoptosis-inducing factor into the nucleus, indicating caspase-independent apoptosis. CONCLUSIONS: BA leads to necrosis of RPE cells and triggers mitochondrial apoptosis through both caspase-dependent and - independent pathways. Extreme caution is suggested in the intraocular use of TA suspensions and meticulous evaluation before

adoption of BA as a preservative in the future development of ophthalmic formulations” (Chang et al. 2011).

Benzyl alcohol isolated from litchi chinensis caused a marked increase in prostaglandin E(2) and nitric oxide production in J774 murine macrophage cells, indicating an inflammatory effect.

Zhou Y et al. 2012. BMC Complement Altern. Med. 12, 12. PubMed, 2013 available at <http://www.ncbi.nlm.nih.gov/pubmed/22380404?dopt=AbstractPlus>

“Benzyl alcohol (BnOH) is widely used as a component of foods, cosmetics, household products and medical products. It is generally considered to be safe for human use, however, it has been connected to a number of adverse effects, including hypersensitivity reactions and neonatal deaths. BnOH is a membrane fluidizing agent that can affect membrane protein activity and cellular processes such as ligand binding to cell surface receptors, endocytosis and degradation of lysosomal cargo. In this study, we examined the effects of BnOH on intracellular transport using Shiga toxin (Stx), diphtheria toxin (DT) and ricin. BnOH caused reduced toxicity of all three toxins at BnOH concentrations that cause membrane fluidization. The reduced toxicity of Stx and ricin was mainly due to inhibition of retrograde transport between endosomes and the trans-Golgi network as BnOH had small effects on cell association and endocytosis of ricin and Stx. Strikingly, BnOH also induced a reversible fragmentation of the Golgi apparatus.” As taken from Simm R et al. 2017. Exp. Cell Res. 357(1), 67-78. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/28450044>

“Molecularly tethered lipid bilayer membranes were constructed on a commercially available chemically modified gold substrate. This is a new and promising product that has allowed the construction of very robust lipid bilayers. Very high resolution electrical impedance spectroscopy (EIS) was used to determine the dielectric structure of the lipid bilayers and associated interfaces. The EIS data were modelled in terms of the dielectric substructure using purpose developed software. The hydrophobic region, where the lipid tails are located, was revealed by the EIS in the frequency range of (1-100) Hz and its thickness was calculated from the capacitance of this region and found to be approximately 3-4 nm. The hydrophilic region, where the polar heads are located, was revealed at higher frequencies and its thickness was estimated to be approximately 1-2 nm. The effect of the local anaesthetic benzyl alcohol (BZA) on the tethered lipid bilayers was investigated. The effect of BZA on the membrane capacitance and conductance allowed the changes in the thickness of the polar head and hydrophobic tails regions to be determined. It was found that the addition of BZA caused a significant increase in the capacitance (corresponding to a decrease in the thickness) of the hydrophobic region and an increase in the membrane electrical conductance. The EIS allowed a distinction between a hydrophobic region in the centre of the bilayer and an outer hydrophobic region. Benzyl alcohol was found to have the largest effect on the outer, hydrophobic region, although the inner hydrophobic region was also consistently affected.” As taken from Alobeedallah H et al. 2016. J. Membr. Biol. 9(6), 833-844. PubMed, 2017 available at <https://www.ncbi.nlm.nih.gov/pubmed/27803961>

“Multidose formulations of biotherapeutics, which offer better dosage management and reduced production costs, require the addition of antimicrobial preservatives (APs). APs have been shown, however, to decrease protein stability in solution and cause protein aggregation. In this report, the effect of 4 APs, m-cresol, phenol, phenoxyethanol, and benzyl alcohol on conformational stability, aggregation propensity, and backbone flexibility of an IgG1 mAb, mAb-4, is investigated. Compared with no preservative control, each of the APs decreased the conformational stability of mAb-4 as measured by differential scanning calorimetry and extrinsic fluorescence spectroscopy. The addition of APs resulted in increased monomer loss and aggregate accumulation at 50°C over 28 days, as monitored by size-exclusion chromatography. The extent of conformational destabilization and protein aggregation of mAb-4 induced by APs followed their calculated octanol-water partition coefficients. Increases in backbone flexibility, as measured by hydrogen exchange, of a region

located in the CH2 domain of the mAb (heavy chain 237-254) in the presence of APs also correlated with hydrophobicity. Based on these results, the destabilizing effect of APs on mAb-4 correlates with the increased hydrophobicity of the APs and their ability to enhance the local backbone flexibility of an aggregation hot spot within the CH2 domain of the mAb.” As taken from Arora J et al. 2017. J. Pharm. Sci. 106(6), 1508-1518. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/28212986>

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
ICLo - Inhibitor Concentration Low	In vitro	Mouse - liver	46 mmol/L/16 H	In Vitro Toxicity Studies - cell membrane integrity: cytoplasmic enzymes leakage (lactate dehydrogenase , ATP enzymes etc.)	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year : 86,253,2015
ICLo - Inhibitor Concentration Low	In vitro	Mouse - liver	9.6 mmol/L/4H	Biochemical - Metabolism (Intermediary) - effect on mitochondrial function	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year : 86,253,2015
ICLo - Inhibitor Concentration Low	In vitro	Human - liver tumor	46 mmol/L/48 H	In Vitro Toxicity Studies - cell membrane integrity: cytoplasmic enzymes leakage (lactate dehydrogenase , ATP enzymes etc.)	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year : 86,253,2015
IC50 - Inhibitor Concentration 50	In vitro	Human lymphocyte	>200 mg/L/45H	In Vitro Toxicity Studies - other assays	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year : 29,901,2015
ICLo - Inhibitor Concentration Low	In vitro	Human - skin	3.60 pph/24H	In Vitro Toxicity Studies - other assays	TOLED5 Toxicology Letters. (Elsevier Science Pub. B.V., POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1977-

					Volume(issue)/page/year : 271,1,2017

As taken from RTECS, 2019.

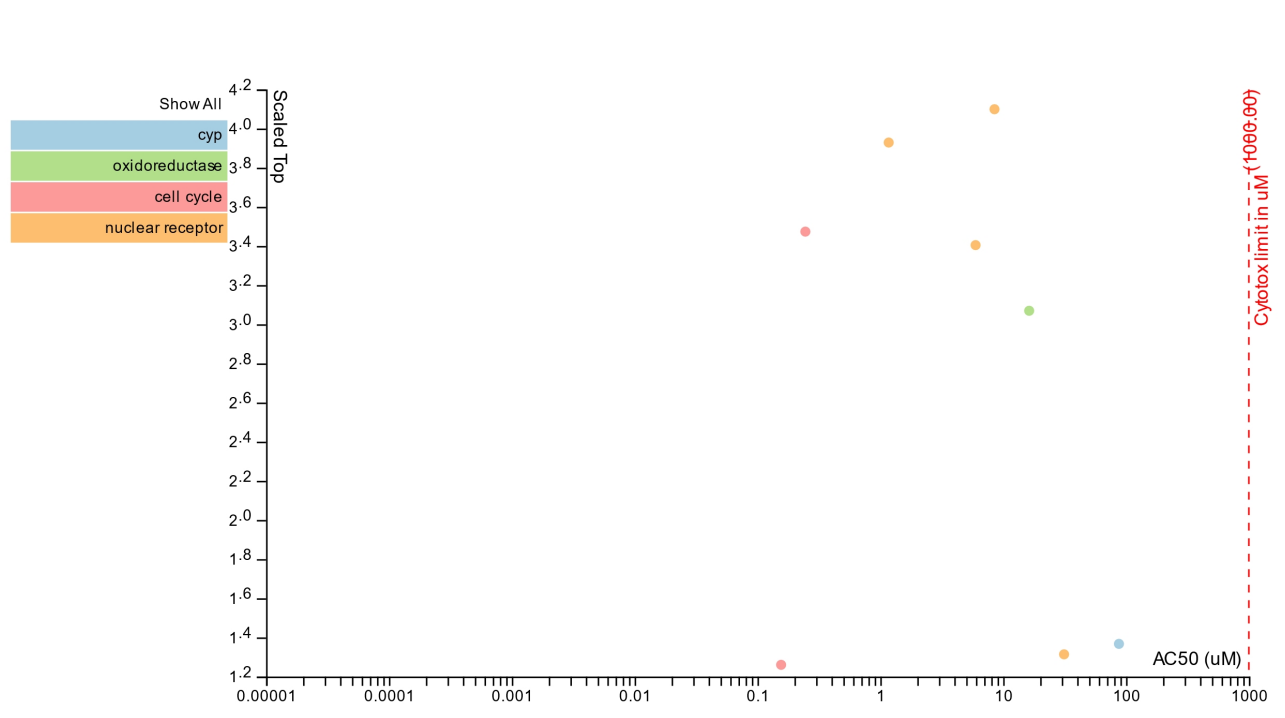
High-throughput Assay Data

The US Environmental Protection Agency (EPA) evaluated benzyl alcohol in a series of high-throughput assays, which are publicly available on the US EPA's CompTox Dashboard (section BIOACTIVITY / sub-section TOXCAST:SUMMARY), available at the following URL: <https://comptox.epa.gov/dashboard>

US EPA provides the following data use considerations for ToxCast data: "The activity of a chemical in a specific assay does not necessarily mean that it will cause toxicity or an adverse health outcome. There are many factors that determine whether a chemical will cause a specific adverse health outcome. Careful review is required to determine the use of the data in a particular decision contexts. Interpretation of ToxCast data is expected to change over time as both the science and analytical methods improve."

A summary of the ToxCast assay data on benzyl alcohol is provided below in Figure 1. Figure 1 proves an overview of the types of assays where activity was noted with this substance. The complete study details are available on US EPA's CompTox Dashboard.

Figure 1



6. Functional effects on

6.1. Broncho/pulmonary system

/HUMAN EXPOSURE STUDIES/ A blinded, controlled study in healthy volunteers who were treated with 3 mL of saline with or without benzyl alcohol (9 mg/mL) found that the benzyl alcohol group had bronchitis with erythema, tracheobronchial mucosal edema, metaplasia, denudation of cilia, and mucosal lymphocytic infiltration on bronchoscopy. [Dart, R.C. (ed). Medical Toxicology. Third Edition, Lippincott Williams & Wilkins. Philadelphia, PA. 2004., p. 1232]

/SIGNS AND SYMPTOMS/ Nebulizers of bacteriostatic saline containing benzyl alcohol as a preservative can cause bronchitis in healthy adults. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

As taken from HSDB, 2018

“Benzyl alcohol has been linked with the risk of severe side effects including breathing problems (called “gasping syndrome”) in young children.”

As taken from EMA, 2019

“After repeated inhalation of B [benzyl alcohol] aerosol (approx. 18 mg B, four times daily in physiological saline solution), one patient developed a severe bronchitis with bloody sputum, which was apparently caused by B. Due to the lung disease that already existed before the treatment with B, this study cannot be used to assess the risk of occupational exposure to B. However, corresponding effects should not be ruled out at workplaces, especially in case of pre-existing respiratory diseases. This is also true for the development of allergic skin diseases after repeated occupational exposure.”

As taken from GESTIS

6.2. Cardiovascular system

“High exposure can cause decrease in blood pressure, ... (Sax 1986).”

As taken from SKYE data bank of environmental chemicals.

“/LABORATORY ANIMALS: Acute Exposure/ Benzyl alcohol displayed antiarrhythmic-antifibrillatory effects when injected iv (0.2-0.4 mL/kg of a 4% solution) into dogs and rats with spontaneous and drug induced arrhythmias. IV injections of high doses caused intravascular hemolysis.[Eichbaum FW, Yasaka WJ; Basic Res Cardiol 71 (4): 355-70 (1976)] **PEER REVIEWED** PubMed Abstract”

/ALTERNATIVE and IN VITRO TESTS/ ... To identify phototoxic effects, 43 fragrances were evaluated in vitro with a photohemolysis test using suspensions of human erythrocytes exposed to radiation sources rich in ultraviolet (UV) A or B in the presence of the test compounds. Hemolysis was measured by reading the absorbance values, and photohemolysis was calculated as a percentage of total hemolysis. ... Moderate UVA-induced hemolysis (5-11%) was found with benzyl alcohol ... The phototoxic effects depended on the concentration of the compounds and the UV doses administered. ...[Placzek M et al; Acta Derm Venereol 87 (4): 312-6 (2007)] **PEER REVIEWED** PubMed Abstract

/ALTERNATIVE and IN VITRO TESTS/ Suspensions of human erythrocytes were incubated with benzyl alcohol, benzoic acid, and sodium benzoate. Each material was tested at 10⁻⁵, 10⁻⁴, and 10⁻³ mol/L. Erythrocytefree samples were also incubated with the test materials and used as controls. Following incubation, suspensions and samples were exposed to varying amounts of ultraviolet A (UVA) light from one of three sources. Hemolysis was measured as a function of absorbance of 550 nm light. None of the three substances produced significant photohemolysis.[Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

As taken from HSDB, 2018

“Benzyl alcohol can cause hemolysis of human and rabbit erythrocytes in the presence of 0.9% NaCl.”

"Benzyl alcohol displays a pronounced antiarrhythmic-antifibrillatory effect, when injected i.v. into dogs and rats with spontaneous or drug-induced arrhythmias. Mechanisms which might be responsible for the antiarrhythmic effect: lengthening of the effective refractory period, Local and general anaesthetic effects, changes of osmolality. The i.v. injection of benzyl- alcohol in high doses, produces intravascular haemolysis."

As taken from IUCLID Dataset (2000), Benzyl alcohol (100-51-6).

6.3. Nervous system

"Ingestion of large volumes results in CNS depression..... Dermal application to humans ... exerts narcotic action. (Sax 1986)."

As taken from SKYE data bank of environmental chemicals.

"Gasping respiration was observed in premature infants who had received benzyl alcohol in medications administered intravenously. The syndrome was characterized by central nervous system dysfunction with hypoactivity, hypotonia, and depression of the sensorium, followed by apnoea, seizure activity, and coma; severe metabolic acidosis; skin breakdown; haematological and hepatic disturbances, including thrombocytopenia, leukopenia, direct hyperbilirubinaemia, hyperammonaemia; hypotension and renal failure; cardiovascular collapse; and death at 6-46 days of age. Infants with this syndrome had received 99-234 mg/kg bw per day of benzyl alcohol, while a matched control group of infants who had received benzyl alcohol but who did not develop the syndrome had received doses of 27-99 mg/kg bw per day. (The median intravenous lethal dose in the rat is 314 mg/kg bw.)" As taken from WHO Food Additives Series 37 available at <http://www.inchem.org/documents/jecfa/jecmono/v37je05.htm>

"/SIGNS AND SYMPTOMS/ Benzyl alcohol is believe to have a role in the increased frequency of cerebral intraventricular hemorrhages and mortality reported in very-low-birth-weight (VLBW) infants (weight < 1000 g) who received flush solutions preserved with benzyl alcohol. An increased incidence of developmental delay and cerebral palsy is also noted in the same VLBW patient population, suggesting a secondary damaging effect of benzyl alcohol. Goldfrank, L.R., Goldfrank's Toxicologic Emergencies 8th Ed. 2006., McGraw-Hill, New York, N.Y., p. 831

"/SIGNS AND SYMPTOMS/ Paraparesis and flaccid paraplegia after epidural or intrathecal administration of more than 7.5 mg of benzyl alcohol as a diluent are suggestive of benzyl alcohol toxicity. Dart, R.C. (ed). Medical Toxicology. Third Edition, Lippincott Williams & Wilkins. Philadelphia, PA. 2004., p. 1231

"/OTHER TOXICITY INFORMATION/ No direct ocular injury from external contact has been reported in human beings, but one death and one case of serious illness with delirium and visual disturbances were supposed to have been caused by absorption of benzyl alcohol from an impure preparation of benzyl benzoate which was employed in massaging the skin Grant, W.M. Toxicology of the Eye. 3rd ed. Springfield, IL: Charles C. Thomas Publisher, 1986., p. 144"

/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ Benzyl alcohol applied daily to guinea pigs with experimental trichophytosis on surface of scarred infected skin produced paralysis in hind limbs after several days. [Wollmann H et al; Pharmazie 22 (8): 455 (1967)] **PEER REVIEWED**
<http://www.ncbi.nlm.nih.gov/pubmed/5601620?dopt=Abstract>
PubMed Abstract

/LABORATORY ANIMALS: Neurotoxicity/ In a study in rats, lumbosacral dorsal root action potential amplitudes were measured after exposure to 0.9% or 1.5% benzyl alcohol in either 0.9% sodium chloride solution or distilled water. Rats exposed to all benzyl alcohol solutions for less than 1 minute had inhibited dorsal root actions potentials. This was attributed to the local anesthetic effects of benzyl alcohol. Nerve function was 50-90% restored after rinsing the nerves with 0.9% sodium chloride solutions. Chronic intrathecal exposure to benzyl alcohol 0.9% over 7 days showed scattered areas of demyelination and early remyelination. The 1.5% benzyl alcohol solution-exposed dorsal nerve roots showed greater changes with widespread areas of demyelination and fatty degeneration of nerve fibers. Goldfrank, L.R., Goldfrank's Toxicologic Emergencies 8th Ed. 2006., McGraw-Hill, New York, N.Y., p. 832

/LABORATORY ANIMALS: Acute Exposure/ Microscopic examination revealed local nerve degeneration when 5% benzyl alcohol was injected into the side of a cat's face. At 10% local anesthesia was produced. OECD; SIDS Initial Assessment Report for SIAM 13. Report on Benzoates: Benzoic acid, Sodium benzoate, Potassium benzoate, Benzyl alcohol (CAS No: 65-85-0, 532-32-1, 582-25-2, 100-51-6) p.300 UNEP Publications (November 2001). Available from, as of November 18, 2014: <http://www.inchem.org/pages/sids.html>

/SIGNS AND SYMPTOMS/ Epidural or intrathecal injection of 7.5 mg or more benzyl alcohol as a diluent has been followed by paraparesis and flaccid paraplegia. Residual symptoms may be present 26 months after the initial injury. Dart, R.C. (ed). Medical Toxicology. Third Edition, Lippincott Williams & Wilkins. Philadelphia, PA. 2004., p. 1231

As taken from HSDB, 2018

"Aseptic meningitis has been observed following intrathecal administration of radiopharmaceuticals that contain benzyl alcohol as a preservative. Cisterna magna injections of benzyl alcohol in concentrations as high as 10 times that normally used did not produce meningitis in adult or immature dogs."

"Injection of benzyl alcohol (700-900 mg/kg, i.p.) caused rapid immobilization of mice. The mice were immobilized within 2 min. and remained unresponsive (no righting reflex, no wink reflex, and no leg reflex) for about 30 min. The immobilizing effect was accompanied by a marked hyperglycemia. Tracer studies indicated that the hyperglycemic effect may have resulted from increased gluconeogenesis."

"Benzyl alcohol used as a stabilizer for antibiotics of aminoglycosid structure is the substance responsible for the displacement of bilirubin from albumin. The free, unbound, unconjugated bilirubin tends to diffuse into the lipid of the brain of young Gunn rats with resultant kernicterus."

"After i.v. injection in mice, benzyl alcohol was found to inhibit TBPS binding and to stimulate GABA receptor mediated C1 influx into brain vesicles."

As taken from IUCLID Dataset (2000), Benzyl alcohol (100-51-6).

"Benzyl alcohol with epinephrine provides prolonged cutaneous anesthesia, although it is not as effective as lidocaine with epinephrine. However, benzyl alcohol is significantly less painful on injection than lidocaine with epinephrine, and it may offer an alternative for local anesthesia." As taken from Wilson L and Martin S. 1999. Ann Emerg Med. 33(5), 495-9, available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=10216324&query_hl=5&itool=pubmed_DocSum

"In short-term studies lethargy and sedation, respiratory problems, staggering, depression and coordination defects were observed (IUCLID, 2000. Ks: 2)." As taken from Bassan A et al. 2012. Reports on toxicokinetics, toxicity and allergenicity data on substances to be evaluated as acceptable previous cargoes for edible fats and oils (NP/EFSA/CONTAM/2011/01) - Batches n. 1, 2 and 3. Available at <http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2012.EN-274/epdf>.

"The antiemetic maropitant, with metacresol as preservative (Cerenia, Zoetis), has been associated with pain after subcutaneous injection in dogs and cats. Recently, a generic formulation containing benzyl alcohol was authorised (Prevomax, Le Vet). Benzyl alcohol is reported to have local anaesthetic properties and reduce injection pain. This study compared local pain after subcutaneous injection of the two maropitant formulations, administered at approximately 4°C and 25°C, to dogs. Thirty-two healthy beagle dogs were enrolled into a blinded, randomised, cross-over study. Dogs received subcutaneous injections of maropitant injection containing metacresol as preservative and maropitant injection containing benzyl alcohol as preservative, both at approximately 4°C and 25°C, with at least three days in between treatments. Injection pain was evaluated by two blinded observers using a visual analogue scale immediately after injection and a simple descriptive scale at two minutes after injection. In healthy beagle dogs, subcutaneous injection of maropitant with benzyl alcohol is significantly less painful than injection of maropitant with metacresol." As taken from Deckers N et al. 2018. Vet. Rec. Open 5(1), e000262. PubMed, 2018 available at <https://www.ncbi.nlm.nih.gov/pubmed/29531751>

"Benzyl alcohol is notably used via intramuscular administration in antibiotics, anti-inflammatory products or neuroleptics for its anaesthetic properties, in order to reduce pain at the injection site. The median concentration is 150 mg per injection (30 mg/ml). The range of injected dose varies from 20 to 600 mg/day."

As taken from EMA, 2017

"Oral short-term or subchronic studies with benzyl alcohol showed neurotoxicity manifested as lethargy in mice (from 500 mg/kg per day) and lethargy or staggering (from 800 mg/kg bw per day) in rats."

As taken from EFSA, 2019

"B [benzyl alcohol] has a local anaesthetic effect on the mucosae and the skin, which can mask possible irritant effects. Thus, 1% B solution in physiological saline solution initially caused pain in the eye, followed by complete anaesthesia within a few minutes."

"After oral ingestion, 5 ml of a 1% B solution caused mild anaesthesia, increased salivation and a tingling sensation after 1-minute oral exposure."

As taken from GESTIS

"This report presents the case of a patient whose inhalation exposure to benzyl alcohol led to clinical manifestations similar to toluene intoxication, including sudden altered mental status, metabolic acidosis, hypokalemia, hypophosphatemia, and hyperammonemia. Toxicity from benzyl alcohol inhalation is quite rare, and hyperammonemia associated with renal tubular dysfunction in poisoning cases has not been reported in the past. (...)

The patient's altered mental status was thought to be caused by benzyl alcohol central neurotoxicity. A mice model study showed that acute benzyl alcohol poisoning causes motor dysfunction, dyspnea, seizures, and death. Furthermore, inhibition of benzyl alcohol metabolism by pretreatment also resulted in death, which suggests that benzyl alcohol itself is toxic. However, the specific mechanism with which benzyl alcohol affects the central nervous system is unknown. (...) The cause of metabolic acidosis and hyperammonemia was thought to be renal tubular dysfunction."

Inada M et al. (2022) Toxic benzyl alcohol inhalation: Altered mental status with metabolic acidosis and hyperammonemia.

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

"In a study to evaluate the effects of short-term intake of benzyl alcohol on alcohol and aldehyde dehydrogenase activities, groups of four male and four female Sprague-Dawley rats received either

a 2% (v/v) solution of benzyl alcohol as the sole drinking fluid ad libitum or deionized water, for 12 consecutive days. Enzymatic activities were determined in the cytoplasmic and mitochondrial fractions of liver tissue obtained from the animals after treatment. Pretreatment with benzyl alcohol decreased cytoplasmic alcohol and aldehyde dehydrogenase activities when ethanol and acetaldehyde, respectively, were the substrates, but had no effect on alcohol dehydrogenase activity when benzyl alcohol was the substrate. The effect on alcohol dehydrogenase activity was seen only in female rats. Similarly, benzyl alcohol pretreatment resulted in decreased activity of aldehyde dehydrogenase in female rats when cetaldehyde was the substrate, but not when benzaldehyde was the substrate" As taken from WHO Food Additives Series 37 available at <http://www.inchem.org/documents/jecfa/jecmono/v37je05.htm>

"/ALTERNATIVE and IN VITRO TESTS/ Local anesthetics and alcohols inhibited mitochondrial electron transport at several points along the chain. N-butanol and benzyl alcohol inhibited each of segments of rat liver and beef heart mitochondrial electron transport chain assayed; these include cytochrome c oxidase, durohydroquinone oxidase, succinate oxidase and dehydrogenase, NADH oxidase, succinate-cytochrome c oxidoreductase, and others. [Chazotte B, Vanderkooi G; Biochim Biophys Acta 636 (2): 153-61 (1981)] **PEER REVIEWED** PubMed Abstract

/OTHER TOXICITY INFORMATION/ Compared to control rats, benzyl alcohol noncompetitively inhibited activity of hepatic alcohol dehydrogenase (L-ADH) of rats maintained for a short term on 5% ethanol. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/OTHER TOXICITY INFORMATION/ Messiha reported that short-term intake of 2% benzyl alcohol in the drinking water resulted in an inhibition of hepatic alcohol dehydrogenase and mitochondrial aldehyde dehydrogenase isoenzyme activities in female rats. The effects were not noted in male rats. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/OTHER TOXICITY INFORMATION/ Studies reported benzyl alcohol to increase activity of membrane-bound Ca²⁺-C-dependent enzymes such as adenylate cyclase and thiol proteinase. Conversely, benzyl alcohol inhibited activities of various glycosyltransferases of the rat liver Golgi membrane. The activities of erythrocyte-bound p-nitrophenylphosphatase and acetylcholinesterase were increased at some concentrations of benzyl alcohol and inhibited by others. The effect on cell membranes was considered the mechanism by which benzyl alcohol inhibited lymphocyte-mediated cytolysis in vitro. Benzyl alcohol induced time-, dose-, and temperature-dependent hemolysis of erythrocytes. [Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)] **PEER REVIEWED** PubMed Abstract

/OTHER TOXICITY INFORMATION/ Benzyl alcohol is a membrane "fluidizer" that affects lipid bilayer structure. It has been demonstrated to act on membranes of erythrocytes and hepatocytes. Nair B; Int J Toxicol 20 Suppl 3: 23-50 (2001)]

As taken from HSDB, 2018

"50 mM benzyl alcohol fluidized proximal brush-border membranes prepared from human small intestine and increased p-nitrophenyl-phosphatase activity in this membrane. This agent also shifted the phase transition temperature of the membrane and breakpoint temperature of this enzymatic activity."

"The in vitro effect of local anesthetic benzyl alcohol was studied using isolated cells from rat stomach. Lower concentrations of the alcohol increased the basal aminopyrine accumulation and

potentiated the secretory response of parietal cells to histamine and dbcAMP. At higher concentrations the alcohol progressively inhibited both the basal 14-C-aminopyrine accumulation and that stimulated by histamine, dbcAMP or carbachol. While a low concentration increased gastric microsomal (H-K)-ATPase activity, higher concentrations inhibited enzyme activity to about 80 % of those activities found in resting parietal cells."

"It was demonstrated that benzyl alcohol, a neutral local anesthetic, inhibits the uptake and degradation of lowdensity lipoprotein and endocytosis of transferrin receptors of guinea-pig leukemic B lymphocytes. This inhibition is very rapid, concentration dependant and reversible by simple washing. Membrane fluidity of the living cells is also modified."

"In vitro studies of human liver alcohol dehydrogenase (ADH) variants revealed that benzyl alcohol is slowly metabolized by beta-2-ADB. Working with this solvent might lead to toxic effects; these could be particularly prominent in individuals possessing the beta-2-ADH if they have a lower capacity to eliminate them, or they could be particularly prominent in those with beta-1-ADH if they quickly convert them into toxic aldehydes."

As taken from IUCLID Dataset (2000), Benzyl alcohol (100-51-6).

"Protein unfolding rates measured by hydrogen exchange were very sensitive to benzyl alcohol concentration, and increased markedly when salt was also added." As taken from Tobler SA et al. 2004. J Pharm Sci. 93(6), 1605-17 available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15124217&query hl=3&itool=pubmed_docsum

"Benzyl alcohol, an antimicrobial preservative, accelerates aggregation and precipitation of recombinant human interleukin-1 receptor antagonist (rhIL-1ra) in aqueous solution. The loss of native monomer during incubation at 37 degrees C was determined by analysis of sample aliquots with size exclusion high performance liquid chromatography (SE-HPLC). Benzyl alcohol caused minor perturbation of the tertiary structure of the protein without changing its secondary structure, documenting that the preservative caused a minor shift in the protein molecular population toward partially unfolded species. Consistent with this conclusion, in the presence of benzyl alcohol the rate of H-D exchange was accelerated and the fluorescence of 1-anilinonaphthalene-8-sulfonic acid in the presence of rhIL1ra was increased. Benzyl alcohol did not alter the free energy of unfolding based on unfolding experiments in urea or guanidine HCl. With differential scanning calorimetry it was determined that benzyl alcohol reduced the apparent Tm of rhIL-1ra, but this effect occurred because the preservative lowered the temperature at which the protein aggregated during heating. Isothermal calorimetry documented that the interaction of benzyl alcohol with rhIL-1ra is relatively weak and hydrophobically driven. Thus, benzyl alcohol accelerates protein aggregation by binding to the protein and favoring an increase in the level of partially unfolded, aggregation-competent species. Sucrose partially inhibited benzyl alcohol-induced aggregation and tertiary structural change." As taken from Zhang Y al. 2004. J Pharm Sci. 93(12), 3076-89 available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15514986&query hl=5&itool=pubmed DocSum

"1. We studied the effects of benzylalcohol on heterologously expressed wild type (WT), paramyotonia congenita (R1448H) and hyperkalaemic periodic paralysis (M1360V) mutant alpha-subunits of human skeletal muscle sodium channels. 2. Benzylalcohol blocked rested channels at -150 mV membrane potential, with an ECR(50) of 5.3 mM in wild type, 5.1 mM in R1448H, and 6.2 mM in M1360V. When blockade was assessed at -100 mV, the ECR(50) was reduced in R1448H (2 mM) compared with both wild type (4.3 mM; P<0.01) and M1360V (4.3 mM). 3. Membrane depolarization before the test depolarization significantly promoted benzylalcohol-induced sodium channel blockade. The values of K(D) for the fast-inactivated state derived from benzylalcohol-induced shifts in steady-state availability curves were 0.66 mM in wild type and 0.58 mM in R1448H. In the presence of slow inactivation induced by 2.5 s depolarizing prepulses, the ECI(50) for benzylalcohol-induced current inhibition was 0.59 mM in wild type and 0.53 mM in R1448H. 4.

Recovery from fast inactivation was prolonged in the presence of drug in all clones. 5. Benzylalcohol induced significant frequency-dependent block at stimulating frequencies of 10, 50, and 100 Hz in all clones. 6. Our results clearly show that benzylalcohol is an effective blocker of muscle sodium channels in conditions that are associated with membrane depolarization. Mutants that enter voltage-dependent inactivation at more hyperpolarized membrane potentials compared with wild type are more sensitive to inhibitory effects at the normal resting potential.” As taken from Haeseler G et al. 2000. Br J Pharmacol. 130(6), 1321-30 available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=10903972&query_hl=5&itool=pubmed_DocSum

“Abstract Aim: To study the effects of intravitreally injected triamcinolone acetonide (TA) and/or its preservative benzyl alcohol (BA) in healthy rabbit retina. Methods: Forty-eight rabbits (aged 4 months, body weight $\approx$ 3 kg) were randomized into four groups (n = 12). They were examined with electroretinography (ERG) prior to drug exposure, and then injected intravitreally with a combination of TA and BA, TA without BA, BA alone or a balanced saline solution (BSS). The electroretinograms were assessed 1 week and 7 weeks post-injection. The rabbits were euthanized and the sectioned retinas were studied. Immunohistochemical analysis was performed on rods, cones, rod bipolar cells, horizontal cells, amacrine cells and Müller cells. Results: Rabbits injected with BA showed a significantly lower rod-mediated b-wave amplitude than the controls 1 week after injection.....Immunohistochemistry revealed that protein kinase C alpha (PKC α) was down-regulated in both the perikarya and the axons of bipolar cells in histological sections from rabbit retina injected with TA + BA, BA and TA. Conclusions: Intravitreal injection of the preservative BA reduces the isolated rod-mediated retinal response in the rabbit, transiently and selectively.....The effects observed are not only limited to retinal function, but also include changes in the expression of PKC α in rod bipolar cells, indicating drug-related interference with normal retinal physiology in the healthy rabbit eye.” As taken from Myers AC et al. 2013. Curr. Eye Res. 38(6), 649-61. PubMed, 2014 available at: <http://www.ncbi.nlm.nih.gov/pubmed/23537282>

“Acetaminophen (APAP) toxicity is the most common cause of acute liver failure in industrialized countries. Understanding the mechanisms of APAP-induced liver injury as well as other forms of sterile liver injury is critical to improve the care of patients. Recent studies demonstrate that danger signaling and inflammasome activation play a role in APAP-induced injury. The aim of these investigations was to test the hypothesis that benzyl alcohol (BA) is a therapeutic agent that protects against APAP-induced liver injury by modulation of danger signaling. APAP-induced liver injury was dependent, in part, on Toll-like receptor (TLR)9 and receptor for advanced glycation endproducts (RAGE) signaling. BA limited liver injury over a dose range of 135-540 μ g/g body weight or when delivered as a pre-, concurrent, or post-APAP therapeutic. Furthermore, BA abrogated APAP-induced cytokines and chemokines as well as high-mobility group box 1 release. Moreover, BA prevented APAP-induced inflammasome signaling as determined by interleukin (IL)-1 β , IL-18, and caspase-1 cleavage in liver tissues. Interestingly, the protective effects of BA on limiting liver injury and inflammasome activation were dependent on TLR4 signaling, but not TLR2 or CD14. Cell-type-specific knockouts of TLR4 were utilized to further determine the protective mechanisms of BA. These studies found that TLR4 expression specifically in myeloid cells (LyzCre-tlr4-/-) were necessary for the protective effects of BA.” As taken from Cai C et al. 2014. Hepatology 60, 990-1002. PubMed, 2014 available at <http://www.ncbi.nlm.nih.gov/pubmed/24798499>

“Acetaminophen (APAP) hepatotoxicity is a serious public health problem in western countries. Current treatment options for APAP poisoning are limited and novel therapeutic intervention strategies are needed. A recent publication suggested that benzyl alcohol (BA) protects against APAP hepatotoxicity and could serve as a promising antidote for APAP poisoning. To assess the protective mechanisms of BA, C56Bl/6J mice were treated with 400 mg/kg APAP and/or 270 mg/kg BA. APAP alone caused extensive liver injury at 6 h and 24 h post-APAP. This injury was attenuated by BA co-treatment. Assessment of protein adduct formation demonstrated that BA inhibits APAP metabolic activation. In support of this, in vitro experiments also showed that BA

dose-dependently inhibits cytochrome P450 activities. Correlating with the hepatoprotection of BA, APAP-induced oxidant stress and mitochondrial dysfunction were reduced. Similar results were obtained in primary mouse hepatocytes. Interestingly, BA alone caused mitochondrial membrane potential loss and cell toxicity at high doses, and its protective effect could not be reproduced in primary human hepatocytes (PHH). We conclude that BA protects against APAP hepatotoxicity mainly by inhibiting cytochrome P450 enzymes in mice. Considering its toxic effect and the loss of protection in PHH, BA is not a clinically useful treatment option for APAP overdose patient.” As taken from Du K et al. 2015. Food Chem. Toxicol. 86, 253-61. PubMed, 2016 available at <http://www.ncbi.nlm.nih.gov/pubmed/26522885>

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 phenyl carbinol 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 phenyl carbinol. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	1,426	Carmines, 2002 & Rustemeier et al., 2002
	1,990	Baker et al., 2004a
	260	JTI KB Study Report(s)
	5,500	
	5,900	
	7,670	
	10,900	Gaworski et al., 2011 & Coggins et al., 2011h
	1,566	Roemer et al., 2014
In vitro genotoxicity	1,426	Carmines, 2002 & Roemer et al., 2002
	1,990	Baker et al., 2004c
	260	Renne et al., 2006
	260	JTI KB Study Report(s)

	5,500	
	7,670	
	4,510	fGLH Study Report (2010)
	10,900	Gaworski et al., 2011 & Coggins et al., 2011h
	1,566	Roemer et al., 2014
In vitro cytotoxicity	1,426	Carmines, 2002 & Roemer et al., 2002
	1,990	Baker et al., 2004c
	260	
	5,500	JTI KB Study Report(s)
	7,670	
	4,510	fGLH Study Report (2010)
	10,900	Gaworski et al., 2011 & Coggins et al., 2011h
	1,566	Roemer et al., 2014
	3,674	Gaworski et al., 1998
	1,426	Carmines, 2002 & Vanscheeuwijck et al., 2002
	1,990	Baker et al., 2004c
	260	Renne et al., 2006
Inhalation study	260	
	5,500	JTI KB Study Report(s)
	7,670	
	10,900	Gaworski et al., 2011 & Coggins et al., 2011h
	1,566	Schramke et al., 2014
Skin painting	3,674	Gaworski et al., 1999
	260	
	5,500	JTI KB Study Report(s)
In vivo genotoxicity	1,566	Schramke et al., 2014
	7,670	JTI KB Study Report(s)

In a pyrolysis study, 94.2% of benzyl alcohol added to cigarettes was transferred intact to the smoke (Purkis et al. 2011).

9. Heated/vapor emissions toxicity

Total particulate matter (TPM) from heated (tobacco or nicotine) product(s) containing Phenyl carbinol 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 Phenyl carbinol 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	117	JTI KB Study Report(s)
In vitro cytotoxicity	117	JTI KB Study Report(s)

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

Endpoint	Tested level (ppm)	Reference
Aerosol chemistry	10,000	Logic (2019a) Labstat International Inc. (2021)
In vitro genotoxicity	10,000	Logic (2019a) Labstat International Inc. (2022)
In vitro cytotoxicity	10,000	Logic (2019a) Labstat International Inc. (2022)
In vivo genotoxicity	400	Logic (2019a)
Inhalation study	400	Logic (2019a)

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

Endpoint	Tested level	Reference
Aerosol chemistry	0.2412 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vitro genotoxicity	0.2412 mg/(tobacco portion; 310 mg)	Logic (2019b)

In vitro cytotoxicity	0.2412 mg/(tobacco portion; 310 mg)	Logic (2019b)
In vivo genotoxicity	0.2412 mg/(tobacco portion; 310 mg)	Logic (2019b)
Inhalation study	0.2412 mg/(tobacco portion; 310 mg)	Logic (2019b)

Aerosol from heated tobacco stick(s) containing Phenyl carbinol was tested in aerosol chemistry and a battery of in vitro test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), the activity of the total particulate matter (TPM) and/or gas vapor phase (GVP) were not increased by the addition of this ingredient when compared to TPM and/or GVP from reference combustible cigarettes. The table below provides the highest tested level(s) and specific endpoint(s):

Endpoint	Tested level (mg/stick)	Reference
Aerosol chemistry	0.35	Labstat International Inc. (2020a) Labstat International Inc. (2021a)
In vitro genotoxicity	0.35	Labstat International Inc. (2020b) Labstat International Inc. (2021b)
In vitro cytotoxicity	0.35	Labstat International Inc. (2020b) Labstat International Inc. (2021b)

“Thousands of electronic cigarette refill fluids are commercially available. The concentrations of nicotine and the solvents, but not the flavor chemicals, are often disclosed on product labels. The purpose of this study was to identify and quantify flavor chemicals in 39 commercial refill fluids that were previously evaluated for toxicity. Twelve flavor chemicals were identified with concentrations ≥ 1 mg/ml: cinnamaldehyde, menthol, benzyl alcohol, vanillin, eugenol, p-anisaldehyde, ethyl cinnamate, maltol, ethyl maltol, triacetin, benzaldehyde, and menthone. Transfer of these flavor chemicals into aerosols made at 3V and 5V was efficient (mean transfer = 98%). We produced lab-made refill fluids containing authentic standards of each flavor chemical and analyzed the toxicity of their aerosols produced at 3V and 5V using a tank Box Mod device. Over 50% of the refill fluids in our sample contained high concentrations of flavor chemicals that transferred efficiently to aerosols at concentrations that produce cytotoxicity. When tested with two types of human lung cells, the aerosols made at 5V were generally more toxic than those made at 3V. These data will be valuable for consumers, physicians, public health officials, and regulatory agencies when discussing potential health concerns relating to flavor chemicals in electronic cigarette products.” As taken from Behar RZ et al. 2018. Sci. Rep. 8(1), 8288. PubMed, 2019 available at:

<https://www.ncbi.nlm.nih.gov/pubmed/29844439>

“Background: Given the high concentrations of nicotine and flavor chemicals in EC (electronic cigarette) fluids, it is important to determine how efficiently they transfer to aerosols, how well they are retained by users (exposure), and if they are exhaled into the environment where they settle of surfaces forming ECEAR (EC exhaled aerosol residue).

Objectives: To quantify the flavor chemicals and nicotine in refill fluids, inhaled aerosols, and exhaled aerosols. Then deduce their retention and contribution to ECEAR.

Methods: Flavor chemicals and nicotine were identified and quantified by GC-MS in two refill fluids, smoking machine-generated aerosols, and aerosols exhaled by 10 human participants (average age 21; 7 males). Machine generated aerosols were made with varying puff durations and two wattages (40 and 80). Participants generated exhale ad libitum; their exhale was quantified, and chemical retention and contribution to ECEAR was modeled.

Results: "Dewberry Cream" had five dominant (≥ 1 mg/mL) flavor chemicals (maltol, ethyl maltol, vanillin, ethyl vanillin, furaneol), while "Cinnamon Roll" had one (cinnamaldehyde). Nicotine transferred well to aerosols irrespective of topography; however, transfer efficiencies of flavor chemicals depended on the chemical, puff volume, puff duration, pump head, and EC power. Participants could be classified as "mouth inhalers" or "lung inhalers" based on their exhaled concentrations of flavor chemicals and nicotine and retention. Lung inhalers had high retention and exhaled low concentrations of EC chemicals. Only mouth inhalers exhaled sufficient concentrations of flavor chemicals/nicotine to contribute to chemical deposition on environmental surfaces (ECEAR).

Conclusion: These data help distinguish two types of EC users, add to our knowledge of chemical exposure during vaping, and provide information useful in regulating EC use."

Khachatoorian C et al. (2022) Tracing the movement of electronic cigarette flavor chemicals and nicotine from refill fluids to aerosol, lungs, exhaled, and the environment.

10. Ecotoxicity

10.1. Environmental fate

Environmental	Fate/Exposure	Summary:
Benzyl alcohol's production and use as a chemical intermediate, in perfumery and flavoring, in textiles and sheet plastics, inks, cosmetics, and drug ingredient may result in its release to the environment through various waste streams. If released to air, a vapor pressure of 9.4×10^{-2} mm Hg at 25 deg C indicates benzyl alcohol will exist solely as a vapor in the atmosphere. Vapor-phase benzyl alcohol will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 2 days. Benzyl alcohol does not contain chromophores that absorb at wavelengths >290 nm and therefore is not expected to be susceptible to direct photolysis by sunlight. If released to soil, benzyl alcohol is expected to have very high mobility based upon a Koc range of <5 to 29. An experimentally derived first-order aerobic biodegradation rate constant of 0.05 days, corresponding to a half-life of about 13 days, suggests that biodegradation is an important environmental fate process. Volatilization from moist soil surfaces is not expected to be an important fate process based upon a Henry's Law constant of 3.37×10^{-7} atm-cu m/mole. Benzyl alcohol is not expected to volatilize from dry soil surfaces based upon a vapor pressure. If released into water, benzyl alcohol is not expected to adsorb to suspended solids and sediment based upon the Koc. Volatilization from water surfaces is not expected to be an important fate process based upon this compound's Henry's Law constant. An estimated BCF of 0.3 suggests the potential for bioconcentration in aquatic organisms is low. Hydrolysis is not expected to be an important environmental fate process since this compound lacks functional groups that hydrolyze under environmental conditions. Occupational exposure to benzyl alcohol may occur through inhalation and dermal contact with this compound at workplaces where benzyl alcohol is produced or used. Monitoring and use data indicate that the general population may be exposed to benzyl alcohol via dermal contact with consumer products containing benzyl alcohol and to a lesser extent via inhalation of ambient air, ingestion of food and drinking water. (SRC) **PEER REVIEWED**		

Artificial	Pollution	Sources:
Benzyl alcohol's production and use as a chemical intermediate, in perfumery and flavoring(1), in textiles and sheet plastics, inks, cosmetics(2) and drug ingredient(3) may result in its release to the environment through various waste streams(SRC). [(1) O'Neil MJ, ed; The Merck Index. 14th ed.		

Whitehouse Station, NJ: Merck and Co., Inc., p. 186 (2006) (2) Lewis RJ Sr, ed; Hawley's Condensed Chemical Dictionary. 13th ed. New York, NY: John Wiley & Sons, Inc., p. 129 (1997) (3) Personal Care Products Council; Cosmeticsinfo.org. Benzyl Alcohol. Available at http://www.cosmeticsinfo.org/ingredient_details.php?ingredient_id=756 as of July 11, 2008.]
PEER REVIEWED

Environmental

Fate:

TERRESTRIAL FATE: Based on a classification scheme(1), Koc values of <5(2) to 29(3) indicate that benzyl alcohol is expected to have very high mobility in soil(SRC). Volatilization of benzyl alcohol from moist soil surfaces is not expected to be an important fate process(SRC) given a Henry's Law constant of 3.37×10^{-7} atm-cu m/mole(4). Benzyl alcohol is not expected to volatilize from dry soil surfaces(SRC) based upon a vapor pressure of 9.40×10^{-2} mm Hg(5). An experimentally derived first-order aerobic biodegradation rate constant of 0.05 days(6), corresponding to a half-life of about 13 days(SRC), suggests that biodegradation is an important environmental fate process in soil(SRC). [(1) Swann RL et al; Res Rev 85: 17-28 (1983) (2) Southworth GR, Keller JL; Water Air Soil Poll 28: 239-48 (1986) (3) Xu F et al; J Environ Qual 30: 1618-23 (2001) (4) Abraham MH et al; J Pharm Sci 83: 1085-100 (1994) (5) Daubert TE, Danner RP; Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, D.C.: Taylor and Francis (1989) (6) Tabak HH, Govind R; in Proc Annu Meet Air Waste Manage Assoc, 85th 8B 92-29.05 (1992)] **PEER REVIEWED**

AQUATIC FATE: Based on a classification scheme(1), Koc values of <5(2) to 29(3) indicate that benzyl alcohol is not expected to adsorb to suspended solids and sediment(SRC). Volatilization from water surfaces is not expected(3) based upon a Henry's Law constant of 3.37×10^{-7} atm-cu m/mole(4). According to a classification scheme(5), an estimated BCF of 0.3(SRC), from its log Kow of 1.10(6) and a regression-derived equation(7), suggests the potential for bioconcentration in aquatic organisms is low(SRC). An experimentally derived first-order aerobic biodegradation rate constant of 0.05 days(8), corresponding to a half-life of about 13 days(SRC), suggests that biodegradation is an important environmental fate process in water(SRC). [(1) Swann RL et al; Res Rev 85: 17-28 (1983) (2) Southworth GR, Keller JL; Water Air Soil Poll 28: 239-48 (1986) (3) Xu F et al; J Environ Qual 30: 1618-23 (2001) (4) Abraham MH et al; J Pharm Sci 83: 1085-100 (1994) (5) Franke C et al; Chemosphere 29: 1501-14 (1994) (6) Hansch C et al; Exploring QSAR. Hydrophobic, Electronic, and Steric Constants. ACS Prof Ref Book. Heller SR, consult. ed., Washington, DC: Amer Chem Soc p. 31 (1995) (7) Meylan WM et al; Environ Toxicol Chem 18: 664-72 (1999) (8) Tabak HH, Govind R; in Proc Annu Meet Air Waste Manage Assoc, 85th 8B 92-29.05 (1992)] **PEER REVIEWED**

ATMOSPHERIC FATE: According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere(1), benzyl alcohol, which has a vapor pressure of 9.4×10^{-2} mm Hg at 25 deg C(2), is expected to exist solely as a vapor in the ambient atmosphere. Vapor-phase benzyl alcohol is degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals(SRC); the half-life for this reaction in air is estimated to be 2 days(SRC), calculated from its rate constant of 2.3×10^{-11} cu cm/molecule-sec at 25 deg C(3). Benzyl alcohol does not contain chromophores that absorb at wavelengths >290 nm(4) and therefore is not expected to be susceptible to direct photolysis by sunlight(SRC). [(1) Bidleman TF; Environ Sci Technol 22: 361-367 (1988) (2) Daubert TE, Danner RP; Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, D.C.: Taylor and Francis (1989) (3) Atkinson R; J Phys Chem Ref Data Monograph 1 (1989) (4) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 8-12 (1990)] **PEER REVIEWED**

Environmental

Biodegradation:

BOD5/COD ratio for benzyl alcohol: 0.091 /From table/ [Lyman, W.J., W.F. Reehl, D.H. Rosenblatt. Handbook of Chemical Property Estimation Methods-Environmental Behavior of Organic Compounds. New York, NY: McGraw-Hill Book Company, 1982., p. 9-64] **PEER REVIEWED**

AEROBIC: Benzyl alcohol underwent 70% of theoretical biological oxygen demand in 5 days under aerobic conditions using an acclimated mixed microbial culture(1). At an initial concn of 250 ppm, benzyl alcohol achieved 29% of the theoretical BOD after 12 hours in a sewage sludge acclimated to this compound, and 31% oxidation in a sludge acclimated to mandelic acid(2). At an initial concn of 500 ppm, it achieved 52%, 42%, and 43% of the theoretical BOD in 12 hours using a settled sewage sludge acclimated to phenol, benzoic acid, and catechol, respectively(2). It is listed as a synthetic organic chemical easily biodegradable by biological sewage treatment(3). Benzyl alcohol at an initial concentration of 500 mg/L was shown to undergo rapid oxygen uptake under aerobic conditions when inoculated with municipal sewage sludge(4,5). Benzyl alcohol achieved 48% of the theoretical BOD in 5 days using a sewage sludge seed(6). Benzyl alcohol underwent 60.8% degradation using an industrial sludge inoculum under aerobic conditions in 5 days(7). Benzyl alcohol, present at 100 mg/L, reached 92-96% of its theoretical BOD in 2 weeks using an activated sludge inoculum at 30 mg/L in the Japanese MITI test(8). An experimentally derived first-order aerobic biodegradation rate constant of 0.05 days was reported(9), corresponding to a half-life of about 13 days(SRC). [(1) Babeu L, Vaishnav DD; J Ind Microbiol 2: 107-15 (1987) (2) McKinney RE et al; Sew Indust Wastes 28: 547-57 (1956) (3) Thom NS and Agg AR; Proc Royal Soc Lond B 189: 347-57 (1975) (4) Marion CV, Malaney, GW; Proc Ind Waste Cong 18: 297-308 (1964) (5) Lutin PA et al; Purdue Univ Eng Bull Ext Series 118: 131-45 (1965) (6) Heukelekian H, Rand MC; J Water Pollut Contr Assoc 29: 1040-53 (1955) (7) Belly RT, Goodhue CT; Proc Int Biodegrad Symp 3: 1103-7 (1976) (8) Chemicals Inspection and Testing Institute; Japan Chemical Industry Ecology - Toxicology and Information Center. ISBN 4-89074-101-1 (1992) (9) Tabak HH, Govind R; in Proc Annu Meet Air Waste Manage Assoc, 85th 8B 92-29.05 (1992)] ****PEER REVIEWED****

ANAEROBIC: Under anaerobic conditions, benzyl alcohol underwent 100% mineralization within 2 weeks when inoculated with a municipal digester sludge(1). Benzyl alcohol at an initial concentration of 50 ppm underwent greater than 75% mineralization to carbon dioxide and methane within 8 weeks using a municipal sewage sludge inocula under anaerobic conditions(2). Using sediment from anoxic salt marsh, 10 mM benzyl alcohol underwent degradation to carbon dioxide and methane after a 2 month incubation period(3). Benzyl alcohol was shown to be readily biodegradable using a synthetic anaerobic sewage sludge(4). [(1) Horowitz A et al; Dev Ind Microbiol 23: 435-44 (1982) (2) Shelton DR, Tiedje JM; App Env Microbiol 47: 850-7 (1984) (3) Balba MTM et al; Biochem Soc Trans 9: 230-1 (1981) (4) Kawahara K et al; Chemosphere 39: 2007-18 (1999)] ****PEER REVIEWED****

Environmental

Abiotic

Degradation:

The rate constant for the vapor-phase reaction of benzyl alcohol with photochemically-produced hydroxyl radicals is 2.29×10^{-11} cu cm/molecule-sec at 25 deg C(1). This corresponds to an atmospheric half-life of about two days at an atmospheric concentration of 5×10^5 hydroxyl radicals per cu cm(1). Benzyl alcohol is not expected to undergo hydrolysis in the environment due to the lack of functional groups that hydrolyze under environmental conditions(2). Benzyl alcohol does not contain chromophores that absorb at wavelengths >290 nm(2) and therefore is not expected to be susceptible to direct photolysis by sunlight(SRC). [(1) Atkinson R; J Phys Chem Ref Data Monograph 1 (1989) (2) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 7-4, 7-5, 8-12 (1990)] ****PEER REVIEWED****

Based on an experimentally determined rate constant for the reaction of benzyl alcohol with alkylperoxy radicals, 2.4 L/mole-s(1) and an estimated alkylperoxy concentration in water of 1×10^{-9} mole/L(2), the half-life for this reaction is 9 years(SRC). The half-life for the reaction of benzyl alcohol with photochemically produced hydroxyl radicals in water can be estimated at approximately 100 days using an experimentally determined rate constant of 8.4×10^{-9} L/mole-s(3) and an optimal hydroxyl radical concentration of 1×10^{-17} mole/L in natural waters(2). Exposure of benzyl alcohol to sunlight for 4 hours in natural water did not produce any detectable oxidizing species (detection limit 1.5 uM), demonstrating that photochemical induced oxidation did not occur within that time frame(4). [(1) Hendry DG et al; J Phys Chem Ref Data 3: 937-78 (1974) (2) Mill T et al; Science 207; 886-7 (1980) (3) Dorfman LM, Adams GE; Reactivity of the Hydroxyl Radical in

Aqueous Solution NSRD-NBS-46 Washington DC: National Bureau of Standards pp 51 (1973) (4) Draper WM, Crosby DG; Arch Environ Contam Toxicol 12: 121-126 (1983)] **PEER REVIEWED**

Environmental

Bioconcentration:

An estimated BCF of 0.3 was calculated in fish for benzyl alcohol(SRC), using a log Kow of 1.10(1) and a regression-derived equation(2). According to a classification scheme(3), this BCF suggests the potential for bioconcentration in aquatic organisms is low(SRC). [(1) Hansch C et al; Exploring QSAR. Hydrophobic, Electronic, and Steric Constants. ACS Prof Ref Book. Heller SR, consult. ed., Washington, DC: Amer Chem Soc p. 31 (1995) (2) Meylan WM et al; Environ Toxicol Chem 18: 664-72 (1999) (3) Franke C et al; Chemosphere 29: 1501-14 (1994)] **PEER REVIEWED**

Soil Adsorption/Mobility:

Experimental Koc values for benzyl alcohol were <5 for three different soils; Apison (0.11% organic carbon), Fullerton (0.06% organic carbon), and Dormont (1.2% organic carbon)(1). An experimental Koc of 15 was determined for benzyl alcohol on a red-brown Australian soil (1.09% organic carbon)(2,3). A log Koc of 1.43 has also been reported(4). According to a classification scheme(5), these Koc values suggest that benzyl alcohol is expected to have very high mobility in soil. [(1) Southworth GR, Keller JL; Water Air Soil Poll 28: 239-48 (1986) (2) Briggs GG; Aust J Soil Res 19: 61-8 (1981) (3) Briggs GG; J Agric Food Chem 29: 1050-9 (1981) (4) Xu F et al; J Environ Qual 30: 1618-23 (2001) (5) Swann RL et al; Res Rev 85: 17-28 (1983)] **PEER REVIEWED**

Volatilization from Water/Soil:

The Henry's Law constant for benzyl alcohol is 3.37×10^{-7} atm-cu m/mole(1). This Henry's Law constant indicates that benzyl alcohol is expected to be essentially nonvolatile from water surfaces(2). Benzyl alcohol's Henry's Law constant indicates that volatilization from moist soil surfaces may not occur(SRC). Benzyl alcohol is not expected to volatilize from dry soil surfaces(SRC) based upon a vapor pressure of 9.4×10^{-2} mm Hg(3). [(1) Abraham MH et al; J Pharm Sci 83: 1085-100 (1994) (2) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 15-1 to 15-29 (1990) (3) Daubert TE, Danner RP; Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, D.C.: Taylor and Francis (1989)] **PEER REVIEWED**

Environmental

Water

Concentrations:

GROUNDWATER: Benzyl alcohol was found at a concn of 170 ug/L in a groundwater sample taken in May 1985 at an unauthorized waste site in New Jersey(1). [(1) USEPA; Superfund Record of Decision. (EPA Region 2). Lang Property, Pemberton Township, New Jersey.Sept 1986. Washington, DC: US EPA. USEPA/ROD/RO2-86/031 (1987)] **PEER REVIEWED**

SURFACE WATER: Benzyl alcohol was identified, not quantified, in samples from Putah Creek, Yolo County, CA collected on November 26, 1993(1). [(1) Umano K et al; Bull Environ Contam Toxicol 56: 558-565 (1996)] **PEER REVIEWED**

RAIN/SNOW/FOG: Benzyl alcohol was detected in March snow samples of urban and rural regions in Russia: in Butovo, Russia at 0.004, Moscow State University, Moscow Russia at 0.11, Shosse Entuziastov, Moscow, Russia at 0.04, and Baikal'sl on Lake Baikal, Siberia (in the vicinity of pulp and paper mill) at 0.30 ug/kg(1). It was not detected in samples from Finland(1). [(1) Poliakova OV et al; Toxicol Environ Chem 75: 181-94 (2000)] **PEER REVIEWED**

Effluent

Concentrations:

Benzyl alcohol has been identified in the waste water effluent from the photographic processing industry(1) and in 6 out of 8 effluent samples from Kraft paper mills located in Georgia at a concentration up to 0.025 mg/L(2). Benzyl alcohol has been qualitatively determined in the secondary effluent from wastewater treatment plants in Illinois(3). The effluent from a Los Angeles county waste water treatment plant contained 500 ug/L of benzyl alcohol(4). Benzyl alcohol was identified, not quantified, in the leachate from a Barcelona, Spain, sanitary landfill(5) and from a municipal refuse disposal site in the Netherlands(6). Benzyl alcohol was identified, not quantified, in

the wastewater of a petrochemical company producing olefins and oxygenated hydrocarbons in Louisiana(7). Benzyl alcohol was found in the effluent of 1 out of 4 test waste incinerators in the US(8). The emission rate of benzyl alcohol from motor vehicle traffic in a tunnel in Los Angeles, CA was reported as 1618.1 ug/L gasoline of gasoline fuel burned(9). Benzyl alcohol was identified, not quantified, in the emissions of various types of furniture coatings(10), some common household wastes(11) and in perfumes(12). [(1) Dagon TJ; J Water Pollut Contrl Fed 45: 2123-35 (1973) (2) Keith LH; Environ Sci Technol 10: 555-64 (1976) (3) Ellis DD et al; Arch Environ Contam Toxicol 11: 373-82 (1982) (4) Gossett RW et al; Mar Pollut Bull 14: 387-92 (1983) (5) Albaiges J et al; Wat Res 20: 1153-9 (1986) (6) Harmsen J; Water Res 17: 699-705 (1983) (7) Keith LH; Sci Total Environ 3: 87-102 (1974) (8) James RH et al; J Proc APCA 77th Ann Meeting: Paper 84-18.5 pp. 1-25 (1984) (9) Fraser MP et al; Environ Sci Technol 32:2051-60 (1998) (10) Salthammer T; Indoor Air 7: 187-97 (1997) (11) Wilkins CK, Larsen K; J High Res Chromatogr 18: 373-77 (1995) (12) Cooper SD et al; J Exposure Anal Environ Epidemiol 5: 57-75 (1995)] **PEER REVIEWED**

Of the total non-methane organic compounds analyzed in 14 whole-air source emission samples from Cairo Egypt collected between June 5 and 10, 1997, benzyl alcohol accounted (% by weight) for: 0.06% (roadway); 0.48% in hot soak, 0.18% in cold start (bus parking garage); 0.01% (motorcycle); 0.02% (petroleum refinery); 0.28% (lead smelter); and 0.28% (cast iron factory); however, these results were in conjunction with p-cymene(1). It was not detected in regular or high grade gasoline headspace, regular or high grade whole gasoline, uncombusted LPG, nor uncombusted natural gas(1). Fireplace emissions from partially seasoned oak tested positive for benzyl alcohol (2). [(1) Doskey PV et al; J Air Waste Manage Assoc 49: 814-22 (1999) (2) Purvis CR et al; Environ Sci Technol 34: 1653-58 (2000)] **PEER REVIEWED**

Sediment/Soil

Concentrations:

SEDIMENT: Benzyl alcohol was detected not quantified in sediments from three river estuaries and a port in Niigata, Japan, sampled in September 1995(1). The compound was detected in samples from the Hokura River (drains industrial and agricultural areas) at a factor of 10+5, the Shinano River (drains urban and agricultural areas) at a factor of 10+5, and the Tainai River (drains industrial and agricultural areas) at a factor of 10+5; concentrations in samples from the Niigata East Port (drains industrial areas) were at a factor of 10+6(1). [(1) Kawata K et al; Bull Environ Contam Toxicol 65: 660-7 (2000)] **PEER REVIEWED**

Atmospheric

Concentrations:

INDOOR: Benzyl alcohol was identified, not quantified, in indoor air from homes in Finland(1). [(1) Kostianinen K; Atmos Environ 29: 693-702 (1995)] **PEER REVIEWED**

SOURCE DOMINATED: Benzyl alcohol concentrations in Los Angeles air sampled on Sept 8-9, 1993 during a severe photochemical smog episode ranged from 2.67-143.97 ng/cu m(1). [(1) Fraser MP et al; Environ Sci Technol 32: 1760-70 (1998)] **PEER REVIEWED**

Milk

Concentrations:

EXPERIMENTAL: When used as a teat dip, concentrations in the milk ranging from 7.6-120 ug/L benzyl alcohol have been determined. When good husbandry practice was followed and the udder dry wiped before milking, mean residues in the milk were reported to be 21 ug/L. [European Medicines Agency (EMA), The European Agency for the Evaluation of Medicinal Products, Veterinary Medicines Evaluation Unit, Committee for Veterinary Medicinal Products; Benzyl Alcohol, Summary Report (1997). Available at the query page: <http://www.emea.europa.eu/pdfs/vet/mrls/Benzylalcohol.pdf> as of July 8, 2008] **PEER REVIEWED**

Other

Environmental

Concentrations:

Benzyl alcohol was identified as a volatile emission from electrical plug-in type of pine-scented air freshener (1). [(1) Liu X et al; Environ Sci Technol 38: 2802-12 (2004)] **PEER REVIEWED**

As taken from HSDB, 2009

The CHEMFATE database provides the following additional soil adsorption data:

SOIA	BENZYL ALCOHOL
CAS#	100-51-6
Soil Adsorp Cns	: .268E 03
Sum. Comments	: AGREES WITH VALUE CALC FROM LOGP
Soil Type	: EASTERN AUSTRALIAN RED-BROWN EARTH
% Org. Content	: 1.09 OM
pH	: 8.0
Concentration	: 10 (MG/L)
Test Cond.	: 10 ML OF TEST CMPD SOLN IN .01 MOL/L CACL2 SHAKEN 2 HR WITH 1-3 G SOIL, ANALYSIS BY UV
Freund. Adsorp.	: .17E 00
KOC	: .268E 03
Remarks	: KOC- CALCULATED USING 1.724 (%OC) = %OM GIVEN IN REF
Abbrev. Ref.	: BRIGGS,GG (1981A)

SOIA	BENZYL ALCOHOL
CAS#	100-51-6
KOC	: 5
Remarks	: KOC REPRESENTS BEST OR AVERAGE VALUE, AS APPROPRIATE. BACK-CALCULATED FROM LOG KOC
Abbrev. Ref.	: SOUTHWORTH,GR & KELLER,JL (1986)
SOIA	BENZYL ALCOHOL
CAS#	100-51-6
KOC	: 27
Remarks	: KOC REPRESENTS BEST OR AVERAGE VALUE, AS APPROPRIATE. BACK-CALCULATED FROM LOG KOC
Abbrev. Ref.	: BRIGGS,GG (1981)

The Ecological Categorization Results from the Canadian Domestic Substances List state that benzenemethanol is not persistent in the environment:

Media of concern leading to Categorization	Water
Experimental Biodegradation half-life (days)	Not Available
Experimental Biodegradation result (%)	96
Reference (biodegradation result)	CHEMICALS INSPECTION AND TESTING INSTITUTE.; BIODEGRADATION AND BIOACCUMULATION DATA OF EXISTING CHEMICALS BASED ON THE CSCL JAPAN.; JAPAN CHEMICAL INDUSTRY ECOLOGY - TOXICOLOGY AND INFORMATION CENTER.ISBN 4-89074-101-1.; 1992
Predicted Ultimate degradation half-life (days)	15
MITI probability of biodegradation	0.7077
TOPKAT probability of biodegradation	1
EPI Predicted Ozone reaction half-life (days)	999
Experimental Atmospheric Oxidation half-life (days)	0.467006307617661
Reference (oxidation)	ATKINSON,R.; KINETICS AND MECHANISMS OF THE GAS-PHASE REACTIONS OF THE HYDROXYL RADICAL WITH ORGANIC COMPOUNDS.; JOURNAL OF PHYSICAL AND CHEMICAL REFERENCE DATA. MONOGRAPH NO. 1.; 1989
EPI Predicted Atmospheric Oxidation half-life (days)	1.296

ROBUST STUDY SUMMARY - Persistence

Item	Yes	No
<u>Reference</u> : Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan, 1992. Chemicals Inspection and Testing Institute (Ed.). Published by: Japan Chemical Industry Ecology-Toxicology & Information Center.		
<u>Test Substance</u> (CAS # and name): The list of the substances is attached (see file on N drive)		
Chemical composition of the substance (including purity, by-products)		X
<u>Method</u>		
References	X	
OECD, EU, national, or other standard method?	X	
Justification of the method/protocol if not a standard method was used	Not applicable	
<u>Test design / conditions</u>		
Study type (photodegradation, hydrolysis, biodegradation, other – <u>specify, do not assess</u>): Biodegradation		
Test type (aerobic or anaerobic - <u>specify, do not assess</u>):		
Test medium (air, water, soil, sediment - <u>specify, do not assess</u>):		
Information on stability of the substance in the media of concern is reported?	X	
Controls (positive or negative) Both	X	
Number of replicates (including controls)		X
Temperature (specify) 25±1 °C	X	
Duration of the experiment (specify) 14 or 28 days	X	

For photodegradation only		
Light source (specify):	Not applicable	
Light spectrum and relative intensity based on sunlight intensity:	Not applicable	
For hydrolysis only		
Measured concentrations reported?	Not applicable	
Basic water properties (pH, hardness, etc.)	Not applicable	
For biodegradation only		
Ready or inherent biodegradation (specify): Both types (see file on N drive)	X	
Innoculum (concentration and source)	X	
Results		
Endpoints: half-life (preferred); degradation, %; etc. (<u>do not assess this item</u>): % biodegradation by BOD or TOC (see file on N drive)		
Information on breakdown products (<u>do not assess this item</u>)		X
<u>Overall score</u> : 8/10 = 80%		
EC Reliability code: 1		
Reliability category (high, satisfactory, low): high		
Comments:		

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

EPISuite provides the following information:

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

Bond Method :	2.17E-007 atm-m3/mole (2.20E-002 Pa-m3/mole)
Group Method:	4.66E-008 atm-m3/mole (4.72E-003 Pa-m3/mole)
Exper Database:	3.37E-07 atm-m3/mole (3.41E-002 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 1.854E-007 atm-m3/mole (1.879E-002 Pa-m3/mole) VP: 0.0535 mm Hg (source: MPBPVP) WS: 4.11E+004 mg/L (source: WSKOWWIN)

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

Log Kow used:	1.10 (exp database)
Log Kaw used:	-4.861 (exp database)
Log Koa (KOAWIN v1.10 estimate):	5.961
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model):	0.9829
Biowin2 (Non-Linear Model) :	0.9878
Biowin3 (Ultimate Survey Model):	3.1422 (weeks)
Biowin4 (Primary Survey Model) :	3.8261 (days)
Biowin5 (MITI Linear Model) :	0.5370
Biowin6 (MITI Non-Linear Model):	0.7077
Biowin7 (Anaerobic Linear Model):	0.7025
Ready Biodegradability Prediction:	YES

Hydrocarbon Biodegradation (BioHCwin v1.01):

Structure incompatible with current estimation method!

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

Vapor pressure (liquid/subcooled):	12.5 Pa (0.094 mm Hg)
Log Koa (Koawin est):	5.961
Kp (particle/gas partition coef. (m3/ug)):	2.39E-007
Mackay model:	2.24E-007
Octanol/air (Koa) model:	

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	8.65E-006
Mackay model:	1.91E-005
Octanol/air (Koa) model:	1.8E-005

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

Hydroxyl Radicals Reaction:

OVERALL OH Rate Constant =	8.2541 E-12 cm3/molecule-sec
Half-Life =	1.296 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life =	15.550 Hrs
Ozone Reaction:	No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi): 1.39E-005 (Junge-Pankow, Mackay avg) 1.8E-005 (Koa method)	

Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	21.46 L/kg (MCI method)
Log Koc:	1.332 (MCI method)
Koc :	13.25 L/kg (Kow method)
Log Koc:	1.122 (Kow method)

Experimental Log Koc: 1.1 (database)

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: 3.37E-007 atm-m³/mole (Henry experimental database)

Half-Life from Model River:	1808 hours (75.32 days)
Half-Life from Model Lake:	1.981E+004 hours (825.3 days)

Removal In Wastewater Treatment:

Total removal:	1.92 percent
Total biodegradation:	0.09 percent
Total sludge adsorption:	1.80 percent
Total to Air:	0.02 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	1.03	11.2	1000
Water	34	360	1000
Soil	64.8	720	1000
Sediment	0.0929	3.24e+003	0

Persistence Time: 424 hr

Distribution of benzyl alcohol according to Fugacity Level III is 1.51% in air, 50.0% in water, 48.4% in soil and 0.0923% in sediment (OECD SIDS Initial Assessment Report, 2001).

The BIODEG database provides the following data:

CAS #	: 100-51-6 BENZYL ALCOHOL
Parameter Type	: Screening Test
Study Biodeg Eval	: BF
Rate	: 85.7
Units	: % DEGRADATION
Test Method	: RADIORESPIROMETRY
Oxygen Condition	: AEROBIC
Analysis Method	: 14-CO2
Incub Time (days	: 5
Microbial Pop	: 25-40 (% SEWAGE)
Inoculum	: SEWAGE
Temp (deg C)	: 22
pH	: 7.3
Reference	: BELLY,RT & GOODHUE,CT (1976)

CAS #	: 100-51-6 BENZYL ALCOHOL

Parameter Type	: Screening Test
Study Biodeg Eva	: BF
Rate	: 33
Units	: %BODT
Test Method	: BOD5
Oxygen Condition	: AEROBIC
Analysis Method	: BOD
Incub Time (days)	: 5
Inoculum	: SEWAGE
Reference	: DORE,M ET AL. (1975)

CAS #	: 100-51-6 BENZYL ALCOHOL
Parameter Type	: Screening Test
Study Biodeg Eval	: BF
Rate	: 48
Units	: %BODT
Test Method	: BOD5
Oxygen Condition	: AEROBIC
Incub Time (days)	: 5
Inoculum	: SEWAGE
Temp (deg C)	: 20
Reference	: HEUKELEKIAN,H & RAND,MC (1955)

CAS #	: 100-51-6 BENZYL ALCOHOL
Parameter Type	: Screening Test
Study Biodeg Eval	: BF
Rate	: 100
Units	: %BODT
Test Method	: WARBURG
Oxygen Condition	: AEROBIC
Analysis Method	: MANO
Incub Time (days)	: 6

Chem Conc (ppm)	: 500
Microbial Pop	: 2000 (MG/L)
Inoculum	: ACTIVATED SLUDGE
Temp (deg C)	: 20
Reference	: LUTIN,PA ET AL. (1965)

CAS #	: 100-51-6 BENZYL ALCOHOL
Parameter Type	: Screening Test
Study Biodeg Eval	: BFA
Rate	: 29
Units	: %BODT
Test Method	: WARBURG
Oxygen Condition	: AEROBIC
Analysis Method	: MANO
Incub Time (days)	: 0.5
Chem Conc (ppm)	: 250
Acclim Per (days)	: 25
Inoculum	: ACTIVATED SLUDGE
Temp (deg C)	: 20
Reference	: LUDZACK,FJ & ETtinger,MB (1960)

CAS #	: 100-51-6 BENZYL ALCOHOL
Parameter Type	: Screening Test
Study Biodeg Eval	: BF
Rate	: 64
Units	: %BODT
Test Method	: WARBURG
Oxygen Condition	: AEROBIC
Incub Time (days)	: 2
Chem Conc (ppm)	: 500
Microbial Pop	: 2500 (MG/L)

Inoculum	: ACTIVATED SLUDGE
Temp (deg C)	: 20
Reference	: MARION,CV & MALANEY,GW (1964)
CAS #	: 100-51-6 BENZYL ALCOHOL
Parameter Type	: Screening Test
Study Biodeg Eval	: BF
Rate	: 52; 42; 43
Units	: %BODT
Test Method	: WARBURG
Oxygen Condition	: AEROBIC
Analysis Method	: BOD
Incub Time (days)	: .5
Chem Conc (ppm)	: 500
Microbial Pop	: 5230; 3310; 6640 (PPM)
Inoculum	: ACTIVATED SLUDGE
Remarks	: MINERAL SALTS, ACCLIMATED IN 24 HR SCAS TEST TO 500 PPM PHENOL; BENZOIC ACID (ADAPTIVE METABOLISM); CATECHOL
Reference	: MCKINNEY,RE ET AL. (1956)
CAS #	: 100-51-6 BENZYL ALCOHOL
Parameter Type	: Screening Test
Study Biodeg Eval	: BFA
Rate	: 31
Units	: %BODT
Test Method	: WARBURG
Oxygen Condition	: AEROBIC
Analysis Method	: MANO
Incub Time (days)	: 0.5

Chem Conc (ppm)	: 250
Acclim Per (days)	: 20
Remarks	: ACCLIMATED - SCAS - MANDELIC ACID
Reference	: MCKINNEY,RE ET AL. (1956)
CAS #	: 100-51-6 BENZYL ALCOHOL
Parameter Type	: Screening Test
Study Biodeg Eval	: BFA
Rate	: 29
Units	: %BODT
Test Method	: WARBURG
Oxygen Condition	: AEROBIC
Analysis Method	: MANO
Incub Time (days)	: 0.5
Chem Conc (ppm)	: 250
Acclim Per (days)	: 20
Remarks	: ACCLIMATED - SCAS - BENZYL ALCOHOL
Reference	: MCKINNEY,RE ET AL. (1956)
CAS #	: 100-51-6 BENZYL ALCOHOL
Parameter Type	: Screening Test
Study Biodeg Eval	: BF
Rate	: 89.2
Units	: %BODT
Test Method	: SEWAGE DIE-AWAY
Oxygen Condition	: AEROBIC
Analysis Method	: BOD
Incub Time (days)	: 5
Chem Conc (ppm)	: 6000
Reference	: WAGNER,R (1976)
CAS #	: 100-51-6 BENZYL ALCOHOL

Parameter Type	: Screening Test
Study Biodeg Eval	: BFA
Oxygen Condition	: ANAEROBIC-WATER
Incub Time (days)	: 60
Inoculum	: ANOXIC SALTMARSH SEDIMENT
Temp (deg C)	: 25
Remarks	: CO2 AND CH4 WERE GENERATED
Reference	: BALBA,MTM ET AL. (1981)
CAS #	: 100-51-6 BENZYL ALCOHOL
Parameter Type	: Screening Test
Study Biodeg Eval	: BFA
Rate	: >75
Units	: %TMETHANE
Oxygen Condition	: ANAEROBIC-WATER
Incub Time (days)	: 56
Chem Conc (ppm)	: 50 (C)
Inoculum	: DIGESTER SLUDGE
Temp (deg C)	: 35
Reference	: SHELTON,DR & TIEDJE,JM (1984)

The CHEMFATE database provides the following data:

MICD	BENZYL ALCOHOL
CAS#	100-51-6
Summary	: NO DEGR
Species Name	:
1	: PSEUDOMONAS AERUGINOSA QMB 1468
2	: ASPERGILLUS VERSICOLR OM 432
Rate	:
1	: NO DEGR, INHIBITION

2	: NO DEGR, INHIBITION
Analyt. Meth.	: GROWTH
Test Cond.	: 2% TEST CMPD, SOLE C SOURCE, MINERAL SALT MEDIUM, SHAKEN, 30 DEGC, 7 AND 23 DAY INCUBATION
Abbrev. Ref.	: STAHL,WH & PESSEN,H (1953)
MICD	BENZYL ALCOHOL
CAS#	100-51-6
Summary	: RAPID DEGR
Species Name	: BACTERIA, ACTINOMYCETES, YEASTS
Rate	: 100% DEGR, SOLE SOURCE OF ENERGY
Test Cond.	: MICROBES ISOLATED FROM ACTIV SLUDGE, 100 MG/L TEST CMPD
Abbrev. Ref.	: KALMYKOVA,GY ET AL. (1980)
MICD	BENZYL ALCOHOL
CAS#	100-51-6
Species Name	:
1	: RHODOTORULA GLUTINIS- VAR GLUTINIS
2	: CANDIDA TROPICALIS
3	: TRICHOSPORON CATANEUM
Rate	:
1	: RAPID DEGR
2	: NO DEGR
3	: DEGR
Abbrev. Ref.	: NEI,N ET AL. (1973)

MICD	BENZYL ALCOHOL
CAS#	100-51-6
Species Name	: TRICHOSPORUM CUTANEUM
Rate	: NOT OXIDIZED

Test Cond.	: WASHED CELL SUSPENSION OF PHENOL OR RESORCINOL ADAPTED MICROBE, 1 UMOL/ML TEST CMPD, PH 7.9, 28 DEGC, INCUBATED UP TO 3 HRS
Abbrev. Ref.	: NEUJAHR,HY & VARGA,JM (1970)
MICD	BENZYL ALCOHOL
CAS#	100-51-6
Summary	: NO DEGR
Species Name	: PSEUDOMONAS SP
Rate	: NO GROWTH
Analyt. Meth.	: GROWTH, WARBURG RESPIROMETER
Test Cond.	: .5 G/L TEST CMPD, MICROBE ACCLIM TO P-TOLUENE SULFONATE AS SOLE C AND S SOURCE, BASAL SALT MEDIUM, PH 7.2, 25 DEGC, INCUBATED 1 HR
Abbrev. Ref.	: FOCHT,DD & WILLIAMS,FD (1970)
NSYD	BENZYL ALCOHOL
CAS#	100-51-6
Summary	: DEGR, NO CHANGE IN DEGR IN PRESENCE OF EFFLUENT
System	: WATER
Rate	: 85.7% AND 88.9% DEGR TO CO2 IN ABSENCE AND PRESENCE OF EFFLUENT RESP
Analyt. Meth.	: 14-CO2 EVOL
Test Cond.	: C-14 SUBSTRATE IN SALT SOLN OR SIMULATED INDUSTRIAL EFFLUENT, ACCLIM ACTIV SLUDGE, PH 7.3 BUFFER, DARK, ROOM TEMP, INCUBATED 5 DAYS IN SEALED SERUM BOTTLE
Abbrev. Ref.	: BELLY,RT & GOODHUE,CT (1976)
NSYD	BENZYL ALCOHOL
CAS#	100-51-6
System	: WATER
Rate	: 89.2% BODT
Analyt. Meth.	: RESPIROMETRIC DILUTION METHOD
Test Cond.	: ACTIV SLUDGE

Abbrev. Ref.	: WAGNER,R (1976)
NSYD	BENZYL ALCOHOL
CAS#	100-51-6
Summary	: DEGR
System	: WATER
Rate	: 100% BODT
Temperature (C)	: 20
Analyt. Meth.	: WARBURG RESPIROMETER
Test Cond.	: ACTIV SLUDGE, 500 MG/L TEST CMPD, 144 HR INCUBATION, SHAKEN
Abbrev. Ref.	: LUTIN,PA ET AL. (1965)
NSYD	BENZYL ALCOHOL
CAS#	100-51-6
Summary	: DEGR
System	: WATER
Temperature (C)	: 20
Analyt. Meth.	: WARBURG
Test Cond.	: 3 ACTIV SLUDGES, 500 MG/L SUBSTRATE, AEROBIC INCUBATION UP TO 7 DAYS
Abbrev. Ref.	: MARION,CV & MALANEY,GW (1964)
NSYD	BENZYL ALCOHOL
CAS#	100-51-6
System	: WATER
Rate	: 33% BODT
Analyt. Meth.	: BOD5 (AFNOR T 90/103 TEST)
Test Cond.	: MICROBES TAKEN FROM 3 POLLUTED SURFACE WATERS
Abbrev. Ref.	: DORE,M ET AL. (1975)

NSYD	BENZYL ALCOHOL
CAS#	100-51-6

Summary	: DEGR
System	: WATER
Rate	: 29% BODT
Analyt. Meth.	: BOD
Test Cond.	: SCAS
Abbrev. Ref.	: LUDZACK,FJ & ETTINGER,MB (1960)
*****NSYD BENZYL ALCOHOL	
CAS#	100-51-6
Summary	: DEGR
System	: WATER
Rate	: 48% BODT
Analyt. Meth.	: BOD
Test Cond.	: BOD5
Abbrev. Ref.	: HEUKELEKIAN,H & RAND,MC (1955)
*****NSYD BENZYL ALCOHOL	
CAS#	100-51-6
Summary	: DEGR
System	: WATER
Rate	: 5.5% BODT
Temperature (C)	: 30
Analyt. Meth.	: WARBURG RESPIROMETER
Test Cond.	: MIXED CULTURE CAPABLE OF RAPID DEGR PHENOL, MINERAL SALTS, 3 HR INCUBATION, REFERENCE PHENOL HAS 70% BODT
Abbrev. Ref.	: CHAMBERS,CW ET AL. (1963)
*****NSYD BENZYL ALCOHOL	
CAS#	100-51-6

Summary	: 1-PROPANOL ADAPTED SLUDGE SHOWED HIGH ACTIVITY
System	: WATER
Abbrev. Ref.	: KALMYKOVA,GY & ROGOVSKAYA,TI (1978)

10.2. Aquatic toxicity

AQUATIC ORGANISMS

Acute/Prolonged Toxicity to Fish

Type:	static
Species:	Leuciscus idus (Fish, fresh water)
Exposure period:	48 hour(s)
Unit:	mg/l
LC0:	630
LC50:	646
Method:	other: Bestimmung der Wirkung von Wasserinhaltsstoffen auf Fische, DIN 38412 Teil 15
Source:	Bayer AG Leverkusen (16)
Type:	static
Species:	Petromyzon marinus
Exposure period:	24 hour(s)
Unit:	mg/l

LC50:	>= 5
Remark:	larvae; screening test
Source:	Bayer AG Leverkusen(17)
Species:	Carassius auratus (Fish, fresh water)
Exposure period:	24 hour(s)
Unit:	mg/l
LC0:	>= 5
Source:	Bayer AG Leverkusen(18)
Species:	Cyprinus carpio (Fish, fresh water)
Exposure period:	48 hour(s)
LC0:	136
Remark:	Testing of acute oral toxicity
Unit:	mg/kg
Source:	Bayer AG Leverkusen (19)
Species:	Lepomis macrochirus (Fish, fresh water)
Exposure period:	24 hour(s)
Unit:	mg/l
LC0:	>= 5

Source:	Bayer AG Leverkusen (18)
Species:	Lepomis macrochirus (Fish, fresh water)
Exposure period:	96 hour(s)
Unit:	mg/l
LC50:	10
Source:	Bayer AG Leverkusen (20)
Species:	Menidia beryllina (Fish, estuary, marine)
Exposure period:	96 hour(s)
Unit:	mg/l
LC50:	15
Source:	Bayer AG Leverkusen (20)
Species:	Pimephales promelas (Fish, fresh water)
Exposure period:	96 hour(s)
Unit:	mg/l
LC50:	460
Source:	Bayer AG Leverkusen (21)
Species:	Salmo trutta (Fish, fresh water, marine)
Exposure period:	24 hour(s)

Unit:	mg/l
LC0:	>= 5
Source:	Bayer AG Leverkusen (18)

Acute Toxicity to Aquatic Invertebrates

Species:	Daphnia magna (Crustacea)
Exposure period:	24 hour(s)
Unit:	mg/l
EC0:	300
EC50:	400
Method:	other: Daphnien–Kurzzeittest, DIN 38412 Teil 11, Bestimmung der Wirkung von Wasserinhaltsstoffen auf Kleinkrebse
Source:	Bayer AG Leverkusen (16)

Species:	Daphnia magna (Crustacea)
Exposure period:	48 hour(s)
Unit:	mg/l
TGK:	360
Method:	other: acute immobilisation test
Source:	Bayer AG Leverkusen (22)

Species:	Daphnia magna (Crustacea)

Exposure period:	24 hour(s)
Unit:	mg/l
EC0:	26
EC50:	55
EC100:	100
Source:	Bayer AG Leverkusen (23)

Toxicity to Aquatic Plants e.g. Algae

Species:	Anabaena cylindrica (Algae)
Exposure period:	3 hour(s)
Unit:	mg/l
EC50:	90
Remark:	blue-green algae
	Inhibition of photosynthesis
Source:	Bayer AG Leverkusen (24)

Species:	Anabaena inaequalis (Algae)
Exposure period:	3 hour(s)
Unit:	mg/l
EC0:	30

Remark:	blue–green algae Inhibition of photosynthesis
Source:	Bayer AG Leverkusen (24)
Species:	Anabaena variabilis (Algae)
Exposure period:	3 hour(s)
Unit:	mg/l
EC50:	35
Remark:	blue–green algae Inhibition of photosynthesis
Source:	Bayer AG Leverkusen (24)

Species:	Chlorella pyrenoidosa (Algae)
Exposure period:	3 hour(s)
Unit:	mg/l
EC50:	95
Remark:	green algae Inhibition of photosynthesis
Source:	Bayer AG Leverkusen (24)

Species:	Haematococcus pluvialis (Algae)
Exposure period:	4 hour(s)
Unit:	mg/l
EC50:	2600

Method:	other: Verfahren nach Tuempling im Warburgapparat
Source:	Bayer AG Leverkusen(16)
Species:	Scenedesmus quadricauda (Algae)
Exposure period:	96 hour(s)
Unit:	mg/l
TGK:	640
Method:	other: cell multiplication inhibition test
Remark:	green algae
Source:	Bayer AG Leverkusen(22)
Species:	Scenedesmus quadricauda (Algae)
Exposure period:	3 hour(s)
Unit:	mg/l
EC50:	79
Remark:	green algae
	Inhibition of photosynthesis
Source:	Bayer AG Leverkusen(24)

Toxicity to Microorganisms e.g. Bacteria

Type:	aquatic

Species:	Escherichia coli (Bacteria)
Exposure period:	48 hour(s)
Unit:	mg/l
EC0:	1000
Method:	other: cell multiplication test
Source:	Bayer AG Leverkusen(22)
Type:	aquatic
Species:	Photobacterium phosphoreum (Bacteria)
Exposure period:	30 minute(s)
Unit:	mg/l
EC50:	71.42
Method:	other: Microtox
Source:	Bayer AG Leverkusen(25)
Type:	aquatic
Species:	Photobacterium phosphoreum (Bacteria)
Exposure period:	5 minute(s)
Unit:	mg/l
EC50:	50

Source:	Bayer AG Leverkusen(26)
Type:	aquatic
Species:	Pseudomonas putida (Bacteria)
Unit:	mg/l
EC10:	658
Method:	other: Test according to Bringmann and Kuehn (cell multiplication inhibition test)
Remark:	Exposure period: 16–18 h
Source:	Bayer AG Leverkusen (16)
Type:	aquatic
Species:	other bacteria: Aerobic heterotrophic
Exposure period:	49 hour(s)
Unit:	mg/l
IC50:	2100
Remark:	Inhibition of respiration; prolonged incubation compared with ISO 8192
Source:	Bayer AG Leverkusen (27)
Type:	aquatic
Species:	other bacteria: Nitrosomonas
Exposure period:	24 hour(s)

Unit:	mg/l
IC50:	390
Method:	other: Inhibition of nitrification, comparable with ISO/DIS 9509
Remark:	Inhibition of N-oxidation
Source:	Bayer AG Leverkusen (27)

As taken from IUCLID Dataset (2000), Benzyl alcohol (100-51-6).

Record for benzenemethanol:

Spec. Sci. Name	Exp. Type	Media Type	Resp. Site	Endpoint	Trend	Effect	Conc (ug/L)
Spec. Name Common	Chem. Anal.	Loc	Obs. Dur. (Days)	BCF	Eff %	Effect Meas.	Appl. Rate
Scenedesmus subspicatus	S	FW		EC03		HIS	F 16000 ug/L
Green Algae			7			PRLF	
Anabaena inaequalis	S	FW		EC50	DEC	POP	A > 100000 ug/L
Blue-Green Algae	U	LAB	0.125			PSYN	
Anabaena inaequalis	S	FW		EC50	DEC	ENZ	A > 100000 ug/L
Blue-Green Algae	U	LAB	0.208			NITG	
Anabaena variabilis	S	FW		EC50	DEC	POP	A > 100000 ug/L
Blue-Green Algae	U	LAB	0.125			PSYN	

Chlorella pyrenoidosa	S	FW		EC50	DEC	POP	A > 100000 ug/L
Green Algae	U	LAB	0.125			PSYN	
Haematococcus pluvialis				EC50		POP	F 2600000 ug/L
Green Algae	U	LAB	NR			GPOP	
Scenedesmus quadricauda	S	FW		EC50	DEC	POP	A > 100000 ug/L
Green Algae	U	LAB	0.125			PSYN	
Anabaena cylindrica	S	FW		EC50	DEC	POP	A > 100000 ug/L
Blue-Green Algae	U	LAB	0.125			PSYN	
Anabaena cylindrica	S	FW		EC50	DEC	ENZ	A > 100000 ug/L
Blue-Green Algae	U	LAB	0.208			NITG	
Daphnia magna				EC50		BEH	F 400000 ug/L
Water Flea	U	LAB	NR			LOCO/	
Leuciscus idus				LC50		MOR	F 646000 ug/L
Ide, Silver Or Golden Orfe	U	LAB	NR			MORT	
Lepomis macrochirus	S	FW		LC50		MOR	F 10000 ug/L

Bluegill	U	LAB	4			MORT	
Menidia beryllina	S	SW		LC50		MOR	F 15000 ug/L
Inland Silverside	U	LAB	4			MORT	
Pimephales promelas	S	FW		LC50		MOR	F 770000 ug/L
Fathead Minnow	U	LAB	0.042			MORT	
Pimephales promelas	S	FW		LC50		MOR	F 770000 ug/L
Fathead Minnow	U	LAB	1			MORT	
Pimephales promelas	S	FW		LC50		MOR	F 770000 ug/L
Fathead Minnow	U	LAB	2			MORT	
Pimephales promelas	S	FW		LC50		MOR	F 480000 ug/L
Fathead Minnow	U	LAB	3			MORT	
Pimephales promelas	S	FW		LC50		MOR	F 460000 ug/L
Fathead Minnow	U	LAB	4			MORT	
Pimephales promelas	S	FW		LT50	INC	MOR	T 10500 ug/L
Fathead Minnow	U	LAB	> 4			MORT	
Pimephales promelas	S	FW		LT50	INC	MOR	T 105000 ug/L

Fathead Minnow	U	LAB	> 4			MORT	
Pimephales promelas	S	FW		LT50	INC	MOR	T 1050000 ug/L
Fathead Minnow	U	LAB	0.013			MORT	
Pimephales promelas	S	FW		NR-LETH	DEC	MOR	T 1050000 ug/L
Fathead Minnow	U	LAB	0.031		100	MORT	
Aedes aegypti	S	FW		EC50*		ITX	F 105 L/ha
Yellow Fever Mosquito	U	LAB	1			IMBL	
Aedes aegypti	S	FW		EC50*		ITX	F 129 L/ha
Yellow Fever Mosquito	U	LAB	1			IMBL	
Aedes aegypti	S	FW		EC50*		MOR	F 160 L/ha
Yellow Fever Mosquito	U	LAB	(1 - 3)			HTCH	
Aedes scutellaris	S	FW		EC50*		ITX	F 110 L/ha
Mosquito	U	LAB	1			IMBL	
Aedes scutellaris	S	FW		EC50*		ITX	F 126 L/ha
Mosquito	U	LAB	1			IMBL	
Aedes scutellaris	S	FW		EC50*		MOR	F 160 L/ha

Mosquito	U	LAB	(1 - 3)			HTCH	
Tetrahymena pyriformis		FW		IC50	DEC	POP	F 891880 (561900-1168900) ug/L
Ciliate	U	LAB	2			PGRT/	
Tetrahymena pyriformis	S	FW		IC50	DEC	POP	F 853470 (686420-1057020) ug/L
Ciliate	U	LAB	2			PGRT/	

As taken from US EPA ECOTOX database

The Ecological Categorization Results from the Canadian Domestic Substances List state that benzenemethanol is not inherently toxic to aquatic organisms:

Pivotal value for iT (mg/l)	55
Experimental result iT (mg/l)	55
Test species iT (Latin)	Daphnia mag
Test species iT (Common)	Water flea
Final EndPoint iT	EC50
Exposure duration iT (hours)	24
Reference iT	Z.Wasser-Abwasser-Forsch. 15(1):1-6 (GER) (ENG ABS) (OECDG Data File)
Toxicity to fathead minnow (LC50 in mg/l) as predicted by Topkat v6.1	205
Toxicity to fish (LC50 in mg/l) as predicted by Oasis Forecast M v1.10	1,545.2308
Toxicity to fish (LC50 in mg/l) as predicted by Aster	557.928625
Toxicity to fish (LC50 in mg/l) as predicted by PNN	170.78953
Toxicity to daphnia (EC50 in mg/l) as predicted by Topkat v6.1	43.2
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	918.99
Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	5.87E+002

ROBUST STUDY SUMMARIES - Inherent Toxicity

Item	Yes	No
Reference: Bringmann G and R. Kühn. 1982. Ergebnisse der Schadwirkung wassergefährdender Stoffe gegen Daphnia magna in einem weiterentwickelten standardisierten Testverfahren. Z. Abwasser Forsch. 15(1982) 1:1-6.		

<u>Test Substance:</u> CAS # and name: CAS # 100-51-6; Benzenemethanol		
*Chemical composition of the substance (including purity, by-products)		x
Persistence/stability of test substance in aquatic solution		x
<u>Method</u>		
References (More details on test).	x	
*OECD, EU, national, or other standard method? Standardised Daphnia toxicity test.	x	
Justification of the method/protocol if not a standard method was used	NA	
*GLP (Good Laboratory Practice) Not applicable.	NA	
<u>Test organisms</u> (specify common and Latin names) Daphnia magna – water flea.		
Latin or both Latin and common names reported?	x	
Life cycle age / stage of test organism Not applicable.	NA	
Sex. Not applicable.	NA	
Length and weight of test organisms. Max. 24 hours old. Not applicable.	NA	
Number of test organisms per replicate. Ten test organisms.	x	
Food type / feeding periods (acclimation/during test) .	x	
<u>Test design / conditions</u>		
Test type – acute or chronic (specify, but <u>do not assess this item</u>): acute 24 h EC ₅₀		
Experiment type (laboratory or field) specified?	x	

System type (static, semi-static, flow-through)?	x	
Negative or positive controls (specify)?		x
Number of replicates (including controls) and concentrations 3 replicates.	x	
Exposure pathways (food, water, both)		x
Exposure duration	x	
*Measured concentrations reported? Nominal conc.		x
Exposure media conditions (temperature, pH, electrical conductivity, hardness, TOC, DOC, DO, major cations and anions; other)	x	
Was pH within 6-9 range? (<u>do not assess this item</u>)	x	
Was temperature within 5-28 °C range? (<u>do not assess this item</u>)	x	
Photoperiod and light intensity	x	
Stock and test solution preparation Dilution series.	x	
Use of emulgators / solubilizers (especially for poorly soluble / unstable substances) Not used.	NA	
Analytical monitoring intervals		x
Statistical methods used: Regression.	x	
<u>Results</u>		
Toxicity values (LC ₅₀ , EC ₅₀ , or IC ₅₀ - EC ₅₀ = 55mg/L		
Other endpoints reported - BCF/BAF, LOEC/NOEC (specify, <u>do not assess this item</u>): No		
*Was toxicity value below the chemical's water solubility?	x	

Other adverse effects (carcinogenicity, mutagenicity, etc. <u>Do not assess this item</u>) No.		
<u>Score</u> : major items – 2/4, 16/22= 73%		
EC Reliability code: 2		
Reliability category (high, satisfactory, low): Satisfactory		
Comments:		
Item	Yes	No
Reference: Dawson, G.W., Jennings, A.L., Drozdowski, D. and E. Rider. 1975/77. The Acute Toxicity of 47 Industrial Chemicals to Fresh and Saltwater Fishes. Journal of Hazardous Materials: 303-318.		
<u>Test Substance</u> (CAS # and name): 100-51-6 (benzyl alcohol)		
*Chemical composition of the substance (including purity, by-products)		X
Persistence/stability of test substance in aquatic solution		X
<u>Method</u>		
References		X
*OECD, EU, national, or other standard method?		X
Justification of the method/protocol if not a standard method was used		
* <u>GLP</u> (Good Laboratory Practice)	n/a	n/a
<u>Test organisms</u> (specify common and Latin names): tidewater silversides (Menidia beryllina)		
Latin or both Latin and common names reported?	X	
Life cycle age / stage of test organism	X	

Sex		X
Length and weight of test organisms	X	
Number of test organisms per replicate		X
Food type / feeding periods (acclimation/during test)	X	
<u>Test design / conditions</u>		
Test type – acute or chronic (specify, but <u>do not assess this item</u>): acute		
Experiment type (laboratory or field) specified?	X	
System type (static, semi-static, flow through)?	X	
Negative or positive controls (specify)?		X
Number of replicates (including controls) and concentrations		X
Exposure pathways (food, water, both)	X	
Exposure duration	X	
*Measured concentrations reported?		X
Exposure media conditions (temperature, pH, electrical conductivity, hardness, TOC, DOC, DO, major cations and anions; other)	X	
Was pH within 6-9 range? (<u>do not assess this item</u>)	X	
Was temperature within 5-28 °C range? (<u>do not assess this item</u>)	X	
Photoperiod and light intensity		X
Stock and test solution preparation		X

Use of emulgators / solubilizers (especially for poorly soluble / unstable substances)	n/a	n/a
Analytical monitoring intervals		X
Statistical methods used		X
<u>Results</u>		
Toxicity values (LC ₅₀ , EC ₅₀ , or IC ₅₀ - specify, <u>do not assess this item</u>): LC ₅₀ = 15 mg/L (96h)		
Other endpoints reported - BCF/BAF, LOEC/NOEC (specify, <u>do not assess this item</u>): No		
*Was toxicity value below the chemical's water solubility?	X	
Other adverse effects (carcinogenicity, mutagenicity, etc. <u>Do not assess this item</u>)		X
<u>Score</u> : major items - 1/4; overall score - 10/23 (44%)		
EC Reliability code: 4		
Reliability category (high, satisfactory, low): not acceptable		
Comments: Three major items answered "no"; overall score 44%. Poor methodology and reporting of data. No controls. High uncertainty. Nominal concentrations. No documentation on chemical purity or by-products. No documentation on water hardness, TOC. Not acceptable for categorization; Reliability code = 4.		
Item	Yes	No
Reference: Dawson, G.W., Jennings, A.L., Drozdowski, D. and E. Rider. 1975/77. The Acute Toxicity of 47 Industrial Chemicals to Fresh and Saltwater Fishes. Journal of Hazardous Materials: 303-318.		
<u>Test Substance</u> (CAS # and name): 100-51-6 (benzyl alcohol)		
*Chemical composition of the substance (including purity, by-products)		X
Persistence/stability of test substance in aquatic solution		X

<u>Method</u>		
References		X
*OECD, EU, national, or other standard method?		X
Justification of the method/protocol if not a standard method was used		
* <u>GLP</u> (Good Laboratory Practice)	n/a	n/a
<u>Test organisms</u> (specify common and Latin names): bluegill sunfish (<i>Lepomis macrochirus</i>)		
Latin or both Latin and common names reported?	X	
Life cycle age / stage of test organism	X	
Sex		X
Length and weight of test organisms	X	
Number of test organisms per replicate		X
Food type / feeding periods (acclimation/during test)	X	
<u>Test design / conditions</u>		
Test type – acute or chronic (specify, but <u>do not assess this item</u>): acute		
Experiment type (laboratory or field) specified?	X	
System type (static, semi-static, flow through)?	X	
Negative or positive controls (specify)?		X
Number of replicates (including controls) and concentrations		X

Exposure pathways (food, water, both)	X	
Exposure duration	X	
*Measured concentrations reported?		X
Exposure media conditions (temperature, pH, electrical conductivity, hardness, TOC, DOC, DO, major cations and anions; other)	X	
Was pH within 6-9 range? (<u>do not assess this item</u>)	X	
Was temperature within 5-28 °C range? (<u>do not assess this item</u>)	X	
Photoperiod and light intensity		X
Stock and test solution preparation		X
Use of emulgators / solubilizers (especially for poorly soluble / unstable substances)	n/a	n/a
Analytical monitoring intervals		X
Statistical methods used		X
<u>Results</u>		
Toxicity values (LC ₅₀ , EC ₅₀ , or IC ₅₀ - specify, <u>do not assess this item</u>): LC ₅₀ = 10 mg/L (96h)		
Other endpoints reported - BCF/BAF, LOEC/NOEC (specify, <u>do not assess this item</u>): No		
*Was toxicity value below the chemical's water solubility?	X	
Other adverse effects (carcinogenicity, mutagenicity, etc. <u>Do not assess this item</u>)		X
<u>Score</u> : major items - 1/4; overall score - 10/23 (44%)		
EC Reliability code: 4		

Reliability category (high, satisfactory, low): not acceptable

Comments: Three major items answered "no"; overall score 44%. Poor methodology and reporting of data. No controls. High uncertainty. Nominal concentrations. No documentation on chemical purity or by-products. No documentation on water hardness, TOC. Not acceptable for categorization; Reliability code = 4.

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

ECOSAR Version 1.11 provides the following aquatic toxicity data for CAS RN 100-51-6:

Values used to Generate ECOSAR Profile

Log Kow: 1.076 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 4.29E+004 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations

Benzyl Alcohols

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Benzyl Alcohols :	Fish	96-hr	LC50	213.875
Benzyl Alcohols :	Daphnid	48-hr	LC50	157.779
Benzyl Alcohols :	Green Algae	96-hr	EC50	48.312
Benzyl Alcohols :	Fish		ChV	15.538 !
Benzyl Alcohols :	Daphnid		ChV	20.804 !
Benzyl Alcohols :	Green Algae		ChV	19.094
Neutral Organic SAR :	Fish	96-hr	LC50	601.014
(Baseline Toxicity) :	Daphnid	48-hr	LC50	313.337
	Green Algae	96-hr	EC50	163.995

	Fish		ChV	53.120
	Daphnid		ChV	24.096
	Green Algae		ChV	35.512

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

DATA SOURCE			
	Year	:	1997
	Bibliographic source	:	Results of Eco-toxicity tests of chemicals conducted by Ministry of the Environment in Japan (- March 2013)
MATERIALS AND METHODS			
Test guideline			
	Guideline	:	OECD Guideline 203 (Fish, Acute Toxicity Test)
	Principles of method if other than guideline	:	These tests are conducted based on OECD-GLP standard and OECD test guidelines. However, because most of these data have not been evaluated by experts, confirmation of test results is needed if these data are used for assessment.
Test materials			
Test material identity			
	Identifier	:	CAS number
	Identity	:	100-51-6
	Identifier	:	common name

	Identity	:	Benzyl alcohol
RESULTS AND DISCUSSION			
Effect concentrations			
	Exposure duration	:	96
	Unit	:	h
	Endpoint	:	LC50
	Effect concentration	:	>100
	Unit of effect concentrations	:	mg/L
	Any other information on results incl. tables	:	Tests conducted before FY 2002 needs confirmation of test results, because some of these tests were conducted using a dispersant.
DATA SOURCE			
	Year	:	1997
	Bibliographic source	:	Results of Eco-toxicity tests of chemicals conducted by Ministry of the Environment in Japan (- March 2013)
MATERIALS AND METHODS			
Test guideline			
	Guideline	:	OECD Guideline 204 (Fish, Prolonged Toxicity Test)
	Principles of method if other than guideline	:	These tests are conducted based on OECD-GLP standard and OECD test guidelines. However, because most of these data have not been evaluated by experts, confirmation of test results is needed if these data are used for assessment.

Test materials			
Test material identity			
	Identifier	:	CAS number
	Identity	:	100-51-6
	Identifier	:	common name
	Identity	:	Benzyl alcohol
RESULTS AND DISCUSSION			
Effect concentrations			
	Exposure duration	:	14
	Unit	:	d
	Endpoint	:	LC50
	Effect concentration	:	> 99
	Unit of effect concentrations	:	mg/L
	Exposure duration	:	14
	Unit	:	d
	Endpoint	:	NOEC
	Effect concentration	:	5.1
	Unit of effect	:	mg/L

	concentrations		
	Any other information on results incl. tables	:	Tests conducted before FY 2002 needs confirmation of test results, because some of these tests were conducted using a dispersant. Financial year the test was conducted.
DATA SOURCE			
Reference			
	Year	:	1997
	Bibliographic source	:	Results of Eco-toxicity tests of chemicals conducted by Ministry of the Environment in Japan (- March 2013)
MATERIALS AND METHODS			
Test guideline			
	Guideline	:	OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)
	Principles of method if other than guideline	:	These tests are conducted based on OECD-GLP standard and OECD test guidelines. However, because most of these data have not been evaluated by experts, confirmation of test results is needed if these data are used for assessment.
Test materials			
Test material identity			
	Identifier	:	CAS number
	Identity	:	100-51-6
	Identifier	:	common name
	Identity	:	Benzyl alcohol

RESULTS AND DISCUSSION		
Effect concentrations		
Exposure duration	:	48
Unit	:	h
Endpoint	:	EC50
Effect concentration	:	230
Unit of effect concentrations	:	mg/L
Any other information on results incl. tables	:	Tests conducted before FY 2002 needs confirmation of test results, because some of these tests were conducted using a dispersant.
DATA SOURCE		
Reference		
Year	:	1997
Bibliographic source	:	Results of Eco-toxicity tests of chemicals conducted by Ministry of the Environment in Japan (- March 2013)
MATERIALS AND METHODS		
Test guideline		
Guideline	:	OECD Guideline 211 (Daphnia magna Reproduction Test)
Principles of method if other than guideline	:	These tests are conducted based on OECD-GLP standard and OECD test guidelines. However, because most of these data have not been evaluated by experts, confirmation of test results is needed if these data are used for assessment.

Test materials			
Test material identity			
	Identifier	:	CAS number
	Identity	:	100-51-6
	Identifier	:	common name
	Identity	:	Benzyl alcohol
RESULTS AND DISCUSSION			
Effect concentrations			
	Exposure duration	:	21
	Unit	:	d
	Endpoint	:	EC50
	Effect concentration	:	66
	Unit of effect concentrations	:	mg/L
	Exposure duration	:	21
	Unit	:	d
	Endpoint	:	NOEC
	Effect concentration	:	51

	Unit of effect concentrations	:	mg/L
	Any other information on results incl. tables	:	Tests conducted before FY 2002 needs confirmation of test results, because some of these tests were conducted using a dispersant.
DATA SOURCE			
Reference			
	Year	:	1997
	Bibliographic source	:	Results of Eco-toxicity tests of chemicals conducted by Ministry of the Environment in Japan (- March 2013)
MATERIALS AND METHODS			
Test guideline			
	Guideline	:	OECD Guideline 201 (Alga, Growth Inhibition Test)
	Principles of method if other than guideline	:	These tests are conducted based on OECD-GLP standard and OECD test guidelines. However, because most of these data have not been evaluated by experts, confirmation of test results is needed if these data are used for assessment.
Test materials			
Test material identity			
	Identifier	:	CAS number
	Identity	:	100-51-6
	Identifier	:	common name
	Identity	:	Benzyl alcohol

Test organisms			
Study design			
Test conditions			
RESULTS AND DISCUSSION			
Effect concentrations			
	Exposure duration	:	72
	Unit	:	h
	Endpoint	:	EC50
	Effect concentration	:	770
	Unit of effect concentrations	:	mg/L
	Basis for effect	:	growth rate
	Exposure duration	:	72
	Unit	:	h
	Endpoint	:	NOEC
	Effect concentration	:	310
	Unit of effect concentrations	:	mg/L
	Basis for effect	:	growth rate

	Exposure duration	:	72
	Unit	:	h
	Endpoint	:	EC50
	Effect concentration	:	500
	Unit of effect concentrations	:	mg/L
	Basis for effect	:	AUG
	Exposure duration	:	72
	Unit	:	h
	Endpoint	:	NOEC
	Effect concentration	:	310
	Unit of effect concentrations	:	mg/L
	Basis for effect	:	AUG
	Any other information on results incl. tables	:	Tests conducted before FY 2002 needs confirmation of test results, because some of these tests were conducted using a dispersant. Growth rata values before 2003 FY are calculated values from original values, because endpoint values of 0 - 72h were not reported.

As taken from J-CHECK, 2017

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

Record for benzenemethanol:

Spec. Name	Sci.	Resp. Site	Media Type	Exp. Type	Dose#	Endpoint	Effect	Signif.	Dose
Spec. Common Name		Exp. Dur. (Days)	Test Loc.	Chem. Anal.	Res. Sample Unit	BAF/BCF	Effect Meas.	Sig. Level	Dose Stat. Meth.
Lactuca sativa			AGR			EC50	REP		NC 1 mM
Lettuce		3					GERM		
Lactuca sativa			AGR	DA		EC50	REP		F 1.0 mM
Lettuce		3	LAB	U			GERM		
Acalymma vittatum			NONE	EN	2	NOEL	AVO	ANOSIG	F 100 mg/eu
Striped Cucumber Beetle		1	FIELDA	U			CHEM	<0.05	

As taken from US EPA ECOTOX database

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
bird - wild	LD50	oral	100mg/kg (100mg/kg)		Toxicology and Applied Pharmacology. Vol. 21, Pg. 315, 1972.

As taken from ChemIDplus (b), available at <https://chem.nlm.nih.gov/chemidplus/>

10.5. All other relevant types of ecotoxicity

EPISuite provides the following information:

Bioaccumulation Estimates (BCFBAF v3.01):

Log BCF from regression-based method:	0.137 (BCF = 1.371 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-1.5734 days (HL = 0.02671 days)
Log BCF Arnot-Gobas method (upper trophic):	0.190 (BCF = 1.549)
Log BAF Arnot-Gobas method (upper trophic):	0.190 (BAF = 1.549)
log Kow used:	1.10 (expkow database)

The Ecological Categorization Results from the Canadian Domestic Substances List state that Benzenemethanol is not bioaccumulative in the environment.

Empirical Log Kow	1.1
Log Kow predicted by KowWin	1.08
Log BAF T2MTL predicted by Gobas	0.238514788983152
Log BCF 5% T2LTL predicted by Gobas	0.20167408817411
Log BCF Max predicted by OASIS	1.28787890301384
Log BCF predicted by BCFWIN	-0.503

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

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12. Other information

- “Benzyl alcohol, benzoic acid and its salts, and benzyl benzoate function mostly as fragrance ingredients/preservatives in cosmetic products. The Cosmetic Ingredient Review Expert Panel previously established concentration limits for benzyl alcohol, benzoic acid, and sodium benzoate in cosmetics and determined that the available data were insufficient to support the safety of these ingredients during inhalation exposure. After reviewing newly available data, it was concluded that benzyl alcohol, benzoic acid and its salts, and benzyl benzoate are safe in the present practices of use and concentration described in this safety assessment.”
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13. Last audited

June 2023

[FOREWORD](#)

[INTRODUCTION](#)

BENZOATES

CAS N°:65-85-0, 532-32-1, 582-25-2, 100-51-6

SIDS Initial Assessment Report for 13th SIAM

(Bern, 7th - 9th November 2001)

Chemical Name: **Benzoates: Benzoic acid, Sodium benzoate,
Potassium benzoate, Benzyl alcohol**

CAS No: **65-85-0, 532-32-1, 582-25-2, 100-51-6**

Sponsor Country: **The Netherlands**

National SIDS Contact Point in Sponsor Country: Mr. Dick Sijm

HISTORY:

In 2001 ICCA asked The Netherlands to be the sponsor country for the benzoates

no testing (X)

testing ()

COMMENTS:

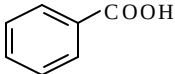
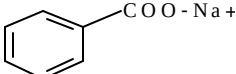
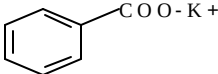
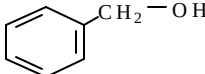
The Benzoates were already discussed in other frameworks such as the WHO. Therefore the original data were not again evaluated. The conclusions of other frameworks are discussed in the SIAR. This SIAR can be considered as a state of the art report on benzoates.

Deadline for circulation:

Date of Circulation:

(To all National SIDS Contact Points and the OECD Secretariat)

SIDS INITIAL ASSESSMENT PROFILE**Benzoates Category**

CAS No.	65-85-0	532-32-1	582-25-2	100-51-6
Chemical Name	Benzoic acid	Sodium benzoate	Potassium benzoate	Benzyl alcohol
Structural Formula				

RECOMMENDATIONS

The chemicals are currently of low priority for further work.

SUMMARY CONCLUSIONS OF THE SIAR

Benzyl alcohol, benzoic acid and its sodium and potassium salt can be considered as a single category regarding human health, as they are all rapidly metabolised and excreted via a common pathway within 24hrs. Systemic toxic effects of similar nature (e.g. liver, kidney) were observed. However with benzoic acid and its salts at higher doses than with benzyl alcohol. For environmental effects the category is less clear, however all are readily biodegradable, non-bioaccumulative and acute toxicity values are similar. For human health all exposure routes are possible, despite benzoic acid and its salts being solids and benzyl alcohol being a liquid. For workers it will mainly be by inhalation and by skin, whereas for consumers it will mainly be by oral and dermal routes.

Human Health

The compounds exhibit low acute toxicity as for the oral and dermal route. The LD50 values are > 2000 mg/kg bw except for benzyl alcohol which needs to be considered as harmful by the oral route in view of an oral LD50 of 1610 mg/kg bw. The 4 hrs inhalation exposure of benzyl alcohol or benzoic acid at 4 and 12 mg/l as aerosol/dust respectively gave no mortality, showing low acute toxicity by inhalation for these compounds.

Benzoic acid and benzyl alcohol are slightly irritating to the skin, while sodium benzoate was not skin irritating. No data are available for potassium benzoate but it is also expected not to be skin irritating. Benzoic acid and benzyl alcohol are irritating to the eye and sodium benzoate was only slightly irritating to the eye. No data are available for potassium benzoate but it is expected also to be only slightly irritating to the eye.

The available studies for benzoic acid gave no indication for a sensitizing effect in animals, however occasionally very low positive reactions were recorded with humans (dermatological patients) in patch tests. The same occurs for sodium benzoate. It has been suggested that the very low positive reactions are non-immunologic contact urticaria. Benzyl alcohol gave positive and negative results in

animals. Benzyl alcohol also demonstrated a maximum incidence of sensitization of only 1% in human patch testing. Over several decades no sensitization with these compounds has been seen among workers.

For benzoic acid repeated dose oral toxicity studies give a NOAEL of 800 mg/kg/day. For the salts values > 1000 mg/kg/day are obtained. At higher doses increased mortality, reduced weight gain, liver and kidney effects were observed.

For benzyl alcohol the long-term studies indicate a NOAEL > 400 mg/kg bw/d for rats and > 200 mg/kg bw/d for mice. At higher doses effects on bodyweights, lesions in the brains, thymus, skeletal muscle and kidney were observed. It should be taken into account that administration in these studies was by gavage route, at which saturation of metabolic pathways is likely to occur.

It can be concluded that benzoic acid and its salts exhibit very low repeated dose toxicity. Benzyl alcohol exhibits low repeated dose toxicity.

All chemicals showed no mutagenic activity in *in vitro* Ames tests. Various results were obtained with other *in vitro* genotoxicity assays. Sodium benzoate and benzyl alcohol showed no genotoxicity *in vivo*. While some mixed and/or equivocal *in vitro* chromosomal/chromatid responses have been observed, no genotoxicity was observed in the *in vivo* cytogenetic, micronucleus, or other assays. The weight of the evidence of the *in vitro* and *in vivo* genotoxicity data indicates that these chemicals are not mutagenic or clastogenic. They also are not carcinogenic in long-term carcinogenicity studies.

In a 4-generation study with benzoic acid no effects on reproduction were seen (NOAEL $\geq$ 750 mg/kg). No compound related effects on reproductive organs (gross and histopathology examination) could be found in the (sub) chronic studies in rats and mice with benzyl acetate, benzyl alcohol, benzaldehyde, sodium benzoate and supports a non-reprotoxic potential of these compounds. In addition, data from reprotoxicity studies on benzyl acetate (NOAEL >2000 mg/kg bw/d; rats and mice) and benzaldehyde (tested only up to 5 mg/kg bw; rats) support the non-reprotoxicity of benzyl alcohol and benzoic acid and its salts.

In rats for sodium benzoate dosed via food during the entire gestation developmental effects occurred only in the presence of marked maternal toxicity (reduced food intake and decreased body weight) (NOAEL = 1400 mg/kg bw). For hamster (NOEL: 300 mg/kg bw), rabbit (NOEL: 250 mg/kg bw) and mice (CD-1 mice, NOEL: 175 mg/kg bw) no higher doses (all by gavage) were tested and no maternal toxicity was observed. For benzyl alcohol: NOAEL = 550 mg/kg bw (gavage; CD-1 mice). LOAEL = 750 mg/kg bw (gavage mice). In this study maternal toxicity was observed e.g. increased mortality, reduced body weight and clinical toxicology. Benzyl acetate: NOEL = 500 mg/kg bw (gavage rats). No maternal toxicity was observed.

Environment

From the data (fish, daphnia, algae, bacteria) it is obvious that neutralization of the pH greatly reduces (up to one order of magnitude) the acute toxicity of benzoic acid. This is also supported by the lower toxicity observed with sodium benzoate. Under environmental relevant conditions therefore the acute toxicity of benzoic acid, sodium benzoate and potassium benzoate for all four trophic levels is > 100 mg/l. Under environmental relevant conditions the acute toxicity of benzyl alcohol for fish, daphnia and bacteria is > 100 mg/l. For algae, an EC 50 3hrs of 95 mg/l is reported. Under environmental relevant conditions, benzoic acid and its salts have very low acute toxicity, whereas benzyl alcohol has low to moderate acute toxicity.

Exposure

Worldwide production capacity of benzoic acid is estimated at 700 kt per year. The major outlet (75%) for benzoic acid is as a chemical intermediate in the production of phenol, which in turn is mainly used to produce caprolactam. The next largest outlet is as a feedstock for sodium benzoate (10%) and chemical synthesis of plasticizers (5%).

Worldwide production capacity of sodium benzoate is estimated at 100 kt per year. The major outlet for sodium benzoate is as preservative in food and beverages (60%). Second most important market is cooling liquids (10%). The main function of sodium benzoate in most applications is as preservative.

Worldwide production capacity of potassium benzoate is estimated at 7 kt per year. It is used as a preservative in nonalcoholic beverages.

Worldwide production capacity of benzyl alcohol is estimated at 50 kt. Major use for benzyl alcohol is as curing agent in epoxy coatings (30%), where it becomes chemically bounded after reaction. Other important uses include the use as a solvent in low concentrations in waterborne coatings (10%) and use in paint strippers (10%) and chemical intermediate for synthesis for benzyl esters that are used in the flavor and fragrance industry (10%). The use in paint strippers is limited to uses in industrial settings.

Benzyl alcohol, benzoic acid and its sodium and potassium salt are also used in pharmaceuticals, cosmetics and/or food. Consumer exposure in these specific applications are controlled by the fact that, for all these applications, specific regulatory frameworks (regional and/or national) with authorization/approval procedures and specific advisory bodies exist (*inter alia*: the US FDA, WHO JECFA, EU SCF, etc), including, on a regular basis, reevaluation of approvals, hazardous properties and factual exposures. According to information from products registers, uses that are not specifically regulated include uses of the substances in different kinds of products e.g. paints, varnishes solvents, cleaning and washing agents, photochemicals and antifreeze agents.

Benzoic acid is a white solid, with a solubility in water of 2.9 g/l and with a vapour pressure of 0.0011 hPa at 20 °C. The log octanol/water partition coefficient was measured to 1.88; the Henry's law constant = 0.0046-0.022 Pa·m³/mol; and the pKa = 4.2. Sodium benzoate and potassium benzoate are white solids, with solubility in water of 556 g/l and with a vapour pressure of <0.0011 hPa at 20 °C. The log octanol/water partition coefficient were measured to -2.269. Benzyl alcohol is a colorless liquid, with a solubility in water of 40 g/l and with a vapour pressure of 0.13 hPa at 20°C.

The log octanol/water partition coefficient was measured to 1.1.

The distribution modeling according to Mackay Level III indicates soil and water to be the favored compartments for the chemicals. However, physical chemical properties and use patterns indicate water to be the main compartment for these substances. None are expected to hydrolyze. All are readily biodegradable. None has bioaccumulative potential.

NATURE OF FURTHER WORK RECOMMENDED

Regarding all the information provided, the substances have low priority for further work.

SIDS Initial Assessment Report (SIAR)

1. IDENTITY

Category name: Benzoates

<u>Chemicals:</u>	<u>CAS#:</u>	<u>Molecular Weight</u>
Benzoic acid	65-85-0	122.12
Sodium benzoate	532-32-1	144.11
Potassium benzoate	582-25-2	160.21
Benzylalcohol	100-51-6	108.4

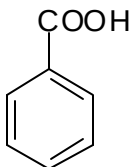
Physico-chemical properties:

Chemical	Appearance	Melting point	Boiling point @ 1013 hPa	Vapor pressure (at 20°C)	octanol/water partition coefficient (LogP)	Water Solubility (at 20°C)	Henry's law constant	pKa
Benzoic acid	White solid	122.4°C	249.2°C	0.0011 hPa	1.88	2.9 g/l	.0046 - .022 Pa*m ³ /mol	4.19
Sodium benzoate	White solid	330.6°C	464.9°C	< 0.001 hPa	-2.269	556 g/l		
Potassium benzoate *	White solid	330.6°C	464.9°C	< 0.001 hPa	-2.269	556 g/l		
Benzyl alcohol	Clear liquid	-15°C	205.3°C	0.13 hPa	1.1	40 g/l		

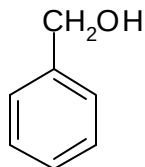
*) No data for Potassium benzoate were available, but they are expected to be the same as for sodium benzoate.

Category Justification:

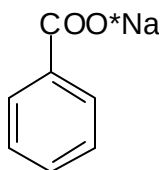
The proposed category of this ICCA HPV Benzoates submission consists of the following chemicals:



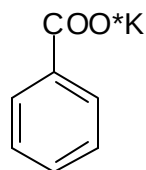
Benzoic Acid
CAS# 65-85-0



Benzyl Alcohol
CAS# 100-51-6

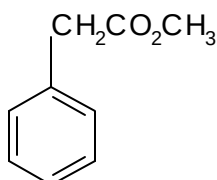


Sodium Benzoate
CAS# 532-32-1

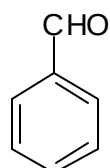


Potassium Benzoate
CAS# 582-25-2

The following chemicals (benzylacetate and benzaldehyde) are being used in this ICCA HPV benzoates submission only for supportive data purposes. They are not as such included in this category submission for reasons stated below:



Benzyl Acetate
CAS# 140-11-4

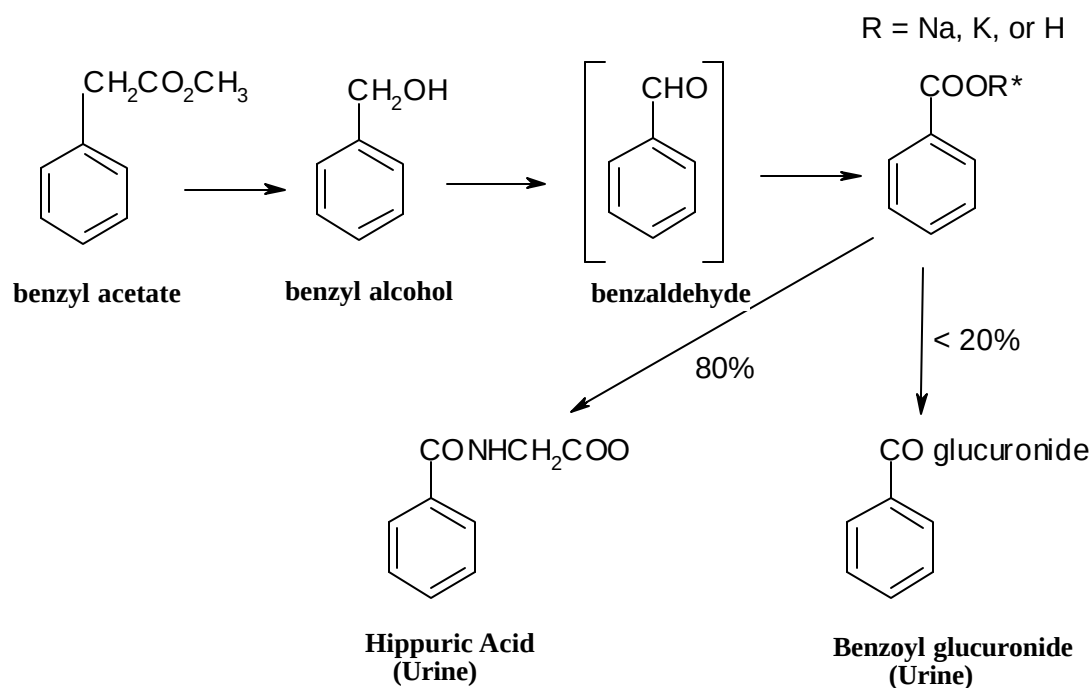


Benzaldehyde
CAS# 100-52-7

Sponsored in the US EPA HPV Program
by the Flavor and Fragrance High Production
Volume Consortia (FFHPVC)

Completed SIDS/SIAR

The common metabolic pathway of all these substances, adapted from JECFA 1997 and the American Conference of Governmental Industrial Hygienists Documentation of the Threshold Limit Values and Biological Exposure Indices, is provided below (ACGIH, 1986):



The sodium and potassium salts of benzoic acid are expected to immediately dissociate and form benzoic acid in an aqueous environment.

The benzylacetate, benzylalcohol, benzaldehyde and benzoic acid and its sodium and potassium salt were considered as a single category regarding human health by JECFA as they are all rapidly metabolized and excreted via a common pathway within 24hrs (JECFA 1997).

Benzyl acetate, the first compound in the metabolic pathway diagram, is very rapidly hydrolyzed by esterases in *several species including man* to benzyl alcohol and acetic acid. The benzylalcohol is then very rapidly metabolized as shown in the above diagram and only at very high dose (> 500 mg/kg/day by oral gavage route) some saturation of metabolic pathways occurs. This is among others very well shown in studies on benzylacetate (see below; from JECFA 1997).

Male B6C3F1 mice and Fischer 344 rats treated either intravenously or orally with ¹⁴C-benzyl acetate. The intravenous dose was equivalent to 10 mg/kg bw for mice and 5 mg/kg bw for rats. For oral administration, benzyl acetate was dissolved in corn oil and administered at doses equivalent to 10, 100, or 1000 mg/kg bw for mice and 5, 50, or 500 mg/kg bw for rats. The compound was readily absorbed from the gastrointestinal tract of both species, and about 90% of the total dose was recovered as urinary metabolites after 24h. A small proportion (0.3-1.3%) of the total dose was excreted in the faeces after both intravenous and oral administration. Elimination of benzyl acetate as carbon dioxide or volatile substances was minimal after intravenous treatment and consequently was not determined after oral treatment. Analysis of tissues of animals sacrificed 24 h after intravenous or oral administration of labelled compound showed no ¹⁴C activity, indicating that elimination of the label was virtually complete by this time. This clearance pattern indicates that benzyl acetate is readily absorbed and excreted after oral administration. The relative amounts of benzyl acetate absorbed, metabolized, and excreted were unaffected by the size or number of doses administered. Repeated treatment of rats with benzyl acetate at 500 mg/kg bw per day for 14 days, followed by a single dose of labelled compound did not change the clearance pattern. More than 90% of the radiolabel in the urine was present as hippuric acid, with minor amounts as benzyl alcohol and benzylmercapturic acid (up to 4%); no unchanged benzyl acetate was found, and the levels of benzoyl glucuronide were not measured.

There was no evidence to suggest saturation or reduction of metabolic capacity in either species over the dose range tested. At much higher dosing the proportion of the dose present as benzoyl glucuronide increased with dose, indicating a limited capacity for glycine conjugation only at extreme high dose levels.

These studies clearly show, that the compound is rapidly absorbed from the gastrointestinal tract of rats and mice, and about 90% of the total dose is recovered as urinary metabolites after 24h. More than 90% of the radio-label in the urine is present as hippuric acid, with minor amounts as benzyl alcohol and benzylmercapturic acid (up to 4%); no unchanged benzyl acetate was found. Only at very high doses, saturation of these pathways will occur.

This clearly shows the rapid pathway of hydrolysis to benzyl alcohol and subsequent oxidation to benzaldehyde to benzoic acid and subsequent conjugation to the hippuric acid.

All supports a very rapid absorption, distribution, biotransformation, and excretion of these substances by the common pathway given above.

Repeated dose toxicity studies (information in this SIAR) reveal only systemic toxic effects (e.g. liver, kidney) of similar nature, at high dose.

For environmental effects the category is less clear, however all are readily biodegradable, non-bioaccumulative and acute toxicity values for water organisms under environmental relevant conditions are similar.

For human health all exposure routes are possible, despite benzoic acid and its salts being solids and benzylalcohol being a liquid. For workers exposure will mainly be by inhalation and by skin, whereas for consumers it will mainly be oral and dermal.

2. GENERAL INFORMATION ON EXPOSURE

Production and use:

Benzoic Acid

Worldwide production capacity is estimated at 700 kt per year. Average operating rate is at max 80% resulting in a production of 560 kt benzoic acid per year. The major outlet (75%) for benzoic acid is in the production of phenol, which in turn is mainly used to produce caprolactam. The next biggest outlet is as a feedstock for sodium benzoate (10%) and chemical synthesis of plasticizers (5%). Benzoic acid is therefore mainly (>80%) used as a chemical intermediate for synthesis of other chemicals, as well as for the production of sodium salt (10%). So it has mainly a controlled use in industrial settings.

Sodium Benzoate

Worldwide production capacity is estimated at 100 kt per year. Average operating rate is at max 75% resulting in a production of 75 kt sodium benzoate per year. The major outlet for sodium benzoate is as a preservative in food and beverages (60%). Second most important market is cooling liquids (10%). The main function of sodium benzoate in most applications is as a preservative.

Potassium Benzoate:

Worldwide production capacity is estimated at 7 kt per year. It is used as a preservative in nonalcoholic beverages.

Benzyl Alcohol

Worldwide production capacity is estimated at 50 kt per year. Average operating rate is at max 80% resulting in a production of 40 kt benzyl alcohol per year. The major use for benzyl alcohol is as a curing agent in epoxy coatings (30%), where it becomes chemically bound after reaction. Other important uses are as a solvent in low concentrations in waterborne coatings (10%), and use in paint strippers (10%) and as chemical intermediate for synthesis of benzyl esters that are used in the Flavor and Fragrance industry (10%). The use in paint strippers is limited to uses in industrial settings.

Benzylalcohol, benzoic acid and its sodium and potassium salt have been used for decades in pharmaceuticals, cosmetics and/or food as preservatives and flavoring/fragrance agents

Information in Product registers:

According to information in Product Registers the substances are used in different kinds of products e.g. paints, varnishes, solvents, cleaning and washing agents, photochemicals and antifreeze agents.

Release into the environment during production and use :

In DSM Geleen The Netherlands, during production, about 650 kg/year of benzylalcohol are emitted into the atmosphere (< 0.01 % of production volume). Based on the amount benzylalcohol discharged to the DSM WWTP, it can be calculated that the influent concentration of the WWTP is at about 1 ug/l. Because of its ready biodegradability and the existing dilution of effluent to the receiving water, the concentration in the receiving water will be < 0.01 ug/l.

In DSM Rotterdam The Netherlands, during production sodium benzoate is emitted to air at < 0.01 % of the production volume. For benzoic acid this is < 0.001 %.

2.1 Environmental Exposure and Fate

Distribution modelling using Mackay Level III (the EPA default: equal releases (10,000 kg/hr) and equal distribution to all compartments was used) indicates water (34.8-50%) and soil (48.4-64.2%) to be the main compartment for all four chemicals. None are expected to volatilize to the atmosphere ($\leq 1.51\%$), nor to adsorb to sediment ($\leq 0.09\%$) (Meylan & Howard, 1999).

However physical chemical properties and use patterns indicate water to be the main compartment for these substances.

Distribution (%) according to Fugacity Level III

Chemical	CAS#	Air	Water	Soil	Sediment
Benzoic acid	65-85-0	0.911	34.8	64.2	0.093
Sodium benzoate	532-32-1	1.45e-007	45.3	54.6	0.0755
Potassium benzoate	582-25-2	1.61 e-007	45.3	54.6	0.0755
Benzyl alcohol	100-51-6	1.51	50.0	48.4	0.0923

Based on structure and organic chemistry rules (e.g. bonding in organic molecules, activation energy, reactivity, transformations, addition, substitution, elimination) no hydrolysis is expected at pH ranges of 4 - 11.

The calculated photodegradation for benzyl alcohol and the benzoates are 50% after 1.3 to 3 days (Meylan and Howard, 1999), and the measured photodegradation for benzoic acid is 90% after 140 minutes (Matthews, 1990).

Biodegradation and Bioaccumulation

All four chemicals are readily biodegradable (> 90% after 28 days) both aerobically (MITI, 1992; Zahn & Wellens, 1980; Salanitro et al., 1988) and anaerobically (Battersby & Wilson, 1989; Horowitz et al., 1982).

(Benzoic acid is used as positive control in OECD Guideline for ready biodegradability testing). From the results of numerous removal experiments the main elimination pathway for the chemicals is biotic mineralization.

The octanol/water partition coefficient of all compounds indicates a low potential for bioaccumulation. This is also supported by the rapid biotransformation and/or excretion of these compounds in urine in mammals.

2.2 Human Exposure

For human health all exposure routes are possible, despite benzoic acid and its salts being solids and benzyl alcohol being a liquid. For workers exposure will mainly be by inhalation and by skin, whereas for consumers it will mainly be oral and dermal

Consumer exposure:

Benzoic acid, benzylalcohol, sodium benzoate and potassium benzoate are widely used in food, cosmetic and pharmaceutical applications as preservatives and flavoring/fragrance agents. Benzoic acid and benzylalcohol are naturally occurring (Merck Index, 1996). Consumer exposure in these specific applications are controlled by specific regulatory frameworks (regional and/or national) with authorization/approval procedures and specific advisory bodies (among others US FDA, WHO JECFA, EU SCF, etc). A re-evaluation of approvals, hazardous properties and factual exposures (among others compliance to the ADI) inclusive, are performed on a regular basis. According to information in Product Registers the substances are used in different kinds of products e.g. paints, varnishes, solvents, cleaning and washing agents, photo chemicals and antifreeze agents. Benzoic acid and sodium benzoate are under re-evaluation at the EU Scientific Committee for Food. From preliminary information (June 2001) re-approval is expected for these substances. The Joint FAO/WHO Expert Committee on Food Additives (JEFCA) has established a group Acceptable Daily Intake (ADI) for benzoic acid and its salts and benzyl alcohol, benzyl acetate and benzaldehyde of 5 mg benzoic acid equivalent/kg bodyweight. This group ADI is based on the structural similarity and common metabolic fate of these chemicals (WHO, 1997).

Worker exposure:

Companies have provisionally advised exposure limits for benzoic acid and its salts as well as for benzyl alcohol. Also the US WEEL (Workplace Environmental Exposure Limit) Committee of the AIHA has set limits for benzyl alcohol at a value of 10-ppm (44 mg/m³) 8hr TWA.

In the several past decades of production, no cases of health complaints (sensitisation inclusive) have occurred.

Also from companies that use the substances no health complaints (sensitisation inclusive) have ever been reported.

3. HUMAN HEALTH

3.1 Effects on Human Health

In general:

- Benzoate from potassium benzoate and sodium benzoate will change from the ionized form to the undissociated benzoic acid molecule under physiological conditions.
- Benzyl acetate, benzyl alcohol and benzaldehyde are all metabolized to benzoic acid and it is therefore reasonable to assume that the results of studies on members of the group will apply to the others.
- All benzyl compounds are rapidly absorbed, and rapidly and completely excreted in the urine. The main transformation of benzoic acid is the formation of hippuric acid.
- It is considered also that data gaps for one substance can be adequately addressed by the existing data for the other compounds.

Only the results of the critical studies are given, but for most endpoints additional studies exist (see full IUCLID documents), that support the results in the critical studies.

3.1.1 Acute Oral Toxicity

Three of the four compounds were tested according to Guideline methods. All demonstrated very low or low toxicity, especially the benzoate salts. Only benzyl alcohol has a LD50 slightly less than 2000mg/kg bw and should therefore be considered as harmful. Although the studies on potassium benzoate were not Guideline studies, these were accepted because the results showed low toxicity, similar to the sodium salt.

Chemical	Species	Protocol	Result	Reference
Benzoic acid	rat	Directive 84/449/EEC	LD50 =2565 mg/kg	IRDC#163-282, 1974
	mouse	OPPTS 870.1100	LD50 =2250 mg/kg	BRL#9348, 1979
Sodium benzoate	rat	Directive 84/449/EEC	LD50 =3140 mg/kg	Loeser, 1977-A; Deuel et al., 1954
	rat	other	LD50 =4070 mg/kg	Smyth & Carpenter, 1948
Benzyl alcohol	rat	Directive 84/449/EEC	LD50 =1610 mg/kg	Loeser, 1978
	rat	other	LD50 =2080 mg/kg	Graham & Kuizenga, 1945; Opdyke, 1973
	mouse	other	LD50 =1580 mg/kg	Jenner, 1964; Opdyke, 1973
Potassium benzoate	rat	other	LD50 = >10,000 mg/kg	Kravets-Bekker & Ivanova, 1970
	mouse		LD50 = >10,000 mg/kg	Kravets-Bekker & Ivanova, 1970
	guinea pig		LD50 = >10,000 mg/kg	Kravets-Bekker & Ivanova, 1970

3.1.2 Acute Dermal Toxicity

Two of the compounds were tested for acute dermal toxicity. Both demonstrated low toxicity.

Chemical	Species	Protocol	Result	Reference
Benzoic acid	rabbit	EPA OTS 798.1100	LD50= > 2000 mg/kg	IRDC#163-282, 1974; Opdyke, 1973
Benzyl alcohol	Rabbit	Other	LD ₅₀ = 2000 mg/kg	NPIRI,1974
	guinea pig	Other	LD ₅₀ = < 5 ml/kg	Jones, 1967; Opdyke, 1973

3.1.3 Acute Inhalation Toxicity

Two of the compounds were tested for acute inhalation toxicity according to Guideline procedures; both demonstrating very low toxicity.

Chemical	Species	Protocol	Result	Reference
Benzoic acid	rat	EPA OTS 798.1150	LC ₅₀ = >12.2 mg/l/4h. No mortality at 12.2 mg/l as dust.	IRDC#163-282, 1974
Benzyl alcohol	rat	OECD Guide-line 403 and GLP	LC ₅₀ = > 4.178 mg/l/4h. No mortality at 4.178 mg/l as aerosol	Bayer AG, 1990

In conclusion: The compounds exhibit low acute toxicity, except benzylalcohol that has an oral LD₅₀ slightly less than 2000 mg/kg bw and should therefore be considered as harmful by the oral route.

3.1.4 Skin Irritation

Three of the compounds were tested for skin irritation according to Guideline procedures; the potassium salt should be similar to the sodium salt, therefore being non-irritating.

Chemical	Species	Protocol	Result	Reference
Benzoic acid	Rabbit	EPA OTS 798.4470	not irritating	IRDC # 163-282
	rabbit	Directive 84/449/EEC	slightly irritating	RCC NOTOX - study no. 0847/1083, 1988.
Sodium benzoate	Rabbit	OECD Guide-line 404	not irritating	RCC NOTOX - study no. 014658
	rabbit	Directive 84/449/EEC	not irritating	Loeser, E., 1977-B
Benzyl alcohol	rabbit	OECD Guide-line 404	not irritating	Bayer AG data, Report No. 19232, 1990
	rabbit	Other	slightly irritating	Smyth, H. F. et al., 1951; reported in US NTP: TR 343, 1989.

3.1.5 Eye Irritation

Three of the compounds were tested for eye irritation according to Guideline procedures; the potassium salt should be similar to the sodium salt, therefore being non- to slightly irritating.

Chemical	Species	Protocol	Result	Reference
Benzoic acid	Rabbit	Directive 84/449/EEC	highly irritating	RCC NOTOX - study no. 0847/1084, 1988
	rabbit	EPA OTS 798.4500	severely irritating	IRDC #163-282
Sodium benzoate	Rabbit	Directive 84/449/EEC	not irritating	Loeser, E., 1977-B
	rabbit	OECD Guide-line 405	slightly irritating	RCC NOTOX - study no. 014669, 1988
Benzyl alcohol	Rabbit	OECD Guide-line 405	moderately irritating	Bayer AG data, Report No. 19232, 1990
	rabbit	Other: limited data	highly irritating	Smyth, H. F. et al., 1951; reported in US NTP: TR 343, 1989

In conclusion: Benzoic acid and benzylalcohol are slightly irritating to the skin, while sodium and potassium benzoate are not skin irritating. Benzoic acid and benzyl alcohol are irritating to eyes, and sodium and potassium benzoate are only slightly irritating to eyes

3.1.6 Sensitization

The available studies for benzoic acid gave no indication for a sensitizing effect in animals, however some weak positive reactions were recorded with the human patch test. Benzyl alcohol was non-sensitizing in the Draize and Guinea Pig Maximization Tests, but a positive sensitizer in the Freund's Complete Adjuvant Test and the guinea pig Open Cutaneous Test and demonstrated a maximum incidence of sensitization of 1% in clinical human patch testing. A clinical dermatological study showed positive patch test reactions in 0.2% of the patients treated with 5% sodium benzoate in petrolatum. It has been suggested that this very low potential of sodium benzoate to elicit a *non-immunologic* contact urticaria may be due to the formation of benzoic acid at skin contact.

Chemical	Species	Protocol	Result	Reference
Benzoic acid	guinea pig	Draize	not sensitizing	BRL #9347, 1979
	guinea pig	Guinea pig maximization test	not sensitizing	Gad, 1986
	human	Patch test	occasional positive result	Rademaker & Forsyth, 1989; Forsbeck & Skog, 1977
Sodium benzoate	Human	Patch test	5 of 2045 patients positive	Brasch, J. et al., 1993
	human	Patch test	nonimmunologic contact urticaria	Nethercott, J.R., 1984
Benzyl alcohol	guinea pig	Draize Test	not sensitizing	Klecak, G. et al., 1977
	guinea pig	Guinea pig maximization test	not sensitizing	Klecak, G. et al., 1977
	guinea pig	Freund's complete adjuvant test	sensitizing	Klecak, G. et al., 1977
	guinea pig	Open epicutaneous test	sensitizing	Klecak, G. et al., 1977
	human	Patch-Test	sensitizing	Malten, K. E. et al., 1984; Mitchell, J. C. et al., 1982; Nethercott, J. R., 1982

In conclusion: No firm conclusion on the sensitizing potential of benzyl alcohol can be made due to the varied results with the various tests. Both benzoic acid and sodium benzoate were non-sensitizing in animal test but showed a very low incidence in humans (patients) tested by the patch test.

CICAD conclusion on benzoic acid and sodium benzoate was: "However, both substances are known to cause non-immunologic immediate contact reactions. This effect is scarce in healthy subjects, while in patients with frequent urticaria or asthma, symptoms or exacerbation of the symptoms were observed".

3.1.7 Repeat Dose Toxicity

Several short term repeated dose toxicity studies are available (see IUCLID documents) on compounds of the group (as well as benzaldehyde and benzyl acetate) and support the outcome and No Observed Adverse Effect Level (NOAEL) of the longer term studies given below.

In a 4-generation study 20 rats/sex/group were dosed continuously by diet with 375 or 750 mg/kg/day **benzoic acid**. In all 4 generations no influence on growth (weight, weight gain and food efficiency (measured by protein efficiency)) and organ weights was found. The animals of the 3rd generation were killed and examined histopathologically after 16 weeks (after lactation of the pups). No histo-pathological findings were found. In the paper, no information is given on the organs investigated, however the robustness of the total study, the reputation of the investigators, as well as the reputation of the Professor who did the histopathologic investigation, a high scientific quality has to be assumed even though the studies were performed many years ago. From other parameters it can be assumed that as a minimum the brains, heart, liver, kidney, testis and spleen were examined.

Feeding of 375 mg/kg/day led to prolongation of survival compared to controls

NOAEL $\geq$ 750 mg/kg/day

(Kieckebusch & Lang, 1960)

Due to missing hematological and clinical chemistry investigations in all studies only a preliminary NO(A)EL of about 800 mg/kg can be derived for rats which is based on the studies from Kieckbusch & Lang (1960), Kreis et al. (1967) and Bio-Fax (1973) (Details to be found in the IUCLID).

A 21 day dermal study with male/female New Zealand white rabbits dosed with 100, 500, or 2500 mg/kg bw **benzoic acid** 5 days/week showed no compound related effects in behavior, body weight organ weights, clinical laboratory tests or survival. Very slight dermal irritation was noted for 1/8 rabbits at the 2500 mg/kg level.

NOAEL = 2500 mg/kg/day

(IRDC# 163-675, 1981)

Four groups of 10 CD rats/sex/group were exposed to 0, 25, 250 or 1200 mg **benzoic acid** dust aerosol/m³ (analytical concentration; MMAD 4.7 μ m) for 6 hours/day and 5 days/week over 4 weeks. At $\geq$ 25 mg/m³ an increased incidence of interstitial cell infiltrate and interstitial fibrosis in the lungs in treated animals compared with controls was seen. *However, there was no clear dose-dependency*. A concentration of $\geq$ 250 mg/m³ resulted in upper respiratory tract irritation and decreased absolute kidney weights in females. In the highest-dose group one rat/sex died and the body weight gain was decreased in males and females. Other effects included a decrease in platelets (males/females), absolute/relative liver weights (males) and trachea/lung weights (females). LOAEC (local effect) = 25 mg/m³ (However no clear dose-response was observed).

NOAEC (systemic) = 25 mg/m³

(IRDC# 163-676, 1981)

In a 10-day study, rats received **sodium benzoate** in feed. At the lowest tested concentration of 1358 mg/kg changes in serum cholesterol levels occurred in females. At doses of 1568 mg/kg and above changes in further serum parameters and an increased relative liver weight were described. Histopathological changes of the liver, increased relative kidney weights and disorders of the central nervous system were seen after dosing via diet with $\approx$ 1800 mg/kg. (Fujitani, 1993)

A 90-day study with male/female Sherman rats given 640, 1280, 3145, or 6290 mg/kg/day USP **sodium benzoate** continuously in feed showed no adverse effects at $\leq$ 3145 mg/kg bw. There was increased mortality (4/8 died); reduced weight gain; increased weight of livers and kidneys; pathological lesions (not specified) in livers and kidneys at 6290 mg/kg bw.

NOAEL = 3145 mg/kg bw/day

(Deuel, 1954)

For mice the NO(A)EL of **sodium benzoate** is higher. According to a 35 day study (by drinking water) no effects were observed at 3000 mg/kg bw. At this dose level also in a chronic study no toxic effects were found in histopathological examinations (see 3.1.9 paragraph 2, Toth, 1984) (Toth, 1984).

A 13-week study with male/female F344/N rats given 50, 100, 200, 400, or 800 mg/kg/day **benzyl alcohol** by *gavage* showed staggering, respiratory difficulty, and lethargy in rats of the high dose group. Hemorrhages occurred around the mouth and nose, and there were histologic lesions in the brain, thymus, skeletal muscle, and kidney. There were reductions in relative weight gain in male rats dosed with 800 mg/kg and in female rats dosed with 200 mg/kg or more. No notable changes in bw gain or compound-related histopathologic lesions were observed in rats from the lower dose groups.

In the 2-y study (see 3.1.9 paragraph 3), however, no notable changes were found on bw or bw gain at 200 or 400 mg/kg/d. The NOAEL in this 2-y rat study was 400 mg/kg/day, the highest dose tested.

NOAEL = 400 mg/kg/day (based on investigated parameters and taking into account the bw results of the 2-y study)

(US NTP Technical Report No. TR 343, 1989)

A 13-week study with male/female B6C3F1 mice given 50, 100, 200, 400, or 800 mg/kg/day **benzyl alcohol** by *gavage* showed staggering in mice dosed with 800 mg/kg, after dosing during the first 2 weeks of the study. Staggering after dosing occurred during the first 2 w of the study in mice dosed with 800 mg/kg. There were reductions in relative weight gain in male mice dosed with 400 or 800 mg/kg, and in female mice dosed with 200 mg/kg or more. No notable changes in bw gain or compound-related histopathologic lesions were observed in mice from the lower dose groups.

In the 2-y study (see 3.1.9 paragraph 4), however no notable changes were found on bw or bw gain at 200 mg/kg/d. The NOAEL in this 2-y mice study was 200 mg/kg/day the highest dose tested.

NOAEL = 200 mg/kg/day (based on reduction of relative weight gain only and taking into account the bw results of the 2-y study).

(US NTP Technical Report No. TR 343, 1989)

It should be noted: these studies were done by gavage (leading to greater toxicity due to the “bolus effect”). The administration of the benzyl compounds by gavage are likely to reveal changes at lower doses compared to studies where the substances are applied in the diet, leading to a distribution in the body over time.

In conclusion: For benzoic acid repeated dose (long-term inclusive) oral toxicity gives a NOAEL of 800 mg/kg/day. For the salts values > 1000 mg/kg/day are obtained. At higher doses increased mortality, reduced weight gain, liver and kidney effects were observed. For benzyl alcohol taking into account also the results of the long-term studies indicate a NOAEL $\geq$ 400 mg/kg bw/d for rats and $\geq$ 200 mg/kg bw/d for mice, however it should be taken into account that in these studies administration was by gavage, at which bolus dosing occurs and saturation of metabolic pathways is likely to occur. At high doses, effects on bodyweights, lesions in the brains, thymus, skeletal muscle and kidney were observed.

It can be concluded that benzoic acid and its salts exhibit very low repeated dose toxicity. Benzyl alcohol exhibits low repeated dose toxicity.

3.1.8 Genetic Toxicity

3.1.8.1 Genetic Toxicity *in vitro*

Benzoic acid was not mutagenic in Ames tests with and without metabolic activation (EGG# 580-192-1-78, 1978). The Sister Chromatid Exchange assay with human lymphocytes was negative - no metabolic activation was used (Jansson, 1988; Tohda, 1980). A Chromosome Aberration study with CHL cells was ambiguous - no metabolic activation was used (Ishidate, et al., 1984). A recombination assay with *Bacillus subtilis* H17 and M45 was positive (reported with minimal documentation in an abstract, Nonaka, 1989).

Sodium benzoate was not mutagenic in Ames tests with and without metabolic activation (Ishidate, et al., 1984). A cytogenetic assay using anaphase preparations of cultured human embryonic lung cells was negative - no metabolic activation was used (FDA PB 245453, 1974). An *Escherichia coli* reverse mutation assay was negative with and without metabolic activation (Prival, 1991). A cytogenetic assay using CHL cells was positive without metabolic activation (Ishidate, et al., 1984; Ishidate & Odashima, 1977). Sister Chromatid Exchange assays using Chinese hamster cells or human lymphocytes were positive without metabolic activation (Abe & Sasaki, 1977; Xing & Zhang, 1990). A recombination assay with *Bacillus subtilis* H17 and M45 was positive (reported with minimal documentation in an abstract, Nonaka, 1989).

Potassium benzoate tested positive in a recombination assay using *Bacillus subtilis* H17 and M45, with and without metabolic activation (Ishizaki & Ueno, 1989).

Benzyl alcohol was not mutagenic in Ames tests with and without metabolic activation (US NTP Technical Report No. TR 343, 1989). *Escherichia coli* reverse mutation assay was negative with and without metabolic activation (Leifer et al., 1981). A cytogenetic assay using CHO cells was negative without metabolic activation and positive with metabolic activation (Anderson et al., 1990; Zeiger et al., 1990). A Sister Chromatid Exchange assay using CHO cells was ambiguous with and without metabolic activation (US NTP Technical Report No. TR 343, 1989). A recombination assay with *Bacillus subtilis* H17 and M45 was positive (reported with minimal documentation, Kuroda et al., 1984).

Summary of (non-Ames) *in vitro* results:

Species (test system)	End-point	Results		Remarks
		without metabolic activation	with metabolic activation	
Benzoic acid				
Human lymphoblastoid cells (transformed by Epstein-Barr virus)	Sister chromatid exchange	Negative	NT	
Bacillus subtilis H17, M45	Recombination assay			tested positive (no further information available, only summary given)
Chinese hamster cells (CHL)	Chromosome aberration	?	NT	result given as negative in: Ishidate et al. (1984)
Sodium benzoate				
Human embryonic lung cells	Anaphase preparation	Negative	NT	
E.coli WP2	Reverse mutation assay	Negative	Negative	
Bacillus subtilis H17, M45	Recombination assay			tested positive (no further information available, only summary given)
Chinese hamster cells (CHL)	Chromosome aberration	Positive	NT	
Chinese hamster cells (DON)	Sister chromatid exchange	Positive?	NT	slight increase without dosage effect
Human lymphocytes	Sister chromatid exchange	Positive	NT	
Potassium benzoate				
Bacillus subtilis H17, M45	Recombination assay			tested positive (limited data)
Benzyl alcohol				
E.coli	Reverse mutation assay	Negative	Negative	
Chinese hamster cells (CHO)	Cytogenetic assay	Negative	Positive	
Chinese hamster cells (CHO)	Sister chromatid Exchange	?	?	
Bacillus subtilis H17, M45	Recombination assay			tested positive (limited data)

? = ambiguous

NT = not tested

In conclusion: Studies of these chemicals in the Ames point mutation assay do not show evidence of mutagenicity.

However, some have been reported to be positive in the less commonly used *Bacillus subtilis* recombination assay. In a number of cases adverse effects on the chromosome could be noticed, however also negative and/or equivocal results were reported.

However many higher-level *in vivo* tests (clastogenicity inclusive) were negative (see 3.1.8.2).

3.1.8.2 Genetic Toxicity *in vivo*

General remark: Since the sodium salt of benzoic acid instantaneously dissociates to the benzoic acid, the studies with sodium benzoate are also representative for benzoic acid and potassium benzoate.

A cytogenic assay in male rats given single or multiple gavage doses of 50, 500, or 5,000 mg/kg **sodium benzoate** showed no significant increase in chromosomal aberrations in the bone marrow.
(FDA PB 245453, 1974)

A dominant lethal assay using male rats given single or multiple gavage doses of 50, 500, or 5,000 mg/kg **sodium benzoate** was non-mutagenic.
(FDA PB 245453, 1974)

Remark: IPCS CICAD 26 (2000) mentioned this dominant lethal assay as a positive result, however evaluation of the raw data in the original report (by experts of the industry consortium and a recent independent review by Prof. R. Kroes) gives no support for this. In addition the authors of the study clearly conclude negative. FDA also evaluated this study as negative. In addition sodium benzoate doesn't contain a structural alert for genotoxicity.

A host mediated assay using male rats given multiple gavage doses of 50, 500, or 5,000 mg/kg **sodium benzoate** showed no elevation of mutant frequencies in *Salmonella typhimurium* G46; no elevation of mutant frequencies in *Salmonella typhimurium* TA 1530; no increase in recombinant frequencies in *Saccharomyces cerevisiae* D3.
(FDA PB 245453, 1974)

A host mediated assay using male rats given a single gavage dose of 50, 500, or 5,000 mg/kg **sodium benzoate** showed an elevation of mutant frequencies in *Salmonella typhimurium* TA 1530 in the intermediate dose level; the other doses were negative.
(FDA PB 245453, 1974)

A Mouse Micronucleus assay using 50, 100, 200 mg/kg **benzyl alcohol** by i.p. injection was negative at all doses tested.
(Hayashi et al., 1988)

A Replicative DNA Synthesis assay using male Fischer 344 rats given a single dose of 0, 300 or 600 mg/kg bw **benzyl alcohol** by gavage was negative at all doses tested.
(Uno et al., 1994) ;

A Replicative DNA Synthesis assay using male B6C3F1 male mice given a single dose of 0, 400 or 800 mg/kg bw **benzyl alcohol** by gavage was negative at all doses tested.
(Miyagawa et al., 1995)

A *Drosophila melanogaster* SRL assay with **benzylalcohol** 5000 ppm (feed) and 8000 ppm (injection) was negative (Foureman, et al., 1994)

Summary of genetic toxicity *in vivo* results:

Species (test system)	End-point	Results	Remarks
Sodium benzoate			
male Sprague Dawley rats	Cytogenetic Assay (bone marrow)	Negative	
male ICR mice	Host-Mediated Assay (tester strains Salmonella typhimurium TA 1530, G 46 and Saccharomyces cerevisiae D3)	Negative	elevated mutant frequency with T A 1530 in the intermediate single gavage dosing only (clear negative after multiple gavage dosing)
male random bred rats	Dominant Lethal Assay	Negative	
Benzyl alcohol			
male mice	Mouse Micronucleus Assay	Negative	
male Fischer 344 rats	Replicative DNA Synthesis	Negative	
male B6C3F1	Replicative DNA Synthesis	Negative	
Drosophila melanogaster	SLR assay	Negative	

In addition data from *in-vivo* genotoxicity studies on **benzyl acetate** and **benzaldehyde** (JECFA report, 1997) are supportive evidence for the non-genotoxicity of benzyl alcohol and benzoic acid and its salts.

Summary genetic toxicity *in vivo* results:

Species (test system)	End-point	Dose	Results	Remarks
Benzaldehyde				
<i>Drosophila melanogaster</i>	Sex-linked recessive lethal mutation	150 ppm (feed), 2500 ppm (injection)	Negative	Woodruff et al. (1985); US NTP (1990)
Benzyl acetate				
<i>Drosophila melanogaster</i>	Sex-linked recessive lethal mutation	300 ppm (feed), 20,000 ppm (injection)	Negative	US National Toxicology Program (1993)
Mouse bone-marrow cells	Chromosomal aberration	325-1700 mg/kg bw (i.p.)	Negative	US National Toxicology Program (1993)
Mouse bone-marrow cells	Micronucleus formation	312-1250 mg/kg bw (i.p.)	Negative	US National Toxicology Program (1993)
Mouse peripheral blood	Micronucleus formation	3130-50 000 ppm in diet	Negative	US National Toxicology Program (1993)
Mouse bone-marrow cells	Sister chromatid exchange	325-1700 mg/kg bw (i.p.)	Negative	US National Toxicology Program (1993)

In conclusion: The compounds exhibit no genotoxicity in several *in-vivo* assays evaluating different endpoints.

3.1.9 Carcinogenicity

In a 2-year carcinogenicity study, groups of 50 male and 52 female Fischer 344 rats, four to five weeks old, received diets containing 1% (500 mg/kg bw per day) or 2% (1000 mg/kg bw per day) **sodium benzoate** for 18-24 months. Controls, consisting of 25 male and 43 female rats, received basal diet. Food intake was adequately controlled to avoid an excess; tap water was available *ad libitum*. Survival was very poor in all groups, due to intercurrent sialodacryoadenitis and mycoplasma infections. All surviving animals were sacrificed between 18 and 25 months, all were autopsied, and various tissues were examined histopathologically. No adverse clinical signs directly attributable to treatment were observed, and only negligible differences in average body weight and mortality rate were seen between the treated and control groups. Although a variety of tumors occurred among treated and control rats of each sex, they were of similar type and incidence.

(Sodemoto & Enomoto, 1980)

Poor survival in all groups, due to infections, limits the usefulness of this study.

A lifelong study using male/female Swiss Albino mice given 2% **sodium benzoate** continuously in drinking water showed no carcinogenic effect.

In the main study, a 2% solution of sodium benzoate (purity, 99%) was administered in the drinking water to groups of 50 male and 50 female five-week-old mice for their lifetime. Groups of 100 males and 100 females were used as untreated controls. Both treated and control animals were 'carefully checked'; their body weights were measured weekly, and gross pathological changes were recorded. The animals were either allowed to die or were sacrificed when moribund. Complete necropsies were performed on all animals, and the liver, spleen, kidneys, bladder, thyroid, heart, pancreas, testes, ovaries, brain, nasal turbinates, at least four lobes of the lungs, and organs with

gross pathological changes were examined histologically. The average daily intake of sodium benzoate was 124.0 mg for males and 119.2 mg for females on the basis of daily water consumption of 6.2 and 5.9 ml, respectively. The dose of sodium benzoate was equivalent to 6200 mg/kg bw per day for males and 5960 mg/kg bw per day for females. Treatment had no effect on survival or the incidence of tumors.

(Toth, 1984).

This study is sufficiently reliable due to the number of animals and detailed histopathological examinations.

In a 2-year carcinogenicity study, **benzyl alcohol** was administered in corn oil by gavage to groups of 50 Fischer 344/N rats of each sex at a dose of 0, 200, or 400 mg/kg bw per day on five days a week for 103 weeks. The rats were observed twice daily, and body weights were recorded weekly for the first 12 weeks and once a month thereafter. Gross necropsy was performed on all animals and 49 tissues and organs, including brain, kidney, pancreas, and skeletal muscle, from all female rats and from male rats in the vehicle control and high-dose groups and those in the other groups that died before 22 months or which had gross lesions were examined histologically. The mean body weights of treated and control animals were comparable throughout the study. No compound-related clinical signs were observed, although a sialodacryoadenitis viral infection was widespread among the study animals in the third month. The survival of treated females was significantly lower than that of vehicle controls: 70% of controls, 34% of low-dose females, and 34% of high-dose females; this was due to a much higher incidence of accidental deaths related to the gavage process. Survival among the male rats was comparable in all groups: 56% of controls, 54% at the low dose, and 48% at the high dose.

Cataracts and retinal atrophy were observed at increased incidences in rats at the high dose. The authors attributed this effect to the proximity of this group of animals to fluorescent light for most of the study. An increased incidence of hyperplasia of the forestomach epithelium was seen (not statistically significant) in male rats: control, 0/48; low dose, 0/19; high dose, 4/50. Hemorrhage and foreign material in the respiratory tract seen in treated rats that died before the end of the study were suggested by the authors to have been the result of either direct deposition of material into the lung during gavage 'accidents' or the anaesthetic properties of benzyl alcohol resulting in reflux of gavage material and aspiration into the lungs. No pancreatic acinar-cell adenomas were reported, and no other effects of treatment were seen at gross necropsy or histopathological examination.

(US National Toxicology Program, 1989)

In a 2-year carcinogenicity study, **benzyl alcohol** (purity, 99%) was given to groups of 50 B6C3F1 mice of each sex, eight to nine weeks of age, at a dose of 0, 100, or 200 mg/kg bw per day in corn oil by gavage on five days a week for 103 weeks. The doses were selected on the basis of those found to induce neurotoxic effects (lethargy and staggering) in short-term studies. The mice were observed twice daily, and their body weights were recorded weekly for the first 12 weeks and once a month thereafter. Gross necropsy was performed on all animals, and 50 tissues and organs, including brain, liver, kidney, and stomach, from all vehicle controls, animals at the high dose, and animals at the other doses that died before 22 months or had gross lesions were examined histologically.

The mean body weights of treated and control mice were comparable throughout the study. The survival of control females was significantly lower than that of animals at the high dose after week 74, but no other differences in survival were seen: 68% of control, 66% of low-dose, and 70% of high-dose males; and 50% of control, 62% of low-dose, and 72% of high-dose females. No significant treatment-related effects were noted at gross necropsy or histopathological examination.

(US National Toxicology Program, 1989).

In conclusion: The compounds exhibit no carcinogenicity.

3.1.10 Toxicity to Reproduction

In a 4-generation study 20 rats/sex/group were dosed continuously by diet with 375 or 750 mg/kg/day **benzoic acid**. In all 4 generations, no effects on fertility (“Fortpflanzung”) and lactation (“Aufzugt der Jungen”) were found. In addition a so-called “Alters Paarung” after 48 weeks gave no influence on start of menopause.

NOAEL (Parental) $\geq$ 750 mg/kg/day

NOAEL (F1 Offspring) $\geq$ 750 mg/kg/day

NOAEL (F2 Offspring) $\geq$ 750 mg/kg/day
(Kieckebusch & Lang, 1960)

In addition data from reprotoxicty studies on benzyl acetate and benzaldehyde (JECFA report 1997) give supportive evidence for the non-reprotoxicity of benzyl alcohol and benzoic acid and its salts.

The potential reproductive toxicity of **benzyl acetate** was assessed by examining sperm morphology, vaginal cytology, and the weights of male reproductive organs at the end of the 13-week feeding study (US National Toxicology Program, 1993) in mice. Dietary levels of 3130-50 000 ppm benzyl acetate (> 3000 mg/kg bw/d) had no effect on the weights of the epididymis, cauda epididymis, or testis or on sperm motility or density or the percent of abnormal sperm. The mean length of the estrous cycle of mice at the high dose was significantly greater than that of the control group. This effect was associated with a significant decrease in body weight.
(Morrissey et al., 1988)

The potential reproductive toxicity of **benzyl acetate** was assessed by examining sperm morphology, vaginal cytology, and the weights of male reproductive organs at the end of the 13-week feeding study in rats. Dietary levels of 3130-50 000 ppm benzyl acetate (> 2000 mg/kg bw/d) had no effect on the weights of the epididymis, cauda epididymis, or testis, on sperm motility, or on the density or percent of abnormal sperm.

(US National Toxicology Program, 1993)

A single study was conducted to examine the potential reproductive toxicity of **benzaldehyde**, and the report was available as a translation from Romanian. A group of 10 rats of breeding age were given 2 mg benzaldehyde in oil (type not specified) by gavage every other day for 32 weeks, equivalent to about 5 mg/kg bw per day. Ten controls were used. Two pregnancies in each rat, one at 75 days and one at 180 days, were studied. The end-points examined included the number of pregnant females, number of offspring born, pup body weight at days 7 and 21 post partum, and pup viability.

At the end of treatment, the body weights of control and treated rats were similar: 265 g and 260 g, respectively. It was reported that fewer females in the group given **benzaldehyde** than in the control group became pregnant; however, no data or statistical analyses were presented. The authors concluded that treatment did not significantly modify any of the parameters studied. No further details were available.

The NOAEL was about 5 mg/kg bw per day.

(Spom et al., 1967)

In addition no compound related effects on reproductive organs (gross and histopathology examination) could be found in the (sub) chronic studies in rats and mice with benzyl acetate, benzyl alcohol, benzaldehyde, sodium benzoate and supports a non-reprotoxic potential of these compounds (see studies in sections on repeated dose toxicity and carcinogenicity).

In conclusion: According to IPCS CICAD 26 (2000) (only evaluating benzoic acid and sodium benzoate), no clear statement on the reproductive effects can be given on basis of the Kieckebusch & Lang (1960) and Toth (1984) studies only. However, critical evaluation of the original paper of

the Kieckebusch & Lang study gives confidence of an adequately performed study although it was performed many years ago. In addition, reprotoxicity studies on benzaldehyde and benzylacetate and the fact that no compound related effects on reproductive organs were found in the (sub)chronic studies with all the compounds supports the lack of reproductive potential. Therefore the available consistent data on compounds in this group (data on benzyl acetate and benzaldehyde inclusive) taken as a whole are sufficient to demonstrate the lack of reprotoxic potential.

3.1.11 Developmental Toxicity

Pregnant Wistar rats were treated on day 9 of gestation with one dose of 510 mg/kg **benzoic acid** in carboxymethylcellulose. Animals were sacrificed on Day 20 of gestation and the uterus observed in situ for implantation and resorption sites. Live fetuses were removed, examined for gross malformations, weighed, and prepared for histopathological examination. Treatment with benzoic acid resulted in no dead or resorbed implants and 3 % abnormal survivors, rates comparable to the control animals.

NOAEL Maternal toxicity: 510 mg/kg bw
NOAEL Teratogenicity: 510 mg/kg bw
(Kimmel et al., 1971)

A 4-generation study with female rats dosed with 375 or 750 mg/kg/day **benzoic acid** during pregnancy and lactation showed no effects on the dams or on the growth and development of the offspring.

NOAEL Maternal toxicity: $\geq$ 750 mg/kg/day
NOAEL Teratogenicity: $\geq$ 750 mg/kg/day
(Kieckebusch & Lang, 1960)

Studies on the developmental toxicity of sodium benzoate administered by gavage to multiple species (rat, mice, rabbit, hamster) were conducted by Food and Drug Research Labs, Inc. (1972):

A study using pregnant Wistar rats, dosed with 1.75, 8, 38 or 175 mg/kg **sodium benzoate** by gavage on Days 6-15 of gestation showed no effect on nidation or on maternal or fetal survival; the number of abnormalities of soft and skeletal tissues did not differ from controls.

NOAEL Maternal toxicity: 175 mg/kg bw
NOAEL Teratogenicity: 175 mg/kg bw
(FDA PB# 221777, 1972)

A study using pregnant CD-1 mice, dosed with 1.75, 8, 38 or 175 mg/kg **sodium benzoate** by gavage on Days 6-15 of gestation showed no effect on nidation or on maternal or fetal survival; the number of abnormalities of soft and skeletal tissues did not differ from controls.

NOAEL Maternal toxicity: 175 mg/kg bw
NOAEL Teratogenicity: 175 mg/kg bw
(FDA PB# 221777, 1972)

A study using pregnant Dutch-belted rabbits, dosed with 2.5, 12, 54 or 250 mg/kg **sodium benzoate** by gavage on Days 6-18 of gestation showed no effect on nidation or on maternal or fetal survival; the number of abnormalities of soft and skeletal tissues did not differ from controls.

NOAEL Maternal toxicity: 250 mg/kg bw
NOAEL Teratogenicity: 250 mg/kg bw
(FDA PB# 221777, 1972)

A study using pregnant Golden hamsters, dosed with 3, 14, 65 or 300 mg/kg **sodium benzoate** by gavage on Days 6-10 of gestation showed no effect on nidation or on maternal or fetal survival; the number of abnormalities of soft and skeletal tissues did not differ from number in controls.

NOAEL Maternal toxicity: 300 mg/kg bw

NOAEL Teratogenicity: 300 mg/kg bw
(FDA PB# 221777, 1972)

A study using pregnant Wistar rats, dosed with 700, 1400, 2800, 5600 mg/kg **sodium benzoate** in the diet during the entire gestation showed no statistical difference in organ and bone abnormalities of fetuses between experimental groups and controls; growth of treated offsprings was similar to controls in rats dosed with 1400 mg/kg/day; reduced food intake and decreased body weight of the pregnant rats especially in the 5600 mg/kg group; 100% perinatal death rate; organ abnormalities of fetuses involved eye, brain and kidneys, in addition abnormalities of the skeletal system were found in rats dosed with ≥ 2800 mg/kg/day. The authors concluded that the effects on the dams and fetuses at the 2800 and 5600 levels were due to reduced maternal feed intake in these groups, leading to malnutrition,

NOAEL Maternal toxicity: 1400 mg/kg bw

NOAEL Teratogenicity: 1400 mg/kg bw
(Onodera et al., 1978)

Fifty female mice were given **benzyl alcohol** at 550 mg/kg bw per day by gavage on days 6-15 of gestation; a further 50 mice received the corn oil vehicle. All dams were allowed to deliver naturally, and pups and dams were observed until day 3 post partum, when the experiment was terminated. Body weight, clinical observations, and mortality were recorded daily throughout treatment and up to day 3 post partum. Mortality was not significantly increased in animals given benzyl alcohol over that in the control group. One treated mouse showing languid behaviour, laboured breathing, and a rough coat died, but no other deaths or clinical signs were reported. Maternal body weight and body-weight gain during treatment and up to day 3 post partum were virtually identical for treated and control animals. All other parameters examined, including gestation index, average number of live pups per litter, and postnatal survival and pup body weight on days 0 and 3 post partum, were not significantly different from the control values. The authors concluded that, at the predicted LD10, benzyl alcohol had no significant effects on the development of CD-1 mice.

NOAEL = 550 mg/kg bw per day

(York et al., 1986; JECFA, 1997).

Benzyl alcohol dissolved in distilled water was administered by gavage at a dose of 750 mg/kg bw per day to 50 CD-1 mice on days 7-14 of gestation; evidence of copulation was considered the first day of gestation. A control group of 50 animals received distilled water only. All animals were allowed to deliver their litters and nurse their pups for three days, at which time necropsies were performed. Maternal body-weight gain and mortality, mating, gestation, numbers of live and dead pups per litter, total litter weight on days 1 and 2 post partum, litter weight change between days 1 and 3 post partum, and pup survival on days 1 and 3 post partum were recorded. During the treatment period, 18 deaths were reported, all of which were attributed to treatment; a further death was reported on day 15 of gestation, the day after treatment was terminated. Clinical signs of toxicity, including hunched posture, tremors, inactivity, prostration, hypothermia, ataxia, dyspnoea, swollen or cyanotic abdomen, and piloerection, were reported in up to 20 mice during treatment. Piloerection was also reported in some animals up to day 3 post partum, but no other clinical signs were seen after the period of administration. No differences were observed in the mating or gestation indices, the total number of resorptions, the mean length of gestation, or the number of live pups per litter between treated and control groups. Maternal body weight, measured on days 4 and 7 of gestation, was not significantly different from control values; however, statistically

significant reductions were reported on day 18 of gestation ($P < 0.001$) and on day 3 post partum ($P < 0.05$). Maternal body-weight gain during days 7-18 of gestation was significantly lower than that of controls ($P < 0.001$). Significant reductions in pup body weight were reported, including a lower mean pup weight per litter on days 1 ($P < 0.01$) and 3 post partum ($P < 0.001$), a mean litter weight change between day 1 and day 3 post partum ($P < 0.05$), and a mean pup weight change between days 1 and 3 post partum ($P < 0.001$). No differences in pup survival were observed by day 3 post partum. The authors concluded that benzyl alcohol may be a reproductive hazard, apparently on the basis of the reductions in pup body weights, an effect that was observed in conjunction with maternal toxicity evidenced by increased mortality, reduced body weights, and clinical toxicity during the period of administration. As effects were seen on the dams and fetuses at the only dose used in this study, there was no NOAEL.

LOAEL = 750 mg/kg bw per day

(US National Institute of Occupational Safety and Health, 1983; Hardin et al., 1987).

In a developmental toxicity study in rats, **benzyl acetate** given by gavage did not show teratogenic effects and on the basis of fetotoxic effects a NOEL of 500 mg/kg/day could be established.

(Ishiguro et al., 1993)

Many of these studies were done by gavage (leading to greater toxicity due to the “bolus effect”). In these studies NOEL of ≥ 500 mg/kg were found.

Thus, studies on reproductive and/or developmental toxicology performed by the administration of the benzyl compounds by gavage are likely to reveal changes at lower doses compared to studies where the substances are applied in the diet, leading to a distribution in the body over time,

In conclusion: The compounds exhibit no developmental toxicity and a NOEL of 500 mg/kg/day can be established for developmental effects for this group of substances

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

The studies used as the basis for the following data did not always state whether effect values were based on nominal or measured concentrations. However, because of the good water solubility, their insignificant volatility and low adsorption potential, all nominal concentrations of the test substances are expected to correspond to effective concentrations even in tests with open systems and longer exposure durations.

Acute toxicity to fish

Chemical	Species	Protocol	Result	Reference
Benzoic acid	<i>Lepomis macrochirus</i>	EPA-660/3-75-009	LC ₅₀ (96 h) =44.6 mg/l LC0 = 180 mg/l (pH control)	UCES#11506-03-85, 1979
	<i>Salmo gairdneri</i>	EPA-660/3-75-009	LC ₅₀ (96 h) =47.3 mg/l	Buzzel et al 1968 UCES#11506-03-84, 1979
	<i>Leuciscus idus</i>	other	LC ₅₀ (48 h) =460 mg/l (pH 7 –8)	Juhnke & Luedemann, 1978
Sodium benzoate	<i>Pimephales promelas</i>	EPA OPP 72-1	LC ₅₀ (96 h) =484 mg/l (pH 7.4, flow-through, measured concentrations)	Geiger et al., 1985
	<i>Pimephales promelas</i>		LC50 (96 h) > 100 mg/l	Ewell et al 1986
Benzyl alcohol	<i>Pimephales promelas</i>	EPA OPP 72-1	LC ₅₀ (96 h) =460 mg/l	Mattson, V.R. et al., EPA-600 /3-76-097, PB-262897, 1976
	<i>Leuciscus idus</i>	DIN 38412 Teil 15	LC ₅₀ (48 h) =646 mg/l	Knie et al., 1983
Benzyl alcohol		Specific acute spill testing (*)	LC50 (96 h) 10 and 15 mg/l	Dawson et al 1975/1977

No data for potassium benzoate were identified, but it should be similar to sodium benzoate.

(*) REMARK: For benzylalcohol two valuable guideline studies gave acute toxicity values > 100 mg/l.

Dawson et al, however reported acute toxicity values 10 – 15 mg/l. Their static tests however were directed to simulate acute spill circumstances. The test substances were pipetted or poured undiluted directly into the aquaria with fish.

So without preparing defined concentrations according to guideline. No analytical monitoring was done. Aeration was not used during the first 24 hrs thus allowing chemicals to act in an uninterrupted state at the onset of the test period.

For environmental relevant conditions and for derivation of a PNECaqua a benzylalcohol acute toxicity (LC50 96 hrs) to fish of > 100 mg/l should therefore be used.

Acute toxicity to aquatic invertebrates

Chemical	Species	Protocol	Result	Reference
Benzoic acid	<i>Daphnia magna</i>	EPA-660/3-75-009	EC ₅₀ (48 h) => 100 mg/l (pH 8.4)	UCES#11506-03-80, 1979
		other	EC ₅₀ (24 h) = 500 mg/l (with neutralization)	Bringmann, & Kuehn, 1982
		other	EC ₅₀ is 102 mg/l (without neutralization)	Bringmann, & Kuehn, 1982
Sodium benzoate Benzyl alcohol	<i>Daphnia magna</i>	other	EC ₅₀ (48 h) => 100 mg/l	Ewell et al., 1986
	<i>Daphnia magna</i>	DIN 38412 Teil 11	EC ₅₀ (24 h) = 400 mg/l	Knie et al., 1983
	<i>Daphnia magna</i>	other	EC ₅₀ (48 h) = 360 mg/l	Bringmann & Kuehn, 1959

No data for potassium benzoate were identified, but it should be similar to sodium benzoate.

Acute toxicity to aquatic plants (algae)

Chemical	Species	Protocol	Result	Reference
Benzoic acid	<i>Scenedesmus quadricauda</i>	other	EC ₅₀ (3 h) = 75 mg/l	Stratton & Corke, 1982
	<i>Scenedesmus quadricauda</i>	cell multiplication inhibition test; static	Inhibition starts at 1630 mg/l (96 hr) (pH = 7)	Bringmann & Kuehn, 1977
	<i>Chlorella pyrenoidosa</i>	other	EC ₅₀ (3 h) = 60 mg/l	Stratton & Corke, 1982
	<i>Anabaena variabilis</i>	other	EC ₅₀ (14d) = >10 mg/l	Stratton & Corke, 1982
Sodium benzoate	Green algae	ECOSAR	EC ₅₀ (96 h) = 478 mg/l	
Benzyl alcohol	<i>Chlorella pyrenoidosa</i>	other	EC ₅₀ (3 h) = 95 mg/l	Stratton & Corke, 1982
	<i>Haematococcus pluvialis</i>	other	EC ₅₀ (4 h) = 2600 mg/l	Knie et al., 1983
	<i>Scenedesmus quadricauda</i>	cell multiplication inhibition test	Inhibition starts at 640 mg/l (96 h)	Bringmann & Kuehn 1959

Remark: The studies are no guideline studies, but despite this shortcoming they indicate a moderate to low acute toxicity. The *Scenedesmus* study of Stratton and Cork was not used because the endpoint is about the inhibition of the photosynthesis and not growth (rate). The blue green algae were left out because they are not directly used for the effect assessment for the aquatic

environment and the endpoint was inhibition of the photosynthesis and not growth (rate). No data for potassium benzoate were identified, but it should be similar to sodium benzoate.

Acute toxicity to micro-organisms (bacteria)

Chemical	Species	Protocol	Result	Reference
Benzoic acid	activated sludge	OECD 209 (respiration inhibition)	EC ₅₀ (3 h) > 1000 mg/l (pH 7.5)	Klecka et al., 1985
	<i>Photobacterium phosphoreum</i>	Static	EC ₅₀ (30 min) = 16.85 mg/l	Kaiser, 1987
	<i>Pseudomonas putida</i>	Static	Inhibition starts at 480 mg/l (16 h) (pH neutral)	Cicad 2000
Sodium benzoate	<i>Achromobacter liquefaciens</i>	other: static	EC ₅₀ (24 h) = > 3000 mg/l	Nikkilae, 1955
	<i>Micrococcus flavus</i>	other: static	EC ₅₀ (24 h) = >500 mg/l	Nikkilae, 1955
Benzyl alcohol	<i>Escherichia coli</i>	cell multiplication inhibition test	EC ₀ (48 h) = 1000 mg/l	Bringmann & Kuhn, 1959
	<i>Pseudomonas putida</i>	cell multiplication inhibition test	EC ₁₀ (16-18 h) = 658 mg/l	Knie et al., 1983

No data for potassium benzoate were identified, but it should be similar to sodium benzoate.

In conclusion:

From the data (fish, daphnia, algae, bacteria) it is obvious that neutralization of the pH greatly reduces (up to one order of magnitude) the acute toxicity of benzoic acid. This is also supported by the lower toxicity observed with the sodium benzoate. Under environmental relevant conditions therefore the acute toxicity of benzoic acid, sodium benzoate and potassium benzoate for all four trophic levels is > 100 mg/l.

Under environmental relevant conditions the acute toxicity of benzylalcohol for fish, daphnia and bacteria is > 100mg/l. For algae an acute EC 50 3hrs of 95 mg/l

Therefore it can be concluded that under environmental relevant conditions, benzoic acid and its salts have very low acute toxicity, whereas benzylalcohol has low to moderate acute toxicity

4.2 Terrestrial Effects

There were no available studies on terrestrial organisms.

IPCS CICAD 26 (2000) concluded for benzoic acid and sodium benzoate: No information on toxic effects of benzoic acid and sodium benzoate on plants, earthworms or other terrestrial organisms or on ecosystems were identified. Only antimicrobial properties were identified preventing bacterial or fungal growth. Based on these data they conclude a low toxicity potential of benzoic acid and sodium benzoate in the terrestrial environment.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Benzylalcohol, benzoic acid and its sodium and potassium salt can be considered as a single category regarding human health, as they are all rapidly metabolised and excreted via a common pathway within 24hrs.

Systemic toxic effects of similar nature (e.g liver, kidney) were observed. However, with benzoic acid and its salts at higher doses than with benzylalcohol. For environmental effects the category is less clear, however all are readily biodegradable, non-bioaccumulative and acute toxicity values are similar.

For human health all exposure routes are possible, despite benzoic acid and its salts being solids and benzylalcohol being a liquid. For workers exposure will mainly be by inhalation and by skin, whereas for consumers it will mainly be by oral and dermal route.

Human Health:

The compounds exhibit low acute toxicity as for the oral and dermal route. The LD50 values are > 2000 mg/kg bw except for benzylalcohol which needs to be considered as harmful by oral route in view of an oral LD50 of 1610 mg/kg bw. The 4 hrs inhalation exposure of benzylalcohol or benzoic acid at 4 and 12 mg/l as aerosol/dust respectively gave no mortality, showing low acute toxicity by inhalation for these compounds.

Benzoic acid and benzyl alcohol are slightly irritating to the skin, while sodium benzoate was not skin irritating. No data are available for potassium benzoate but it is also expected not to be skin irritating. Benzoic acid and benzyl alcohol are irritating to the eye and sodium benzoate was only slightly irritating to the eye. No data are available for potassium benzoate but it is expected also to be only slightly irritating to the eye.

The available studies for benzoic acid gave no indication for a sensitizing effect in animals, however occasionally very low positive reactions were recorded with humans (dermatological patients) in patch tests. The same occurs for sodium benzoate. It has been suggested that the very low positive reactions are a non-immunologic contact urticaria. Benzyl alcohol gave positive and negative results in animals. Benzyl alcohol also demonstrated a maximum incidence of sensitization of only 1% in human patch testing. Over several decades no sensitization with these compounds has been seen among workers.

For benzoic acid repeated dose oral toxicity studies give a NOAEL of 800 mg/kg/day. For the salts values > 1000 mg/kg/day are obtained. At higher doses increased mortality, reduced weight gain, liver and kidney effects were observed.

For benzyl alcohol the long-term studies indicate a NOAEL $\geq$ 400 mg/kg bw/d for rats and $\geq$ 200 mg/kg bw/d for mice. At higher doses effects on bodyweights, lesions in the brains, thymus, skeletal muscle and kidney were observed. It should be taken into account that administration in these studies was by gavage route, at which saturation of metabolic pathways is likely to occur. It can be concluded that benzoic acid and its salts exhibit very low repeated dose toxicity. Benzylalcohol exhibits low repeated dose toxicity.

All chemicals showed no mutagenic activity in *in vitro* Ames tests. Various results were obtained with other *in vitro* genotoxicity assays.

Sodium benzoate and benzyl alcohol showed no genotoxicity *in vivo*.

While some mixed and/or equivocal *in vitro* chromosomal/chromatid responses have been observed, no genotoxicity was observed in the *in vivo* cytogenetic, micronucleus, or other assays. The weight of the evidence of the *in vitro* and *in vivo* genotoxicity data indicates that these chemicals are not mutagenic or clastogenic. They also are not carcinogenic in long-term carcinogenicity studies. In addition data from *in-vivo* genotoxicity studies on benzyl acetate and benzaldehyde (JECFA report, 1997) support the non-genotoxicity of benzylalcohol and benzoic acid and its salts.

Carcinogenicity studies (2-year) with sodium benzoate and benzyl alcohol showed no evidence of carcinogenic activity.

In a 4-generation study with benzoic acid no effects on reproduction were seen (NOAEL $\geq$ 750 mg/kg). No compound related effects on reproductive organs (gross and histopathology examination) could be found in the (sub) chronic studies in rats and mice with benzyl acetate, benzyl alcohol, benzaldehyde, sodium benzoate and supports a non-reprotoxic potential of these compounds. In addition, data from reprotoxicity studies on benzyl acetate (NOAEL >2000 mg/kg bw/d; rats and mice) and benzaldehyde (tested only up to 5 mg/kg bw; rats) support the non-reprotoxicity of benzyl alcohol and benzoic acid and its salts.

In rats for sodium benzoate dosed via food during the entire gestation developmental effects occurred only in the presence of marked maternal toxicity (reduced food intake and decreased body weight) (NOAEL = 1400 mg/kg bw). For hamster (NOEL : 300 mg/kg bw), rabbit (NOEL :250 mg/kg bw) and mice (CD-1 mice, NOEL : 175 mg/kg bw) no higher doses (all by gavage) were tested and no maternal toxicity was observed. For benzyl alcohol: NOAEL= 550 mg/kg bw (gavage; CD-1 mice). LOAEL = 750 mg/kg bw (gavage mice). In this study maternal toxicity was observed e.g. increased mortality, reduced body weight and clinical toxicology. Benzyl acetate: NOEL = 500 mg/kg bw (gavage rats). No maternal toxicity was observed.

Environment:

From the data (fish, daphnia, algae, bacteria) it is obvious that neutralization of the pH greatly reduces (up to one order of magnitude) the acute toxicity of benzoic acid. This is also supported by the lower toxicity observed with sodium benzoate. Under environmental relevant conditions therefore the acute toxicity of benzoic acid, sodium benzoate and potassium benzoate for all four trophic levels is > 100 mg/l.

Under environmental relevant conditions the acute toxicity of benzylalcohol for fish, daphnia and bacteria is > 100mg/l. For algae an acute EC 50 3hrs of 95 mg/l is reported.

Therefore it can be concluded that under environmental relevant conditions benzoic acid and its salts have very low acute toxicity, whereas benzylalcohol has low to moderate acute toxicity.

Exposure:

Worldwide production capacity of benzoic acid is estimated at 700 kt per year. The major outlet (75%) for benzoic acid is as a chemical intermediate in the production of phenol, which in turn is mainly used to produce caprolactam. The next largest outlet is as a feedstock for sodium benzoate (10%) and chemical synthesis of plasticizers (5%).

Worldwide production capacity of sodium benzoate is estimated at 100 kt per year. The major outlet for sodium benzoate is as preservative in food and beverages (60%). Second most important market is cooling liquids (10%). The main function of sodium benzoate in most applications is as preservative.

Worldwide production capacity of potassiumbenzoate is estimated at 7 kt per year. It is used as a preservative in nonalcoholic beverages.

Worldwide production capacity of benzyl alcohol is estimated at 50 kt. Major use for benzylalcohol is as curing agent in epoxy coatings (30%), where it becomes chemically bound after reaction. Other important uses include the use as a solvent in low concentrations in waterborne coatings (10%) and use in paint strippers (10%) and chemical intermediate for synthesis for benzyl esters that are used in the flavor and fragrance industry (10%). The use in paint strippers is limited to uses in industrial settings.

Benzylalcohol, benzoic acid and its sodium and potassium salt are also used in pharmaceuticals, cosmetics and/or food. Consumer exposure in these specific applications are controlled by the fact that for all these applications specific regulatory frameworks (regional and/or national) with authorization/approval procedures and specific advisory bodies exist (among others US FDA, WHO JECFA, EU SCF, etc), with on regular basis reevaluation of approvals, hazardous properties and factual exposures inclusive. According to information from products registers uses that are not specifically regulated includes uses of the substances in different kinds of products e.g. paints, varnishes solvents, cleaning and washing agents, photochemicals and antifreeze agents.

Benzoic acid is a white solid, with solubility in water of 2.9 g/l and with a vapor pressure of 0.0011 hPa at 20 °C. The octanol/water partition coefficient was measured to 1.88; the Henry's law constant = 0.0046-0.022 Pa·m³/mol; and the pKa = 4.2.

Sodium benzoate and potassium benzoate are white solids, with solubility in water of 556 g/l and with a vapor pressure of <0.0011 hPa at 20 °C. The octanol/water partition coefficient were measured to -2.269.

Benzyl alcohol is a colorless liquid, with solubility in water of 40 g/l and with a vapor pressure of 0.13 hPa at 20 °C. The octanol/water partition coefficient was measured to 1.1.

The distribution modeling according to Mackay Level III indicates soil and water to be the favored compartments for the chemicals. None are expected to hydrolyze. All are classified as readily biodegradable. None has bioaccumulative potential.

5.2 Recommendations

Several of the toxicological studies on benzyl alcohol and benzoic acid and its salts were carried out some years ago and do not always fulfill for 100% present-day guidelines. However, well-known research groups and/or test laboratories ran the studies according to scientific standards and or accepted protocols at that time. They did appear to be acceptable studies for evaluation. Also, all were peer-reviewed and published in high quality scientific literature. Most of them have been reviewed and accepted by other fora like FDA, JECFA, and IPCS as acceptable studies. In addition, there is good consistency in the individual data for a substance in the group as well as between members of the group (benzyl acetate and benzaldehyde data inclusive). Therefore, taken as a whole, the available studies give a robust database for hazard assessment and hazard evaluation of these compounds and further studies are not indicated. The JECFA Committee (1997) concluded that the data reviewed for compounds in this group were sufficient to demonstrate lack of teratogenic, reproductive or carcinogenic potential. Consequently, the Committee concluded that further studies were not required.

Taking into account the rapid biodegradability, the low bioaccumulation potential, the low to moderate toxicity to most aquatic species, and the rapid metabolism of these substances, these substances will pose a minimal risk to the aquatic environment.

Taking into account the rapid metabolism and excretion, the non-bioaccumulation, the low toxicity after acute and repeated exposures, the non-reprotoxicity, the non-genotoxicity and the non-carcinogenicity, the low irritating and non- to very low sensitizing properties of these substances, as well as the controlled (industrial settings) and /or regulated (pharma, cosmetics and /or food) uses, these substances will pose a minimal risk to humans (workers and consumers).

Therefore these substances have low priority for further work.

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I U C L I D D a t a S e t

Existing Chemical	ID: 65-85-0
CAS No.	65-85-0
EINECS Name	benzoic acid
EC No.	200-618-2
TSCA Name	Benzoic acid
Molecular Formula	C7H6O2

Producer Related Part
Company: Bayer Corporation
Creation date: 21-OCT-1999

Substance Related Part

Company:	Bayer Corporation
Creation date:	21-OCT-1999

Memo: Bayer Corporation

Printing date: 14-FEB-2002
Revision date:
Date of last Update: 14-FEB-2002

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Chapter (profile):      Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile): Reliability: without reliability, 1, 2,
                        3, 4
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Flags (profile):
  Flags:      without flag, confidential, non
              confidential, WGK(DE), TA-Luft (DE),
              Material Safety Dataset, Risk Assessment,
              Directive 67/548/EEC, SIDS
```

1. GENERAL INFORMATION

DATE: 14-FEB.-2002
SUBSTANCES ID: 65-85-0**1.0.1 Applicant and Company Information**

Type: lead organisation
Name: American Chemistry Council (formerly Chemical
Manufacturers Association, HPV Benzoates Panel
Street: 1300 Wilson Boulevard
Town: 22209 Arlington, VA
Country: United States
14-AUG-2001

Type: cooperating company
Name: ATOFINA Chemicals, Inc.
Country: United States
14AUG-2001

Type: cooperating company
Name: Bayer Corporation
Country: United States
14-AUG-2001

Type: cooperating company
Name: DSM Special Products
Country: Netherlands
13-DEC-2000

Type: cooperating company
Name: Noveon, Inc.
Country: United States
14-AUG-2001

Type: cooperating company
Name: Velsicol Chemical Corporation
Country: United States

21-MAY-2001

Type: lead organisation
Name: American Chemistry Council, Benzoates Panel
16-JAN-2001

1.0.2 Location of Production Site, Importer or Formulator**1.0.3 Identity of Recipients****1.0.4 Details on Category/Template**

1.1.0 Substance Identification**1.1.1 General Substance Information****1.1.2 Spectra****1.2 Synonyms and Tradenames****1.3 Impurities****1.4 Additives****1.5 Total Quantity****1.6.1 Labelling****1.6.2 Classification****1.6.3 Packaging****1.7 Use Pattern****1.7.1 Detailed Use Pattern****1.7.2 Methods of Manufacture****1.8 Regulatory Measures****1.8.1 Occupational Exposure Limit Values****1.8.2 Acceptable Residues Levels****1.8.3 Water Pollution****1.8.4 Major Accident Hazards****1.8.5 Air Pollution****1.8.6 Listings e.g. Chemical Inventories**

1.9.1 Degradation/Transformation Products**1.9.2 Components****1.10 Source of Exposure****1.11 Additional Remarks****1.12 Last Literature Search**

Type of Search: Internal and External
Date of Search: 07-SEP-1999

Remark: Only HPV endpoints: TOXLINE data base and
internal studies.

14-AUG-2001

1.13 Reviews

2.1 Melting Point

Value: = 122.4 degree C

Method: other: measured

Test substance: other TS: benzoic acid; purity not noted

Reliability: (2) valid with restrictions

Data from Handbook or collection of data

Flag: Critical study for SIDS endpoint

14-AUG-2001

(1) (2)

Value: = 122 degree C

15-JAN-2001

(3)

Value: = 121.7 degree C

15-JAN-2001

(4)

2.2 Boiling Point

Value: = 249.2 degree C at 1013 hPa

Method: other: measured

Test substance: other TS: benzoic acid; purity not noted

Reliability: (2) valid with restrictions

Data from Handbook or collection of data

Flag: Critical study for SIDS endpoint

14-AUG-2001

(1) (5)

Value: = 250 degree C at 1013 hPa

Reliability: (2) valid with restrictions

15-JAN-2001

(2)

Value: = 249 degree C at 1013 hPa

15-JAN-2001

(4)

2.3 Density

Type: density

Value: = 1.2659 at 15 degree C

Method: other:

Reliability: (2) valid with restrictions
Data from Handbook or collection of data
Flag: Critical study for SIDS endpoint
14-AUG-2001 (1)

Type: density
Value: = 1.321 g/cm³ at 20 degree C
15-JAN-2001 (6)

2.3.1 Granulometry

2.4 Vapour Pressure

Value: = .0011 hPa at 20 degree C
Method: other (measured): Handbook Value
Reliability: (2) valid with restrictions
Data from Handbook or collection of data
Flag: Critical study for SIDS endpoint
14-AUG-2001 (7)

Value: = .0053 hPa at 20 degree C
Flag: Critical study for SIDS endpoint
15-JAN-2001 (8)

2.5 Partition Coefficient

log Pow: = 1.88
Method: other (measured): centrifugal distribution chromatography
Year: 1988
Reliability: (2) valid with restrictions
Meets generally accepted scientific method and is described in sufficient detail
Flag: Critical study for SIDS endpoint
14-AUG-2001 (9)

log Pow: = 1.9
Method: other (calculated): CLOGP-3.63 (1991)
Year: 1991

Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
26-JAN-2001 (10)

log Pow: = 1.93

Method: other (measured): gemessen, Schuettelmethode,
spektralphotometrischeKonzentrationsbestimmung
15-JAN-2001 (11)
log Pow: 1.81 - 1.88

Method: other (measured): gemessen, Schuettelmethode,
spektralphotometrischeKonzentrationsbestimmung
14-AUG-2001 (12)

2.6.1 Solubility in different media

Solubility in: Water
Value: = 2.931 g/l at 20 degree C

Method: other: similar to OECD Guideline 105
Test substance: other TS: Research grade benzoic acid (Merck)

Method: According to Pal, A., Maity, S.K., & Lahiri,
S.C. J. Indian Chem. Soc. (1983) 60:475.

Remark: pH-Value: no data
Result: 2.45 g/l at 15 degree C (0.0210 mol/l at 288K)
2.93 g/l at 20 degree C (0.0240 mol/l at 293K)
3.47 g/l at 25 degree C (0.0284 mol/l at 298K)

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
Well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
14-FEB-2002 (13)

Solubility in: Water
Value: = 2.91 g/l at 20 degree C

Remark: pH-value: no data
14-FEB-2002 (14)

2.6.2 Surface Tension

2.7 Flash Point

Value: = 121 degree C

Method: other: no data

Remark: nicht angegeben

15-JAN-2001

(2)

2.8 Auto Flammability

Value: = 574 degree C at 1013 hPa

Method: other

Year: 1990

GLP: no

15-JAN-2001

(15)

2.9 Flammability

Remark: Not applicable.

14-AUG-2001

2.10 Explosive Properties

Remark: Dust explosions possible. LEL 0.95 % and UEL
8.2 %

14-AUG-2001

2.11 Oxidizing Properties

Remark: Not applicable.

14-AUG-2001

2.12 Dissociation Constant

2.13 Viscosity

2.14 Additional Remarks

Remark: Henry-constant (Pa * m3/mol):
0.0046 - 0.022 (calculated as quotient of
vapour pressure and water solubility at 20
degree C)

Flag: Critical study for SIDS endpoint

14-AUG-2001

Remark:	Dissociation-constant (25 degree C): pKa = 4.1951	
Flag:	Critical study for SIDS endpoint	
14-AUG-2001		(16)
Remark:	Dissociation-constant (20 degree C): pKa = 4.21	
Flag:	Critical study for SIDS endpoint	
14-AUG-2001		(17)
Remark:	Dissociation-constant pKa (25 degree C): 3.99-4.205 (various methods; summarized values)	
14-AUG-2001		(18)
Remark:	Begin of sublimation at ca. 100 degree C. At ca. 150 degree C formation of anhydride, at ca. 370 degree C decarboxylation. Volatile with steam.	
14-AUG-2001		(2)
Remark:	pH-value: 3,1 at 1 g/l water(roomtemperature)	
14-AUG-2001		(19)
Remark:	pH-value: 2.8(saturated solution, 25 degree C)	
14-AUG-2001		(2)

3.1.1 Photodegradation

Type: other:mineralization in aqueous TiO₂
Light source: other:20W NEC blacklight blue fluorescent tube
Light spect.: ≤ 350 nm
Conc. of subst.: 50 mg/l at 40 degree C

INDIRECT PHOTOLYSIS

Sensitizer: other: aqueous TiO₂
Conc. of sens.: 40 mg/l
Degradation: 90 % after 140 minute(s)

Method: other(measured):mineralization in aqueous TiO₂
Year: 1990
GLP: no data
Test substance: other TS: benzoic acid, purity not noted

Remark: Photochemical dissociation of benzoic acid by
Irradiation with UV light if fixed on solid
carriers:-90 % mineralization in aqueous
TiO₂- suspension after 2-3 h of irradiation
with sunlight on 1 m² water
surface(concentration 50 mg/l related to test
substance)
This endpoint has been studied several times
by several other investigators/groups and all
support the result of the study mentioned
above.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
Well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
14-FEB-2002 (20)

Type: other: calculated
Light source: Sun light
Conc. of subst.: at 25 degree C
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1560000 molecule/cm³
Rate constant: ca. .0000000000001242 cm³/(molecule * sec)
Degradation: 50 % after 8.6 day(s)

Method: other (calculated): AOPWin version 1.89
Year: 1999
Test substance: other TS: molecular structure

- Reliability: (2) valid with restrictions
Accepted calculation method
- Flag: Critical study for SIDS endpoint
14-AUG-2001 (21)
- Remark: UV-Spectrum lambda max (nm):
227.5 (Methanol; lg epsilon: 4.27)
222 (Methanol/KOH; lg epsilon: 4.07)
15-JAN-2001 (22)
- Remark: photochemical dissociation of benzoic acid by
UV-irradiation if fixed on solid carriers
(SiO₂): -10.2 % mineralization after 17 h
irradiation with light(lambda > 290 nm)(no data
concerning concentration)
15-JAN-2001 (23)
- Remark: photochemical dissociation of benzoic acid by
Irradiation with UV light if fixed on solid
carriers:- 67 % mineralization in aqueous ZnO-
suspension after 24 h of irradiation with
sunlight (concentration 100-200 mg/l
related to DOC)
15-JAN-2001 (24)
- Remark: Formation of a small amount of photochemical
aerosols after irradiation of some cristalls
of benzoic acid with a deuterium lamp (180 <
lamda < 400 nm) in a laboratory reactor.
15-JAN-2001 (25)

3.1.2 Stability in Water

- Result: Based on structure and organic chemistry rules
(e.g. bonding in organic molecules, activation
energy, reactivity, transformations, addition,
substitution, elimination) no hydrolysis will
occur at pH ranges 4 - 11.
26-JAN-2001

3.1.3 Stability in Soil

- Remark: Not available.
14-AUG-2001

3.2.1 Monitoring Data (Environment)

Remark: Not available.
14-AUG-2001

3.2.2 Field Studies**3.3.1 Transport between Environmental Compartments**

Type: adsorption
Media: water - soil
Method: other: see below

Method: 14C-labeled benzoic acid (767MBq mmol⁻¹) of radiochemical purity greater than 98.5% was prepared in 0.01 M calcium nitrate in concentrations of 0.01, 0.1, 1.0, 10 mg/l. The solutions were added to three types of autoclaved, dry soils (2 g) and allowed to equilibrate on a mechanical shaker for 72 hrs at 6C.
The soil types were sandy till, clayey till, and melt water sand.
The suspension was allowed to settle and the supernatant liquid tested for 14C activity.
Adsorption constants were determined.

Result: No adsorption was observed for benzoic acid in melt water sand and clayey till; very low adsorption was observed in sandy till(K=0.23).

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-AUG-2001 (26)

Type: fugacity model level III
Media: other: air - water - soil - sediment

Method: other: EPIWin Modeling Program

Remark: Modeling was performed using equal releases (10,000 kg/hr) and equal distribution to all compartments.

3. ENVIRONMENTAL FATE AND PATHWAYS

DATE: 14-FEB-2002

SUBSTANCES ID: 65-85-0

Result:	Distribution (percent)	Half-Life (hr)	Emissions (kg/hr)	Fugacity (atm)
Air	0.911	207	1000	2.3 e-011
Water	34.8	360	1000	6.11e-013
Soil	64.2	360	1000	1.22e-011
Sediment	0.093	1.44e+003	0	4.73e-013

Persistence Time: 421 hr

Reaction Time: 516 hr

Advection Time: 2.28e+003 hr

Percent Reacted: 81.5

Percent Advected

Reliability: (2) valid with restrictions
Accepted calculation method

Flag: Critical study for SIDS endpoint

14-FEB-2002

(21)

3.3.2 Distribution**3.4 Mode of Degradation in Actual Use**

Remark: Benzoic acid is readily biodegradable, and in production and use in chemical industry it is biodegraded in a waste water treatment plant. In many species, benzoic acid is rapidly absorbed, conjugated with glycine and excreted as hippuric acid.

23-OCT-1995

3.5 Biodegradation

Type: aerobic

Inoculum: activated sludge, industrial, non-adapted

Concentration: 1000 mg/l related to COD (Chemical Oxygen Demand)

508 mg/l related to Test substance

Degradation: > 90 % after 2 day(s)

Method: OECD Guide-line 302 B "Inherent biodegradability: Modified Zahn-Wellens Test"

Year: 1981

GLP: no data

Test substance: other TS: reagent grade benzoic acid

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint
14-FEB-2002 (27)

Type: anaerobic
Inoculum: anaerobic sludge
Concentration: 73 mg/l related to Test substance
Contact time: 28 day(s)
Degradation: 96 - 100 % after 7 day(s)

Method: other: see below
GLP: no data
Test substance: other TS: commercial grade benzoic acid,
purity > 95%

Method: A 10% anaerobic sludge inoculum was transferred to 160 ml serum bottles previously amended with 50 ppm Carbon (related to test substance) using strict anaerobic techniques. Methane production from test bottles vs. controls monitored weekly for 4 weeks or until net production occurred. At that time, the bottles were amended again with the same substrate and methane production monitored to confirm the observation. All data were obtained from duplicate bottles. Methane was measured using a flame ionization detector on a Perkin-Elmer Model 900 GC equipped with a 3-m Tenax-G.C. column

Remark: 96 % mineralisation (CH₄-Production) in 1 week with sludge from Jackson, MI waste-treatment plant 100 % mineralisation (CH₄-Production) in 2 weeks with sludge from Adrian, MI waste-treatment plan

Test condition: The test bottles were incubated at 35 degree C in the dark. Substrates were kept under an atmosphere of 90% N₂ and 10% H₂

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-AUG-2001 (28)

Type: anaerobic

Concentration: 50 µg/l related to DOC (Dissolved Organic Carbon)

Contact time: 2 month

Degradation: > 75 % after 2 month

Method: other: see below

GLP: no data

Test substance: other TS: benzoic acid, purity not noted

Method: Sludge samples collected from primary and Secondary anaerobic digesters were diluted to 10% and incubated anaerobically with 50 ug Carbon per ml (related to test substance). All compounds were tested in triplicate. Gas production was measured by gas chromatography and by a pressure transducer. Biodegradation was determined by net increase in gas pressure in bottles amended with test chemicals over non-amended controls.

Result: Degradation is expressed as percentage of Theoretical Methane production based on the stoichiometry of degradation.

Test condition: The test bottles were incubated at 35 degree C in the dark.
Substrates were kept under atmospheres of 10% CO₂ and 90% N₂.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, Well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint

14-AUG-2001 (29)

Type: aerobic

Inoculum: activated sludge, industrial

Degradation: 86.9 % after 5 day(s)

Test substance: other TS: benzoic acid-1-14C (0.026mC/mg) obtained from NewEngland Nuclear Corporation, Boston, Massachusetts.

Method: Radio-respirometric study using radio-labeled chemicals by activated sludge and in a complex photographic processing effluent using acclimated industrial sludge.
Concentration of test substance was 0.1 or 0.2ml of radioactive substrate(27,000-400,000 dpm). Samples were incubated in the dark at ambient temperature.

Result: 14C02 recovery without effluent = 68.2% after
5 days 14C02 recovery in presence of effluent
= 86.9% after 5 days

30-JAN-2001 (30)

Inoculum: activated sludge, domestic
Concentration: 10 mg/l related to Test substance
Degradation: 74 % after 5 day(s)

Method: other: BOD test; 20 degree C; pH 7.0; minimal
medium

Remark: Degradation after 20 d: 78 %
t 1/2 for TOC: 1 d
BOD: 2 d
no lag phase

14-FEB-2002 (31)

Inoculum: activated sludge, non-adapted
Concentration: 100 mg/l related to Test substance

Method: other: Respirometer, 20 degree C; pH 7

Remark: Degradation after 65-80 h: 61-69 %; 5-20 h lag
phase

14-FEB-2002 (32)

Inoculum: activated sludge, domestic
Concentration: 500 mg/l related to Test substance
Degradation: after 6 day(s)

Method: other: Warburg-Respirometer, 20 degree C

Remark: Measured O₂-consumption (graphically
determined; considering endogenous
respiration): ca. 525-750 mg/l
= ca. 1050-1500 mg O₂/g substance (ThOD 1967
mg O₂/g substance)

15-JAN-2001 (33)

Inoculum: activated sludge, non-adapted
Concentration: 500 mg/l related to Test substance

Method: other: Warburg-Respirometer; 20 degree C

Remark: Measured O₂-consumption (graphically determined;

	Considering endogenous respiration): after 1 d ca. 410 mg/l = ca. 820 mg O ₂ /g substance (ThOD 1967 mg O ₂ /g substance). Benzoic acid had an initial toxic effect on two of three samples of activated sludge from different communal purification plants, after 24 hours degradation started in these samples, too.
15 - JAN - 2001	(34)
Inoculum:	activated sludge, adapted
Concentration:	200 mg/l related to COD (Chemical Oxygen Demand)
Degradation:	99 % after 5 day(s)
Method:	other: aerobic degradation, 20 degree C
Remark:	Concentration related to 101.7 mg substance/l 20 days adaption, degradation 88.5 mg COD/g.h
14 - FEB - 2002	(35)
Inoculum:	activated sludge, domestic
Concentration:	16 mg/l related to Test substance
Degradation:	100 % after 1 day(s)
Method:	other: aerobic degradation, static, 30 degree C; pH 7.3
Remark:	Substance specific analysis
14 - FEB - 2002	(36)
Inoculum:	activated sludge, domestic
Concentration:	.059 mg/l related to Test substance
Degradation:	99.5 % after 7 day(s)
Method:	other: aerobic degradation; 29 degree C; measurement of radioactivity(C ¹⁴ labelled at the carboxygroup)(CO ₂ -formation)
Remark:	Test with trace concentrations
15 - JAN - 2001	(37)
Inoculum:	activated sludge, industrial
Concentration:	150 mg/l related to Test substance
Degradation:	86 % after 1 day(s)

Method: other: aerobic degradation; semi-continuous;
25-30 degree C; pH 7; parameter: TOC

Remark: 1 day acclimation
15-JAN-2001 (38)

Inoculum: activated sludge, domestic, adapted
Concentration: 1000 mg/l related to COD (Chemical Oxygen
Demand)

Degradation: 97 % after .2 day(s)

Method: other: aerobic degradation; static; test
temperature 30 degree C ; pH 7.2

Remark: Concentration equivalent to 508 mg substance/l
20 days adaptation with glucose as additional
C-source

14-FEB-2002 (39)

Inoculum: other bacteria: obligatory anaerobic species
from sludge of the first purification step

Concentration: 300 mg/l related to Test substance

Degradation: 91 % after 18 day(s)

Method: other: anaerobic degradation, enrichment
culture; 35 degree C; parameter: gas
production

Remark: 8 days lag phase
Degradation after 18 d: 91 +- 7.8 %

14-FEB-2002 (40)

Inoculum: other bacteria: anaerobic sludge, domestic

Concentration: 50 mg/l related to Test substance

Degradation: after 21 day(s)

Method: other: anaerobic degradation, static, 35
degree C, adding of test substance in solid
form; parameter: gas production

Remark: Degradation: 110.5 %

14-FEB-2002 (41)

Inoculum: other bacteria: anaerobic sludge, domestic,
washed

Concentration: 50 mg/l related to Test substance

Degradation: 89.5 % after 35 day(s)

Method: other: anaerobic degradation, static, 35 degree C, adding of test substance in solid form; parameter: gas production

15 - JAN - 2001 (41)

Inoculum: other bacteria: anaerobic laboratory sludge, adapted

Concentration: 24 mg/l related to Test substance

Degradation: 86 - 93 % after 23 day(s)

Method: other: anaerobic degradation, static, parameter: gas production, 37 degree C

15 - JAN - 2001 (42)

Inoculum: other bacteria: activated sludge, domestic/industrial sewage

Concentration: .8 mg/l related to Test substance

Degradation: > 71.5 % after 5 day(s)

Method: other: closed bottle-test

15 - JAN - 2001 (19)

Inoculum: activated sludge, domestic

Concentration: 700 mg/l related to Test substance

Degradation: 76 % after 5 day(s)

Method: other: respirometric determination of BOD; 20 degree C

15 - JAN - 2001 (43)

3.6 BOD5, COD or BOD5/COD Ratio

Method:

Year:

Method:

Remark: BOD5/COD ratio is 0.72, indicating readily biodegradation.

14 - AUG - 2001 (15)

3.7 Bioaccumulation

BCF: 3.16

Method: other: BCF Program (v2.13)
Year: 1999
Test substance: other TS: molecular structure

Result: Estimated Log BCF = 0.500 (BCF = 3.162)
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
14-AUG-2001 (21)

Remark: Based on the log P and the fact that many
species absorb benzoic acid rapidly and
rapidly metabolize it to hippuric acid that is
excreted in urine, no bioaccumulation is
indicated.
15-JAN-2001

3.8 Additional Remarks

Remark: Soil sorption coefficient Kd at 50 ug/l
Loamy sand : 0.4 m depth: 1.92
Sand : 18.9 m depth: 0.62
23-OCT-1995 (44)

Remark: Biomagnification factors (modell ecosystem)
(0.01-0.1 ppm; radiolabelled):
Gambusia affinis (mosquito fish) 21
Daphnia magna 1772
Oedogonium cardiacum (green algae) 102
Culex quinquifasciatus (midge, larvae) 138
Physa (snail) 2786
Duration of test: 48 h
Fishes were added after 24 h; no
differentiation between bioaccumulation
and magnification.
There is no evidence whether a plateau was
achieved; the depuration rate is unknown.
23-OCT-1995 (45)

Remark: Bioconcentration factor:
Selenastrum capricornutum (green algae) 7.6
23-OCT-1995 (46)

Remark: Bioconcentration factors:
Leuciscus idus (golden orfe) < 10 (fresh weight) (3 d)
Chlorella fusca (green algae) < 10 (fresh weight) (1 d)
activated sludge 1300 (dry weight) (5 d)
There is no evidence whether a plateau was achieved;
the depuration rate is unknown.
23-OCT-1995 (23)

Remark: Bioconcentration factor (calculated):
Oncorhynchus mykiss (rainbow trout, muscle) 14
23-OCT-1995 (47)

Remark: Degradation in soil:
Half life in soil: 35 d
(Determination of mineralization by
radioactive labelling)
(loamy sand/sand, independent of depth 3-18 m)
23-OCT-1995 (44)

Remark: Degradation in soil:
Inoculum: soil microorganisms ("septic tank
tile fields")
Method: anaerobic degradation, static;
parameter: 14 CO₂;
20 degree C
Concentration: 1 mg/kg related to soil
Half life: 18.2 h
23-OCT-1995 (48)

Remark: Degradation in sea water:
Inoculum: sea water
Method: Determination of BOD

Concentration: 2 mg/l related to test
substance
Degradation after 5 d: 74.9 %
No further information about test conditions
14-AUG-2001 (49)

Remark: Degradation in sea water:
Inoculum: sea water (New York, USA)

14-AUG-2001	Method: aerobic degradation, static; 29 degree C; measurement of radioactivity of the 14C-labelled substance(at carboxyl group) Concentration: 0.059 mg/l related to test substance Degradation after 7 d: 98.7 % Determination with trace concentrations (50)
23-OCT-1995	Remark: Degradation in marine ecosystems: Benzoic acid can be degraded by different marine yeasts (9 of 12 tested species: <i>Saccharomyces rosei</i>, <i>S. italicus</i>, <i>S. chevaliero</i>, <i>Cryptococcus laurentii</i>, <i>C. luteolus</i>, <i>C. neoformans</i>, <i>Rhodotorulus rubra</i>, <i>R. glutinis</i>, <i>Hansenula anomala</i>). No information about test conditions. (50)
23-OCT-1995	Remark: Elimination in rainwater: Inoculum: rainwater Methode: aerobic degradation; 22 degree C Concentration: 0.001 mg/l related to test substance Degradation after 7 d: 22-40 % Degradation after 45 d: 100 % (51)
23-OCT-1995	Remark: Inoculum: Basische Parabraunerde (ueber p-Hydroxybenzoesaeure isoliertes Inokulum) Method: aerobic degradation, static, room temperature Concentration: 20 mg test substance/kg soil Degradation after 3 d: 40 % Degradation after 7 d: 44 % Degradation after 70 d: 63 % related to the release of labelled CO₂ in % applied radioactivity (labelled benzene ring) (52)
	Remark: Inoculum: soil microorganisms (loamy sand) Method: aerobic degradation, static, 30 degree C, pH = 7.3 Concentration: 16 mg/l related to test substance Degradation after 1 d: 100 % substance specific analysis

23-OCT-1995

(36)

Remark: Inoculum: soil microorganisms (sandy soil in 18.3 m depth)
Method: aerobic degradation, static, 24 degree C
Concentration: 0.05 mg/kg related to test substance
Degradation after 15 d: 40 %
Half life: 35 d (graphically determined)

14-AUG-2001

(44)

Remark: Inoculum: soil microorganisms (loam)
Method: aerobic degradation, static, 25 degree C
Concentration: 25 mg/l related to test substance
Degradation after 1 d: 100 %
The cleavage of the benzene ring was detected by UV adsorption.

14-AUG-2001

(53)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: static
Species: *Lepomis macrochirus* (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
NOEC: 10
LC50: 44.6

Method: other: Test conducted according to EPA-660/3-75-009 except that replicate concentrations were not used.

Year: 1975

GLP: no data

Test substance: other TS: technical grade benzoic acid

Remark: Higher LC50s were seen with other species.

Result: 24 hr LC50 = >56.0 mg/l; 48 hr LC50 = 46.0 mg/l; 72 hr LC50 = 46.0 mg/l

Test condition: Purified, deionized ater reconstituted to Ph of 7.49, total hardness of 44 mg/l CaCO₃, total alkalinity of 31 mg/l CaCO₃.

Reliability: (2) valid with restrictions
Guideline study with acceptable restrictions

Flag: Critical study for SIDS endpoint

14-FEB-2002 (54)

Type: static
Species: *Salmo gairdneri* (Fish, estuary, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
NOEC: 10
LC50: 47.3

Year: 1979

GLP: no data

Test substance: other TS: technical grade benzoic acid

Method: Test conducted according to EPA-660/3-75-009 except that replicate concentrations were not used.

Result: 24 hr LC50 = 47.3 mg/l; 48 hr LC50 = 47.3 mg/l; 72 hr LC50 = 47.3 mg/l

Test condition: Purified, deionized water reconstituted to pH of 7.44, total hardness of 36 mg/l CaCO₃, total alkalinity of 27 mg/l CaCO₃.
Reliability: (2) valid with restrictions Guideline study with acceptable restrictions
Flag: Critical study for SIDS endpoint
14-AUG-2001 (55)

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no data
LC0: 400
LC50: 460
LC100: 600

Method: other: Fish test acc. to Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung L15
Year: 1976
GLP: no data
Test substance: other TS: benzoic acid, purity not noted

Remark: pH 7 - 8
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
14-AUG-2001 (56)

Type: static
Species: Lepomis macrochirus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring:
LC0: 180

Method: other: aerated; 19.5-20.5 degree C; pH control
15-JAN-2001 (31)

Species: Carassius auratus (Fish, fresh water)
Unit: mg/l Analytical monitoring:
LC100: 200

Method: other: no data

Remark: exposure period: 7-96 h
15-JAN-2001 (57)

Species: Lepomis humilis (Fish, fresh water)
Exposure period: 1 hour(s)
Unit: mg/l Analytical monitoring:
LC100: 550 - 570

Method: other: no data

15-JAN-2001 (57)

4.2 Acute Toxicity to Aquatic Invertebrates

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no data
EC0: 260
EC50: 500
EC100: 1000

Method: other: Immobilization test at 20 degree C; pH 8.0

Year: 1982

GLP: no data

Test substance: other TS: benzoic acid, purity not noted

Remark: standardized culture
without neutralization EC0 : 77 mg/l
EC50 : 102 mg/l
EC100: 136 mg/l

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-AUG-2001 (58)

Type: static

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
NOEC: 100
EC50: > 100

Method: other: EPA-660/3-75-009
Year: 1979
GLP: no data
Test substance: other TS: technical grade benzoic acid
Test condition: The water was vigorously aerated and determined by analysis to have pH of 8.45, total hardness of 250 mg/l CaCO₃, total alkalinity of 141 mg/l CaCO₃.
Reliability: (2) valid with restrictions
Guideline study
Flag: Critical study for SIDS endpoint
14-FEB-2002 (59)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
EC0: 540
EC50: 1540

Method: other: Immobilization test
(neutralization); 20-22 degree C;
pH 7.6 - 7.7

Remark: wild population
06-JUN-2001 (60)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
EC50: 300

Method: other: Immobilization test acc. to Bringmann & Kuehn

15-JAN-2001 (61)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Scenedesmus quadricauda (Algae)
Endpoint: other: Inhibition of photosynthesis
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring: no data
EC50: 75

Method: other: see below

Year: 1982
GLP: no data
Test substance: other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA

Method: Photosynthesis was assayed by following the uptake of (14C)O₂ from NaH(14C)O₂. Plastic culture flasks contained 9.9ml cell suspension (containing 1.0 E+5 algal cells/ml), 0.1ml radioisotope, and 0.1ml of test chemical. The flasks were incubated for 3 hours and photosynthetic activity assayed. Five replicates of five concentrations, ranging from 0 to 100 mg/ml, were used. Per cent inhibition was calculated relative to photosynthetic activity in the controls. EC₅₀ values were determined by probit (Finney DJ. 1971. Probit Analysis, 3rd ed). Analyses for significant differences (p=0.05) were performed using Dunnett's test (Winer BJ. 1971. Stat. Prin. in Exp. Design, 2nd ed).

Test condition: 20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, Well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-FEB-2002 (62)

Species: Scenedesmus quadricauda (Algae)
Exposure period: 8 day(s)
Unit: mg/l Analytical monitoring:
TGK : 1630

Method: other: static, inhibition of cell multiplication; 27 degree C; pH 7

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, Well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-AUG-2001 (63)

Species: Scenedesmus quadricauda (Algae)
Endpoint: growth rate
Exposure period: 14 day(s)
Unit: mg/l Analytical monitoring:

EC50: > 10

Test substance: other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA

Method: Growth was assessed by measuring the absorbance of cultures with time using a Bausch and Lomb Spectronic 20 spectrophotometer.
The wavelength employed (420 nm) was determined by the method of Sorokin C. (1973. Handbook of Phycological Methods).
Sidearm flasks containing 94.9ml of medium and 0.1 ml of test chemical were inoculated with 5 ml of an active culture (containing 6.5 E+4 cyanobacterial and 1.0 E+5 algal cells per ml) and incubated for 12 - 14 days.
Five replicates of five concentrations of test chemical, ranging from 0 to 10 mg/ml, were used. Optical densities of treated cultures were determined daily and per cent inhibition was calculated relative to the controls.
Growth rates were determined by Sorokin C (1973) and EC50 values were determined by probit (Finney DJ. 1971. Probit Analysis, 3rd ed).

Test condition: 20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-AUG-2001 (62)

Species: Chlorella pyrenoidosa (Algae)
Endpoint: other: inhibition of photosynthesis
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring: no data
EC50: 60

Method: other: see below
Year: 1982
GLP: no data

Test substance: other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA

Method:	Photosynthesis was assayed by following the uptake of (14C)O₂ from NaH(14C)O₂. Plastic culture flasks contained 9.9ml cell suspension (containing 1.0 E+5 algal cells/ml), 0.1ml radioisotope, and 0.1ml of test chemical. The flasks were incubated for 3 hours and photosynthetic activity assayed. Five replicates of five concentrations, ranging from 0 to 100 mg/ml, were used. Per cent inhibition was calculated relative to photosynthetic activity in the controls. EC₅₀ values were determined by probit (Finney DJ. 1971. Probit Analysis, 3rd ed). Analyses for significant differences (p=0.05) were performed using Dunnett's test (Winer BJ. 1971. Stat. Prin. in Exp. Design, 2nd ed).		
Test condition:	20 degree C; 12 h light/dark-cycle; light intensity 7000 lux		
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, Well documented and acceptable for assessment		
Flag:	Critical study for SIDS endpoint		
14-AUG-2001			(62)
Species:	Chlorella pyrenoidosa (Algae)		
Endpoint:	growth rate		
Exposure period:	14 day(s)		
Unit:	mg/l	Analytical monitoring:	
EC ₅₀ :	> 10		
Test substance:	other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA		
Method:	Growth was assessed by measuring the absorbance of cultures with time using a Bausch and Lomb Spectronic 20 spectrophotometer. The wavelength employed(420 nm) wasdetermined by the method of Sorokin C. (1973. Handbook of Phycological Methods). Sidearm flasks containing 94.9ml of medium and 0.1 ml of test chemical were inoculated with 5 ml of an active culture (containing 6.5 E+4 cyanobacterial and 1.0 E+5 algal cells per ml) and incubated for 12 - 14 days.		

Five replicates of five concentrations of test chemical, ranging from 0 to 10 mg/ml, were used. Optical densities of treated cultures were determined daily and per cent inhibition was calculated relative to the controls. Growth rates were determined by Sorokin C (1973) and EC50 values were determined by probit (Finney DJ.1971. Probit Analysis, 3rd ed).

Test condition: 20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-AUG-2001 (62)

Species: *Anabaena variabilis* (Algae)
Endpoint: growth rate
Exposure period: 14 day(s)
Unit: mg/l Analytical monitoring: no data
EC50: > 10

Method: other: see below
GLP: no data
Test substance: other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA

Method: Growth was assessed by measuring the absorbance of cultures with time using a Bausch and Lomb Spectronic 20 spectrophotometer. The wavelength employed (420nm) was determined by the method of Sorokin C. (1973. Handbook of Phycological Methods). Sidearm flasks containing 94.9ml of medium and 0.1 ml of test chemical were inoculated with 5 ml of an active culture (containing 6.5 E+4 cyanobacterial and 1.0 E+5 algal cells per ml) and incubated for 12 - 14 days. Five replicates of five concentrations of test chemical, ranging from 0 to 10 mg/ml, were used. Optical densities of treated cultures were determined daily and per cent inhibition was calculated relative to the controls.

Growth rates were determined by Sorokin C (1973) and EC50 values were determined by probit (Finney DJ. 1971. Probit Analysis, 3rd ed).

Test condition: 20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
Well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-AUG-2001 (62)

Species: Anabaena cylindrica (Algae)
Endpoint: other: inhibition of photosynthesis
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring:
EC50: 60

Test substance: other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA

Method: Photosynthesis was assayed by following the uptake of (14C)O2 from NaH(14C)O2. Plastic culture flasks contained 9.9ml cell suspension (containing 1.0 E+5 algal cells/ml), 0.1ml radioisotope, and 0.1ml of test chemical. The flasks were incubated for 3 hours and photosynthetic activity assayed. Five replicates of five concentrations, ranging from 0 to 100 mg/ml, were used. Per cent inhibition was calculated relative to photosynthetic activity in the controls. EC50 values were determined by probit (Finney DJ. 1971. Probit Analysis, 3rd ed). Analyses for significant differences (p=0.05) were performed using Dunnett's test (Winer BJ. 1971. Stat. Prin. in Exp. Design, 2nd ed).

Test condition: 20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Reliability: (2) valid with restrictions
23-MAY-2001 (62)

Species: Microcystis aeruginosa (Algae, blue, cyanobacteria)
Exposure period: 8 day(s)

Unit: mg/l Analytical monitoring:
TGK : 55

Method: other: inhibition of cell multiplication at 27 degree C; pH 7

15-JAN-2001 (64)

Species: Anabaena inaequalis (Algae)
Endpoint: growth rate
Exposure period: 14 day(s)
Unit: mg/l Analytical monitoring:
EC50: 9

Test substance: other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA

Method: Growth was assessed by measuring the absorbance of cultures with time using a Bausch and Lomb Spectronic 20 spectrophotometer. The wavelength employed (600 nm) was determined by the method of Sorokin C. (1973. Handbook of Phycological Methods). Sidearm flasks containing 94.9ml of medium and 0.1 ml of test chemical were inoculated with 5 ml of an active culture (containing 6.5 E+4 cyanobacterial and 1.0 E+5 algal cells per ml) and incubated for 12 - 14 days. Five replicates of five concentrations of test chemical, ranging from 0 to 10 mg/ml, were used. Optical densities of treated cultures were determined daily and per cent inhibition was calculated relative to the controls. Growth rates were determined by Sorokin C (1973) and EC50 values were determined by probit (Finney DJ. 1971. Probit Analysis, 3rd ed).

Test condition: 20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Reliability: (2) valid with restrictions

13-DEC-2000 (62)

Species: Anabaena cylindrica (Algae)
Endpoint: growth rate
Exposure period: 14 day(s)
Unit: mg/l Analytical monitoring:

EC50: > 10

Test substance: other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA

Method: Growth was assessed by measuring the absorbance of cultures with time using a Bausch and Lomb Spectronic 20 spectrophotometer. The wavelength employed (600 nm) was determined by the method of Sorokin C. (1973. Handbook of Phycological Methods). Sidearm flasks containing 94.9ml of medium and 0.1 ml of test chemical were inoculated with 5 ml of an active culture (containing 6.5 E+4 cyanobacterial and 1.0 E+5 algal cells per ml) and incubated for 12 - 14 days. Five replicates of five concentrations of test chemical, ranging from 0 to 10 mg/ml, were used. Optical densities of treated cultures were determined daily and per cent inhibition was calculated relative to the controls. Growth rates were determined by Sorokin C (1973) and EC50 values were determined by probit (Finney DJ. 1971. Probit Analysis, 3rd ed).

Test condition: 20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Reliability: (2) valid with restrictions

06-SEP-2000 (62)

Species: Anabaena inaequalis (Algae)

Endpoint: other: Inhibition of photosynthesis

Exposure period: 3 hour(s)

Unit: mg/l Analytical monitoring:

EC50: 5

Test substance: other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA

Method: Photosynthesis was assayed by following the uptake of (14C)O2 from NaH(14C)O2. Plastic culture flasks contained 9.9ml cell suspension (containing 1.0 E+5 algal cells/ml), 0.1ml radioisotope, and 0.1ml of test chemical. The flasks were incubated for 3 hours and photosynthetic activity assayed.

Five replicates of five concentrations, ranging from 0 to 100 mg/ml, were used.
Per cent inhibition was calculated relative to photosynthetic activity in the controls. EC50 values were determined by probit (Finney DJ. 1971. Probit Analysis, 3rd ed).
Analyses for significant differences ($p=0.05$) were performed using Dunnett's test (Winer BJ. 1971. Stat. Prin. in Exp. Design, 2nd ed).

Test condition: 20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Reliability: (2) valid with restrictions
14-AUG-2001 (62)

Species: Anabaena variabilis (Algae)
Endpoint: other: Inhibition of photosynthesis
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring:
EC50: 55

Method: other: inhibition of photosynthesis;
20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Test substance: other TS: >95% pure, purchased from Aldrich Chemical Co. Milwaukee, Wisconsin, USA

Method: Photosynthesis was assayed by following the uptake of $(^{14}\text{C})\text{O}_2$ from $\text{NaH}(^{14}\text{C})\text{O}_2$. Plastic culture flasks contained 9.9ml cell suspension (containing 1.0×10^5 algal cells/ml), 0.1ml radioisotope, and 0.1ml of test chemical. The flasks were incubated for 3 hours and photosynthetic activity assayed.
Five replicates of five concentrations, ranging from 0 to 100 mg/ml, were used.
Per cent inhibition was calculated relative to photosynthetic activity in the controls. EC50 values were determined by probit (Finney DJ. 1971. Probit Analysis, 3rd ed).
Analyses for significant differences ($p=0.05$) were performed using Dunnett's test (Winer BJ. 1971. Stat. Prin. in Exp. Design, 2nd ed).

Test condition: 20 degree C; 12 h light/dark-cycle; light intensity 7000 lux

Reliability: (2) valid with restrictions

14 -AUG-2001

(62)

4.4 Toxicity to Microorganisms e.g. Bacteria

Species: activated sludge
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring:
EC50: > 1000

Method: OECD Guide-line 209 "Activated Sludge,
Respiration Inhibition Test"
Year: 1984
Test substance: other TS: benzoic acid; purity not noted

Remark: pH 7,5
Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
14 -AUG-2001 (62)

Species: Photobacterium phosphoreum (Bacteria)
Exposure period: 30 minute(s)
Unit: mg/l Analytical monitoring:
EC50: 16.85

Method: other: static at 15 degree C; Microtox-Test
Test substance: other TS: benzoic acid; purity not noted

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
14 -AUG-2001 (66)

Species: Pseudomonas putida (Bacteria)
Exposure period: 16 hour(s)
Unit: mg/l Analytical monitoring:
TGK : 480

Method: other: static; 25 degree C; pH 7
Test substance: other TS: benzoic acid; purity not noted

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
Well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint

14-AUG-2001 (63)

Species: Pseudomonas fluorescens (Bacteria)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
EC0: 1000

Method: other: Bestimmung der biologischen
Schadwirkung toxischer Abwaessergegen
Bakterien. DEV, L 8 (1968) modifiziert

14-AUG-2001 (19)

Species: other bacteria: Pseudomonas Stamm Berlin
Exposure period: 1 hour(s)

Unit: mg/l Analytical monitoring:
EC10: 50

Method: other: Oxygen consumption test acc. to Robra,
GWf-Wasser/Abwasser 117,80-86 (1976)

14-AUG-2001 (61)

Species: other bacteria: population of microorganisms
from communal sewage
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
Tlm : 500

Method: other: static, inhibition of cell
multiplication; 37 degree C; pH 6.9

14-AUG-2001 (31)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

Remark: No data available.
14-AUG-2001

4.5.2 Chronic Toxicity to Aquatic Invertebrates

Remark: No data available.
14-AUG-2001

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

4.6.2 Toxicity to Terrestrial Plants

Remark: No data available.
14-AUG-2001

4.6.3 Toxicity to Soil Dwelling Organisms

Remark: No data available.
14-AUG-2001

4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

Remark: No data available.
14-AUG-2001

4.7 Biological Effects Monitoring

Remark: No data available.
14-AUG-2001

4.8 Biotransformation and Kinetics

Remark: No data available.
14-AUG-2001

4.9 Additional Remarks

Remark: Toxicity to protozoa:
TT (Chilomonas paramecium): 48 h EC5 356 mg/l
(cell multiplication) pH 6,9
23-OCT-1995 (67)

Remark: Toxicity to protozoa:
Entosiphon sulcatum 72 h EC5: 218 mg/l
(cell multiplication)
23-OCT-1995 (68)

Remark: Toxicity to protozoa:
Uronema parduczi 20 h TT: 31 mg/l, pH 6.9
(cell multiplication)

23-OCT-1995 (69)

Remark: Toxicity to yeast: 6 w MIC (pH 3.5; 25 degree C
adapted non-adapted
-Saccharomyces cerevisiae St 1297 170 mg/l 100 mg/l
-Kluveromyces fragilis 173 125
-Kloeckera apiculata 188 125
-Hansenula anomala 223 140
-Candida crusei 440 300
-Saccharomycodes ludwigii 650 300
-Schizosaccharomyces pombe 567 325
-Zygosaccharomyces bailii 1250 600

23-OCT-1995 (70)

Remark: Toxicity to fungi:
Fusarium oxysporum:
Test concentration: 610 mg/l
Growth inhibition at
pH 4.0 : 83.5 %
pH 4.8 : 74.6 %
pH 5.6 : 57.9 %
pH 6.4 : 39.5 %
pH 7.2 : 23.7 %

23-OCT-1995 (71)

Remark: Antimicrobial effects (pH 6):
minimal microbicide minimal inhib.
Conc. (MMC) conc. (MIC)
(serial dilution
test)
-Aspergillus niger 1000 mg/l 500-1000 mg/l
-Candida albicans 1200 500-1000
-Escherichia coli 160 100-200
-Klebsiella pneumoniae 160 100-200
-Penicillium notatum 1000 500-1000
-Pseudomonas aeruginosa 160 200-500
-Pseudomonas cepacia 160
-Pseudomonas fluorescens 160 200-500
-Staphylococcus aureus 20 50-100

23-OCT-1995 (72)

5.0 Toxicokinetics, Metabolism and Distribution

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Sex: male/female
No. of Animals: 50
Vehicle: other: corn oil
Value: 2565 mg/kg bw

Method: Directive 84/449/EEC, B.1 "Acute toxicity (oral)"

GLP: no data
Test substance: other TS: technical grade benzoic acid

Method: 25 male and 25 female Spartan rats weighing 200 to 250 grams were used for this study. The test compound was suspended in corn oil and administered orally at the following dosage levels: 500, 1250, 1984, 3150, and 5000 mg/kg. Five rats of each sex were used at each dosage level. Volumes of 10 ml/kg bw were administered at all dosage levels. All rats were observed for mortality continuously during the first 4 hours after dosing, at 24 hours and once daily thereafter for a total of 14 days. Body weights were recorded initially and at 14 days.

Result: All surviving rats, males and females, exhibited normal body weight gains during the 14 day observation period. The acute oral LD50 of benzoic acid in male albino rats was calculated to be 2742 mg/kg (2279-3299 mg/kg). The acute oral LD50 of benzoic acid in female albino rats was calculated to be 2360 mg/kg (2042-2726 mg/kg). A combined acute oral LD50 for benzoic acid in male and female albino rats was calculated to be 2565 mg/kg (2292-2870 mg/kg).

LD50 calculations were done according to WR Thompson. 1947.Bact. Rev. 11:115-145.

Dose level (mg/kg)	Mortality
500	0/5
1250	0/5
1984	0/5
3150	4/5
5000	5/5

Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint

14-FEB-2002 (73)

Type: LD50
Species: mouse
Sex: male/female
No. of Animals: 60
Vehicle: other: Tween 80 (1.5%)
Value: 2250 mg/kg bw

Method: EPA OPPTS 870.1100
Year: 1979
GLP: no data
Test substance: other TS: Commercial Grade benzoic acid
(Velsicol lot #52829055)

Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint

14-FEB-2002 (74)

Type: LD50
Species: rat
Value: = 1700 mg/kg bw

26-JAN-2001 (75)

Type: LD50
Species: rat
Value: = 3040 mg/kg bw

26-JAN-2001 (4)

5. TOXICITY

DATE: 14-FEB.-2002
SUBSTANCES ID: 65-85-0

Type: LD50
Species: rat
Value: = 2530 mg/kg bw

26-JAN-2001 (76)

Type: LD50
Species: mouse
Value: = 1940 mg/kg bw

26-JAN-2001 (77)

Type: LD50
Species: mouse
Value: = 2370 mg/kg bw

26-JAN-2001 (78)

5.1.2 Acute Inhalation Toxicity

Type: LC50
Species: rat
Sex: male/female
No. of Animals: 10
Exposure time: 4 hour(s)
Value: > 12.2 mg/l

Method: EPA OTS 798.1150
Year: 1974
GLP: no data
Test substance: other TS: technical grade benzoic acid

Method: Ten rats (4 units of 2 or 3 rats/unit to prevent piling) were placed in a sealed 59.1 liter glass chamber and exposed to a dynamic atmosphere containing the dust of the test material. A Wright Dust Feeder controlled addition of The test substance; airflow regulated by a flowmeter. The rats were observed continuously during the 4-hour exposure, and for a period of 14 days following exposure.

Result: All of the rats survived the 4-hour exposure and the 14-day observation period. Signs during the exposure period included occasional increased motor activity and slight erythema. At the conclusion of exposure, 1 rat exhibited salivation.

At 24 hours and through the 14-day observation period, all rats appeared normal and exhibited normal body weight gains.
Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
14-FEB-2002 (73)

Type: LC50
Species: rat
Exposure time: 1 hour(s)
Value: > .026 mg/l

Remark: exposure to vapor
generalized inactivity, lacrimation at 0.026 mg/l/1h, no mortality
15-JAN-2001 (4)

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Sex: male/female
No. of Animals: 4
Vehicle: other: neat
Value: > 2000 mg/kg bw

Method: EPA OTS 798.1100
Year: 1974
GLP: no data
Test substance: other TS: technical grade benzoic acid

Method: The test compound was applied once only to a shaved area of the back of each rabbit at a dose of 2000 mg/kg bw.
The skin of 1 male and 1 female was abraded with a scalpel blade prior to test application.
The area was wrapped with a gauze bandage and occluded with plastic wrap.
The bandages were removed and the backs washed 24 hours after application.
The rabbits were observed for a period of 14 days.

Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
14-FEB-2002 (73)

Type: LD50

5. TOXICITY

DATE: 14-FEB.-2002
SUBSTANCES ID: 65-85-0

Species: rabbit
Value: > 10000 mg/kg bw

Remark: mortality: 0/5
15-JAN-2001 (4)

Type: LD50
Species: rabbit
Value: > 5000 mg/kg bw

Remark: mortality: no information
15-JAN-2001 (79)

5.1.4 Acute Toxicity, other Routes

Type: LD50
Species: mouse
Route of admin.: i.p.
Value: = 1460 mg/kg bw

23-MAR-2001 (80)

5.2 Corrosiveness and Irritation**5.2.1 Skin Irritation**

Species: rabbit
Concentration: undiluted
Exposure: Semiocclusive
Exposure Time: 4 hour(s)
No. of Animals: 6
PDII: 0
Result: not irritating
EC classificat.: not irritating

Method: EPA OTS 798.4470
GLP: no data
Test substance: other TS: benzoic acid, technical flakes

Remark: Primary Skin Irritation and Corrosive Hazard
(Title 49, Transportation, Chapter 1)

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint
14-AUG-2001 (73)

Species: rabbit
Concentration: undiluted
Exposure: Semiocclusive

Exposure Time:	4 hour(s)
No. of Animals:	3
PDII:	.5
Result:	slightly irritating
EC classificat.:	not irritating
Method:	Directive 84/449/EEC, B.4 "Acute toxicity (skin irritation)"
GLP:	yes
Test substance:	other TS: benzoic acid, purity not noted
Method:	The flank site of 3 albino rabbits was exposed to 0.5 g of the test substance moistened with 0.25 ml Milli-R0 water for 4 hours using semi-occlusive dressings.
Result:	The primary skin irritation index amounted to 0.5; based on these results, the test substance should be considered as minimally irritating to the skin; According to Annex VI of EEC Council Directive 67/548/EEC (amended by Directive 83/467/EEC), the test substance need not be labelled as a skin irritant.
Reliability:	(1) valid without restriction Guideline study
Flag:	Critical study for SIDS endpoint
14-AUG-2001	(81)
Species:	rabbit
Method:	other: see remarks
Remark:	irritation score: 1.66/8.00 single application of 500 mg dry powder (no further information), response scored at 24 h and 72 h
23-MAR-2001	(4)
Species:	rabbit
Concentration:	undiluted
Exposure Time:	24 hour(s)
No. of Animals:	2
Result:	not irritating
Method:	other:
Test substance:	other TS: benzoic acid, purity not noted
Method:	2 animals; application of 500 mg/animal at the inner side of the ear for 24 h
13-MAR-2001	(82)

Species: human

Method: other: see remarks

Remark: Chamber-Scarification-Test
threshold irritating concentration:
1) normal skin: 30 % in ethanol
2) scarified skin: 7.5 % in ethanol: moderate irritations;
application of 15 % in ethanol leads to marked irritation with erosions

23-MAR-2001 (83) (84)

Species: human

Remark: intermittent exposure, total dose applied: 22 mg, duration of exposure: 3 days
irritation classified as moderate

23-MAR-2001 (85)

Species: human

Method: other: see remarks

Remark: 16 mM benzoic acid (in petrolatum) produced an Erythematous reaction in 12 of 13 healthy volunteers on the cheek and in 6 subjects on the forehead, neck and upper back.
8 mM and 4 mM benzoic acid produced only a reaction on cheek.open application method

23-MAR-2001 (86)

Species: human

Method: other: see remarks

Remark: benzoic acid (in 50 % aqueous isopropanol) was applied to the medial cheek of adult volunteers; a 2 % solution led to wheals (11/11), a 0.04 % solution to erythema (11/11) and pruritus (4/11)

23-MAR-2001 (87)

Species: human

Method: other: see remarks

Remark: non-immunologic immediate contact reactions
30-45 min after application skin-test with 10
ul doses of 50, 100, 250, 500 or 1000 mM
benzoic acid in various vehicles (emollient
cream, petrolatum, 2-propyl alcohol/water-
mixture (1:1), abs. ethyl alcohol, synthetic
lanolin substitute), openly applied on the
back of 11 healthy subjects and 3 patients
with psoriasis, eczema, and rosacea resp. for
15 min

23-MAR-2001

(88)

5.2.2 Eye Irritation

Species: rabbit
Concentration: undiluted
Dose: .1 ml
Exposure Time: 1 hour(s)
Comment: rinsed after (see exposure time)
No. of Animals: 8
Result: corrosive
EC classificat.: risk of serious damage to eyes

Method: EPA OTS 798.4500
GLP: no data
Test substance: other TS: benzoic acid, technical flakes

Remark: Group I, consisting of 5 rabbits, were exposed
to the test compound for 5 minutes; 3 rabbits
in Group II were exposed to the test substance
for 24 hours.
Following the exposure period, the treated
eyes were washed with a gentle continuous
stream of water for 2 minutes.
Eye Irritation Test in Albino Rabbits (21 CFR,
Part 191)

Result: Both Group I (5 minute exposure) and Group II
(24 hrs exposure) - an extremely irritating
and corrosive substance.

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint

14-AUG-2001

(73)

Species: rabbit
Concentration: undiluted
Dose: 77 other: mg
Result: highly irritating
EC classificat.: irritating

Method: Directive 84/449/EEC, B.5 "Acute toxicity (eye irritation)"

GLP: yes

Test substance: other TS: benzoic acid, purity not noted

Remark: Based on Draize score of 35 the test substance should be classified as severely irritating according to the scheme of Kay & Calandra; according to Annex VI of EEC Council Directive 67/548/EEC (amended by Directive 83/467/EEC), the test substance should be labelled as an eye irritant. instillation of approx. 77 mg in the eye

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint

14-AUG-2001 (89)

Species: rabbit

Method: other: see remarks

Remark: irritation score: 65.0/110

23-MAR-2001 single application of 100 mg dry powder, responses scored at 24, 48 or 72 h (4)

Species: rabbit

Result: slightly irritating

Method: other: OECD Guideline 405

23-MAR-2001 (90)

Species: rabbit

Result: moderately irritating

Method: other: see remark

Remark: 2 animals; instillation of 50 mg/animal into The conjunctival sac

23-MAR-2001 (82)

5.3 Sensitization

Type: Draize Test

Species: guinea pig

Concentration 1st: Induction 500 undiluted occlusive epicutaneous

2nd:Challenge 500 undiluted occlusive epicutaneous
No. of Animals: 10
Result: not sensitizing
Classification: not sensitizing

Method: EPA OPP 81-6
Year: 1959
GLP: yes
Test substance: other TS: benzoic acid, purity not noted

Result: During induction and challenge, the grand mean for erythema and edema at 24 and 48 hours was 0. Based on this study, it was concluded that Benzoic acid is neither an irritant nor a sensitizer when applied to guinea pigs.

Reliability: (1) valid without restriction
GLP guideline study

Flag: Critical study for SIDS endpoint

14-AUG-2001

(91)

Type: Guinea pig maximization test
Species: guinea pig
Concentration 1st: Induction 10 % intracutaneous
2nd: Induction 20 % semiocclusive
3rd: Challenge 20 % semiocclusive
Result: not sensitizing
Classification: not sensitizing

Method: OECD Guide-line 406 "Skin Sensitization"
GLP: no data
Test substance: other TS: benzoic acid, purity not noted

Remark: test concentrations: intradermal injection
10 %, topical induction 20 %, challenge 20 %

Reliability: (1) valid without restriction
GLP guideline study

Flag: Critical study for SIDS endpoint

14-AUG-2001

(92)

Type: Buehler Test
Species: guinea pig
Result: not sensitizing

Test substance: other TS: benzoic acid; purity not noted

Remark: test concentrations: induction 20 %, challenge 20 %

14-AUG-2001

(92)

Type: Mouse local lymphnode assay

Species: mouse
Result: not sensitizing

Test substance: other TS: benzoic acid; purity not noted

Remark: test concentrations: 5, 10 or 20 %
14-AUG-2001 (93)

Type: Mouse ear swelling test
Species: mouse
Result: not sensitizing

Test substance: other TS: benzoic acid; purity not noted

Remark: test concentrations: induction 20 %, challenge 20 %
14-AUG-2001 (92)

Type: other: see remarks
Species: guinea pig
Result: sensitizing

Method: other: ear swelling test

Remark: groups of five guinea pigs were challenged by applying various concentrations of benzoic acid to both sides of the earlobe. The thickness of the ear was measured at various time intervals. Benzoic acid was positive (concentration-dependent effect).
14-AUG-2001 (94)

Type: other: see remarks
Species: human

Method: other: patch-test
Test substance: other TS: benzoic acid; purity not noted

Remark: 3 workers of a pharmaceutical plant with transient urticaria after exposure to sodium benzoate and 3 previously unexposed healthy control subjects were tested. All subjects reacted to benzoic acid at 0.25 % in aqueous solution under occlusion. 1 worker and 2 controls reacted to sodium benzoate at 0.5 % in saline under occlusion, but none reacted to sodium benzoate at 0.5 % in aqueous solution. All 3 workers reacted in a closed patch test to benzoic acid at 5 % in petrolatum.

The time course of the responses to benzoic acid and sodium benzoate was similar in controls and workers.
The potential of sodium benzoate to elicit Nonimmunologic contact urticaria may be due to the formation of benzoic acid at skin contact.

14-AUG-2001 (95)

Type: other: see remarks
Species: human

Method: other: patch-test

Remark: 3/5 patients with chronic urticaria developed positive skin reactions in a patch test with benzoic acid (5 % in petrolatum).

14-AUG-2001 (96)

Type: other: see remarks
Species: human

Method: other: patch-test
Test substance: other TS: benzoic acid; purity not noted

Remark: In a patch test with benzoic acid (5 % in petrolatum), 108/113 patients showed no reaction and 5/113 patients showed a 1+ reaction.
Benzoic acid was not classified as a sensitizer.

14-AUG-2001 (97)

Type: other: see remarks

Species: human

Method: other: patch-test

Remark: In a study of cosmetic intolerance with patients tested for possible contact dermatitis, 34 (0.7 %) of all patients and 1 (0.6 %) patient with pure allergy to cosmetics reacted positive.

14-AUG-2001 (98)

Type: other: see remark
Species: human

Method: other: patch-test
Test substance: other TS: benzoic acid; purity not noted

Remark: a baker developed dermatitis from flours which contained traces of benzoic acid; patch tests showed contact type eczematous hypersensitivity to benzoic acid (6 % in petrolatum).

14-AUG-2001 (99)

Type: other: see remark
Species: human

Method: other: patch-test
Test substance: other TS: benzoic acid; purity not noted

Remark: 40 children (under 12 years old) were tested for contact urticaria against food additives. 14 of them reacted positive to benzoic acid (no further information).

Reliability: (3) invalid
Documentation insufficient for assessment

14-AUG-2001 (100)

Type: other: see remarks
Species: human

Method: other: skin-prick-test

Remark: 23 out of 91 subjects suffering from chronic or recurrent urticaria were tested in a skin test: 10/23 positive subjects (at least one histamine equivalent skin test reaction) reacted to benzoic acid (5 % in petrolatum).

14-AUG-2001 (101)

Type: other: see remarks
Species: human

Method: other: oral provocation test

Remark: a chemical worker suffered from allergic reactions of increasing intensity while being constantly exposed to benzoic acid during work. After oral exposure to sodium benzoate (500 mg) he suffered a severe anaphylactic shock. He showed similar but milder reaction after consuming food containing benzoic acid.

14-AUG-2001 (102)

Type: other: see remarks
Species: human
Method: other: oral provocation test
Remark: only one out of 7 subjects with a positive skin test for benzoic acid showed a positive response (itching, wealing) after repeated oral exposure

14-AUG-2001 (101)

Type: other: see remarks
Species: human
Method: other: oral provocation test
Remark: to patients suffering from asthma benzoic acid was given orally (no details reported); approx. 50 % of the subjects showed asthmatic hypersensitivity, rhinitis and urticaria.

14-AUG-2001 (103)

Type: other: see remarks
Species: human
Method: other: patch-test
Remark: 7 patients with recurrent episodes of erythema multiforme were found to be sensitive to benzoic acid. Advice on avoidance of benzoic acid resulted in resolution of attacks in 4 patients (3 patients were not able to adhere to an exclusion diet).

14-AUG-2001 (104)

5.4 Repeated Dose Toxicity

Type: Chronic
Species: rat Sex: male/female
Strain: no data
Route of administration: oral feed
Exposure period: generation 1 and 2: lifelong,
generation 3: 16 weeks,
generation 4: until breeding
Frequency of treatment: continuously in diet
Post exposure period: no
Doses: 0.5 or 1 % in diet (approx. 375 or 750 mg/kg/day)

Control Group:	yes	
NOAEL:	750 mg/kg bw	
Year:	1960	
GLP:	no	
Test substance:	other TS: benzoic acid, purity not noted	
Method:	A robust protocol according to standards at That time was used. Taking into account the reputation of the investigators a high quality has to be assumed.	
Remark:	40 rats/group; initial body weight: 40-50 g The mean compound consumption was calculated according to Lehman, A.J., Assoc. Food Drug Off. Q. Bull. 18, 66 (1954).	
Result:	In all 4 generations no influence on growth (weight, weight gain and food efficiency (measured by protein efficiency))and organ weights was found. In all 4 generations, no effects on fertility ("Forzplanzung")and lactation ("Aufzugt der Jungen")was found. The animals of the 3rd generation were killed and examined histopathological after 16 weeks (after lactation of the pups.)No histopathological findings were found. In the paper no information is given on the organs investigated, however due to the robustness of the total study, the reputation of the investigators, as well as the reputation of the Professor who did the histopathologic investigation, a high quality has to be assumed. From other parameters it can be assumed that as a minimum the brains, heart, liver, kidney, testis and were examined. Feeding of 0.5 % led to prolongation of survival compared to controls. In addition a so-called "Alters Paarung" after 48 weeks gave no influence on start of menopause.	
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment	
Flag:	Critical study for SIDS endpoint	
14-FEB-2002		(105)
Type:	Sub-chronic	
Species:	rat	Sex: male
Strain:	no data	
Route of administration:	oral feed	
Exposure period:	28 days	

Frequency of treatment: continuously in diet

Post exposure period: no

Doses: 760, 3800 or 7600 ppm (approx. 65, 324.1 or 647.5 mg/kg/day)

Control Group: yes

NOAEL: 647.5 mg/kg bw

Method: other

GLP: no data

Test substance: other TS: benzoic acid; purity not noted

Remark: 10 rats/group; initial body weight: 120 g
mean feed consumption: 85.5; 85.3 or 85.2 g/kg/d

Result: no deaths or signs of intoxication during experiment, no significant gross pathological lesions at autopsy

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, Well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint

14-FEB-2002

(4)

Species: rabbit Sex: male/female

Strain: New Zealand white

Route of administration: dermal

Exposure period: 21 days

Frequency of treatment: 5 days/week for 3 weeks

Doses: 100, 500, 2500 mg/kg bw

Control Group: yes, concurrent vehicle

NOAEL: 2500 mg/kg bw

GLP: yes

Test substance: other TS: benzoic acid, purity not noted

Method: Four male and four female rabbits were used in each treatment group and in the control group. The skin of one-half of the animals was abraded and the others left intact. Benzoic acid was applied 5 days a week for 3 weeks at dosage levels of 100, 500, 2500 mg/kg bw. The rabbits were observed daily for signs of dermal irritation and changes in general behavior and appearance. Individual body weights were recorded weekly. Hematologic and biochemical studies were conducted once in the pretest period and again

at 21 days of the study. Gross and histopathology was performed on liver, kidneys, thyroid/parathyroid, heart, lung, ovaries, testes, adrenals as well as most gastrointestinal tract and neurological organs.

Result: Very slight dermal irritation was noted for one rabbit at the 2500 mg/kg dosage level. No compound-related effects were seen in general behavior and appearance, body weight, clinical laboratory tests, organ weights, or survival.

Reliability: (1) valid without restriction
Meets generally accepted scientific method and is described in sufficient detail

Flag: Critical study for SIDS endpoint
14-AUG-2001 (106)

Type: Sub-chronic
Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of administration: inhalation
Exposure period: 4 weeks
Frequency of treatment: 6 h/d; 5 d/w
Post exposure period: none
Doses: 0, 25, 250, 1200 mg/m³
Control Group: yes
NOAEL: 25 mg/m³
LOAEL: 250 mg/m³

Year: 1981
GLP: yes
Test substance: other TS: technical grade benzoic acid

Method: Four groups of rats (10 animals/sex/group) were exposed to a dust aerosol of benzoic acid at concentrations of 0, 25, 250, 1200 mg/m³, 6 hrs/day, 5 days/week, 4 consecutive weeks. The animals were observed twice daily, pharmacotoxic signs observed weekly, and their body weights recorded prior to exposure and weekly thereafter.

Animals found in a moribund condition were sacrificed. After 4 weeks of exposure, all surviving animals were necropsied and biochemical, hematologic, organ weights and histopathologic evaluations were conducted.

Result: No compound-related gross lesions were seen in any animal from any dose group.
Compound-related microscopic lesions, consisting of an increase of inflammatory cell infiltrate and an increase in the incidence, intensity, and extent of interstitial fibrosis in lungs of rats from all dose groups (but not dose related), were observed.
1200 mg/3: 1 animal/sex died; decreased body weight; decrease in platelets; decreased absolute and relative weights of liver (m) and trachea/lung (f); no significant difference in biochemical parameters.
>= 250 mg/m3: upper respiratory tract irritation, decreased absolute and relative weights of kidney (f).
0 - 250 mg/m3: No deaths; no effects on weight gain; no significant effects on organ weights, biochemical or hematologic parameters.

Test condition: The concentration was generated as a dust aerosol with an IRAD dust generator.
The test material (white flakes) was ground in an Oster blender to produce a more respirable particle. Actual exposure concentration was determined by gravimetric techniques.
Particle size distribution was determined using Andersen 8 stage cascade impactor.
Average particle size was 4.7um.

Reliability: (1) valid without restriction
Meets generally accepted scientific method and is described in sufficient detail

Flag: Critical study for SIDS endpoint
14-FEB-2002 (107)

Species: mouse
Sex: male/female
Strain: other: cross bred white mice
Route of administration: gavage
Exposure period: 12 weeks
Frequency of treatment: once daily
Post exposure period: no
Doses: 80 mg/kg/day
Control Group: yes

Test substance: other TS: analytical grade benzoic acid

Method: 50 mice/sex (initial body weight: 8-10 g) received benzoic acid by oral intubation.
Observations for general condition, behavior, survival, food consumption, and weight gain were recorded daily.

Result: reduced weight gain without reduced food intake; mortality rate at week 10: 32 % in males and females
Reliability: (3) invalid
Flag: No histopathology or clinical chemistry
14-AUG-2001 Critical study for SIDS endpoint (108)

Species: rat Sex: male
Strain: Wistar
Route of administration: oral feed
Exposure period: 5 days
Frequency of treatment: continuously in diet
Post exposure period: 19 or 30 days
Doses: 3 % in diet (approx. 2250 mg/kg/day)
Control Group: yes

Remark: 15 rats; initial body weight: 60 g
the mean compound consumption was calculated according to Lehman, A.J., Assoc. Food Drug Off. Q. Bull. 18, 66 (1954)
Result: growth retardation; histologically demonstrable brain damage (necrosis of parenchymal cells of the stratum granulosum of the fascia dentata and the cortex of the lobus piriformis) still present after 35 days
Flag: Critical study for SIDS endpoint
14-AUG-2001 (109)

Species: rat Sex: male/female
Strain: Wistar
Route of administration: oral feed
Exposure period: 72 weeks
Frequency of treatment: continuously in diet
Post exposure period: no data
Doses: 1.5 % in diet (approx. 1125 mg/kg/day)
Control Group: yes

Remark: 20 m + 30 f (dosed group), 13 m + 12 f (control); initial body weight: 50-60 g
the mean compound consumption was calculated according to Lehman, A.J., Assoc. Food Drug Off. Q. Bull. 18, 66 (1954)
Result: reduced food intake, growth retardation, increased mortality rate (15/50 vs. 3/25 in the control)
14-AUG-2001 (110)

Species: rat Sex: male
Strain: Wistar
Route of administration: oral feed
Exposure period: 7 - 35 days
Frequency of treatment: continuously in diet
Post exposure period: no
Doses: 1.1 % in diet (approx. 825 mg/kg/day)
Control Group: yes

Remark: 5-10 rats/group
the mean compound consumption was calculated
according to Lehman, A.J., Assoc. Food Drug
Off. Q. Bull. 18, 66 (1954)
Result: reduced food intake, growth retardation, no
pathological findings

15-JAN-2001 (109)

Species: rat Sex: male
Strain: Wistar
Route of administration: oral feed
Exposure period: 5 days
Frequency of treatment: continuously in diet
Post exposure period: no
Doses: 3 % in diet (approx. 2250 mg/kg/day)
Control Group: yes

Remark: 5-10 rats/group; initial body weight: approx.
60 g the mean compound consumption was
calculated according to Lehman, A.J., Assoc.
Food Drug Off. Q. Bull. 18, 66 (1954)
Result: after 4-5 days disorders of central nervous
system: excitation, ataxia, tonic-clonic
convulsions; after 3-5 days brain damage was
demonstrable histologically (necrosis of
parenchymal cells of the stratum granulosum of
the fascia dentata and the cortex of the lobus
piriformis)

15-JAN-2001 (109)

Species: rat Sex: male/female
Strain: Wistar
Route of administration: oral unspecified
Exposure period: 72 weeks
Frequency of treatment: once daily
Post exposure period: no
Doses: 40 mg benzoic acid/kg/day and 80 mg
sodium bisulphite/kg/day
Control Group: yes

5. TOXICITY

DATE: 14-FEB.-2002

SUBSTANCES ID: 65-85-0

Test substance: other TS: analytical gradeRemark: 50 rats/sex; initial body weight: 100-120 g;
testResult: reduced weight gain, kidney function and the
reaction on stress factors were altered (no
further information); the erythrocyte
sedimentation rate was increased

14-AUG-2001

(108)

Species: rat Sex: male/female
Strain: Wistar
Route of administration: oral unspecified
Exposure period: 72 weeks
Frequency of treatment: once daily
Post exposure period: no data
Doses: 40 mg/kg/day
Control Group: yes

Remark: 10 rats/sex; initial body weight: 100-120 g;
test substance: analytical gradeResult: the rats developed some tolerance to a single
add. application of 4000 mg sodium benzoate/kg
given terminally, the mortality rate was 25 %

15-JAN-2001

(108)

Species: mouse Sex: male/female
Strain: no data
Route of administration: gavage
Exposure period: 12 weeks

Frequency of treatment: once daily

Post exposure period: no

Doses: 80 mg benzoic acid/kg/day and 160 mg
sodium bisulphite/kg/day

Control Group: yes

Remark: 100 mice/group; initial body weight: 8-10 g;
test substance: analytical gradeResult: reduced weight gain without reduced food
intake; mortality rate at week 10: 70 % in
males and 62 % in females

15-JAN-2001

(108)

Species: mouse Sex: male/female
Strain: no data
Route of administration: oral unspecified
Exposure period: 68 weeks

5. TOXICITY

DATE: 14-FEB.-2002

SUBSTANCES ID: 65-85-0

Frequency of treatment: once daily
Post exposure period: no data
Doses: 40 mg/kg/day
Control Group: yes

Remark: 25 mice/sex (initial body weight 10-15 g) or
25 mice/sex (initial body weight 16-20 g) were
tested; test substance: analytical grade
Result: no effects were reported

15-JAN-2001
(108)

(108)

Species: mouse Sex: male/female
Strain: no data
Route of administration: oral unspecified
Exposure period: 68 weeks
Frequency of treatment: once daily
Post exposure period: no data
Doses: 40 mg benzoic acid/kg/day and 80 mg
sodium
bisulphite/kg/day
Control Group: yes

Remark: 25 mice/sex (initial body weight 10-15 g) or
25 mice/sex (initial body weight 16-20 g) were
tested; test substance: analytical grade

Result: reduced weight gain without reduced food
intake; mortality rate at week 32: 56-65 % in
males and 45-72 % in females

15-JAN-2001

(108)

Species: cat Sex: male
Strain: no data
Route of administration: oral feed
Exposure period: 15 days
Frequency of treatment: continuously in diet
Post exposure period: no data
Doses: 100 or 200 mg/kg/day
Control Group: yes

Remark: 4 cats/group were tested; initial body weight:
1.7-2.27 kg

Result: no effects were observed

15-JAN-2001

(111)

Species: cat Sex: male
Strain: no data

Route of administration: oral feed
Exposure period: 3-4 days
Frequency of treatment: continuously in diet
Post exposure period: no data
Doses: 0.5 % in diet (approx. 300-420 mg/kg/day)
Control Group: yes

Remark: 4 cats were tested; initial body weight:
1.42-2.0 kg
Result: convulsions, hyperaesthesia, apprehension,
swollen hepatocytes with infiltrations of
macrophages and fibroblasts, swollen kidney
tubules, no pathological findings in brain and
spinal cord; mortality: 2/4

15-JAN-2001 (111)

Species: cat Sex: male
Strain: no data
Route of administration: oral feed
Exposure period: 23 days
Frequency of treatment: continuously in diet
Post exposure period: no data

Doses: 0.25 % in diet (approx. 130-160
mg/kg/day)
Control Group: yes

Remark: 4 cats were tested; initial body weight: 3.2-
4.0 kg
Result: no effects were observed

15-JAN-2001 (111)

5.5 Genetic Toxicity 'in Vitro'

Type: Salmonella typhimurium reverse mutation
assay
System of testing: TA 98, TA100, TA 1535, TA1537, TA1538
Concentration: 0, 20, 100, 500, 1000, 2000ug/plate
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 471
Year: 1983
GLP: no data
Test substance: other TS: technical grade benzoic acid

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint
14-FEB-2002 (112)

Type: other: Sister chromatid exchange
System of testing: human lymphocytes
Concentration: 0 to 2.0 mM
Cytotoxic Concentration: no data
Metabolic activation: without
Result: negative

Method: other: similar to OECD Guide-line 479
Year: 1986
Test substance: other TS: benzoic acid, purity = 99%
(estimated by NMR)

Reliability: (2) valid with restrictions
Comparable to Guideline study with acceptable
restrictions

Flag: Critical study for SIDS endpoint

14-AUG-2001 (113)

Type: other: Sister chromatid exchange
System of testing: human lymphoblastoid cells transformed by
Epstein- Barr virus (NL2, NL3, NL4)

Concentration: 0.001, 0.003, 0.01, 0.03 M
Cytotoxic Concentration: 0.03 M
Metabolic activation: without
Result: negative

Method: OECD Guide-line 479
Year: 1986
Test substance: other TS: benzoic acid purchased from Kanto
Chemical Co., Tokyo, Japan

Test condition: Test done only without metabolic activation.

Reliability: (2) valid with restrictions
Guideline study with acceptable restrictions

Flag: Critical study for SIDS endpoint
14-AUG-2001 (114)

Type: other: Chromosomal aberration test
System of testing: Chinese hamster fibroblast cell line
(CHL)
Concentration: up to 10 mg/plate
Metabolic activation: without
Result: ambiguous

Test substance: other TS: benzoic acid, >99% pure

Method: The study was carried out using a Chinese Hamster fibroblast cell line (CHL) which were exposed to the test substance at one of three dose levels for 24 and 48 hr. No metabolic activation systems were applied. Chromosome preparations were made following treatment with Colcemid. A hundred well-spread metaphases were observed per plate and the incidence of polyploid cells and cells with chromosome aberrations was recorded.

Result: At 48 hr, there was an incidence of 1% polyploid cells and 8% cells with structural aberration (incidence between 5-9.9% is considered equivocal).

Reliability: (2) valid with restrictions

Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-FEB-2002 (115)

Type: Bacillus subtilis recombination assay

System of testing: Bacillus subtilis H17, M45
Metabolic activation: no data
Result: positive

Test substance: no data

Method: An overnight culture of B. subtilis, H17 and M45, was mixed with test solutions and incubated for 30 minutes at 37 degree C. After treatment viable cells were counted and the ratio of 50% survival concentrations were calculated.

Result: Benzoic acid showed DNA damaging potential although it had been negative in the Ames test.

Reliability: (4) not assignable
insufficient documentation (abstract only)

Flag: Critical study for SIDS endpoint
06-JUN-2001 (116)

Type: other: Salmonella microsome assay
System of testing: S. typhimurium TA 98, TA 100, TA 1535, TA 1536, TA 1537, TA 1538
Metabolic activation: with and without
Result: negative

Remark: insufficient documentation
12-JAN-2001 (117)

Type:	other: Salmonella microsome assay	
System of testing:	S. typhimurium TA 97, TA 98, TA 100, TA 1535, TA 1537	
Metabolic activation:	with and without	
Result:	negative	
12-JAN-2001		(118)
Type:	other: Salmonella microsome assay	
System of testing:	S. typhimurium TA 98, TA 100, TA 1535, TA 1537	
Metabolic activation:	with and without	
Result:	negative	
11-JAN-2001		(119)
Type:	other: Mitotic recombination	
System of testing:	Saccharomyces cerevisiae D3	
Metabolic activation:	with and without	
Result:	negative	
Remark:	insufficient documentation	
11-JAN-2001		(117)
Type:	other: Chromosomal aberration test	
System of testing:	Chinese hamster fibroblast cell line (CHL)	
Metabolic activation:	without	
Result:	ambiguous	
11-JAN-2001		(120)
Type:	other: umu test	
System of testing:	S. typhimurium TA 1535/pSK1002	
Metabolic activation:	with and without	
Result:	negative	
11-JAN-2001		(121)

5.6 Genetic Toxicity 'in Vivo'

Remark: See IUCLID data set on sodium benzoate (CAS# 532-32-1).
Data on sodium benzoate reveal no in vivo genotoxicity.
Therefore no in vivo genotoxicity study for benzoic acid is indicated.

14-FEB-2002

5.7 Carcinogenicity

Remark: See IUCLID data set on sodium benzoate (CAS# 532-32-1).
Data on sodium benzoate reveal no in vivo genotoxicity.
Therefore no in vivo genotoxicity study for benzoic acid is indicated.

Flag: Critical study for SIDS endpoint
14-FEB-2002

5.8.1 Toxicity to Fertility

Type: other: 4 generation study
Species: rat
Sex: male/female

Strain: no data
Route of administration: other: oral feed (first 8 weeks paired feed technique; afterwards ad libitum)

Exposure Period: generation 1 and 2: lifelong;
generation 3: 16 weeks;
generation 4: until breeding

Frequency of treatment: continuously in diet
Doses: 0.5 or 1 % in diet (approx. 375 or 750 mg/kg/day)

Control Group: yes
NOAEL Parental: ≥ 750 mg/kg bw
NOAEL F1 Offspring: ≥ 750 mg/kg bw
NOAEL F2 Offspring: ≥ 750 mg/kg bw

Year: 1960
GLP: no
Test substance: other TS: benzoic acid, purity not noted

Method: A robust protocol, according to standards at that time, was used. Taking into account the reputation of the investigators a high quality has to be assumed.

Remark: 40 (20 M = 20 F) rats/group; initial body weight: 40-50 g.
The mean compound consumption was calculated according to Lehman, A.J., Assoc. Food Drug Off. Q. Bull. 18, 66 (1954).

Result: In all 4 generations no influence on growth (weight, weight gain and food efficiency (measured by protein efficiency)) and organ

weights was found.
In all 4 generations, no effects on fertility ("Forzplanzung") and lactation ("Aufzugt der Jungen") was found. The animals of the 3rd generation were sacrificed and examined histopathologically after 16 weeks (after lactation of the pups.) No remarkable histopathological findings were found.
In the paper no information is given on the organs investigated, however the robustness of the total study, the reputation of the investigators, as well as the reputation of the Professor who did the histopathologic investigation, a high quality has to be assumed. From other parameters it can be assumed that as a minimum the brains, heart, liver, kidney, testis and were examined.
Feeding of 0.5 % led to prolongation of survival compared to controls. In addition a so-called "Alters Paarung" after 48 weeks gave no influence on start of menopause.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint

14-AUG-2001 (105)

5.8.2 Developmental Toxicity/Teratogenicity

Species: rat Sex: female
Strain: Wistar
Route of administration: gavage
Exposure period: single application
Frequency of treatment: at day 9 of gestation
Duration of test: 20 days
Doses: 510 mg/kg
Control Group: no
NOAEL Maternal Toxicity: 510 mg/kg bw
NOAEL Teratogenicity: 510 mg/kg bw

Method: other: Kimmel et al. (1971)
GLP: no data
Test substance: other TS: benzoic acid, purity not noted

Method: Pregnant Wistar rats were treated on day 9 of gestation with one dose of benzoic acid in carboxymethylcellulose. Animals were sacrificed on day 20 of gestation and the uterus observed in

situ for implantation and resorption sites. Live fetuses were removed, examined for gross malformations, weighed, and prepared for histological examination. Skeletal examination was carried out under low magnification.

Remark: Group I was dosed with 510 mg/kg.
Group II was dosed with 510 mg/kg; then 2 h later: 250 or 500 mg/kg acetylsalicylic acid

Result: Treatment with benzoic acid alone resulted in no dead or resorbed implants and 3 % abnormal survivors, rates comparable to the control animals.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-AUG-2001 (122)

Species: rat Sex: male/female
Strain: no data
Route of administration: other: oral feed (first 8 weekspaired feed technique;
Exposure period: generation 1 and 2: lifelong;
generation 3: 16 weeks;
Frequency of treatment: continuously in diet
Duration of test: lifelong
Doses: 0.5 or 1 % in diet (approx. 375 or 750 mg/kg/day)
Control Group: yes
NOAEL Maternal Toxicity: >= 750 mg/kg bw
NOAEL Teratogenicity: 750 mg/kg bw

Year: 1960

Test substance: other TS: benzoic acid, purity not noted

Method: A robust protocol, according to standards at that time, was used. Taking into account the reputation of the investigators a high quality has to be assumed.

Remark: The mean compound consumption was calculated according to Lehman, A.J., Assoc. Food Drug Off. Q. Bull. 18, 66 (1954).

Result: The study demonstrated no effects on the dams or on the growth and development of the offspring.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,

Flag: well documented and acceptable for assessment
Critical study for SIDS endpoint
14-FEB-2002 (105)

Remark: See IUCLID data set on sodium benzoate
(CAS# 532-32-1).
Data on sodium benzoate reveal no in vivo
genotoxicity.
Therefore no in vivo genotoxicity study for
benzoic acid is indicated.

14-FEB-2002

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

5.10 Exposure Experience

Remark: Single oral doses of 1-1.5 g resulted in
dyspepsia, Nausea and vomiting.
23-OCT-1995 (123)

Remark: A systemic inhibitory effect of UV light
(UVA and UVB) on non-immunologic immediate
contact reactions to benzoic acid was found
in healthy volunteers.
23-OCT-1995 (124)

Remark: Effects of infra-red and laser irradiation
were studied on non-immunologic immediate
contact reactions to benzoic acid.
The strength of the contact urticaria was
increased.
23-OCT-1995 (125)

Remark: Daily oral doses of benzoic acid of < 0.5 g
or sometimes up to 4 g/d did not induce
adverse effects in man.
23-OCT-1995 (126)

Remark: Metabolism in humans:
Percutaneous absorption of ¹⁴C-labelled
benzoic acid (4 ug/cm²; area: 2.5 cm²) was
lower in aged subjects (> 65 years) than in
young (18-40 years): cumulative dose
absorbed within 7 days was 19.5 vs. 36.2 %.

The diminished surface lipid content of old skin implies a diminished dissolution medium.

23-OCT-1995 (127)

5.11 Additional Remarks

Type: Metabolism

Remark: The transdermal absorption of benzoic acid was studied in excised human skin and compared to absorption in living man. In equivalent time, the total absorption (% of applied dose) was 42.6 % (in vivo) or 44.9 % (in vitro).

15-JAN-2001 (128)

Type: Metabolism

Remark: The percutaneous absorption and the excretion of benzoic acid were tested in female weanling yorkshire swine (approx.20 kg) after topical and intravenous administration. After i.v. injection of 200 ug (10 uCi)/pig 84.5 % of 14C-activity were excreted with urine and 4.6 % in faeces within 6 days; the radiolabel recovery in carcass was 0.1 %. After topical application of the same dose the radiolabel recovery within 6 days (% of applied dose) was in urine 20 %, faeces 2.9 %, carcass 0.8 %, border 40.2 %, dosed skin 12.2 % and adjacent skin 9.1 %.

23-OCT-1995 (129)

Type: Metabolism

Remark: A concentration of 4 ug/cmE+2 of 14C-labelled benzoic acid was applied to the shaved backs of guinea pigs. The percutaneous absorption was determined from urinary and fecal excretion. Absorption of benzoic acid was similar to published human absorption data (no further information). The percutaneous absorption of 14C-labelled benzoic acid was studied in the Mexican hairless dog and compared to human data. Total absorption and maximum absorption rates were greater in humans than in hairless dogs. Surface counting experiments showed that benzoic acid persisted on the dog skin far

longer than on human skin (no further information). The percutaneous absorption of increasing topical doses of benzoic acid was determined in the Rhesus monkey and humans (dosage: 4, 40, 2000 ug/cmE+2; dose absorbed: monkey 59.2 %, 3.6 %, 17.4 %; human 42.6 %, 25.7 %, 14.4 %). In vivo percutaneous absorption was similar, also the dose-response curve was similar in the two species (no further information).

23-OCT-1995 (130)

Type: Metabolism

Remark: Damaging the skin (tape stripping, irritation, delipidization) increased absorption of benzoic acid dissolved in acetone (200 ug/ml, 50 uCi; topical application: 4 ug/cm2) in hairless guinea pigs: 71.1/73.4/94.1 % vs. 34.2 % absorbed in the group with intact skin.

23-OCT-1995 (131)

Type: Metabolism

Remark: The effect of topical application of benzoic acid on the in vivo percutaneous absorption was tested in 4 rhesus monkeys. Daily applications of 4 ug/cmE+2 were given for 14 days, the 1st and the 8th application used 14C-labelled test substance. To quantify absorption, urine was collected and assayed for radioactivity. The penetration results are expressed as the percentage of the applied dose absorbed, i.e. (% of topical dose eliminated in urine / % of i.v. dose eliminated in urine)*100. After 1st dose 85 % and after 8th dose 89 % were found. No significant change in percutaneous absorption from that following the initial dose was observed following the 8th dose of a multidose regimen.

23-OCT-1995 (132)

Type: Metabolism

Remark: In vitro, the permeation of benzoic acid was measured across isolated stratum corneum, stratum corneum and epidermis, and split-thickness skin. The stratum corneum was shown

- to be the rate limiting barrier and the flux was proportional to the concentration of the undissociated compound.
- 23-OCT-1995 (133)
- Type: Metabolism
- Remark: The percutaneous absorption and metabolism of benzoic acid was determined through hairless guinea pig skin in vitro. The absorption within 48 h was greater through nonviable skin (60.1 % of applied dose) than through viable skin(49.5%). 6.9 % of absorbed dose (2 ug/cm2) were conjugated with glycine to form hippuric acid.
- 23-OCT-1995 (134)
- Type: Metabolism
- Remark: After s.c. administration of radiolabelled benzoic acid to maternal rats it was found, that the acidic compound penetrated the placental barrier readily. The fetal t_{1/2} values were in general lower than those for the corresponding maternal tissues. The fetal blood-brain barrier was penetrated more readily than the adult one for the tested compound.
- 14-AUG-2001 (135)
- Remark: After a single i.p. injection of 410 umol 14C-labelled benzoic acid/kg to female Wistar rats 90 % of the applied 14C-activity was excreted in urine and 1.3 % in bile within 3 hours, mainly as hippuric acid. After 24 hours the excretion was approx. 100 %.
- 23-OCT-1995 (136)
- Remark: Benzoic acid is detoxicated by some mammalian species mainly by conjugation with glycine to form hippuric acid. There is a marked species difference in the efficiency of the process. After an oral dose of 50 mg 14C-benzoic acid most species excreted 50-100 % of radioactivity in the urine within 24 hours. In the turtle and gecko excretion was slower (39 % in 3 days).

In herbivorous and omnivorous species (rhesus, squirrel and capuchin monkeys, pig, rabbit, rat, mouse, guinea pig, hamster, lemming, gerbil) benzoic acid was excreted in the urine almost entirely as hippuric acid, though 10-20 % of the total ¹⁴C-activity appeared as free benzoic acid in pigs and squirrel monkeys within 24 hours, possibly as a result of the decomposition of benzoyl glucuronide. In the 2 men given 1 mg benzoic acid/kg, almost all the urinary metabolite was hippuric acid, with 97 % of the radioactivity excreted within 4 hours and virtually 100 % within 12 hours.

In the carnivorous animals tested (dog, cat, ferret) the main metabolite was hippuric acid, with the dog and ferret excreting also some benzoyl glucuronide. In the hedgehog, an insectivore, a similar excretion occurred. The Indian fruit bat (*Pteroptus gigantus*) excreted 70-80 % of benzoic acid as the glucuronide and the remainder as free acid within 24 hours. The pigeon excreted mainly hippuric acid and in the chick, turtle and gecko the major metabolite was ornithuric acid. When the dose of benzoic acid in the ferret was raised to 200 and 400 mg/kg, the proportion excreted as glucuronide was markedly increased. During the metabolism of benzoic acid, the relative amount of conjugation with glycine and with glucuronic acid varies from species to species and may depend to some extent upon the magnitude of the dose.

14-AUG-2001

(137)

Remark:

In many species, benzoic acid is rapidly absorbed, conjugated with glycine and excreted as hippuric acid.

There appears to be no accumulation of benzoic acid at low doses, but one limiting factor in the biosynthesis of hippuric acid is the availability of glycine: once the glycine pool is exhausted (after application of high doses), an additional metabolite, benzoyl glucuronide, is excreted in the urine of some species (no further information available).

14-AUG-2001

(138)

Remark: 5 days after i.p. injection of 1 ml (4 ug) labeled benzoic acid in saline to female hairless guinea pigs, 92.1 % of the administered dose was excreted in urine.

14-AUG-2001 (131)

Remark: In most animals, the conversion of benzoic acid to hippuric acid has been found to occur in kidney, with conversion possible in the liver when kidney malfunction exists. The monkey metabolized benzoic acid only in the liver (no further information available).

14-AUG-2001 (139)

Remark: After a single i.v. injection of 2.0 to 2.2 mg ¹³C-labelled benzoic acid/kg to male Wistar rats 85 - 99 % of the applied ¹³C-activity was excreted as hippuric acid in urine within 120 minutes after application.

14-AUG-2001 (140)

Remark: In an in vitro study, the nitrosation of methylurea to form N-nitrosomethylurea by benzoic acid at a concentration of 10, 50 or 100 mM was not reduced (101, 108 or 102-110 % compared to control).

14-AUG-2001 (141)

Remark: Regional differences in percutaneous absorption of benzoic acid were tested in vitro (face, abdomen, back, forearm, thigh, lower leg, dorsal foot, dorsal hand, palm and sole).
A trend of increasing permeability from truncal to acral sites was observed (exception: palmar/plantar skin).

23-OCT-1995 (142)

Remark: Benzoic acid was positive in the microsomal degranulation assay, if microsomes were prepared at low 'g' force (10000).

In the test with rough endoplasmatic reticulum prepared at high 'g' forces (≥ 105000) it was negative. The degranulation assay tests the ability of a chemical to dissociate polysomes and ribosomes from the endoplasmatic reticulum.

14-AUG-2001 (143)

Remark: Benzoic acid (purity 99,9 %; 2 % solution in phosphate buffered saline) was administered i.v. (jugular catheter) to two male F 344 rats at approx. 2 mg/l for a total dose of 108 mg. The substance caused no neuroexcitation.

14-AUG-2001 (144)

Remark: The application of benzoic acid (1 % in diet [approx. 450-890 mg/kg/d]) for 1 day to 4 male cats (initial body weight: 1.06- 1.70 kg) resulted in convulsions, aggression, hyperaesthesia, swollen hepatic cells with centrilobular vacuolation, infiltration of inflammatory cells, and marked distension of the kidney glomeruli. No pathological findings in brain and spinal cord. Mortality: 1/4 control group: yes

14-AUG-2001 (111)

Remark: Other: In a screening with COMPACT (computer-optimized molecular parametric analysis of chemical toxicity) benzoic acid was predicted as a potential substrate for cytochrome P450 IIE.

14-AUG-2001 (145)

6.1 Analytical Methods**6.2 Detection and Identification**

7.1 Function

7.2 Effects on Organisms to be Controlled

7.3 Organisms to be Protected

7.4 User

7.5 Resistance

8.1 Methods Handling and Storing**8.2 Fire Guidance****8.3 Emergency Measures****8.4 Possib. of Rendering Subst. Harmless****8.5 Waste Management****8.6 Side-effects Detection****8.7 Substance Registered as Dangerous for Ground Water****8.8 Reactivity Towards Container Material**

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10.1 End Point Summary**10.2 Hazard Summary****10.3 Risk Assessment**

I U C L I D D a t a S e t

(SODIUM BENZOATE: CAS N°: 532-32-1)

Existing Chemical ID: 532-32-1
CAS No. 532-32-1
EINECS Name sodium benzoate
EINECS No. 208-534-8
TSCA Name Benzoic acid, sodium salt
Molecular Formula C7H6O2.Na

Producer Related Part

Company: Bayer Corporation
Creation date: 21-OCT-1999

Substance Related Part

Company: Bayer Corporation
Creation date: 21-OCT-1999

Memo: Bayer Corporation

Printing date: 10-AUG-2001

Revision date:

Date of last Update: 10-AUG-2001

Number of Pages: 68

Chapter (profile): Chapter: 1, 2, 3, 4, 5, 7

Reliability (profile): Reliability: without reliability, 1, 2, 3, 4

Flags (profile): Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1. GENERAL INFORMATION

DATE: 10-AUG-2001
SUBSTANCE ID: 532-32-1**1.0.1 OECD and Company Information**

Type: lead organisation
Name: American Chemistry Council (formerly Chemical Manufacturers Association), Benzoates HPV Panel
Street: 1300 Wilson Boulevard
Town: 22209 Arlington, VA
Country: United States

09-AUG-2001

Type: cooperating company
Name: ATOFINA Chemicals, Inc.
Country: United States

09-AUG-2001

Type: cooperating company
Name: Bayer Corporation
Country: United States

09-AUG-2001

Type: cooperating company
Name: DSM Fine Chemicals
Country: Netherlands

03-JAN-2001

Type: cooperating company
Name: Noveon, Inc.
Country: United States

09-AUG-2001

Type: cooperating company
Name: Velsicol Chemical Corporation
Country: United States

26-MAY-2000

1.0.2 Location of Production Site**1.0.3 Identity of Recipients**

1.1 General Substance Information**1.1.0 Details on Template****1.1.1 Spectra****1.2 Synonyms****1.3 Impurities****1.4 Additives****1.5 Quantity****1.6.1 Labelling****1.6.2 Classification****1.7 Use Pattern****1.7.1 Technology Production/Use****1.8 Occupational Exposure Limit Values****1.9 Source of Exposure****1.10.1 Recommendations/Precautionary Measures****1.10.2 Emergency Measures****1.11 Packaging****1.12 Possib. of Rendering Subst. Harmless****1.13 Statements Concerning Waste**

1.14.1 Water Pollution**1.14.2 Major Accident Hazards**

Legislation:
Substance listed:
10-JUL-2000

1.14.3 Air Pollution

Classified by:
Labelled by:
Number:
Class of danger:
10-JUL-2000

1.15 Additional Remarks**1.16 Last Literature Search**

Type of Search: Internal and External
Date of Search: 07-SEP-1999

Remark: Only HPV endpoints: TOXLINE data base and
internal studies.
09-AUG-2001

1.17 Reviews**1.18 Listings e.g. Chemical Inventories**

2.1 Melting Point

Value: > 300 degree C
Method: other: measured
Remark: Carbonisation at temperature > 500 degree C
Reliability: (2) valid with restrictions
Data from Handbook or collection of data
Flag: Critical study for SIDS endpoint
09-AUG-2001 (1) (2)

Value: 330.6 degree C
Method: other: (calculated) MPBPWIN (v1.31) Program;
Adapted Joback Method
Year: 1999
Testsubstance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
09-AUG-2001 (3)

Value: 410 - 430 degree C
Method: other
Remark: DSM datasheet.
26-JAN-2001

2.2 Boiling Point

Value: 464.9 degree C
Method: other: (calculated) MPBPWIN (v1.31) Program ;
Adapted Stein and Brown Method
Year: 1999
Testsubstance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
09-AUG-2001 (3)

2.3 Density

Type: relative density
Value: = 1.44 g/cm3
Flag: Critical study for SIDS endpoint
26-JAN-2001 (4) (5)

Type: bulk density
 Value: 650 kg/m3
 Remark: thickened
 Source: DSM Special Products B.V. GeleenECB -
 Existing Chemicals Ispra (VA)
 26-MAY-2000 (6)

Type: bulk density
 Value: 350 kg/m3
 Remark: not thickened
 Source: DSM Special Products B.V. GeleenECB -
 Existing Chemicals Ispra (VA)
 26-MAY-2000 (6)

2.3.1 Granulometry

2.4 Vapour Pressure

Value: .00000000489 hPa at 25 degree C
 Method: other (calculated): MPBPWIN (v1.31) Program;
 Modified Grain Method
 Year: 1999
 Testsubstance: other TS: molecular structure
 Result: 3.67E-009 mm Hg; 4.89E-09 hPa
 Reliability: (2) valid with restrictions
 Accepted calculation method
 Flag: Critical study for SIDS endpoint
 09-AUG-2001 (3)

2.5 Partition Coefficient

log Pow: -2.269
 Method: other (calculated): Log Kow(version 1.65
 estimate)
 Year: 1999
 Testsubstance: other TS: molecular structure
 Reliability: (2) valid with restrictions
 Accepted calculation method
 Flag: Critical study for SIDS endpoint
 09-AUG-2001 (7)

log Pow: = -2.13
 Method: other (calculated): CLogP
 Year:
 Testsubstance: other TS: molecular structure

Remark: Calculated according to C. Hansch et al 1985.
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
23-MAR-2001

2.6.1 Water Solubility

Value: 556 g/l at 20 degree C
Method: other
Remark: pH-value: about 8.
Reliability: (2) valid with restrictions
Data from Handbook or collection of data
Flag: Critical study for SIDS endpoint
09-AUG-2001 (8) (9)

Value: 630 g/l at 20 degree C
pH: 7
26-JAN-2001 (6) (6)

Remark: concentrated solutions react neutral
diluted solutions react weakly alkaline (pH 8)
26-JAN-2001 (10)

2.6.2 Surface Tension

2.7 Flash Point

Value: > 100 degree C
Type: closed cup
Method: other: DIN 51758
Year:
Reliability: (1) valid without restriction
Meets National standards method (AFNOR/DIN)
09-AUG-2001 (6)

2.8 Auto Flammability

2.9 Flammability

2.10 Explosive Properties

Result:
Remark: Can form explosive mixtures with air.
09-AUG-2001

2.11 Oxidizing Properties**2.12 Additional Remarks**

Remark: At a rel. humidity of > 50% the salt is
hygroscopic and it dissolves at r. F.-values >
85 %

23-OCT-1995 (10)

Remark: UV spectrum lambda max (nm):
225 (water; lg epsilon: n.a.)

23-OCT-1995 (11)

Remark: pH value ca. 7.5 at 10 g/l water

23-OCT-1995 (6)

3.1.1 Photodegradation

Type: air
Conc. of subst.: at 25 degree C
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1560000 molecule/cm3
Rate constant: .00000000000017775 cm3/(molecule * sec)
Degradation: 50 % after 72.2 hour(s)
Method: other (calculated): AOP Program (v1.89)
Year: 1999 GLP:
Test substance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
09-AUG-2001 (3)

Type:
Method:
Year: GLP:
Test substance:
Remark: See IUCLID on benzoic acid (CAS# 65-85-0); the photodegradation of the sodium salt should be similar.
09-AUG-2001

3.1.2 Stability in Water

Type:
Method:
Year: GLP:
Test substance:
Remark: Based on structure and organic chemistry rules (e.g. bonding in organic molecules, activation energy, reactivity, transformations, addition, substitution, elimination) no hydrolysis will occur at pH ranges 4 - 11.
Flag: Critical study for SIDS endpoint
26-JAN-2001

3.1.3 Stability in Soil

3.2 Monitoring Data (Environment)

Type of

measurement:

Medium:

Method:

Concentration

Remark:

See IUCLID on benzoic acid (CAS# 65-85-0); the data on the sodium salt should be similar.

09-AUG-2001

3.3.1 Transport between Environmental Compartments

Type:

fugacity model level III

Media:

other: air - water - soil - sediment

Air (Level I):

Water (Level I):

Soil (Level I):

Biota (L.II/III):

Soil (L.II/III):

Method:

other: EPIWin Modeling Program

Year: 1999

Result:

	Distribution (percent)	Half-Life (hr)	Emissions (kg/hr)	Fugacity (atm)
Air	1.45e-007	144	1000	4.83e-019
Water	45.3	360	1000	1.38e-020
Soil	54.6	360	1000	6.16e-019
Sediment	0.0755	1.44e+003	0	1.15e-020

Persistence Time: 421 hr

Reaction Time: 520 hr

Advection Time: 2.21e+003 hr

Percent Reacted: 80.9

Percent Advected: 19.1

Reliability:

(2) valid with restrictions

Accepted calculation method

Flag:

Critical study for SIDS endpoint

09-AUG-2001

(12)

3.3.2 Distribution**3.4 Mode of Degradation in Actual Use**

Remark:

In many species benzoic acid sodium salt is rapidly absorbed and rapidly metabolized namely conjugated with glycine and excreted as hippuric acid in the urine.

The substance is readily biodegradable, and is biodegraded within chemical industry via a waste water treatment plant.

09-AUG-2001

3.5 Biodegradation

Type: aerobic
 Inoculum: activated sludge, domestic
 Concentration: 50 mg/l related to Test substance
 Degradation: ca. 90 % after 7 day
 Result: readily biodegradable
 Method: OECD Guide-line 301 B "Ready Biodegradability:
 Modified Sturm

Test (CO₂ evolution)"
 Year: 1981 GLP: no data
 Test substance: other TS: sodium benzoate, purity not noted
 Remark: Sodium benzoate is the recommended "readily biodegradable reference substance" for OECD Guideline studies.
 This endpoint has been studied several times by several other investigators/groups and all support the result of the study mentioned above.

Test condition: 25 degree C
 Reliability: (1) valid without restriction
 Guideline study
 Flag: Critical study for SIDS endpoint

09-AUG-2001 (13) (14)

Type: anaerobic
 Inoculum: other bacteria: anaerobic sewage, domestic and industrial
 Concentration: 50 mg/l related to DOC (Dissolved Organic Carbon)
 Degradation: 93 % 7.5 after 7 day
 Method: other: see below
 Year: GLP: no data
 Test substance: other TS: technical grade sodium benzoate purchased from Aldrich Chemical Co. , UK

Method: 2-3 g sludge plus sodium benzoate (concentration equivalent to 50 mg Carbon/liter or 85 mg substance/l).
 Controls and tests done in triplicate.
 Temperature = 35 degree C.
 Measured gas production (CH₄ + CO₂).

3. ENVIRONMENTAL FATE AND PATHWAYS

DATE: 10-AUG-2001

SUBSTANCE ID: 532-32-1

Remark: retard lag 2 d
 Result: Degradation is expressed as percentage of theoretical methane production based on the stoichiometry of degradation.
 Reliability: (2) valid with restrictions
 Meets generally accepted scientific standards, well documented and acceptable for assessment
 Flag: Critical study for SIDS endpoint
 09-AUG-2001 (15)

Type: aerobic
 Inoculum: other: suspension from marine aquarium filters
 Concentration: 10 mg/l related to DOC (Dissolved Organic Carbon)
 Degradation: > 97 % after 28 day
 Result: readily biodegradable
 Testsubstance: 2 day 20 %
 4 day 45 %
 6 day 55 %
 8 day 70 %
 20 day 85 %
 Method: OECD Guide-line 301 B "Ready Biodegradability:
 Modified Sturm Test (CO₂ evolution)"
 Year: 1981 GLP:
 Test substance:
 Method: Guideline adapted to use seawater as test medium and inoculum
 Reliability: (1) valid without restriction
 03-JAN-2001 (16)

Type: anaerobic
 Inoculum: other bacteria: anaerobic sewage, domestic, 2 weeks preincubated
 Concentration: related to Test substance
 Method: other: anaerobic degradation, static, 35 degree C, parameter: gas production
 Year: GLP:
 Test substance:
 Remark: concentration: 50/60/90 mg/l
 degradation : 47/49/28 d = 60.5/82.7/74 %
 26-JAN-2001 (17)

Type: aerobic
 Inoculum: domestic sewage, non-adapted
 Contact time: 28 day
 Degradation: 84 % after 14 day
 Result: readily biodegradable

3. ENVIRONMENTAL FATE AND PATHWAYS

DATE: 10-AUG-2001

SUBSTANCE ID: 532-32-1

Test substance: 14 day 84 %
 28 day 92 %
 Method: Directive 84/449/EEC, C.7 "Biotic degradation
 - modified MITI test"
 Year: 1982 GLP:
 Test substance: other TS: purchased from Sigma Chemicals
 Reliability: (1) valid without restriction
 19-MAY-2000 (17)

Type: aerobic
 Inoculum: other: microorganisms already present in
 seawater
 Concentration: 11.6 mg/l related to DOC (Dissolved Organic
 Carbon)
 Contact time: 61 day
 Degradation: 80.5 % after 20 day
 Result: readily biodegradable
 Test substance: 5 day 57.4 %
 10 day 72.8 %
 30 day 83.4 %
 50 day 91.7 %
 61 day 96.4 %

Method: OECD Guide-line 301 A (new version) "Ready
 Biodegradability: DOC Die Away Test"
 Year:
 GLP:
 Test substance: no data
 09-MAY-2000 (19)

Type: aerobic
 Inoculum:
 Degradation: 100 % after 28 day
 Result: readily biodegradable
 Method: OECD Guide-line 301 D "Ready Biodegradability:
 Closed Bottle Test"
 Year: GLP:
 Test substance: no data
 09-MAY-2000 (20)

Type:
 Inoculum: activated sludge, non-adapted
 Concentration: 100 mg/l related to Test substance
 Degradation: 84 % after 10 day
 Method: Directive 84/449/EEC, C.7 "Biotic degradation
 - modified MITI test"
 Year:
 GLP:
 Test substance:

3. ENVIRONMENTAL FATE AND PATHWAYS

DATE: 10-AUG-2001

SUBSTANCE ID: 532-32-1

Remark: degradation after 10 d: 64 - 98 % (n=14)
 after 28 d: 75 - 111 % (n=14)
 0 d lag phase EG-Ringtest 1981-82
 26-JAN-2001 (21)

Type:
 Inoculum:
 Degradation: 88 % after 28
 Test substance: 60 day 95 %
 Method: OECD Guide-line 301 A (new version) "Ready
 Biodegradability: DOC Die Away Test"
 Year: GLP:
 Test substance: no data
 09-MAY-2000 (20)

Type:
 Inoculum: other bacteria: purification plant outflow
 mixed with a soil suspension
 Concentration: 5 mg/l related to Test substance
 Method: other: Respirometer-Test (Closed Bottle Test)
 Year: GLP:
 Test substance:
 Remark: degradation after 30 d: 75 - 111 % ThSB
 54-89 %: Medium without NH₄ Cl
 71-130%: Medium with NH₄ Cl
 26-JAN-2001 (22)

Type:
 Inoculum: other bacteria: anaerobic laboratory-sewage,
 adapted
 Concentration: 300 mg/l related to Test substance
 Degradation: 98 % after 4 day
 Method: other: anaerobic degradation, static
 Year: GLP:
 Test substance:
 Remark: parameter: gasproduction
 Test condition: 35 degree, enrichment culture
 26-JAN-2001 (23)

Type:
 Inoculum: other bacteria: anaerobic sewage, domestic,
 washed
 Concentration: 50 mg/l related to Test substance
 Degradation: 49.8 % after 61 day
 Method: other: anaerobic degradation, static,
 35 degree C, parameter: gasproduction
 Year: GLP:
 Test substance:

Remark: concentration: 60/60 mg/l
degradation : 35/56 d = 95.3/96.5 %
26-JAN-2001 (17)

Type:
Inoculum: other bacteria: methanogenic sewage laboratory culture, benzoate-adapted
Concentration: 3000 mg/l related to Test substance
Degradation: ca. 99 % after 5 day
Method: other: anaerobic degradation, static, 37 degree C, analytical control of concentration, pH 6.7-6.9
Year: GLP:
Test substance:
26-JAN-2001 (24)

Type:
Inoculum: other bacteria: anaerobic sewage from a purification plant of woodmanufacturing industry, benzoate-adapted
Concentration: 307 mg/l related to Test substance
Degradation: ca. 99 % after 2 day
Method: other: anaerobic degradation, static, analytical control of concentration
Year: GLP:
Test substance:
Remark: Original data of concentration: 2.13 mM
Test condition: 37 degree C
26-JAN-2001 (24)

Type:
Inoculum: other bacteria: anaerobic enrichment culture (fen), adapted
Concentration: 2306 mg/l related to Test substance
Degradation: 100 % after 4 day
Method: other: anaerobic degradation, static, parameter: gas production by GC, 39degree C, pH 6.7
Year: GLP:
Test substance:
26-JAN-2001 (25)

3.6 BOD5, COD or BOD5/COD Ratio

Remark: No data.
09-AUG-2001

3.7 Bioaccumulation

Species:
Exposure period:
Concentration:
BCF: 3.16
Elimination:
Method: other: (calculated) BCF Program (v2.13)
Year: 1999 GLP:
Test substance: other TS: molecular structure
Remark: Based on the log P and its rapid
metabolization and excretion in many species
no bioaccumulation is indicated.
Result: Estimated Log BCF = 0.500 (BCF = 3.162)
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
09-AUG-2001 (3)

Species:
Exposure period:
Concentration:
BCF:
Elimination:
Method:
Year: GLP:
Test substance:
Remark: Based on the log P and its rapid
metabolization and excretion in many species
no bioaccumulation is indicated.
09-AUG-2001

3.8 Additional Remarks

Remark: Aerobic degradation in sea water:
Inoculum: sea water; salinity 18,6 %,
20 degree C
Method: Modified OECD Screening Test, OECD
Guideline 301 E adopted 12 May 81, EG-
Richtlinie 84/449/EWG, part C.3 im EG-
Amtsblatt L 251, ISO 7824 (1984)
Concentration: 20 mg/l DOC
degradation after 28d: 100 %
degradation after 12d: 95 %
23-OCT-1995 (26)

Remark: Aerobic degradation in sea water:
Inoculum: sea water (38.7 o/oo), 20 degree C
Method: shake flask test - die away-test;
parameters: DOC initial concentration: 20 mg/l
related to Test substance; 11.6 mg DOC/l (1)
40 mg/l resp. 23.2 mg/l related to DOC (2)
degradation: after 5/20/61 d: 57.4/80.5/96.4 %;
lag time: 4 d (1)
after 5/20/61 d: 30.8/72.8/98.0 %;
lag time: 3 d (2)

23-OCT-1995 (27)

Remark: Anaerobic degradation in lake water:
Inokulum: sediment (eutrophic lake)
Method: anaerobic degradation, semistatic;
28 resp. 37 degree C; pH 7,4 - 7,6
Concentration: 724 mg/l related to Test
substance
Degradation after 20 d: ca. 100 %
Remark: 50% (w/v) sediment in culture medium
started after 4 h (only in undiluted
sediment), complete transformation
to methane, detection of C14 sodium
benzoate (ring-labelled); adaption
to aliphatic fatty acids

23-OCT-1995 (28)

Remark: Anaerobic degradation in laboratory aquifer
column:
Method: continuous, room temperature, contents
of column:
30 % material of water-bearing soil
sediment/
70 % slate-debris
Concentration: 28.1 mg/l related to Test
substance
Degradation: > 95 %
Remark: Degradation after adaptation phase of
1 week to m-Xylol; 30 degree C; flow through
time: 2.6 cm/h, length of column: 25 cm

23-OCT-1995 (29)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: flow through
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
NOEC: > 245
EC50 : 484
Method: EPA OPP 72-1
Year: GLP: no data
Test substance: other TS: sodium benzoate, 99+% purity
Method: pH was adjusted to approximate that of Lake Superior water (pH 7.8) with NaOH or HCL. Compound analyses were done by HPLC: all exposure chambers at 0, 24, 48, 72 and 96 hr. Fathead minnows used in this experiment were cultured at US EPA Environmental Research Laboratory, Duluth, MN and University of Wisconsin - Superior campus. 20 fish/concentration and control. Behavior and toxic signs were noted at 4,24,48,72 and 96 hours and used to calculate EC50.
Remark: Affected fish were hyperactive and lost equilibrium prior to death. No effect data were recorded. Individual lengths and weights of the test fish were not recorded, however the measured mean weight was 230 mg. Alkalinity increased with increasing toxicant concentration. This endpoint had been studied by another investigator and reported results similar to the study mentioned above.
Test condition: temperature =23.9 degree C (+/-0.3); dissolved oxygen = 7.0 mg/l; pH=7.37; hardness = 43.4 mg/l CaCO3; alkalinity = 80.9mg/l CaCO3; tank volume = 7.3 liter; average measured concentrations 101, 163, 245, 400, 680 mg/l
Reliability: (1) valid without restriction
Guideline study
Flag: Critical study for SIDS endpoint
09-AUG-2001 (30) (31)

Type: static
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: > 100
Method: other: see below
Year: GLP: no data
Test substance: other TS: sodium benzoate, reagent-grade
Method: 10 fish/dose were exposed to a solution of the
test substance for 96 hours (a total of seven
aquatic species were tested simultaneously).
Biological observations and determinations of
temperature, dissolved oxygen and pH were done
daily. Survival, condition, and behavior were
recorded.
The LC50 values were estimated by interpolation
Method (Stephan, CE, ASTM STP 634, FL Mayer & JL
Hamelink (eds.) pps 65-84).
Test condition: 20 degree C; pH 6.5-8.5; 16 hr light/day;
size of minnows = 200-500 mg; food was withheld
for 24 hr prior to exposure; tests were done in
duplicate.
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
well documented and acceptable for assessment
09-AUG-2001 (32)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: > 100
Method: other: see below
Year: GLP: no data
Test substance: other TS: sodium benzoate, reagent grade
Method: 10 organisms/dose were exposed to a solution
of the test substance for 96 hours (a total of
seven aquatic species were tested
simultaneously).
Biological observations and determinations of
temperature, dissolved oxygen and pH were done
daily.

Survival, condition, and behavior were recorded. The LC50 values were estimated by interpolation method (Stephan, CE, ASTM STP 634, FL Mayer & JL Hamelink (eds.) pps 65-84).

Remark: This endpoint had been studied by another investigator and reported results similar to the study mentioned above.

Test condition: 20 degree C; pH 6.5-8.5; 16 hr light/day; Daphnia were at first and second larval instar; food was withheld for 24 hr prior to exposure; tests were done in duplicate.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
09-AUG-2001 (33) (32)

Type: static

Species: Gammarus fasciatus (Crustacea)

Exposure period: 96 hour(s)

Unit: mg/l Analytical monitoring: no

EC50: > 100

Method: other: see below

Year: GLP: no data

Test substance: other TS: sodium benzoate, reagent grade

Method: 10 organisms/dose were exposed to a solution of the test substance for 96 hours (a total of seven aquatic species were tested simultaneously). Testing concentrations were 0.1, 1.0, 10, and 100 mg/l. Biological observations and determinations of temperature, dissolved oxygen and pH were done daily. Survival, condition, and behavior were recorded. The LC50 values were estimated by interpolation method (Stephan, CE, ASTM STP 634, FL Mayer & JL Hamelink (eds.) pps 65-84).

Test condition: 20 degree C; pH 6.5-8.5; 16 hr light/day; Gammarus weighed approximately 7 mg at testing; food was withheld for 24 hr prior to exposure; tests were done in duplicate.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, Well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
09-AUG-2001 (32)

Type: static
Species: Asellus intermedius (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: > 100
Method: other: see below
Year: GLP: no data
Test substance: other TS: sodium benzoate, reagent grade
Method: 10 organisms/dose were exposed to a solution of the test substance for 96 hours (a total of seven aquatic species were tested simultaneously). Testing concentrations were 0.1, 1.0, 10, and 100 mg/l. Biological observations and determinations of temperature, dissolved oxygen and pH were done daily. Survival, condition, and behavior were recorded. The LC50 values were estimated by interpolation Method (Stephan, CE, ASTM STP 634, FL Mayer & JL Hamelink (eds.) pps 65-84).
Test condition: 20 degree C; pH 6.5-8.5; 16 hr light/day; organisms weighed approximately 12 mg at testing; food was withheld for 24 hr prior to exposure; tests were done in duplicate.
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment.
09-AUG-2001 (32)

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring:
EC50: < 650
Method: other: no data
Year: GLP:
Test substance:
Test condition: 25 degree C
09-AUG-2001 (34)

Type: static
Species: other aquatic mollusc: Helisoma trivolvis
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: > 100
Method: other: see below

Year:	GLP: no data
Test substance:	other TS: sodium benzoate, reagent grade
Method:	10 organisms/dose were exposed to a solution of the test substance for 96 hours (a total of seven aquatic species were tested simultaneously). Testing concentrations were 0.1, 1.0, 10, and 100 mg/l. Biological observations and determinations of temperature, dissolved oxygen and pH were done daily. Survival, condition, and behavior were recorded. The LC50 values were estimated by interpolation method (Stephan, CE, ASTM STP 634, FL Mayer & JL Hamelink (eds.) pps 65-84).
Test condition:	20 degree C; pH 6.5-8.5; 16 hr light/day; organisms weighed approximately 180 mg at testing; food was withheld for 24 hr prior to exposure; tests were done in duplicate.
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment.
09-AUG-2001	(32)

Type:	static
Species:	other aquatic worm: <i>Dugesia tigrina</i>
Exposure period:	96 hour(s)
Unit:	mg/l
EC50:	> 100
Method:	other: see below
Year:	GLP: no data
Test substance:	other TS: sodium benzoate, reagent grade
Method:	10 organisms/dose were exposed to a solution of the test substance for 96 hours (a total of seven aquatic species were tested simultaneously). Testing concentrations were 0.1, 1.0, 10, and 100 mg/l. Biological observations and determinations of temperature, dissolved oxygen and pH were done daily. Survival, condition, and behavior were recorded. The LC50 values were estimated by interpolation method (Stephan, CE, ASTM STP 634, FL Mayer & JL Hamelink (eds.) pps 65-84).
Test condition:	20 degree C; pH 6.5-8.5; 16 hr light/day; organisms weighed approximately 6 mg at testing; food was withheld for 24 hr prior to exposure; tests were done in duplicate.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
well documented and acceptable for assessment.
09-AUG-2001 (32)

Type: static
Species: other aquatic worm: *Lumbriculus variegatus*
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: > 100
Method: other: see below
Year: GLP: no data
Test substance: other TS: sodium benzoate, reagent grade
Method: 10 organisms/dose were exposed to a solution of
the test substance for 96 hours (a total of seven
aquatic species were tested simultaneously).
Testing concentrations were 0.1, 1.0, 10, and 100
mg/l.
Biological observations and determinations of
temperature, dissolved oxygen and pH were done
daily.
Survival, condition, and behavior were recorded.
The LC50 values were estimated by interpolation
Method (Stephan, CE, ASTM STP 634, FL Mayer & JL
Hamelink (eds.) pps 65-84).
Test condition: 20 degree C; pH 6.5-8.5; 16 hr light/day;
organisms weighed approximately 6 mg at testing;
food was withheld for 24 hr prior to exposure;
tests were done in duplicate.
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
well documented and acceptable for assessment
09-AUG-2001 (32)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: green algae
Endpoint:
Exposure period: 96 hour(s)
Unit: g/l Analytical monitoring: no
EC50: 430
Method: other: (calculated) ECOSAR Program (v0.99e)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Result: ECOSAR Class: Neutral Organics Organism: Green Algae
Predicted 96-hr EC50 = 4.3e+005 mg/l
(> saturation)

Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (12)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type:
Species: other bacteria: *Achromobacter liquefaciens*
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
EC50: >= 3000
Method: other: static, 22 degree C, pH 7
Year: GLP:
Test substance: other TS
Remark: 7 d-EC0 >= 3000 mg/l
Test substance: sodium benzoate; purity not noted
Flag: Critical study for SIDS endpoint
10-AUG-2001 (35)

Type:
Species: other bacteria: *Micrococcus flavus*
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
EC50: > 500
Method: other: static, 22 degree C, pH 7
Year: GLP:
Test substance: other TS
Remark: 7 d-EC0 >= 3000 mg/l
Test substance: sodium benzoate; purity not noted
Flag: Critical study for SIDS endpoint
10-AUG-2001 (35)

Type:
Species: other bacteria: *Sarcina flava*
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
EC50: < 100
Method: other: static, 22 degree C, pH 7
Year: GLP:
Test substance: other TS
Remark: 7 d-EC0 >= 3000 mg/l
Test substance: sodium benzoate; purity not noted
Flag: Critical study for SIDS endpoint
10-AUG-2001 (35)

4. ECOTOXICITY

DATE: 10-AUG-2001

SUBSTANCE ID: 532-32-1

Type:
Species: other bacteria: *Micrococcus luteus*
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
EC50: 500
Method: other: static, 22 degree C, pH 7
Year: GLP:
Test substance:
Remark: 7 d-EC0 500 mg/l
10-AUG-2001 (35)

Type:
Species: other bacteria: *Sarcina lutea*
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
EC50: < 100
Method: other: static, 22 degree C, pH 7
Year: GLP:
Test substance:
Remark: 7 d-EC0 1000 mg/l
10-AUG-2001 (35)

4.5 Chronic Toxicity to Aquatic Organisms**4.5.1 Chronic Toxicity to Fish**

Species:
Endpoint:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: No data. Based on the low acute toxicity and the readily biodegradation no relevant chronic toxicity is expected.
10-AUG-2001

4.5.2 Chronic Toxicity to Aquatic Invertebrates

Species:
Endpoint:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: No data.
10-AUG-2001

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Soil Dwelling Organisms

Type:
Species:
Endpoint:
Exposure period:
Unit:
Method:
Year: GLP:
Test substance:
Remark: No data.
10-AUG-2001

4.6.2 Toxicity to Terrestrial Plants

Species:
Endpoint:
Expos. period:
Unit:
Method:
Year: GLP:
Test substance:
Remark: No data.
10-AUG-2001

4.6.3 Toxicity to other Non-Mamm. Terrestrial Species

Species:
Endpoint:
Expos. period:
Unit:
Method:
Year: GLP:
Test substance:
Remark: No data.
10-AUG-2001

4.7 Biological Effects Monitoring

Remark: No data.
10-AUG-2001

4.8 Biotransformation and Kinetics

Type:
Remark: Rapid absorbtion and metabolisation and excretion. Conjugation with glycine and excreted in urine as hippuric acid.
10-AUG-2001

4.9 Additional Remarks

Remark: Carcinogenicity in fishes (*Oryzias latipes*): no tumor incidence up to concentration of 80000 mg/kg in food (ca. 8 g sodium benzoate salt/kg fish and day); proliferation of tissue in the bile-duct (observation period 24 weeks) 13/50 fishes died after an exposure period of 12-24 weeks
23-OCT-1995 (36)

Remark: Toxicity to fungi: MIC:
100 mg/l (*Talaromyces flavus*, 35 d, pH 3.5)
> 600 mg/l (*Talaromyces flavus*, 35 d, pH 5.4)
23-OCT-1995 (37)

Remark: Toxicity to fungi: MIC (at room temperature):
100 mg/l (*Byssoschlamys fulva*, 16 d, pH 3.5)
23-OCT-1995 (38)

Remark: Toxicity to fungi: Depending on temperature (21, 30 oder 37 degree C) and concentration of sodium benzoate (0, 200, 300, 400 oder 500 mg/l) production of biomass by *Byssoschlamys nivea* was reduced in apple- and grapefruit juice up to an exposure period of 105 days.
23-OCT-1995 (39) (40)

Remark: Toxicity to fungi:
no visible growth of:
Saccharomyces cerevisiae Willia anomala Penicillium glaucum
pH 2.6 200 mg/l 120 mg/l 600 mg/l
5 2000 mg/l 1000 mg/l 4000 mg/l
7 30000 mg/l 20000 mg/l 60000 mg/l
Method: n.a.
Test duration: n.a.

4. ECOTOXICITY

DATE: 10-AUG-2001

SUBSTANCE ID: 532-32-1

23-OCT-1995

(41)

Remark:

Toxicity to yeast:

no visible growth of:

Saccharomyces ellipsoideus pH 3.5 500 mg/l
5.0 5000 mg/l
6.5 >25000 mg/l

Method: n.a.

Test duration: n.a.

23-OCT-1995

(42)

5.1 Acute Toxicity**5.1.1 Acute Oral Toxicity**

Type: LD50
 Species: rat
 Strain: no data
 Sex: male/female
 Number of Animals: 5
 Vehicle: water
 Value: = 3450 mg/kg bw
 Method: other: see below
 Year: GLP: no data
 Test substance: other TS: USP Sodium benzoate, purchased from Merck
 Method: 5 animals/sex/group; animals did not fast prior to treatment; animals observed for 14 days.
 Reliability: (2) valid with restrictions
 Meets generally accepted scientific standards, well documented and acceptable for assessment
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (43)

Type: LD50
 Species: rat
 Strain: Sherman
 Sex: no data
 Number of Animals: 6
 Vehicle: no data
 Value: = 4070 mg/kg bw
 Method: other: see below
 Year: GLP: no
 Test substance: other TS: sodium benzoate, purity not noted
 Method: Groups of 6 rats were given single oral doses differing by a factor of 10. Animals were observed for morbidity and mortality.
 Reliability: (2) valid with restrictions
 Meets generally accepted scientific standards, well documented and acceptable for assessment
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (44)

Type: LD50

Species: rat
 Strain:
 Sex:
 Number of Animals:
 Vehicle:
 Value: = 3140 mg/kg bw
 Method: Directive 84/449/EEC, B.1 "Acute toxicity (oral)"
 Year: GLP: no data
 Test substance: other TS: sodium benzoate, purity not noted
 Reliability: (1) valid without restriction
 Guideline study
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (45)

Type: LD50
 Species: rat
 Strain:
 Sex: male/female
 Number of Animals: 70
 Vehicle:
 Value: = 2100 mg/kg bw
 Method:
 Year: GLP: no data
 Test substance: other TS: USP Sodium benzoate, purchased from Merck
 Method: Animals fasted 18 h prior to treatment; dosed by gavage; observed for 5 days.
 Reliability: (2) valid with restrictions
 30-JAN-2001 (43)

5.1.2 Acute Inhalation Toxicity

Type:
 Species:
 Strain:
 Sex:
 Number of Animals:
 Vehicle:
 Exposure time:
 Value:
 Method:
 Year: GLP:
 Test substance:

Remark: See IUCLID dataset on benzoic acid
(CAS# 65-85-0); the loss of acidity due to the
sodium salt should decrease toxicity.

10-AUG-2001

5.1.3 Acute Dermal Toxicity

Type:
Species:
Strain:
Sex:
Number of
Animals:

Vehicle:

Value:

Method:

Year:

GLP:

Test substance:

Remark: See IUCLID dataset on benzoic acid
(CAS# 65-85-0); the loss of acidity due to the
sodium salt should decrease toxicity.

10-AUG-2001

5.1.4 Acute Toxicity, other Routes

Type: LD50

Species: rat

Strain:

Sex:

Number of
Animals:

Vehicle:

Route of admin.: i.v.

Value: = 1714 mg/kg bw

Method:

Year:

GLP:

Test substance:

10-AUG-2001

(46)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit

Concentration:

Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: not irritating
EC classificat.:
Method: OECD Guide-line 404 "Acute Dermal
Irritation/Corrosion"
Year: 1981 GLP: yes
Test substance: other TS: sodium benzoate; purity not noted
Reliability: (1) valid without restriction
GLP guideline study
Flag: Critical study for SIDS endpoint
10-AUG-2001 (47)

Species: rabbit
Concentration:

Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: not irritating
EC classificat.:
Method: other: see remarks
Year: GLP:
Test substance: other TS: sodium benzoate; purity not noted
Remark: application of dry powder (500 mg/animal) for
24 h; responses were scored at end of
treatment and after 48 h
Flag: Critical study for SIDS endpoint
10-AUG-2001 (48)

Species: rat
Concentration:

Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: irritating
EC classificat.:

Method: other: intradermal; see remark
 Year: GLP:
 Test substance: other TS: sodium benzoate; purity not noted
 Remark: sodium benzoate (dose 0.1 ml; 0, 10, 20 %
 saline solution) was tested for intradermal
 irritation in male Wistar rats.
 Radioactive indicator was used to quantify the
 biological response (increase of permeability
 of blood capillaries).
 At low concentrations (1 %) little irritation
 and at higher levels (≥ 3 %) significant
 irritation was recorded. The degree of
 irritation was dose-dependent.

10-AUG-2001 (49)

5.2.2 Eye Irritation

Species: rabbit
 Concentration:
 Dose:
 Exposure Time:
 Comment:
 Number of
 Animals:
 Result: slightly irritating
 EC classificat.:
 Method: OECD Guide-line 405 "Acute Eye
 Irritation/Corrosion"
 Year: 1987 GLP: yes
 Test substance: other TS: sodium benzoate; purity not noted
 Remark: according to EEC Directive 84/449/EEC, Annex V
 of the EEC Directive 67/548/EEC no labelling
 as eye irritant
 Draize score: 9.3
 Reliability: (1) valid without restriction
 GLP guideline study
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (50)

Species: rabbit
 Concentration:
 Dose:
 Exposure Time:
 Comment:
 Number of
 Animals:
 Result: not irritating

EC classificat.:

Method: Directive 84/449/EEC, B.5 "Acute toxicity (eye irritation)"

Year:

GLP:

Test substance: other TS: sodium benzoate; purity not noted
 Remark: application of dry powder (50 mg/animal) for 24 h; responses were scored at 24 h, 48 h and 72 h; postexposure observation time: 7 d

Reliability: (1) valid without restriction

Guideline study

Flag: Critical study for SIDS endpoint

10-AUG-2001

(48)

5.3 Sensitization

Type: Patch-Test

Species: human

Number of
Animals:

Vehicle:

Result:

Classification:

Method: other: patch-test

Year:

GLP:

Test substance: other TS: sodium benzoate; purity not noted
 Remark: 5 of 2045 patients of dermatological clinics developed positive reactions to the treatment with 5% sodium benzoate in petrolatum.

Flag: Critical study for SIDS endpoint

10-AUG-2001

(51)

Type: Patch-Test

Species: human

Number of
Animals:

Vehicle:

Result:

Classification:

Method: other: patch-test

Year:

GLP:

Test substance: other TS: sodium benzoate; purity not noted
 Remark: 3 workers of a pharmaceutical plant with transient urticaria after exposition to sodium benzoate and 3 previously unexposed healthy control subjects were tested.

All subjects reacted to benzoic acid at 0.25 % in aqueous solution under occlusion. 1 worker and 2 controls reacted to sodium benzoate at 0.5 % in saline under occlusion, but none reacted to sodium benzoate at 0.5 % in aqueous solution.

All 3 workers reacted in a closed patch test to benzoic acid at 5 % in petrolatum. The time course of the responses to benzoic acid and sodium benzoate was similar in controls and workers.

The potential of sodium benzoate to elicit nonimmunologic contact urticaria may be due to the formation of benzoic acid at skin contact.

Flag: Critical study for SIDS endpoint
10-AUG-2001 (52)

Type: other: oral provocation test
Species: human
Concentration: Challenge 100 other: mg other: oral
Number of Animals: 81
Vehicle:
Result: not sensitizing
Classification: not sensitizing
Method: other
Year: GLP: no
Test substance: other TS: sodium benzoate; purity not noted
Remark: Oral challenge test: double blind challenge; 81 persons who claimed to suffer from a food-related intolerance. No sensitisation found.

Flag: Critical study for SIDS endpoint
10-AUG-2001 (53) (54)

Type: other
Species: human
Concentration: Challenge 50 other: mg other: oral
Challenge 500 other: mg other: oral
Number of Animals:
Vehicle: other: none
Result: ambiguous
Classification:
Method: other: oral challenge
Year: GLP: no
Test substance: other TS: sodium benzoate; purity not noted

5. TOXICITY

DATE: 10-AUG-2001

SUBSTANCE ID: 532-32-1

Remark: Various oral challenge tests; patients suffering from asthma or rhinitis dosed with 50-500 mg benzoic acid sodium salt orally. Result : 15/157; 11/531; 10/46 positive
10-AUG-2001 (55) (56)

Type: other: see remarks
Species: human

Number of
Animals:

Vehicle:

Result:

Classification:

Method:

other: double-blind oral challenge test

Year:

GLP:

Test substance:

Remark: A patient with Melkersson-Rosenthal syndrome reacted positive to sodium benzoate (50 mg). no further information available

10-AUG-2001 (57)

Type: other: see remarks
Species: human

Number of
Animals:

Vehicle:

Result:

Classification:

Method:

other: gastric challenge test

Year:

GLP:

Test substance:

Remark: in a double-blind placebo-controlled study 25 children with severe atopic dermatitis were challenged with food and food additives, applied by nasogastric tube. 3/6 patients challenged with sodium benzoate showed a response. Reactions were exacerbations of isolated skin symptoms in all 3 and additionally abdominal pain in association with rash in one child.

10-AUG-2001 (58)

Type: other: see remarks
Species: human

Number of
Animals:

Vehicle:

5. TOXICITY

DATE: 10-AUG-2001

SUBSTANCE ID: 532-32-1

Result:

Classification:

Method: other: oral challenge test

Year: GLP:

Test substance:

Remark: in 21 patients (5-64 years old) with severe atopic eczema oral challenge tests with food additives were performed.
4/19 patients reacted to sodium benzoate (10, 50, 100, 300 mg; administered in gelatine capsules) with exacerbation of symptoms (flare up of atopic eczema, anaphylactoid reactions, generalized pruritus).

10-AUG-2001

(59)

Type: other: see remarks

Species: human

Number of
Animals:

Vehicle:

Result:

Classification:

Method: other: oral provocation test

Year: GLP:

Test substance:

Remark: a chemical worker suffered from allergic reactions of increasing intensity while being constantly exposed to benzoic acid during work. After oral exposure to sodium benzoate (500 mg) he suffered a severe anaphylactic shock. He showed similar but milder reaction after consuming food containing benzoic acid.

10-AUG-2001

(60)

Type: other: see remarks

Species: human

Number of
Animals:

Vehicle:

Result:

Classification:

Method: other: oral provocation test

Year: GLP:

Test substance:

Remark: In a 19-year-old girl with no medical history apart from atopic asthma during infancy, a severe anaphylaxis was observed after eating food which mainly contained sodium benzoate as food additive. The patient remained symptom-free during a sodium benzoate free diet. In the oral provocation test (single oral application of 20 mg sodium benzoate) a localized urticaria (arms) and generalised itching was observed. In a second oral challenge (application of 160 mg sodium benzoate), a higher tolerance level was noted.

10-AUG-2001 (61)

Type: other: see remarks
Species: human
Number of Animals:
Vehicle:
Result:
Classification:
Method: other: oral provocation test
Year: GLP:
Test substance:
Remark: after a single oral application of 20 mg sodium benzoate, 2/10 patients with asthma and 2/7 patients with atopic dermatitis reacted positive; observed were bronchial obstruction/meteorism, nausea or dermatitis resp.

10-AUG-2001 (62)

5.4 Repeated Dose Toxicity

Species: rat Sex: male/female
Strain: Sherman
Route of admin.: oral feed
Exposure period: 90 d
Frequency of treatment: continuously in diet
Post. obs. period: no
Doses: 1, 2, 4 or 8 % in diet (approx. 640-6290 mg/kg/day)
Control Group: yes
NOAEL: 3145 mg/kg bw

LOAEL:	6290 mg/kg bw	
Method:	other: see below	
Year:		GLP: no data
Test substance:	other TS: USP sodium benzoate purchased from Merck	
Method:	Male rats (weighing 212 -430 grams) and female rats (weighing 163 to 267 grams) were dosed by gavage after being fasted for 18 hours. Animals were observed for 5 days (time interval chosen because all survivors were gaining weight and in "satisfactory nutritional condition").	
Result:	<= 4 % in diet: no adverse effects; 8 % in diet: increased mortality (4/8 died); reduced weight gain; increased weight of livers and kidneys; pathological lesions(not specified) in livers and kidneys	
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(43)
Species:	rat	Sex: male/female
Strain:	Sherman	
Route of admin.:	oral feed	
Exposure period:	30 d	
Frequency of treatment:	continuously in diet	
Post. obs. period:	no data	
Doses:	16-1090 mg/kg/day	
Control Group:	yes	
NOAEL:	> 1090 mg/kg	
Method:	other: see below	
Year:		GLP: no data
Test substance:	other TS: sodium benzoate, purity not noted	
Method:	Groups of 10 rats (5 males, 5 females) were administered doses of sodium benzoate by oral feed for thirty days. Animals were observed for weight gain, appetite, morbidity and mortality. Surviving animals were necropsied. Adrenal, upper intestine, kidney, liver, and spleen were examined.	
Remark:	10 rats/group This endpoint has been studied several times by several other investigators/groups and all reported results similar to the study mentioned above.	

Result:	No adverse effects were observed.	
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment	
Flag:	Critical study for SIDS endpoint	(63) (44)
10-AUG-2001		
Species:	mouse	Sex: male/female
Strain:	other: Albino Swiss	
Route of admin.:	drinking water	
Exposure period:	35 days	
Frequency of treatment:	continuously in drinking water	
Post. obs. period:	no data	
Doses:	0.5; 1; 2; 4 or 8 % in drinking water	
Control Group:	yes	
NOAEL:	2 %	
LOAEL:	4 %	
Method:	other: Toth, B. (1984)	
Year:		GLP: no data
Test substance:	other TS: sodium benzoate, purity not noted	
Remark:	"By taking into account four parameters (survival rate, body weight, chemical consumption, histological changes), the 2% dose level was found suitable for the lifelong treatment."	
Result:	8 %: 4/4 males and 4/4 females died within 3 weeks; 4 %: 3/4 males and 3/4 females died during the treatment period and the body weight of surviving mice was substantially reduced.	
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment	
Flag:	Critical study for SIDS endpoint	(64)
10-AUG-2001		
Species:	rat	Sex: male/female
Strain:	other: F344/Ducrj	
Route of admin.:	oral feed	
Exposure period:	10 d	
Frequency of treatment:	continuously in diet	
Post. obs. period:	no	
Doses:	1.81; 2.09 or 2.4 % in diet (approx. 1358, 1568 or 1800 mg/kg/d)	
Control Group:	yes	

LOAEL:	1358 mg/kg bw	
Method:		
Year:		GLP:
Test substance:	other TS: sodium benzoate, purity not noted	
Remark:	The mean compound consumption was calculated according to Lehman, Food Drug Off. Q. Bull. 18, 66 (1954).	
Result:	At the lowest tested concentration of 1358 mg/kg changes in serum chlolesterol levels occurred in females. At doses of 1568 mg/kg and above changes in further serum parameters and an increased relative liver weight were described. Histopathological changes of the liver, increased relative kidney weights and disorders of the central nervous system were seen after dosing via diet with > 1800 mg. 1/6 male rat in the 2.4 %-group, who developed increased sensitivity to stimuli and convulsions, died.	
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment.	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(65)
Species:	mouse	Sex: male/female
Strain:	B6C3F1	
Route of admin.:	oral feed	
Exposure period:	10 d	
Frequency of treatment:	continuously in diet	
Post. obs. period:	no	
Doses:	2.08; 2.5 or 3 % in diet (approx. 3012, 3750 or 4500 mg/kg/d)	
Control Group:	yes	
NOAEL:	3750 mg/kg bw	
LOAEL:	4500 mg/kg bw	
Method:	other: see below	
Year:		
GLP:	no data	
Test substance:	other TS: sodium benzoate (specific grade) purchased from Wako Pure Chemical Ind., Osaka, Japan	
Method:	Sodium benzoate, mixed with the powdered diet, was fed to groups of 12 mice (6 males, 6 females) for 10 days.	

Animals were observed for body weight gain and clinical signs 5 day/ week.
At the end of the experiment, surviving animals were necropsied. Organ weights, clinical chemistry and histological examinations were performed.

Remark: The mean compound consumption was calculated according to Lehman, Food Drug Off. Q. Bull. 18, 66 (1954).

Result: All mice in the 3.0 %-group showed increased sensitivity to stimuli and 1/5 male and 2/5 females showed convulsions; 2/5 females died; liver weights of males and females and kidney weights of females were dose-dependently increased; histopathologic examination showed enlarged hepatocytes, single cell necrosis and vacuolation of hepatocytes in all livers from males; no histopathologic changes of the kidney were described; serum cholesterol, lipid levels and cholinesterase were increased in males.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
10-AUG-2001 (65)

Species: rat Sex: male/female
Strain: Fischer 344
Route of admin.: oral feed
Exposure period: 18-24 months
Frequency of treatment: continuously in diet
Post. obs. period: no
Doses: 1 or 2 % in diet
Control Group: yes
NOAEL: 2 %
Method: other: OECD 451
Year: GLP: no data
Test substance: other TS: sodium benzoate, purity not noted

Remark: Mean compound consumption:
2 % in diet: m: 280 +- 9.8 mg/d
f: 202 +- 10.5 mg/d

Result: No adverse clinical signs in treated rats; no differences in average body weight and mortality in comparison to controls.

Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment	
Flag:	Critical study for SIDS endpoint	(66)
10-AUG-2001		
Species:	rat	Sex: male/female
Strain:	Sherman	
Route of admin.:	oral feed	
Exposure period:	28 d	
Frequency of treatment:	continuously in diet	
Post. obs. period:		
Doses:	2 or 5 % in diet (see remarks)	
Control Group:	other: no data	
LOAEL:	2002 - 2357 mg/kg bw	
Method:	other: see below	
Year:		GLP: no data
Test substance:	other TS: sodium benzoate, food grade	
Method:	Food grade sodium benzoate was incorporated into the basal diet at concentrations of 2% and 5%. The rats were weighed individually twice a week and were inspected daily for signs of toxicity. Food consumption for each group was recorded weekly and the drug intake as mg/kg bw was calculated using the average body weights for each group. Fisher's T test for small samples was used as a test for significant differences between body weights for the various groups.	
Remark:	6 rats/group; initial body weight: 40-50 g; mean compound consumption: 2 % in diet: m: 2002 - 2357 mg/kg/day f: 2171 - 2396 mg/kg/day 5 % in diet: m: 5686 mg/kg/day f: 7780 mg/kg/day	
Result:	2 %: slight depression of body weight gain only in males 5 %: urine incontinence, convulsions, 100 % mortality after 2nd week	
Reliability:	(3) invalid Significant methodological deficiencies	
10-AUG-2001		(67)
Species:	rat	Sex: male/female
Strain:	Fischer 344	
Route of admin.:	oral feed	
Exposure period:	42 d	

Frequency of treatment: continuously in diet
 Post. obs. period: no data
 Doses: 0.5; 1; 2; 4 or 8 % in diet (approx. 375-6000 mg/kg/day)
 Control Group: yes
 Method: other: see below
 Year: GLP:
 Test substance: other TS: sodium benzoate, purity not noted; supplied by National Institute of Hygienic Sciences pellets in the basal diet
 Method: 10 rats/group; initial body weight: 110-150 g; the mean compound consumption was calculated according to Lehman, Food Drug Off. Q. Bull. 18, 66 (1954); Animals were administered diets containing various concentrations of sodium benzoate for 6 weeks. Survival rate, growth, food intake, behavior and general status were observed during the feeding period. Morphological examinations were carried out.
 Result: 2 % in diet (approx. 1500 mg/kg/day): maximum tolerated dose;
 >= 4 % in diet (approx. >= 3000 mg/kg/day): mortality 10/11 or 10/10; atrophy of the spleen and lymph nodes at autopsy.

10-AUG-2001 (66)

Species: rat Sex: no data
 Strain: no data
 Route of admin.: oral feed
 Exposure period: until death (see below)
 Frequency of treatment: continuously in diet
 Post. obs. period: no
 Doses: 5 % in diet (approx. 3750 mg/kg/day)
 Control Group: yes
 Method:
 Year: GLP:
 Test substance: other TS: benzoic acid
 Remark: the mean compound consumption was calculated according to Lehman, Food Drug Off. Q. Bull. 18, 66 (1954)
 Result: 19/28 young rats (initial body weight: 62-70 g) died during the first 2 weeks; all others died 1 week later; reduced food intake, diarrhea, intestinal haemorrhage and crusted blood in the nose at autopsy.

10-AUG-2001

(68)

Species: rat Sex: no data
Strain: no data
Route of admin.: oral feed
Exposure period: no data
Frequency of treatment: continuously in diet
Post. obs. period: no data
Doses: 5 % in diet (approx. 3750 mg/kg/day)
Control Group: other: no data
Method:
Year: GLP:
Test substance: other TS: benzoic acid
Remark: the mean compound consumption was calculated according to Lehman, Food Drug Off. Q. Bull. 18, 66 (1954)
Result: 4/5 adult rats (initial body weight: 221-232 g) died during 4-5 weeks; body weight was reduced to 161 g

10-AUG-2001

(68)

Species: rat Sex: male
Strain: no data
Route of admin.: oral feed
Exposure period: 23 weeks
Frequency of treatment: continuously in diet
Post. obs. period: no
Doses: 5 % in diet (approx. 3750 mg/kg/d)
Control Group: yes
Method:
Year: GLP:
Test substance: other TS: sodium benzoate, purity not noted
Remark: Basic diet: low casein diet; the study was done to investigate the effect of several xenobiotics on the growth retardation provoked in rats by sodium benzoate; the data presented here are the results of the "long-term" positive control group.
The mean compound consumption was calculated according to Lehman, Food Drug Off. Q. Bull. 18, 66 (1954).
Result: marked growth inhibition, occasionally restlessness, irritability, tremors

10-AUG-2001

(69)

5. TOXICITY

DATE: 10-AUG-2001

SUBSTANCE ID: 532-32-1

Species:	dog	Sex: male/female
Strain:	other: fox terrier	
Route of admin.:	oral feed	
Exposure period:	<= 250 days	
Frequency of treatment:	once daily	
Post. obs. period:		
Doses:	0.1 - 7 g/animal/day	
Control Group:	other: no data	
Method:		
Year:		GLP:
Test substance:		
Result:	0.1 - < 7 g/animal/day: no toxic effect; 7 g/animal/day (approx. 1 g/kg/day): toxic dose (ataxia, tonoclonic convulsions, vomiting, death)	

26-JAN-2001

(70)

5.5 Genetic Toxicity 'in Vitro'

Type:	Ames test	
System of testing:	Salmonella typhimurium TA 92, TA 94, TA 98, TA 100, TA 1535, TA 1537	
Concentration:	0-3 mg/plate	
Cytotoxic Conc.:		
Metabolic activation:	with and without	
Result:	negative	
Method:	OECD Guide-line 471 "Genetic Toxicology: Salmonella thyphimurium Reverse Mutation Assay"	
Year:	1983	GLP: no data
Test substance:	other TS: samples obtained from Japan Food Additives Association; purity = 99% analysed at Ministry of Health and Welfare of Japan	
Remark:	This endpoint has been studied by several other investigators/groups and all support the result of the study mentioned above.	
Reliability:	(1) valid without restriction Guideline study	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(71) (72)

Type:	Cytogenetic assay
System of testing:	cultured human embryonic lung cells

Concentration: 2.0, 20.0, 200.0 ug/ml
 Cytotoxic Conc.:
 Metabolic
 activation: without
 Result: negative
 Method: other: anaphase preparations
 Year: GLP: no data
 Test substance: other TS: FDA 71-37 supplied by Food and Drug Administration
 Remark: This endpoint has been studied by several other investigators/groups and all support the result of the study mentioned above.
 Reliability: (2) valid with restrictions
 Meets generally accepted scientific standards, well documented and acceptable for assessment
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (73) (74)

Type: other: Chromosomal aberration test
 System of
 testing: Chinese hamster fibroblast cell line (CHL)
 Concentration: 0 - 2 mg/plate
 Cytotoxic Conc.:
 Metabolic
 activation: without
 Result: positive
 Method: other: similar to OECD Guideline 473
 Year: 1983 GLP: no data
 Test substance: other TS: samples obtained from Japan Food Additives Association; purity = 99% analysed at Ministry of Health and Welfare of Japan

Reliability: (2) valid with restrictions
 Comparable to Guideline study with acceptable restrictions
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (75) (72)

Type: other: E. coli reversion mutation assay
 System of
 testing: E. coli WP2
 Concentration: no data
 Cytotoxic Conc.: no data
 Metabolic
 activation: with and without
 Result: negative
 Method: EPA OTS 798.5100
 Year: GLP: no data
 Test substance: other TS: sodium benzoate, purchased from Baker; purity not noted

Reliability:	(1) valid without restriction Guideline study	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(76)
Type:	other: Sister chromatid exchange	
System of testing:	Chinese hamster cell line (Don)	
Concentration:	0.001 to 0.01 M / plate	
Cytotoxic Conc.:	no data	
Metabolic activation:	without	
Result:	ambiguous	
Method:	other: see below	
Year:		GLP: no data
Test substance:	other TS: sodium benzoate, supplied by National Institute of Hygienic Sciences, Japan; purity not noted	
Method:	Sodium benzoate was dissolved in Hank's balanced salt solution to desired concentrations. All cultures were kept in complete darkness at 37 degree C for 26 hours (two cell cycles) and 0.25 ug colchicine/ml added for final 2 hours. Cells were collected and stained by acridine orange technique for fluorescence or modified FPG (fluorecence plus Giemsa) for Giemsa.	
Remark:	The number of SCE per cell was determined on the basis of 20-50 intact metaphases without gross chromosome aberrations. slight increase in SCE/cell, but no dosage effect	
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(77)
Type:	other: Sister chromatid exchange	
System of testing:	human lymphocytes	
Concentration:		
Cytotoxic Conc.:		
Metabolic activation:	without	
Result:	positive	
Method:		
Year:		GLP:
Test substance:		
Remark:	only one concentration (10E-2 M) tested	

Reliability: (3) invalid
 Significant methodological deficiencies
 Flag: Critical study for SIDS endpoint
 10-AUG-2001
 (78)

Type: other: Inhibition of DNA synthesis
 System of testing: Vicia faba root meristems
 Concentration:
 Cytotoxic Conc.:
 Metabolic
 activation: without
 Result: positive
 Method:
 Year: GLP:
 Test substance:
 Remark: other observed effects:
 a. concentration-dependent decrease in mitotic figures;
 b. concentration-dependent increase in anaphase bridges;
 c. premature chromosome condensation heading to pycnotic nuclei; d. chromatin erosion in interphase nuclei

Reliability: (3)invalid
 Unsuitable test system
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (79)

Type: Bacillus subtilis recombination assay
 System of testing: Bacillus subtilis H17, M45
 Concentration:
 Cytotoxic Conc.:
 Metabolic
 activation: no data
 Result: positive
 Method:
 Year: GLP:
 Test substance: no data
 Method: An overnight culture of B. subtilis, H17 and M45, was mixed with test solutions and incubated for 30 minutes at 37 degree C. After treatment, viable cells were counted and the ratio of 50% survival concentrations were calculated.
 Result: Sodium benzoate showed DNA damaging potential although it had been negative in the Ames test.

Reliability:	(4) not assignable Documentation insufficient for assessment; abstract only	
Flag:	Critical study for SIDS endpoint	(80)
10-AUG-2001		
Type:	Bacillus subtilis recombination assay	
System of testing:	Bacillus subtilis H17, M45	
Concentration:	6-20 mg/disk, in water	
Cytotoxic Conc.:		
Metabolic activation:	with and without	
Result:	ambiguous	
Method:		
Year:		GLP:
Test substance:		
10-AUG-2001		(81)
Type:	Ames test	
System of testing:	Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538	
Concentration:		
Cytotoxic Conc.:		
Metabolic activation:	with and without	
Result:	negative	
Method:		
Year:		GLP:
Test substance:		
11-JAN-2001		(76)
Type:	Ames test	
System of testing:	Salmonella typhimurium, TA 98, TA100, TA1537	
Concentration:		
Cytotoxic Conc.:		
Metabolic activation:	with and without	
Result:	negative	
Method:		
Year:		GLP:
Test substance:		
01-SEP-2000		(82)
Type:	other: Chromosomal aberration test	
System of testing:	Chinese hamster cell line (Don)	
Concentration:		

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Cytotoxic Conc.:

Metabolic

activation: without
Result: positive

Method:

Year: GLP:

Test substance:

11-JAN-2001 (77)

Type: other: Chromosome aberration test

System of

testing: Chinese hamster fibroblast cell line (CHL)

Concentration:

Cytotoxic Conc.:

Metabolic

activation: with

Result: positive

Method:

Year: GLP:

Test substance: other TS: purity not given

Method: other: Ishidate M. and Odashima S. Mutation
Res. 48: 337-354(1977) and Matsuoka A. et al.
Mutation Res. 66: 277-290 (1979)

01-SEP-2000 (82)

Type: other: Sister chromatid exchange

System of

testing: Vicia faba root tip cells

Concentration:

Cytotoxic Conc.:

Metabolic

activation: without
Result: positive

Method:

Year: GLP:

Test substance:

Remark: only one concentration (10E-2 M) tested

11-JAN-2001 (78)

5.6 Genetic Toxicity 'in Vivo'

Type: Cytogenetic assay

Species: rat

Sex: male

Strain: no data

Route of admin.: gavage

Exposure period: single application

Doses: 50, 500 or 5000 mg/kg

Result: negative

Method:	EPA OTS 798.5385	
Year:		GLP: yes
Test substance:	other TS: compound FDA 71-37, sodium benzoate, as supplied by the Food and Drug Administration	
Result:	no detectable significant aberrations of the bone marrow metaphase chromosomes	
Reliability:	(1) valid without restriction GLP guideline study	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(74)
Type:	Cytogenetic assay	
Species:	rat	Sex: male
Strain:	no data	
Route of admin.:	gavage	
Exposure period:	once daily for 5 consecutive days	
Doses:	50, 500 or 5000 mg/kg	
Result:	negative	
Method:	EPA OPPTS 870.5385	
Year:		GLP: yes
Test substance:	other TS: compound FDA 71-37, sodium benzoate, as supplied by the Food and Drug Administration	
Result:	no detectable significant aberrations of the bone marrow chromosomes	
Reliability:	(1) valid without restriction GLP guideline study	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(74)
Type:	Dominant lethal assay	
Species:	rat	Sex: male
Strain:	no data	
Route of admin.:	gavage	
Exposure period:	single application	
Doses:	50, 500 or 5000 mg/kg	
Result:	negative	
Method:	EPA OPPTS 870.5450	
Year:		GLP: yes
Test substance:	other TS: compound FDA 71-37, sodium benzoate, as supplied by the Food and Drug Administration	
Reliability:	(1) valid without restriction GLP guideline study	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(74)

Type:	Dominant lethal assay	
Species:	rat	Sex: male
Strain:	no data	
Route of admin.:	gavage	
Exposure period:	once daily for 5 consecutive days	
Doses:	50, 500 or 5000 mg/kg	
Result:	negative	
Method:	EPA OPPTS 870.5450	
Year:		GLP: yes
Test substance:	other TS: compound FDA 71-37, sodium benzoate, as supplied by the Food and Drug Administration	
Reliability:	(1) valid without restriction GLP guideline study	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(74)

Type:	other: Host mediated assay	
Species:	mouse	Sex: male
Strain:	no data	
Route of admin.:	gavage	
Exposure period:	single application	
Doses:	50, 500 or 5000 mg/kg	
Result:	negative	
Method:	other: EPA	
Year:		GLP: yes
Test substance:	other TS: compound FDA 71-37, sodium benzoate, as supplied by the Food and Drug Administration	
Result:	No elevation of mutant frequencies in Salmonella Typhimurium G46 and no increase in recombinant frequencies in Saccharomyces cerevesiae D3	
Reliability:	(1) valid without restriction GLP guideline study	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(74)

Type:	other: Host mediated assay	
Species:	mouse	Sex: male
Strain:	no data	
Route of admin.:	gavage	
Exposure period:	single application	
Doses:	50, 500 or 5000 mg/kg	
Result:	negative	
Method:	other: EPA	
Year:		GLP: yes
Test substance:	other TS: compound FDA 71-37, sodium benzoate, as supplied by the Food and Drug Administration	

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Result: elevation of mutant frequencies in *Salmonella typhimurium* TA 1530 in the intermediate dose level; Not dose dependent and negative at multiple dose exposure.

Reliability: (1) valid without restriction
GLP guideline study

Flag: Critical study for SIDS endpoint
10-AUG-2001 (74)

Type: other: Host mediated assay

Species: mouse Sex: male

Strain: no data

Route of admin.: gavage

Exposure period: once daily for 5 consecutive days

Doses: 50, 500 or 5000 mg/kg

Result: negative

Method: other: EPA

Year: GLP: yes

Test substance: other TS: compound FDA 71-37, sodium benzoate, as supplied by the Food and Drug Administration

Result: no elevation of mutant frequencies in *Salmonella Typhimurium* G46; no elevation of mutant frequencies in *Salmonella typhimurium* TA 1530; no increase in recombinant frequencies in *Saccharomyces cerevisiae* D3

Reliability: (1) valid without restriction
GLP guideline study

Flag: Critical study for SIDS endpoint
10-AUG-2001 (74)

5.7 Carcinogenicity

Species: rat Sex: male/female

Strain: Fischer 344

Route of admin.: oral feed

Exposure period: 18-24 months

Frequency of treatment: continuously in diet

Post. obs. period: no

Doses: 1 or 2 % in diet (see remarks)

Result: negative

Control Group: yes

Method: OECD Guide-line 451 "Carcinogenicity Studies"

Year: GLP: no data

Test substance: other TS: sodium benzoate, purity not noted

Method:	Groups of 50 male and 52 female Fischer 344 rats, four to five weeks old, received diets containing 1% (500 mg/kg bw per day) or 2% (1000 mg/kg bw per day) sodium benzoate for 18-24 months. Controls, consisting of 25 male and 43 female rats, received basal diet.	
	Food intake was adequately controlled to avoid an excess; tap water was available ad libitum.	
	Mean compound consumption:	
	1 % in diet: m: 141 +- 9.7 mg/d	
	f: 102 +- 11.8 mg/d	
	2 % in diet: m: 280 +- 9.8 mg/d	
	f: 202 +- 10.5 mg/d	
Remark:	about 40 rats including control animals died during the first 16 months of the experimental period (pneumonia with abscess) about 100 rats including control animals died after 16 months of hemorrhagic pneumonia (infection)	
Result:	Survival was very poor in all groups, due to intercurrent sialodacryoadenitis and mycoplasma infections. All surviving animals were sacrificed between 18 and 25 months, all were autopsied, and various tissues were examined histopathologically. No adverse clinical signs directly attributable to treatment were observed, and only negligible differences in average body weight and mortality rate were seen between the treated and control groups. Although a variety of tumours occurred among treated and control rats of each sex, they were of similar type and incidence; no evidence of carcinogenicity.	
Reliability:	(1) valid without restriction	
	Guideline study	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001	(66)	
Species:	mouse	Sex: male/female
Strain:	other: Albino Swiss	
Route of admin.:	drinking water	
Exposure period:	lifelong	
Frequency of treatment:	continuously in drinking water	
Post. obs. period:	no data	
Doses:	2 % in drinking water	
Result:	negative	
Control Group:	yes	

Method:	other: see below	
Year:		GLP: no data
Test substance:	other TS: sodium benzoate, purity not noted	
Method:	In the main study, a 2% solution of sodium benzoate (purity, 99%) was administered in the drinking-water to groups of 50 male and 50 female five-week-old mice for their lifetime. Groups of 100 males and 100 females were used as untreated controls. Both treated and control animals were 'carefully checked'; their body weights were measured weekly, and gross pathological changes were recorded. The animals were either allowed to die or were sacrificed when moribund. Complete necropsies were performed on all animals, and the liver, spleen, kidneys, bladder, thyroid, heart, pancreas, testes, ovaries, brain, nasal turbinates, at least four lobes of the lungs, and organs with gross pathological changes were examined histologically.	
Remark:	50 males and 50 females were treated; 99 males and 99 females served as controls; average daily intake: 119.2 mg (f) or 124.0 mg (m)	
Result:	The average daily intake of sodium benzoate was 124.0 mg for males and 119.2 mg for females on the basis of daily water consumption of 6.2 and 5.9 ml, respectively. The dose of sodium benzoate was equivalent to 6200 mg/kg bw per day for males and 5960 mg/kg bw per day for females. Treatment had no effect on survival or the incidence of tumours.	
Reliability:	(2) valid with restrictions This study is sufficiently reliable due to a sufficient number of animals and a detailed histopathological examination.	
Flag:	Critical study for SIDS endpoint	
10-AUG-2001		(64)
Species:	rat	Sex: male
Strain:	Fischer 344	
Route of admin.:		
Exposure period:		
Frequency of treatment:		
Post. obs. period:		
Doses:		
Result:		
Control Group:		
Method:		

Year:	GLP:
Test substance:	
Remark:	DEN-PH model; final, general protocol: Group 1: single i.p. dose of diethylnitrosamine, repeated treatment with the test compound from week 2, hepatectomy at week 3, sacrifice at week 8. Group 2: single i.p. dose of diethylnitrosamine, hepatectomy at week 3, sacrifice at week 8. Group 3: single i.p. dose of saline, repeated treatment with the test compound from week 2, sacrifice at week 8. The enhancing effects of chemicals on induction of preneoplastic form of glutathione S-transferase positive foci was measured by comparing the GST-P positive foci in liver slices of treated and control animals.
Result:	positive
26-JAN-2001	(83)

5.8 Toxicity to Reproduction

Type:	other: 2 year carcinogenicity study
Species:	rat Sex: male/female
Strain:	Fischer 344
Route of admin.:	oral feed
Exposure Period:	18 - 24 months
Frequency of treatment:	continuously in diet
Duration of test:	24 months
Doses:	1 or 2 % in diet
Control Group:	yes
NOAEL Parental:	2 %
Method:	other: OECD 451
Year:	GLP: no data
Test substance:	other TS: sodium benzoate, purity not noted
Result:	No evidence of compound related effects in the testes or ovaries of treated rats.
Reliability:	(2) valid with restrictions In the 2 yr feeding study, reproductive organs were examined macroscopically and histologically.
Flag:	Critical study for SIDS endpoint
10-AUG-2001	(66)

Species: Sex:
Strain:
Route of admin.:
Exposure Period:
Frequency of treatment:
Duration of test:
Doses:
Control Group:
Method:
Year: GLP:
Test substance:
Remark: A 4-generation reprotoxicity test with benzoic acid revealed no reproductive effects. Therefore no indication for reproductive toxicity testing for the benzoic acid sodium salt. See IUCLID on benzoic acid (CAS# 65-85-0); the data on the sodium salt should be similar.
Flag: Critical study for SIDS endpoint
10-AUG-2001

5.9 Developmental Toxicity/Teratogenicity

Species: rat Sex: female
Strain: Wistar
Route of admin.: gavage
Exposure period: Day 6-15 of gestation
Frequency of treatment: once daily
Duration of test:
Doses: 1.75; 8; 38 or 175 mg/kg/d
Control Group: yes
NOAEL Maternalt.: ≥ 175 mg/kg bw
NOAEL Teratogen.: ≥ 175 mg/kg bw
Method: EPA OPPTS 870.3700
Year: GLP: no data
Test substance: other TS: sodium benzoate, purity not noted
Remark: This endpoint has been studied several times by several other investigators/groups and all reported results similar to the study mentioned above.
Result: no effect on nidation or on maternal or fetal survival; the number of abnormalities of soft and skeletal tissues did not differ from number in controls; maternal toxicity was not reported at any dose applied

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint (84) (85)
10-AUG-2001

Species: rat Sex: female
Strain: Wistar
Route of admin.: oral feed
Exposure period: whole gestation period (20 d)
Frequency of treatment: continuously in diet
Duration of test:
Doses: 1, 2, 4 or 8 % in diet (700 to 5600 mg/kg)
Control Group: yes
NOAEL Maternalt.: = 1400 mg/kg bw
NOAEL Teratogen.: = 1400 mg/kg bw
Method: other
Year: GLP: no data
Test substance: other TS: sodium benzoate, purity not noted
Remark: The mean food consumption was calculated from graph:
 <= 2 % in diet: approx. 20 mg/kg/day
 4 % in diet: approx. 12 mg/kg/day
 8 % in diet: approx. 2.5 mg/kg/day
 The mean compound consumption was calculated from graph:
 1 % in diet: approx. 700 mg/kg/day
 2 % in diet: approx. 1400 mg/kg/day
 4 % in diet: approx. 2800 mg/kg/day
 8 % in diet: approx. 5600 mg/kg/day

Result: A study using pregnant Wistar rats, dosed with 700, 1400, 2800, 5600 mg/kg sodium benzoate in the diet during the entire gestation showed no statistical difference in organ and bone abnormalities of fetuses between experimental groups and controls; growth of treated offsprings was similar to controls in rats dosed with 1400 mg/kg/day; reduced food intake and decreased body weight of the pregnant rats especially in the 5600 mg/kg group; 100% perinatal death rate; organ abnormalities of fetuses involved eye, brain and kidneys, in addition abnormalities of the skeletal system were found in rats dosed with >2800 mg/kg/day.

Conclusion:	The authors concluded that the effects on the dams and fetuses at the 2800 and 5600 levels were due to reduced maternal feed intake in these groups, leading to malnutrition.	
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment	
Flag:	Critical study for SIDS endpoint	(86)
10-AUG-2001		
Species:	mouse	Sex: female
Strain:	CD-1	
Route of admin.:	gavage	
Exposure period:	Day 6-15 of gestation	
Frequency of treatment:	once daily	
Duration of test:		
Doses:	1.75; 8; 38 or 175 mg/kg/d	
Control Group:	yes	
NOAEL Maternalt.:	>= 175 mg/kg bw	
NOAEL Teratogen.:	>= 175 mg/kg bw	
Method:	EPA OPPTS 870.3700	
Year:		GLP: no data
Test substance:	other TS: sodium benzoate, purity not noted	
Result:	No effect on nidation or on maternal or fetal survival; the number of abnormalities of soft and skeletal tissues did not differ from controls; maternal toxicity was not reported at any dose applied.	
Reliability:	(1) valid without restriction Guideline study	
Flag:	Critical study for SIDS endpoint	(85)
10-AUG-2001		
Species:	rabbit	Sex: female
Strain:	other: Dutch-belted	
Route of admin.:	gavage	
Exposure period:	Day 6-18 of gestation	
Frequency of treatment:	once daily	
Duration of test:		
Doses:	2.5; 12; 54 or 250 mg/kg/d	
Control Group:	yes	
NOAEL Maternalt.:	250 mg/kg bw	
NOAEL Teratogen.:	250 mg/kg bw	
Method:	EPA OPPTS 870.3700	
Year:		GLP: no data

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Test substance: other TS: sodium benzoate, purity not noted
 Result: No effect on nidation or on maternal or fetal survival; the number of abnormalities of soft and skeletal tissues did not differ from controls; maternal toxicity was not reported at any dose applied.
 Reliability: (1) valid without restriction
 Guideline study
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (85)

Species: hamster Sex: female
 Strain: other: golden; outbred
 Route of admin.: gavage
 Exposure period: Day 6-10 of gestation
 Frequency of treatment: once daily
 Duration of test:
 Doses: 3, 14, 65 or 300 mg/kg/d
 Control Group: yes
 NOAEL Maternalt.: 300 mg/kg bw
 NOAEL Teratogen.: 300 mg/kg bw
 Method: EPA OPPTS 870.3700
 Year: GLP: no data

Test substance: other TS: sodium benzoate, purity not noted
 Result: No effect on nidation or on maternal or fetal survival; the number of abnormalities of soft and skeletal tissues did not differ from controls; maternal toxicity was not reported at any dose applied.
 Reliability: (1) valid without restriction
 Guideline study
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (85)

Species: rat Sex: female
 Strain: Sprague-Dawley
 Route of admin.: i.p.
 Exposure period: day 9-11 of gestation
 Frequency of treatment: once daily
 Duration of test:
 Doses: 100, 315 or 1000 mg/kg/d
 Control Group: other: sodium chloride 90 or 600 mg/kg/d
 NOAEL Teratogen.: 315 mg/kg bw
 Method:
 Year: GLP:

Test substance:
Remark: no further information available
Result: 1000 mg/kg: increased rate of in utero deaths,
reduced fetal body weight
26-JAN-2001 (87)

Species: rat Sex: female
Strain: Sprague-Dawley
Route of admin.: i.p.
Exposure period: day 12-14 of gestation
Frequency of treatment: once daily
Duration of test:
Doses: 100, 315 or 1000 mg/kg/d
Control Group: other: sodium chloride 90 or 600 mg/kg/d
NOAEL Teratogen.: 315 mg/kg bw
Method:
Year: GLP:

Test substance:
Remark: no further information available
Result: 1000 mg/kg: reduced fetal body weight,
increased rate of in utero deaths, gross
anomalies in fetuses
26-JAN-2001 (87)

Species: hen Sex:
Strain: Leghorn
Route of admin.: other
Exposure period: once
Frequency of treatment: single injection in eggs
Duration of test:
Doses: highest level tested: 5 mg/egg
Control Group: yes
Method:
Year: GLP:
Test substance:
Remark: Fresh fertile eggs were used, 4 test
conditions were used: injection via the air
cell and via the yolk twice, preincubation 0 h
and 96 h; total number of eggs treated:
approx. 100.
Result: LD50 (injection via air cell at 96 h):
4.74 mg/egg; no teratogenic effects in the
developing chicken embryo.
26-JAN-2001 (88)

Species: hen Sex:
 Strain: other: Ross I stock
 Route of admin.: other
 Exposure period: once
 Frequency of treatment: single injection
 Duration of test:
 Doses: highest level tested: 0.1 mg/embryo
 Control Group: yes
 Method: other: Chick Embryotoxicity Screening Test (CHEST)
 Year: GLP:
 Test substance:
 Result: no embryotoxicity was observed at a concentration of 100 ug/embryo
 26-JAN-2001 (89)

Species: other: chick embryo neural retina cells Sex:
 Strain:
 Route of admin.: other: in vitro
 Exposure period: 24 hours
 Frequency of treatment: single treatment
 Duration of test: 7 days
 Doses: up to cytolethal or solubility limit
 Control Group: yes
 Method: other: Chick embryo retina cell (CERC) assay
 Year: GLP:
 Test substance: other TS: purchased from Sigma Chemical
 Method: The chemical was dissolved in Gibco medium 199 or DMSO and adjusted to pH 7.2. At least five concentrations were tested, with six flasks per concentration.
 7-10 E+06 cells were dispersed in 3ml culture medium, plus the test chemical, and incubated for 18-24 hours.
 Cell aggregates were counted and the medium changed to Gibco 199 without the test chemical. The cells were cultured for an additional 6 days. Protein content was measured by the Lowry method and glutamine synthetase activity measured by a spectrophotometric assay.
 Statistics: pairwise comparisons among treatment groups were done by ANOVA and concentration-response relationships analyzed by general linear methods (SAS, 1987). A chemical was classified as active if there was a significant concentration-dependent decrease in glutamine

synthetase activity, protein content or aggregate size; or increasing trend in aggregate number and at least one concentration group that was significantly different ($p < 0.05$) from the control. Result: Sodium benzoate was classified as inactive in the CERC assay with LOEL at $>34.7\text{mM}$. 19-MAY-2000 (90)

5.10 Other Relevant Information

Type: Metabolism
Remark: The experimental study on the inducibility of the hepatic and renal hippurate-synthesizing system by gradually increasing daily i.p. doses (125-375 mg/kg, given between 17 and 21 days) of sodium benzoate to mice showed no effects. Sodium benzoate did not induce its own metabolizing system. 23-OCT-1995 (91)

Type: Metabolism
Remark: A 15 mM aqueous solution of sodium benzoate was shown to inhibit in vitro the noradrenaline-induced aggregation of platelets from healthy volunteers by blocking the cyclo-oxygenase-thromboxane enzyme system. 23-OCT-1995 (92)

Type: Metabolism
Remark: Six female volunteers (case I) and three male volunteers (case II) were orally given (case I) 33 or 66 mg sodium benzoate in a soft drink or (case II) a sodium benzoate solution at a dosage of 20 mg/kg bw.. In case I, 66 to 86 % of the administered dose was excreted in urine within 3 hours as hippuric acid (maximum at 0 to 30 minutes); in case II, approx. 89 % of the administered dose was excreted in urine within 5 hours as hippuric acid (maximum at 0 to 1 hour). In case I, the concentration of hippuric acid recovered to the predose level after 3 hours, while in case II the concentration of hippuric acid did not recover to the predose level within 5 hours. 23-OCT-1995 (93)

Type: Metabolism

Remark:	After i.p. injection of 2.5 to 10 mmol sodium benzoate/kg bw in male Sprague-Dawley rats, changes in metabolic levels of the liver and in amino acid levels in liver and plasma were noted.
23-OCT-1995	(94)
Type:	Metabolism
Remark:	Sodium benzoate inhibited the dissolution of hydrochlorothiazide (HCT) in vitro. In bioavailability studies with 6 male volunteers, the rate of increase in mean urine volume after intake of HCT-sodium benzoate was 6:1 compared to HCT alone.
23-OCT-1995	(95)
Type:	Metabolism
Remark:	In an in vitro study with gastric mucosa from patients with asthma, atopic eczema and urticaria, the release of histamine and prostaglandin was significantly increased by sodium benzoate at a concentration of 0.4 %. The mucosa of control persons did not react to sodium benzoate.
23-OCT-1995	(62)
Type:	Metabolism
Remark:	In experiments with isolated rat hepatocytes and mitochondria, sodium benzoate at concentrations from 0 to 2.0 mM inhibited gluconeogenesis (max. 67 %) and urea production (max. 52 %) in a dose-dependent manner by depletion of acetyl CoA.
23-OCT-1995	(96)
Type:	Toxicity:
Remark:	I.p. injection of 7.5 mmol/kg ammonium acetate alone produced 10 % mortality in male Swiss albino mice. Subsequent i.p. administration of 7.5 mmol/kg sodium benzoate resulted in 100 % mortality. Pretreatment of mice with carbamyl glutamate (2-20 mmol/kg), a structural analogue of N-acetyl glutamate, reduced mortality to 20 %. The protective effect of carbamyl glutamate is accompanied by an increase in urea production and of carbamyl phosphate synthetase activity.
10-AUG-2001	(97)

Type:

Remark: Effect on ammonia levels:
Male SD-rats received i.p. injections of saline, L-norvaline (1 mmol/kg), L-methionine-SR-sulfoximine (250 umol/kg), sodium benzoate (2.5-10 mmol/kg) in saline, either alone or in combination. L-norvaline and L-methionine-SR-sulfoximine caused an increase in the concentration of ammonia in plasma and in liver (interference with urea and glutamine formation). Subsequent injection of sodium benzoate failed to alleviate ammonia levels, and on the contrary, caused further increase. Sodium benzoate itself resulted in higher levels of ammonia in plasma and liver. Application of glycine did not lower ammonia levels indicating that other factors besides glycine may also be necessary for the removal of sodium benzoate.

10-AUG-2001

(98)

Type:

Remark: Liver perfusion:
In isolated perfused rat liver (livers of male Wistar rats, body weight 120-150 g), addition of sodium benzoate to the perfusion medium led to a rapid and marked stimulation of glutamate release from the liver (maximal glutamate efflux: 0.9 umol/min/g), which was fully reversible. Benzoate concentrations as low as 15 uM were effective to stimulate glutamate release significantly. Simultaneously benzoate inhibits urea and glutamine synthesis and diminishes hepatic ammonia uptake.

10-AUG-2001

(99)

5.11 Experience with Human Exposure

Remark: case-report: A 34 year old man reported in 1985 recurrent swelling of the upper lips and gums associated with the presence of a fissured tongue since he was 10 years old. In 1980 episodes became more frequent and were caused by the ingestion of different foods, including wine, sausages, and "hot foods". Each time, remission occurred spontaneously within 2 weeks. The patient reacted positive in a double-blind challenge test with sodium benzoate (see chapter 4.3).

23-OCT-1995	Upon elimination of sodium benzoate and another food additive, tartrazine, from the usual diet, complete remission of the clinical manifestation occurred.	(57)

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Syracuse Research Corporation. Environmental Science
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13212-2510
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- (6) safety data sheet Bayer AG, Leverkusen, 07.01.92
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7.1 End Point Summary**7.2 Hazard Summary****7.3 Risk Assessment**

I U C L I D D a t a S e t

(POTASSIUM BENZOATE; CAS: 582-25-2)

Existing Chemical	ID: 582-25-2
CAS No.	582-25-2
EINECS Name	potassium benzoate
EINECS No.	209-481-3
Molecular Formula	C7H6O2.K

Producer Related Part
Company: Bayer Corporation
Creation date: 21-OCT-1999

Substance Related Part

Company:	Bayer Corporation
Creation date:	21-OCT-1999

Memo: Bayer Corporation

Printing date: 10-AUG-2001
Revision date:
Date of last Update: 10-AUG-2001

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Chapter (profile):	Chapter: 1, 2, 3, 4, 5, 7
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SID

1.0.1 OECD and Company Information

Type: lead organisation
Name: American Chemistry Council (formerly Chemical Manufacturers Association), Benzoates HPV Panel
Street: 1300 Wilson Boulevard
Town: 22209 Arlington, VA
Country: United States

10-AUG-2001

Type: cooperating company
Name: ATOFINA Chemicals, Inc
Country: United States

10-AUG-2001

Type: cooperating company
Name: Bayer Corporation
Street: 100 Bayer Road
Town: PA 15205-9741 Pittsburgh
Country: United States

06-JUL-2000

Type: cooperating company
Name: DSM Fine Chemicals
Country: Netherlands

06-JUL-2000

Type: cooperating company
Name: Noveon, Inc.
Country: United States

10-AUG-2001

Type: cooperating company
Name: Velsicol Chemical Corporation
Country: United States

06-JUL-2000

1.0.2 Location of Production Site**1.0.3 Identity of Recipients**

1.1 General Substance Information**1.1.0 Details on Template****1.1.1 Spectra****1.2 Synonyms****1.3 Impurities****1.4 Additives****1.5 Quantity****1.6.1 Labelling****1.6.2 Classification****1.7 Use Pattern****1.7.1 Technology Production/Use****1.8 Occupational Exposure Limit Values****1.9 Source of Exposure****1.10.1 Recommendations/Precautionary Measures****1.10.2 Emergency Measures****1.11 Packaging****1.12 Possib. of Rendering Subst. Harmless****1.13 Statements Concerning Waste**

1.14.1 Water Pollution**1.14.2 Major Accident Hazards****1.14.3 Air Pollution****1.15 Additional Remarks****1.16 Last Literature Search**

Type of Search: Internal and External

Date of Search: 07-SEP-1999

Remark: Only HPV endpoints: TOXLINE data base and
internal studies

10-AUG-2001

1.17 Reviews**1.18 Listings e.g. Chemical Inventories**

2.1 Melting Point

Value: 330.6 degree C
Method: other: (calculated) MPBPWIN (v1.31) Program;
Adapted Joback
Method
Year: 1999
GLP: no
Testsubstance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (1)

2.2 Boiling Point

Value: 464.9 degree C
Method: other: (calculated) MPBPWIN (v1.31) Program;
Adapted Stein and Brown Method
Year: 1999
GLP: no
Testsubstance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (1)

2.3 Density

2.3.1 Granulometry

2.4 Vapour Pressure

Value: .00000000489 hPa at 25 degree C
Method: other (calculated): MPBPWIN (v1.31) Program;
Modified Grain Method
Year: 1999
GLP: no
Testsubstance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (1)

2.5 Partition Coefficient

log Pow: -2.269
Method: other (calculated): Log Kow(version 1.65 estimate)
Year: 1999
GLP: no
Testsubstance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (1)

2.6.1 Water Solubility

Value: 556 g/l at 20 degree C
Method: other
Testsubstance: other TS: sodium benzoate
Reliability: (2) valid with restrictions
Data from Handbook or collection of data
Flag: Critical study for SIDS endpoint
10-AUG-2001 (2)

Value: > 1000 g/l at 25 degree C
Method: other: (calculated) WSKOW v1.36 Program
Year: 1999
GLP: no
Testsubstance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (1)

2.6.2 Surface Tension

2.7 Flash Point

2.8 Auto Flammability

2.9 Flammability

2.10 Explosive Properties**2.11 Oxidizing Properties****2.12 Additional Remarks**

3.1.1 Photodegradation

Type: air
Conc. of subst.: at 25 degree C
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1560000 molecule/cm3
Rate constant: .0000000000017775 cm3/(molecule * sec)
Degradation: 50 % after 72.2 hour(s)
Method: other (calculated): AOP Program (v1.89)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (1)

3.1.2 Stability in Water

Type:
Method:
Year: GLP:
Test substance:
Remark: Based on structure and organic chemistry rules
(e.g. bonding in organic molecules, activation
energy, reactivity, transformations, addition,
substitution, elimination) no hydrolysis will
occur at pH ranges 4 - 11.
26-JAN-2001

3.1.3 Stability in Soil

3.2 Monitoring Data (Environment)

3.3.1 Transport between Environmental Compartments

Type: fugacity model level III
Media: other: air - water - soil - sediment
Air (Level I):
Water (Level I):
Soil (Level I):
Biota (L.II/III):
Soil (L.II/III):
Method: other: EPIWin Modeling Program

Year:				
Result:	Distribution	Half-Life	Emissions	Fugacity
	(percent)	(hr)	(kg/hr)	(atm)
Air	1.61e-007	144	1000	4.83e-019
Water	45.3	360	1000	1.38e-020
Soil	54.6	360	1000	6.16e-019
	Sediment	0.0755	1.44e+003	0
	Persistence Time: 421 hr			
	Reaction Time: 520 hr			
	Advection Time: 2.21e+003 hr			
	Percent Reacted: 80.9			
	Percent Advected: 19.1			
Reliability:	(2) valid with restrictions			
	Accepted calculation method			
Flag:	Critical study for SIDS endpoint			
10-AUG-2001				

(1)

3.3.2 Distribution**3.4 Mode of Degradation in Actual Use****3.5 Biodegradation**

Type:	aerobic
Inoculum:	
Degradation:	80.9 % after 22 day
Method:	other: (calculated) Fugacity Level III
Year:	1999
	GLP: no
Test substance:	other TS: molecular structure
Reliability:	(2) valid with restrictions
	Accepted calculation method
Flag:	Critical study for SIDS endpoint
10-AUG-2001	

(1)

Type:	aerobic
Inoculum:	activated sludge, domestic
Concentration:	50 mg/l related to Test substance
Degradation:	ca. 90 % after 7 day
Result:	readily biodegradable
Method:	OECD Guide-line 301 B "Ready Biodegradability:
Modified Sturm Test (CO ₂ evolution)"	

Year: 1981 GLP: no data
 Test substance: other TS: sodium benzoate
 Remark: See IUCLID on sodium benzoate (CAS# 532-32-1); the biodegradation of the potassium salt would be similar to the sodium salt.
 Test condition: temperature = 25 degree C
 Reliability: (1) valid without restriction
 Guideline study
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (3)

Type: anaerobic
 Inoculum: other bacteria: anaerobic sewage, domestic and industrial
 Concentration: 50 mg/l related to DOC (Dissolved Organic Carbon)
 Degradation: 93 % after 7 day
 Method: other: see below
 Year: GLP: no data
 Test substance: other TS: sodium benzoate
 Method: 2-3 g sludge plus sodium benzoate (concentration equivalent to 50 mg Carbon/liter or 85 mg substance/l).
 Controls and tests done in triplicate.
 Temperature = 35 degree C.
 Measured gas production (CH₄ + CO₂).
 Remark: See IUCLID on sodium benzoate (CAS# 532-32-1); the biodegradation of the potassium salt would be similar to the sodium salt.
 Result: Degradation is expressed as percentage of theoretical methane production based on the stoichiometry of degradation.
 Reliability: (2) valid with restrictions
 Flag: Critical study for SIDS endpoint
 10-AUG-2001 (4)

Type:
 Inoculum:
 Method:
 Year: GLP:
 Test substance:
 Remark: See IUCLID on benzoic acid (CAS# 65-85-0); the potassium salt is expected to immediately dissociate and form benzoic acid in an aqueous environment.
 10-AUG-2001

3.6 BOD5, COD or BOD5/COD Ratio

3.7 Bioaccumulation

Species:
Exposure period:
Concentration:
BCF: 3.16
Elimination:
Method: other: (calculated) BCF Program (v2.13)
Year: GLP: no
Test substance: other TS: molecular structure
Result: Estimated Log BCF = 0.500 (BCF = 3.162)

Log Kow (estimated) : 1.87
Log Kow (experimental): 1.87
Log Kow used by BCF estimates: 1.87

Equation Used to Make BCF estimate:
Log BCF = 0.50 (Ionic; Log Kow dependent)
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (1)

3.8 Additional Remarks

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: other: ECOSAR calculations
Species: other: fresh water fish
Exposure period: 96 hour(s)
Unit: g/l Analytical monitoring: no
LC50: > 1000
Method: other: ECOSAR (v 0.99)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Remark: ECOSAR class: Neutral organics. Chemical may
not be soluble enough to measure the predicted
effect.

Result:	ECOSAR Class	Organism	Duration	End Pt	mg/L
=====					
	Neutral Organic SAR: Fish (Baseline Toxicity)		14-day	LC50	1.13e+006
	Neutral Organics: Fish		96-hr	LC50	1.23e+006
	Neutral Organics: Fish		14-day	LC50	1.13e+006
	Neutral Organics: Fish		30-day	ChV	79360.031
Reliability:	(2) valid with restrictions Accepted calculation method				
Flag:	Critical study for SIDS endpoint				
10-AUG-2001	(1)				

Type:
Species:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: See IUCLID on sodium benzoate (CAS# 532-32-1);
the toxicity of the potassium salt would be
similar to the sodium salt.
10-AUG-2001

4.2 Acute Toxicity to Aquatic Invertebrates

Type:
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: g/l Analytical monitoring: no
EC50: 978
Method: other: ECOSAR (v 0.99)
Year: 1999 GLP: no
Test substance: other TS: molecular structure
Remark: ECOSAR class: Neutral organics. Chemical may not be soluble enough to measure the predicted effect.

Result: ECOSAR	Class	Organism	Duration	End Pt	mg/L

Neutral Organics: Daphnid	48-hr	LC50	9.78e+005
Neutral Organics: Daphnid	16-day	EC50	7746.435
Neutral Organics: Mysid Shrimp	96-hr	LC50	7.45e+006
Reliability: (2) valid with restrictions			
Accepted calculation method			
Flag: Critical study for SIDS endpoint			
10-AUG-2001	(1)		

Type:
Species:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: See IUCLID on sodium benzoate (CAS# 532-32-1); the toxicity of the potassium salt would be similar to the sodium salt.
Flag: Critical study for SIDS endpoint
10-AUG-2001

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: Green Algae
Endpoint: biomass
Exposure period: 96 hour(s)
Unit: g/l Analytical monitoring: no
EC50: 478
Method: other: ECOSAR (v 0.99)
Year: 1999 GLP: no

Test substance: other TS: molecular structure
Remark: ECOSAR class: Neutral organics.
Result: ECOSAR Class Organism Duration End Pt mg/L

=====

Neutral Organics: Green Algae	96-hr	EC50	4.78e+005
Neutral Organics: Green Algae	96-hr	ChV	4053.982

Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
10-AUG-2001 (1)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type:
Species:
Exposure period:
Unit: Analytical monitoring:
Method:
Year: GLP:
Test substance:
Remark: See IUCLID on sodium benzoate (CAS# 532-32-1);
the toxicity of the potassium salt would be
similar to the sodium salt.
10-AUG-2001

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

4.5.2 Chronic Toxicity to Aquatic Invertebrates

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Soil Dwelling Organisms

4.6.2 Toxicity to Terrestrial Plants

4.6.3 Toxicity to other Non-Mamm. Terrestrial Species

4.7 Biological Effects Monitoring

4.8 Biotransformation and Kinetics

4.9 Additional Remarks

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain:
Sex:
Number of Animals:
Vehicle:
Value: > 10000 mg/kg bw
Method:
Year: GLP:
Test substance: other TS: potassium benzoate; purity not noted
Reliability: (4) not assignable
Original reference in foreign language
Flag: Critical study for SIDS endpoint
10-AUG-2001 (5)

Type: LD50
Species: mouse
Strain:
Sex:
Number of Animals:
Vehicle:
Value: > 10000 mg/kg bw
Method:
Year: GLP:
Test substance: other TS: potassium benzoate; purity not noted
Reliability: (4) not assignable
Original reference in foreign language
Flag: Critical study for SIDS endpoint
10-AUG-2001 (5)

Type: LD50
Species: guinea pig
Strain:
Sex:
Number of Animals:
Vehicle:
Value: > 10000 mg/kg bw
Method:
Year: GLP:

Test substance: other TS: potassium benzoate; purity not noted
Reliability: (4) not assignable
Flag: Original reference in foreign language
10-AUG-2001 Critical study for SIDS endpoint (5)

5.1.2 Acute Inhalation Toxicity

Type:
Species:
Strain:
Sex:
Number of
Animals:
Vehicle:
Exposure time:
Value:
Method:
Year: GLP:
Test substance:
Remark: See IUCLID on benzoic acid (CAS# 65-85-0); the
loss of acidity due to the potassium salt
should decrease toxicity.
10-AUG-2001

5.1.3 Acute Dermal Toxicity

Type:
Species:
Strain:
Sex:
Number of
Animals:
Vehicle:
Value:
Method:
Year: GLP:
Test substance:
Remark: See IUCLID on benzoic acid (CAS# 65-85-0); the
loss of acidity due to the potassium salt
should decrease toxicity.
10-AUG-2001

5.1.4 Acute Toxicity, other Routes

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species:

Concentration:

Exposure:

Exposure Time:

Number of
Animals:

PDII:

Result:

EC classificat.:

Method:

Year:

GLP:

Test substance:

Remark: See IUCLID on sodium benzoate (CAS# 532-32-1);
the irritating ability of the potassium salt
would be similar to the sodium salt.

10-AUG-2001

5.2.2 Eye Irritation

Species:

Concentration:

Dose:

Exposure Time:

Comment:

Number of
Animals:

Result:

EC classificat.:

Method:

Year:

GLP:

Test substance:

Remark: See IUCLID on sodium benzoate (CAS# 532-32-1);
the irritating ability of the potassium salt
would be similar to the sodium salt.

10-AUG-2001

5.3 Sensitization

5.4 Repeated Dose Toxicity

Species: Sex:
Strain:
Route of admin.:
Exposure period:
Frequency of treatment:
Post. obs. period:
Doses:
Control Group:
Method:
Year: GLP:
Test substance:
Remark: See IUCLID on sodium benzoate (CAS# 532-32-1);
the toxicity of the potassium salt would be
similar to the sodium salt.
10-AUG-2001

5.5 Genetic Toxicity 'in Vitro'

Type: Bacillus subtilis recombination assay
System of testing: Bacillus subtilis H17, M45
Concentration: 1-20 mg/disk; vehicle: water and ethanol
(1:1)
Cytotoxic Conc.:
Metabolic activation: with and without
Result: positive
Method:
Year: GLP:
Test substance: other TS: potassium benzoate; purity not noted
Result: Authors judged results as positive.
Reliability: (3) invalid
Significant methodological deficiencies: one
dose tested
Flag: Critical study for SIDS endpoint
10-AUG-2001 (6)

Type:
System of
testing:
Concentration:

Cytotoxic Conc.:

Metabolic

activation:

Result:

Method:

Year:

GLP:

Test substance:

Remark: See IUCLID on sodium benzoate (CAS# 532-32-1);
the toxicity of the potassium salt would be
similar to the sodium salt.

10-AUG-2001

5.6 Genetic Toxicity 'in Vivo'

Type: Cytogenetic assay

Species:

Sex:

Strain:

Route of admin.:

Exposure period:

Doses:

Result:

Method:

Year:

GLP:

Test substance:

Remark: See IUCLID on sodium benzoate (CAS# 532-32-1);
the toxicity of the potassium salt would be
similar to the sodium salt.

10-AUG-2001

5.7 Carcinogenicity

Species:

Sex:

Strain:

Route of admin.:

Exposure period:

Frequency of
treatment:

Post. obs.
period:

Doses:

Result:

Control Group:

Method:

Year:

GLP:

Test substance:

Remark: See IUCLID on sodium benzoate (CAS# 532-32-1);
the toxicity of the potassium salt would be
similar to the sodium salt.

10-AUG-2001

5.8 Toxicity to Reproduction

Type:

Species:

Sex:

Strain:

Route of admin.:

Exposure Period:

Frequency of

treatment:

Duration of test:

Doses:

Control Group:

Method:

Year:

GLP:

Test substance:

Remark: A 4-generation reprotoxicity test with benzoic
acid revealed no reproductive effects. Therefore
no indication for reprotoxicity for the benzoic
acid potassium salt.
See IUCLID on benzoic acid (CAS# 65-85-0); the
loss of acidity due to the potassium salt should
decrease toxicity.

10-AUG-2001

5.9 Developmental Toxicity/Teratogenicity

Species:

Sex:

Strain:

Route of admin.:

Exposure period:

Frequency of

treatment:

Duration of test:

Doses:

Control Group:

Method:

Year:

GLP:

Test substance:

Remark: See IUCLID on sodium benzoate (CAS# 532-32-1);
the toxicity of the potassium salt would be
similar to the sodium salt.

10-AUG-2001

5.10 Other Relevant Information

5.11 Experience with Human Exposure

- (1) Meylan W. and Howard P. 1999. EPIWin Modeling Program. Syracuse Research Corporation. Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510
- (2) Budavari, S. (ed.), The Merck Index. An encyclopedia of chemicals, drugs, and biologicals. 11th ed., Rahway, New Jersey, 1357 (1989)
- (3) Salanitro, J.P. et al., Water Sci. Technol. 20, 125-130 (1988)
- (4) Battersby, N.S. & Wilson, V., Appl. Environ. Microbiol. 55: 433-439 (1989)
- (5) Kravets-Bekker A.A. & Ivanova O.P. 1970. Faktory Vnesh. Sredy Ikh Znachenie Zdorov'ya Naseleniya No.2, 125: in BIBRA Toxicity Profiles, BIBRA International, Great Britain.
- (6) Ishizaki, M. & Ueno, S., J. Food Hyg. Soc. Japan 30, 447-451 (1989)

7.1 End Point Summary

7.2 Hazard Summary

7.3 Risk Assessment

I U C L I D D a t a S e t

(BENZYL ALCOHOL; CAS: 100-51-6)

Existing Chemical ID: 100-51-6
CAS No. 100-51-6
EINECS Name benzyl alcohol
EC No. 202-859-9
TSCA Name Benzenemethanol
Molecular Formula C7H8O

Producer Related Part

Company: Bayer Corporation
Creation date: 15-JUL-1999

Substance Related Part

Company: Bayer Corporation
Creation date: 15-JUL-1999

Memo: Bayer Corporation

Printing date: 14-FEB-2002
Revision date:
Date of last Update: 14-FEB-2002

Number of Pages: 82

Chapter (profile): Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile): Reliability: without reliability, 1, 2, 3, 4
Flags (profile): Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 Applicant and Company Information

Type: lead organisation
Name: American Chemistry Council, Benzoates Panel
Street: 1300 Wilson Boulevard
Town: 22209 Arlington, VA
Country: United States

14-DEC-2000

Type: cooperating company
Name: B.F. Goodrich
Country: United States

26-MAY-2000

Type: cooperating company
Name: Bayer Corporation
Country: United States

14-DEC-2000

Type: cooperating company
Name: DSM Fine Chemicals
Country: Netherlands

14-DEC-2000

Type: cooperating company
Name: Elf Atochem NA
Country: United States

26-MAY-2000

Type: cooperating company
Name: Velsicol Chemical Corporation
Country: United States

26-MAY-2000

Type: lead organisation
Name: American Chemistry Council, Benzoates Panel

16-JAN-2001

1.0.2 Location of Production Site, Importer or Formulator**1.0.3 Identity of Recipients****1.0.4 Details on Category/Template****1.1.0 Substance Identification****1.1.1 General Substance Information****1.1.2 Spectra****1.2 Synonyms and Tradenames****1.3 Impurities****1.4 Additives****1.5 Total Quantity****1.6.1 Labelling****1.6.2 Classification****1.6.3 Packaging****1.7 Use Pattern****1.7.1 Detailed Use Pattern****1.7.2 Methods of Manufacture****1.8 Regulatory Measures****1.8.1 Occupational Exposure Limit Values****1.8.2 Acceptable Residues Levels**

1.8.3 Water Pollution**1.8.4 Major Accident Hazards****1.8.5 Air Pollution****1.8.6 Listings e.g. Chemical Inventories****1.9.1 Degradation/Transformation Products****1.9.2 Components****1.10 Source of Exposure****1.11 Additional Remarks****1.12 Last Literature Search****1.13 Reviews**

2.1 Melting Point

Value: -15.2 degree C

Method: other: Handbook value
Test substance: other TS: benzyl alcohol, purity not noted

Reliability: (2) valid with restrictions
Data from Handbook or collection of data
Flag: Critical study for SIDS endpoint
14-FEB-2002 (1)

Value: -15.3 degree C

Test substance: other TS: benzyl alcohol, purity not noted
12-FEB-2002 (2)

2.2 Boiling Point

Value: 205.3 degree C at 1013 hPa

Method: other: Handbook value
Test substance: other TS: benzyl alcohol, purity not noted

Reliability: (2) valid with restrictions
Data from Handbook or collection of data
Flag: Critical study for SIDS endpoint
14-FEB-2002 (1)

Value: 205.4 degree C at 1013 hPa
19-JAN-2001 (2)

2.3 Density

Type: density
Value: 1.041 g/cm³ at 24 degree C

Method: other: Handbook value
Test substance: other TS: benzyl alcohol, purity not noted

Reliability: (2) valid with restrictions
Data from Handbook or collection of data

Flag: Critical study for SIDS endpoint
14-FEB-2002 (1)

Type: density
Value: 1.0442 g/cm³ at 22.5 degree C

19-JAN-2001 (2)

2.3.1 Granulometry

2.4 Vapour Pressure

Value: .03 hPa at 20 degree C

Test substance: other TS: benzyl alcohol, purity not noted

Flag: Critical study for SIDS endpoint
12-FEB-2002 (2)

Value: .09 hPa at 30 degree C

Test substance: other TS: benzyl alcohol, purity not noted

Flag: Critical study for SIDS endpoint
12-FEB-2002 (2)

Value: .67 hPa at 50 degree C

Flag: Critical study for SIDS endpoint
19-JAN-2001 (2)

2.5 Partition Coefficient

log Pow: 1.1

Method: other (calculated): Leo, A.: CLOGP-3.54 MedChem
Software 1989. Daylight, Chemical Information
Systems, Claremont, CA 91711, USA

Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint

06 - JUN - 2001 (3)

log Pow: 1.1

Method: other (measured)

Remark: experimentally determined

Flag: Critical study for SIDS endpoint

14 - FEB - 2002 (4)

2.6.1 Solubility in different media

Solubility in: Water

Value: 40 g/l at 20 degree C

Flag: Critical study for SIDS endpoint

14 - FEB - 2002 (5)

Solubility in: Water

Value: 44 g/l at 50 degree C

Flag: Critical study for SIDS endpoint

14 - FEB - 2002 (5)

2.6.2 Surface Tension

2.7 Flash Point

Value: 101 degree C

Type: closed cup

Method: other: DIN 51758

19 - JAN - 2001 (5)

2.8 Auto Flammability

Value:

Remark: ignition temperature: 435 degree C

19 - JAN - 2001 (2)

2.9 Flammability**2.10 Explosive Properties**

Result: other: explosive limits: lower 1.3 % by vol.,
upper 13.0 % by vol. at 170 degree C and 1.013
bar

19-JAN-2001

(2)

2.11 Oxidizing Properties**2.12 Dissociation Constant****2.13 Viscosity****2.14 Additional Remarks**

3.1.1 Photodegradation

Type: air
Light source: Sun light
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 1560000
Rate constant: .0000000000082541 cm³/(molecule * sec)
Degradation: 50 % after 1.3 day(s)

Method: other (calculated): AOPWin version 1.89
Year: 1999
GLP: no
Test substance: other TS: molecular structure

Remark: Experimental Database Structure Match:
experimental OH rate constant= 22.9 E-12
cm³/molecule-sec.

Reliability: (2) valid with restrictions
Accepted calculation method

Flag: Critical study for SIDS endpoint
14-FEB-2002 (6)

3.1.2 Stability in Water

Remark: Based on structure and organic chemistry rules
(e.g. bonding in organic molecules, activation
energy, reactivity, transformations, addition,
substitution, elimination) no hydrolysis will
occur at pH ranges 4 - 11.

Flag: Critical study for SIDS endpoint
26-JAN-2001

3.1.3 Stability in Soil**3.2.1 Monitoring Data (Environment)****3.2.2 Field Studies**

3.3.1 Transport between Environmental Compartments

Type: fugacity model level III
 Media: other: other: air - water - soil - sediment
 Method: other: EPIWin Modeling Program

Remark: Modeling was performed using equal releases (10,000 kg/hr) and equal distribution to all compartments.

Result:	Distribution (percent)	Half-Life (hr)	Emissions (kg/hr)	Fugacity (atm)
Air	1.51	11.2	1000	2.95e-011
Water	50.0	360	1000	6.71e-012
Soil	48.4	360	1000	1.7 e-010
Sediment	0.0923	1440	0	5.52e-012

Persistence Time: 287 hr
 Reaction Time: 353 hr
 Advection Time: 1.54e+003 hr
 Percent Reacted: 81.3
 Percent Advected:

Reliability: (2) valid with restrictions
 Flag: Critical study for SIDS endpoint
 14-FEB-2002

(6)

3.3.2 Distribution**3.4 Mode of Degradation in Actual Use****3.5 Biodegradation**

Type: aerobic
 Inoculum: activated sludge
 Concentration: 100 mg/l
 Degradation: 92 - 96 % after 28 day(s)

Method: OECD Guide-line 301 C "Ready Biodegradability:
 Modified

MITI Test (I)"

Year: 1981

GLP: no data

Test substance: other TS: benzyl alcohol, purity not noted

Remark: slugde conc.: 30 mg/l

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
14-FEB-2002 (7)

Type: aerobic
Inoculum: predominantly domestic sewage
Degradation: > 90 % after 30 day(s)
Method: OECD Guide-line 301 D "Ready Biodegradability:
Closed Bottle Test"
Year: 1972
GLP: no
Test substance: other TS: benzyl alcohol, purity not noted

Remark: related to BOD
Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
29-JAN-2001 (8)

Type: anaerobic
Inoculum: anaerobic sludge
Contact time: 28 day(s)
Degradation: 100 % after 7 day(s)
Result: readily biodegradable

Method: other: see below
Year: 1982
GLP: no data
Test substance: other TS: commercial grade benzyl alcohol,
purity > 95%

Method: A 10% anaerobic sludge inoculum was transferred to 160 ml serum bottles previously amended with 50 ppm carbon (related to test substance) using strict anaerobic techniques. Methane production from test bottles vs. controls was monitored weekly for 4 weeks or until net production occurred. At that time, the bottles were amended again with the same substrate and methane production monitored to confirm the observation. All data were obtained from duplicate bottles. Methane was measured using a flame ionization detector on a Perkin-Elmer Model 900 GC equipped with a 3-m Tenax-G.C. column.

Remark:	100 % mineralisation (CH ₄ -Production) in 1 week with sludge from Jackson, MI waste-treatment plant 100 % mineralisation (CH ₄ -Production) in 2 weeks with sludge from Adrian, MI waste-treatment plant
Test condition:	The test bottles were incubated at 35 degree C in the dark. Substrates were kept under an atmosphere of 90% N ₂ and 10% H ₂
Reliability:	(2) valid with restrictions
Flag:	Critical study for SIDS endpoint
23-MAR-2001	(9)
Type:	anaerobic
Inoculum:	domestic sewage
Concentration:	50 µg/l related to DOC (Dissolved Organic Carbon)
Contact time:	2 month
Degradation:	> 75 % after 2 month
Method:	other: see below
Year:	1984
GLP:	no data
Test substance:	other TS: benzyl alcohol, purity not noted
Method:	Sludge samples collected from primary and secondary anaerobic digesters were diluted to 10 % and incubated anaerobically with 50 ug Carbon per ml (related to test substance). All compounds were tested in triplicate. Gas production was measured by gas chromatography and by a pressure transducer. Biodegradation was determined by net increase in gas pressure in bottles amended with test chemicals over non-amended controls.
Result:	Degradation is expressed as percentage of theoretical methane production based on the stoichiometry of degradation.
Test condition:	The test bottles were incubated at 35 degree C in the dark. Substrates were kept under atmospheres of 10% CO ₂ and 90% N ₂ .
Reliability:	(2) valid with restrictions
Flag:	Critical study for SIDS endpoint
23-MAR-2001	(10)
Type:	aerobic
Degradation:	62 % after 5 day(s)

Method:	OECD Guide-line 301 D	"Ready Biodegradability:
Closed		
GLP:	Bottle Test"	
	no	
Remark:	related to ThOD	
19-JAN-2001		(11)
Type:	aerobic	
Degradation:	77 % after 20 day(s)	
Method:	OECD Guide-line 301 D	"Ready Biodegradability:
Closed		
GLP:	Bottle Test"	
	no	
Remark:	related to ThOD	
19-JAN-2001		(11)
Type:	aerobic	
Inoculum:	activated sludge, adapted	
Degradation:	95 % after 28 day(s)	
Method:	other: Closed bottle test	
Remark:	Test concentration: 2 - 5 mg/l	
	Degradation related to ThOD	
19-JAN-2001		(12)
Type:	aerobic	
Inoculum:	domestic sewage	
Degradation:	89.2 % after 5 day(s)	
Method:	other: respirometric diluting method	
GLP:	no	
Remark:	related to ThOD	
19-JAN-2001		(13)
Type:	aerobic	
Inoculum:	activated sludge, industrial	
Degradation:	88.9 % after 5 day(s)	
Test substance:	other TS	

Method: Radio-respirometric study using radio-labeled chemicals by activated sludge and in a complex photographic processing effluent using acclimated industrial sludge. Concentration of test substance was 0.1 or 0.2ml of radioactive substrate(27,000-400,000 dpm). Samples were incubated in the dark at ambient temperature.

Remark: ¹⁴C02 recovery without effluent = 85.7% after 5 days
¹⁴C02 recovery in presence of effluent = 88.9% after 5 days

Test substance: benzyl-alcohol-7-¹⁴C (carbinol-¹⁴C) obtained from New England Nuclear Corporation, Boston, Massachusetts.

17 - JAN - 2001 (14)

Type: aerobic
Degradation: 85 % after 5 day(s)

GLP: no

Remark: related to ThOD
19 - JAN - 2001 (15)

Remark: The activity of degradation is at a concentration of 100 mg/l not hindered in a model plant (Ascomat)
19 - JAN - 2001 (8)

Remark: Biodegradation characteristics: biodegraded completely in a short time by general microorganisms.
19 - JAN - 2001 (16)

3.6 BOD5, COD or BOD5/COD Ratio

Method:
Year:
Method:
Remark: ThOD: 2515.1 mg/l
19 - JAN - 2001 (13)

3.7 Bioaccumulation

BCF: .31

Method: other: (calculated) BCF Program (v2.13)
Year: 1999
Test substance: other TS: molecular structure

Result: Estimated Log BCF = -0.503 (BCF = 0.3141)
Reliability: (2) valid with restrictions
Accepted calculation method
Flag: Critical study for SIDS endpoint
14-FEB-2002 (6)

3.8 Additional Remarks

Remark: ThOD 2520 mg/g
COD 2520 mg/g
BOD5 1560 mg/g
Influence on biological purification plants:
adapted 1180 mg/l degradable
27-MAY-1993 (17)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: static
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: 460

Method: other: see below
Year: 1976
GLP: no data
Test substance: other TS: reagent grade benzyl alcohol
purchased from Curtin Matheson Scientific,
Inc.

Method: Juvenile fathead minnows were obtained from Environmental Reserach Laboratory, Duluth. All fish used for the test were 4 to 8 weeks of age, 1.1 to 3.1 cm in length, and acclimated for at least 48 hr before testing. Test solutions were prepared by adding a weighed amount of chemical to 4 liters of Lake Superior water (all concentrations are nominal). Water temperature during the test was 18-22 degree C. Range-finding tests were done and definitive tests were conducted with 10 fish per container, 20 fish per concentration. Complete immobilization was considered the biological endpoint and equated with death. Standard graphical procedures were followed to determine LC50 (American Public Health Assn., 1971) Analyses of test water was done for dissolved oxygen and pH at the beginning and 1 or 2 times during the test.

Result: 1 hour LC50 = 770 mg/l
24 hour LC50 = 770 mg/l
48 hour LC50 = 770 mg/l
72 hour LC50 = 480 mg/l
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

23-MAR-2001

(18)

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
LC0: 630
LC50: 646
LC100: 662

Method: other: Bestimmung der Wirkung von
Wasserinhaltsstoffen auf
Fische, DIN 38412 Teil 15
Year: 1983
GLP: no
Test substance: other TS: benzyl alcohol, purity not noted

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
12-FEB-2002 (19)

Type: static
Species: Petromyzon marinus
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: >= 5

GLP: no

Remark: larvae; screening test
17-JAN-2001 (20)

Species: Carassius auratus (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
LC0: >= 5

17-JAN-2001 (21)

Species: Cyprinus carpio (Fish, fresh water)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
LC0: 136

GLP: no

Remark: Testing of acute oral toxicity
Unit: mg/kg

17 - JAN - 2001 (22)

Species: Lepomis macrochirus (Fish, fresh water)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
LC0: >= 5

17 - JAN - 2001 (21)

Species: Lepomis macrochirus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring:
LC50: 10

Remark: The static test was directed to simulate acute spill circumstances. The test substances were pipetted or poured undiluted directly into the aquaria with fish. There was no preparation of defined concentrations according to guideline. No analytical monitoring was done. Aeration was not used during the first 24 hrs thus allowing chemicals to act in an uninterrupted state at the onset of the test period.

Reliability: (4) not assignable
Significant methodological deficiencies

12 - FEB - 2002 (23)

Species: Menidia beryllina (Fish, estuary, marine)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring:
LC50: 15

Remark: The static test was directed to simulate acute spill circumstances. The test substances were pipetted or poured undiluted directly into the aquaria with fish. There was no preparation of defined concentrations according to guideline. No analytical monitoring was done. Aeration was not used during the first 24 hrs thus allowing chemicals to act in an uninterrupted state at the onset of the test period.

Reliability: (4) not assignable
Significant methodological deficiencies

12 - FEB - 2002 (23)

Species: Salmo trutta (Fish, fresh water, marine)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring:
LC0: >= 5

17-JAN-2001 (21)

4.2 Acute Toxicity to Aquatic Invertebrates

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
EC0: 300
EC50: 400
EC100: 500

Method: other: Daphnien-Kurzzeittest, DIN 38412 Teil
11, Bestimmung der Wirkung von
Wasserinhaltsstoffen auf Kleinkrebse

Year: 1983

GLP: no

Test substance: other TS: benzyl alcohol, purity not noted

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
14-FEB-2002 (19)

Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
TGK : 360

Method: other: acute immobilisation test
GLP: no

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint
06-JUN-2001 (24)

Species: Daphnia magna (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no
EC0: 26
EC50: 55
EC100: 100

GLP: no

Reliability: (2) valid with restrictions
16-JAN-2001 (25)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Chlorella pyrenoidosa (Algae)
Endpoint: other: Inhibition of photosynthesis
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring: no data
EC50: 95

Method: other: see below
Year: 1982
GLP: no data
Test substance: other TS: benzyl alcohol purchased from
Aldrich Chemical Co. Wisconsin, USA. Purity >
95%

Method: Photosynthesis was assayed by following the uptake of $^{14}\text{C}\text{O}_2$ from $\text{NaH}^{14}\text{CO}_3$ (Amersham/Searle, Ontario, Canada). Plastic culture flasks containing 9.9 ml of cell suspension (1.0×10^5 cells/ml), 0.1 ml radioisotope and 0.01 ml of test chemical were incubated for 3 hours. Five concentrations, ranging from 0 to 100 ppm, were tested and replicated five times. Photosynthetic activity was assayed according to Stratton et al. (1979) Appl. Environ. Microbiol. 38: 537-43. Per cent inhibition values were calculated relative to photosynthetic activity in the solvent controls and EC50 values determined by Probit analysis. Analyses for significant differences were performed using Dunnett's test and Duncan's multiple range test.

Test condition: Cultures were maintained in a liquid nitrogen-free medium at 20 degree C and a light intensity of 7000 lux on a 12 hour light-dark cycle.

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

14-FEB-2002

(26)

Species: Haematococcus pluvialis (Algae)
Endpoint: other: Inhibition of photosynthesis
Exposure period: 4 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: 2600

Method: other: according to Tuempling v.W. (Fortschritte
Der Wasserchemie. 14 S: 205-213 (1972) using a
Warburg apparatus

GLP: no
Test substance: other TS: benzyl alcohol, purity not noted

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

29-JAN-2001

(19)

Species: Scenedesmus quadricauda (Algae)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring:
TGK : 640

Method: other: cell multiplication inhibition test

Remark: green algae
Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint

06-JUN-2001

(24)

Species: Anabaena cylindrica (Algae)
Endpoint: other: Inhibition of photosynthesis
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring:
EC50: 90

Remark: blue-green algae

17-JAN-2001

(26)

Species: Anabaena inaequalis (Algae)
Endpoint: other: Inhibition of photosynthesis
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring:
EC0: 30

Remark: blue-green algae (26)
17-JAN-2001

Species: Anabaena variabilis (Algae)
Endpoint: other: Inhibition of photosynthesis
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring:
EC50: 35

Remark: blue-green algae (26)
17-JAN-2001

Species: Scenedesmus quadricauda (Algae)
Endpoint: other: Inhibition of photosynthesis
Exposure period: 3 hour(s)
Unit: mg/l Analytical monitoring:
EC50: 79

GLP: no

Remark: green algae (26)
17-JAN-2001

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: aquatic
Species: Escherichia coli (Bacteria)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
EC0: 1000

Method: other: cell multiplication test
GLP: no

Reliability: (2) valid with restrictions
Flag: Critical study for SIDS endpoint (24)
06-JUN-2001

Type: aquatic
Species: Pseudomonas putida (Bacteria)
Unit: mg/l Analytical monitoring: no
EC10: 658

Method: other: Test according to Bringmann and Kuehn
(cell multiplication inhibition test)

GLP:	no	
Remark:	Exposure period: 16-18 h	
Reliability:	(2) valid with restrictions	
Flag:	Critical study for SIDS endpoint	
06-JUN-2001		(19)
Type:	aquatic	
Species:	Photobacterium phosphoreum (Bacteria)	
Exposure period:	30 minute(s)	
Unit:	mg/l	Analytical monitoring: no
EC50:	71.42	
Method:	other: Microtox	
GLP:	no	
19-JAN-2001		(27)
Type:	aquatic	
Species:	Photobacterium phosphoreum (Bacteria)	
Exposure period:	5 minute(s)	
Unit:	mg/l	Analytical monitoring: no
EC50:	50	
GLP:	no	
19-JAN-2001		(28)
Type:	aquatic	
Species:	other bacteria: Aerobic heterotrophic	
Exposure period:	49 hour(s)	
Unit:	mg/l	Analytical monitoring:
IC50 :	2100	
GLP:	no	
Remark:	Inhibition of respiration;	
	prolonged incubation compared with ISO 8192	
19-JAN-2001		(29)
Type:	aquatic	
Species:	other bacteria: Nitrosomonas	
Exposure period:	24 hour(s)	
Unit:	mg/l	Analytical monitoring:
IC50 :	390	

Method: other: Inhibition of nitrification, comparable
with ISO/DIS 9509
GLP: no

Remark: Inhibition of N-oxidation
19-JAN-2001 (29)

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

4.5.2 Chronic Toxicity to Aquatic Invertebrates

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

4.6.2 Toxicity to Terrestrial Plants

4.6.3 Toxicity to Soil Dwelling Organisms

4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

4.7 Biological Effects Monitoring

4.8 Biotransformation and Kinetics

4.9 Additional Remarks

Remark:

Aedes aegypti, eggs	(72h)	LD50	160 l/ha	
		LD90	251 l/ha	
Aedes aegypti, larval stage L1	(24h)	LD50	105 l/ha	
		LD90	132 l/ha	Aedes
aegypti, larval stage L3-L4	(24h)	LD50	129 l/ha	
		LD90	184 l/ha	
Aedes scutellaris, eggs	(72h)	LD50	160 l/ha	
		LD90	265 l/ha	
Aedes scutellaris, larval stage L1	(24h)	LD50	110 l/ha	
		LD90	151 l/ha	
Aedes scutellaris, larval stage L3-L4	(24h)	LD50	126 l/ha	
		LD90	172 l/ha	
19-JAN-2001				(30)

5.0 Toxicokinetics, Metabolism and Distribution

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Sex: male
Value: = 1610 mg/kg bw

Method: other
GLP: no data
Test substance: other TS: benzyl alcohol, purity not noted

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
12-FEB-2002 (31)

Type: LD50
Species: mouse
Sex: male/female
No. of Animals: 10
Vehicle: other: corn oil
Value: = 1580 mg/kg bw

Method: other: see below
GLP: no data
Test substance: other TS: commercial grade benzyl alcohol

Method: Mice were dosed on full stomachs by intubation.
All animals were observed for toxic signs and
time of death for 2 weeks.
The LD50 was computed by the method of
Litchfield & Wilcoxon(1949).
Remark: Toxic signs: depression, death
Reliability: (2) valid with restrictions
Meets generally accepted scientific standards,
well documented and acceptable for assessment
Flag: Critical study for SIDS endpoint
12-FEB-2002 (32) (33)

Type:	LD50
Species:	rat
Strain:	other: Osborne-Mendel
Sex:	male/female
No. of Animals:	10
Vehicle:	other: neat
Value:	= 1230 mg/kg bw
Test substance:	other TS: commercial grade benzyl alcohol
Method:	Groups of 10 young adult Osborne-Mendel rats, evenly divided by sex were fasted for approximately 18 hrs prior to treatment. Animals were dosed by intubation. All animals were observed for toxic signs and time of death for 2 weeks. The LD50 was computed by the method of Litchfield & Wilcoxon (1949).
Remark:	Toxic signs: depression, excitability, coma, death
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment
12-FEB-2002	(32)
Type:	LD50
Species:	rat
Value:	= 2080 mg/kg bw
Method:	other: no data
GLP:	no data
Test substance:	other TS: benzyl alcohol, purity not noted
Reliability:	(4) not assignable Secondary literature; Original reference not available
Flag:	Critical study for SIDS endpoint
12-FEB-2002	(34) (33)
Type:	LD50
Species:	rabbit
Value:	= 1040 mg/kg bw
12-FEB-2002	(34) (35)
Type:	LD50

Species: rat
Value: = 3100 mg/kg bw
16 - JAN-2001 (36)

Type: LDLo
Species: rat
Value: ca. 1040 - 3120 mg/kg bw
16 - JAN-2001 (37)

Type: LD50
Species: mouse
Value: = 1150 mg/kg bw
16 - JAN-2001 (38)

Type: LDLo
Species: mouse
Value: ca. 1040 mg/kg bw
16 - JAN-2001 (37)

Type: LDLo
Species: guinea pig
Value: ca. 1040 - 2600 mg/kg bw
16 - JAN-2001 (37)

5.1.2 Acute Inhalation Toxicity

Type: LC50
Species: rat
Exposure time: 4 hour(s)
Value: > 4.178 mg/l
Method: other
GLP: no data
Test substance: other TS: benzyl alcohol, purity not noted
Flag: Critical study for SIDS endpoint
12-FEB-2002 (39)
Type: LC50
Species: rat

Exposure time: 4 hour(s)
Value: ca. 8.8 mg/l

Remark: Extrapolation according to Haber`s law: LC50
(8h) = 1000 ppm.
19-JAN-2001 (36)

Type: LC50
Species: rat
Exposure time: 4 hour(s)
Value: > .9 mg/l

Remark: LC33 (4h) = 200 ppm.
19-JAN-2001 (40)

Type: LC50
Species: rat
Sex: no data
No. of Animals: 6
Vehicle: other: neat
Exposure time: 4 hour(s)
Value: 8.9 mg/l

Test substance: no data

Result: Exposure to 2000 ppm kills either 2/6, 3/6 or
4/6 rats.
Therefore benzyl alcohol is considered to be
of moderate toxicity.
07-SEP-2000 (41)

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Value: = 2000 mg/kg bw

Method: other
GLP: no data
Test substance: other TS: benzyl alcohol, purity not noted

Flag: Critical study for SIDS endpoint
29-JAN-2001 (42)

Type: LD50
Species: guinea pig
Value: < 5 ml/kg bw

Method: other
GLP: no data

Test substance: other TS: benzyl alcohol, purity not noted

Flag: Critical study for SIDS endpoint
29-JAN-2001 (43) (35)

5.1.4 Acute Toxicity, other Routes

Type: LD50
Species: rat
Route of admin.: i.p.
Value: > 400 - 800 mg/kg bw

19-JAN-2001 (44)

Type: LD50
Species: mouse
Strain: CD-1
Sex: male
Route of admin.: i.p.
Value: = 1000 mg/kg bw

Remark: Acute toxicity after 4 h.
14-FEB-2002 (45)

Type: LD50
Species: mouse
Strain: CD-1
Sex: male
Route of admin.: i.p.
Value: = 650 mg/kg bw

Test substance: other TS: benzyl alcohol, purity not noted

Remark: Acute delayed toxicity after 7 d.
14-FEB-2002 (45)

Type: LD50
Species: guinea pig
Route of admin.: i.p.
Value: > 400 - 800 mg/kg bw

19-JAN-2001 (44)

Type: LD50
Species: rat
Route of admin.: s.c.
Value: = 1700 mg/kg bw

Test substance: other TS: benzyl alcohol, purity not noted

14 -FEB-2002 (46)

Type: LD50
Species: mouse
Route of admin.: s.c.
Value: = 950 mg/kg bw

19 -JAN-2001 (38)

Type: other: LDLO
Species: rabbit
Route of admin.: s.c.
Value: ca. 2080 mg/kg bw

19 -JAN-2001 (37)

Type: LD50
Species: rat
Route of admin.: i.v.
Value: = 314 mg/kg bw

19 -JAN-2001 (47)

Type: LD50
Species: rat
Route of admin.: i.v.
Value: = 53 mg/kg bw

Remark: Rapid injection
19 -JAN-2001 (47)

Type: LD50
Species: mouse
Route of admin.: i.v.
Value: = 324 mg/kg bw

19 -JAN-2001 (48)

Type: LD50
Species: mouse
Route of admin.: i.v.
Value: ca. 105 mg/kg bw

Remark: LD50 value depends on speed of injection
19 -JAN-2001 (49)

Type: LD50
Species: mouse
Route of admin.: i.v.
Value: = 1460 mg/kg bw

19 - JAN - 2001 (47)

Type: other: LDLO
Species: mouse
Strain: CD-1
Sex: male
Route of admin.: i.v.
Value: ca. 135 mg/kg bw

14 - FEB - 2002 (50)

Type: other: LDLO
Species: dog
Route of admin.: i.v.
Value: ca. 50 mg/kg bw

19 - JAN - 2001 (47)

Type: LD50
Species: rat
Route of admin.: other
Value: = 410 mg/kg bw

Remark: Application: intra-arterial.
19 - JAN - 2001 (47)

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Result: not irritating

Method: OECD Guide-line 404 "Acute Dermal
Irritation/Corrosion"
GLP: no data
Test substance: other TS: benzyl alcohol, purity not noted

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
14 - FEB - 2002 (49)

Species: rabbit
Concentration: 10 other: mg
Exposure Time: 24 hour(s)
Result: slightly irritating

Method:	other: see remarks	
GLP:	no data	
Test substance:	other TS: benzyl alcohol, purity not noted	
Flag:	Critical study for SIDS endpoint	
14-FEB-2002		(51) (36)
Species:	rabbit	
Exposure:	Open	
Exposure Time:	24 hour(s)	
Result:	moderately irritating	
Method:	other: see remarks	
Remark:	Exposure time: 24 h, clipped skin, 100	
mg/animal, open,	observation time: 72 h.	
14-FEB-2002		(52)
Species:	rabbit	
Result:	not irritating	
Method:	other: see remarks	
Remark:	Exposure time: 24 h, ear, ca. 500 mg/animal,	
19-JAN-2001	semi-occlusive, observation time: 7 d.	(53)
Species:	guinea pig	
Result:	moderately irritating	
Method:	other: see remarks	
Remark:	Exposure time: 24 h, depilated skin, dose:	
19-JAN-2001	undiluted material, no other data, open,	
	observation time: no data.	(44)
Species:	guinea pig	
Result:	slightly irritating	
Method:	other: see remarks	
Remark:	Exposure time: 24 h, clipped flank,	
19-JAN-2001	dose: 8 mg/animal (30 % in unspecified	
	solvent), open, observation time: no data.	(54)
Species:	guinea pig	
Result:	slightly irritating	

Method: other: see remarks

Remark: Exposure time: 24 h, shaved flanks,
dose: 26 mg/animal (25 % unspecified solvent),
intradermally, observation time: no data.
19-JAN-2001 (55)

Species: guinea pig
Result: not irritating

Method: other: see remarks

Remark: Exposure time: 24 h, clipped skin,
100 mg/animal, open, observation time: 72 h.
19-JAN-2001 (52)

Species: human
Result: irritating

Method: other: Closed Patch Test

Remark: Observation time: 24/48 h, 0.05 % in either
ethanol or a cream base produced irritation in
18 of 614 subjects.
19-JAN-2001 (56)

Species: human
Result: irritating

Method: other: Uncovered Patch Test

Remark: 0.5 % in petrolatum induced contact urticaria
in 7 of 32 volunteers.
19-JAN-2001 (57)

Species: human
Result: slightly irritating

Method: other: Patch Test

Remark: Exposure time: 48 h, ca. 50 mg/person (30 % in
acetone), observation time: up to 120 h.
19-JAN-2001 (52)

Species: other: Male nude mouse
Result: highly irritating

Method: other: see remarks

Remark: Exposure time: 24 h, 10 % in purified water,
occlusive, observation time: no data.
19-JAN-2001 (58)

Species: other: mini-pig
Result: not irritating

Method: other: Patch Test

Remark: Exposure time: 48 h, clipped skin,
50 mg/animal, observation time: no data.
19-JAN-2001 (52)

5.2.2 Eye Irritation

Species: rabbit
Result: moderately irritating

Method: OECD Guide-line 405 "Acute Eye
Irritation/Corrosion"
GLP: no data
Test substance: other TS: benzyl alcohol, purity not noted

Reliability: (1) valid without restriction
Flag: Critical study for SIDS endpoint
14-FEB-2002 (49)

Species: rabbit
Result: highly irritating

Method: other: see remarks
GLP: no data
Test substance: other TS: benzyl alcohol, purity not noted

Remark: Exposure time: 24 h, dose: 750 microg., no other
data.
Flag: Critical study for SIDS endpoint
14-FEB-2002 (51) (36)

Species: rabbit
Concentration: 4 %
Result: not irritating

Method: other: see remarks
Test substance: other TS: benzyl alcohol, purity not noted

Remark: 4 % aqueous solution, tested for stability, no
other data.
Flag: Critical study for SIDS endpoint
14-FEB-2002 (59)

Species: rabbit
Result: not irritating
Method: other: see remarks
Remark: Exposure time: 4 d, 2 drops of a 0.08 %
aqueous solution, no other data.
19-JAN-2001 (38)

Species: rabbit
Result: moderately irritating
Method: other: see remarks
Remark: ca. 100 mg/animal, observation time: 7 d.
19-JAN-2001 (53)

5.3 Sensitization

Type: Draize Test
Species: guinea pig
Result: not sensitizing
Test substance: other TS: benzyl alcohol, purity not noted
Flag: Critical study for SIDS endpoint
14-FEB-2002 (54)

Type: Guinea pig maximization test
Species: guinea pig
Result: not sensitizing
Test substance: other TS: benzyl alcohol, purity not noted
Flag: Critical study for SIDS endpoint
14-FEB-2002 (54)

Type: Freund's complete adjuvant test
Species: guinea pig
Result: sensitizing
Test substance: other TS: benzyl alcohol, purity not noted
Flag: Critical study for SIDS endpoint
14-FEB-2002
(54)

Type:	Open epicutaneous test	
Species:	guinea pig	
Result:	sensitizing	
Test substance:	other TS: benzyl alcohol, purity not noted	
Flag:	Critical study for SIDS endpoint	
14-FEB-2002		(54)
Type:	Patch-Test	
Species:	human	
Result:	sensitizing	
Test substance:	other TS: benzyl alcohol, purity not noted	
Remark:	Maximum incidence of sensitization: 1 %.	
Flag:	Critical study for SIDS endpoint	
14-FEB-2002		(60) (61) (62)
Type:	Patch-Test	
Species:	human	
Result:	sensitizing	
Test substance:	other TS: benzyl alcohol, purity not noted	
14-FEB-2002		(63) (64)
Type:	Patch-Test	
Species:	human	
Result:	ambiguous	
Test substance:	other TS: benzyl alcohol, purity not noted	
14-FEB-2002		(57)
Type:	Patch-Test	
Species:	human	
Test substance:	other TS: benzyl alcohol, purity not noted	
Remark:	Two patients with contact dermatitis were found to be sensitised by benzyl alcohol: 1 per cent in petrolatum	
14-FEB-2002		(65)
Type:	Patch-Test	
Species:	human	
Test substance:	other TS: benzyl alcohol, purity not noted	

Remark: A previously to balsam of Peru sensitised patient reacted on patch testing with benzyl alcohol: 0.5 per cent in olive oil.
14-FEB-2002 (66)

Type: other
Species: laboratory animal

Method: other: additional animal studies are reported

Test substance: other TS: benzyl alcohol, purity not noted
14-FEB-2002 (68) (69) (70) (67)

Type: other
Species: human

Method: other: additional data
Test substance: other TS: benzyl alcohol, purity not noted
14-FEB-2002 (71) (72) (73) (74) (75) (76) (77) (78) (79) (80)
(81) (82) (83) (84) (85) (86) (87) (88) (89) (90)
(91) (92) (93) (94) (95) (96)

Type: other: Application to shaved skin
Species: guinea pig
Result: not sensitizing

Test substance: other TS: benzyl alcohol, purity not noted
14-FEB-2002 (38)

Type: other: Intradermal application
Species: guinea pig
Result: not sensitizing

Test substance: other TS: benzyl alcohol, purity not noted
14-FEB-2002 (38)

Type: other: Maximization Test
Species: human
Result: not sensitizing

Test substance: other TS: benzyl alcohol, purity not noted
14-FEB-2002 (97)

5.4 Repeated Dose Toxicity

Type: Sub-chronic
Species: rat Sex: male/female
Strain: other: F344/N
Route of administration: gavage
Exposure period: 13 w

Frequency of treatment: daily
Post exposure period: no
Doses: 50, 100, 200, 400, 800 mg/kg/d
Control Group: yes
NOAEL: 400 mg/kg bw

Year: 1981
GLP: yes
Test substance: other TS: technical grade benzyl alcohol
(purity =99%)

Method: Groups of 10 rats of each sex were administered 0, 50, 100, 200, 400, or 800 mg/kg benzyl alcohol in corn oil by gavage, 5 days/week for 13 weeks (dose volume = 5 ml/kg). Rats were housed five/cage with feed and water available ad libitum. Animals were observed twice daily; moribund animals were sacrificed. Animal weights were recorded weekly. At the end of the study, survivors were sacrificed. A necropsy was performed on all animals; histologic exams performed on all vehicle controls and animals in the 800 mg/kg group. Brains were examined from rats in the 400 mg/kg group.

Remark: Biochemistry and hematology studies were not performed.

Result: 8/10 male rats dosed with 800 mg/kg died during w 7 and 8. Rats of the high dose group exhibited clinical signs indicative of neurotoxicity including staggering, respiratory difficulty, and lethargy. Hemorrhages occurred around the mouth and nose, and there were histologic lesions in the brain, thymus, skeletal muscle, and kidney. There were reductions in relative weight gain in male rats dosed with 800 mg/kg and in female rats dosed with 200 mg/kg or more. No notable changes in bw gain or compound-

related histopathologic lesions were observed in rats from the lower dose groups. In the 2-y study, however, no notable changes were found on bw or bw gain at 200 or 400 mg/kg/d.

NOAEL = 400 mg/kg/day (based on investigated parameters and taking into account the bw results of 2-y study)

Reliability: (1) valid without restriction
GLP, Comparable to Guideline study

Flag: Critical study for SIDS endpoint

14-FEB-2002 (98)

Type: Sub-chronic
Species: mouse Sex: male/female
Strain: B6C3F1
Route of administration: gavage
Exposure period: 13 w
Frequency of treatment: daily
Post exposure period: no
Doses: 50, 100, 200, 400, 800 mg/kg/d
Control Group: yes
NOAEL: 200 mg/kg bw

Year: 1981
GLP: yes

Test substance: other TS: technical grade benzyl alcohol (purity =99%)

Method: Groups of 10 mice of each sex were administered 0, 50, 100, 200, 400, or 800 mg/kg benzyl alcohol in corn oil by gavage, 5 days/week for 13 weeks (dose volume = 5 ml/kg). Mice were housed five/cage with feed and water available ad libitum. Animals were observed twice daily; moribund animals were sacrificed. Animal weights were recorded weekly. At the end of the study, survivors were sacrificed. A necropsy was performed on all animals; histologic exams performed on all vehicle controls and animals in the 800 mg/kg group. Brains were examined from mice in the 400 mg/kg group and from all mice dying before the end of the study.

Remark: Biochemistry and hematology studies were not performed.

Result: Staggering after dosing occurred during the first 2 w of the study in mice dosed with 800 mg/kg.

There were reductions in relative weight gain in male mice dosed with 400 or 800 mg/kg, and in female mice dosed with 200 mg/kg or more. No notable changes in bw gain or compound-related histopathologic lesions were observed in mice from the lower dose groups. In the 2-y study, however no notable changes were found on bw or bw gain at 200 mg/kg/d. NOAEL = 200 mg/kg/day (based on investigated parameters and taking into account the bw results of 2-y study)

Reliability: (1) valid without restriction
GLP, Comparable to Guideline study

Flag: Critical study for SIDS endpoint

14-FEB-2002 (98)

Type: Chronic
Species: rat Sex: male/female
Strain: Fischer 344
Route of administration: gavage
Exposure period: 103 weeks
Frequency of treatment: 5 d/w
Post exposure period: no
Doses: 200, 400 mg/kg/d
Control Group: yes
NOAEL: 400 mg/kg bw

Year: 1981
GLP: yes
Test substance: other TS: technical grade benzyl alcohol (purity = 99%)

Method: Groups of 50 rats of each sex were administered 0, 200, or 400 mg/kg benzyl alcohol in corn oil by gavage, 5 days/week for 103 weeks. The rats were placed on the study at 8-9 weeks of age. All animals were observed twice daily and clinical signs recorded at least once per month. Body weights were recorded once per week for the first 12 weeks, then once a month thereafter. Animals found moribund and those surviving to the end of the study were humanely killed. Necropsy was performed on all animals; histological exams performed on all female rats and vehicle controls, and high dose rats that died before month 22, and male rats with gross lesions.

Remark: Biochemistry and hematology studies were not performed.
Result: No effect on bw gain or mortality was observed. No apparent compound-related non-neoplastic responses were observed.
Reliability: (1) valid without restriction
GLP, Comparable to Guideline study
Flag: Critical study for SIDS endpoint
14-FEB-2002 (98)

Type: Chronic
Species: mouse Sex: male/female
Strain: B6C3F1
Route of administration: gavage
Exposure period: 103 w
Frequency of treatment: 5 d/w
Post exposure period: no
Doses: 100, 200 mg/kg/d
Control Group: yes
NOAEL: 200 mg/kg bw

Method: other: OECD 451
Year: 1981
GLP: yes
Test substance: other TS: technical grade benzyl alcohol
(purity = 99%)

Method: Benzyl alcohol (purity, 99%) was given to groups of 50 B6C3F1 mice of each sex, eight to nine weeks of age, at a dose of 0, 100, or 200 mg/kg bw per day in corn oil by gavage on five days a week for 103 weeks. The doses were selected on the basis of those found to induce neurotoxic effects (lethargy and staggering) in short-term studies.
The mice were observed twice daily, and their body weights were recorded weekly for the first 12 weeks and once a month thereafter. Gross necropsy was performed on all animals, and 50 tissues and organs, including brain, liver, kidney, and stomach, from all vehicle controls, animals at the high dose, and animals at the other doses that died before

22 months or had gross lesions were examined histologically.
Remark: Biochemistry and hematology studies were not performed.

Result: The mean body weights of treated and control mice were comparable throughout the study. The survival of control females was significantly lower than that of animals at the high dose after week 74, but no other differences in survival were seen: 68% of control, 66% of low-dose, and 70% of high-dose males; and 50% of control, 62% of low-dose, and 72% of high-dose females. No significant treatment-related effects were noted at gross necropsy or histopathological examination. No increase was seen in the incidence of hepatocellular or forestomach neoplasia.

Reliability: (1) valid without restriction
GLP guideline study

Flag: Critical study for SIDS endpoint
14-FEB-2002 (98)

Type: Sub-acute
Species: mouse Sex: male/female
Strain: B6C3F1
Route of administration: oral feed
Exposure period: 10 d
Frequency of treatment: continuously in diet
Post exposure period: no
Doses: 2.08; 2.5 or 3 % in diet (approx. 3012, 3750 or 4500 mg/kg/d)
Control Group: yes
NOAEL: 3750 mg/kg bw
LOAEL: 4500 mg/kg bw

GLP: no data
Test substance: other TS: sodium benzoate (specific grade)
purchased from Wako

Method: Sodium benzoate, mixed with the powdered diet, was fed to groups of 12 rats (6 males, 6 females) for 10 days. Animals were observed for body weight gain and clinical signs 5 day/ week.

Remark: At the end of the experiment, surviving animals were necropsied. Organ weights, clinical chemistry and histological examinations were performed.
Benzyl alcohol will rapidly be metabolized to benzaldehyde and so to benzoic acid (sodium benzoate is the salt of benzoic acid). Therefore the data of sodium benzoate can also be supportive in the repeat dose endpoint.

Result: the mean compound consumption was calculated according to Lehman, Food Drug Off. Q. Bull. 18, 66 (1954)
All mice in the 3.0 %-group showed increased sensitivity to stimuli and 1/5 male and 2/5 females showed convulsions; 2/5 females died; liver weights of males and females and kidney weights of females were dose-dependently increased; histopathologic examination showed enlarged hepatocytes, single cell necrosis and vacuolation of hepatocytes in all livers from males; no histopathologic changes of the kidney were described; serum cholesterol, lipid levels and cholinesterase were increased in males.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-FEB-2002 (99)

Type: Sub-acute
Species: rat Sex: male/female
Strain: other: F344/N
Route of administration: gavage
Exposure period: 16 d
Frequency of treatment: daily
Post exposure period: no
Doses: 125, 250, 500, 1000, 2000 mg/kg/d
Control Group: no data specified

Test substance: other TS: technical grade benzyl alcohol
(purity = 99%)

Remark: No. of animals: 5/sex/dose.

Result: All male and female rats dosed with 2000 mg/kg died. 2/5 male and 3/5 female rats dosed with 1000 mg/kg died. Rats in the 2 highest dose groups were lethargic after dosing.
Other toxic responses in these 2 dose groups included blood around the mouth and nose, subcutaneous hemorrhages, and blood in the urinary and gastrointestinal tract. Animals administered lower doses had no compound-related histologic lesions.

14-FEB-2002 (98)

Type: Sub-acute
Species: mouse Sex: male/female

Strain: B6C3F1
Route of administration: gavage
Exposure period: 16 d
Frequency of treatment: daily
Post exposure period: no data specified
Doses: 125, 250, 500, 1000, 2000 mg/kg/d
Control Group: no data specified

Test substance: other TS: technical grade benzyl alcohol
(purity = 99%)

Remark: No. of animals: 5/sex/dose.
Result: All male and female mice dosed with 2000 mg/kg died. 1/5 male and 2/5 female mice dosed with 1000 mg/kg died. Mice of each sex in the 2 highest dose groups were lethargic after dosing. Other toxic responses in these 2 dose groups included blood around the mouth and nose, subcutaneous hemorrhages, and blood in the urinary and gastrointestinal tract and in the urinary bladder. Animals administered lower doses had no compound-related histologic lesions.
14-FEB-2002 (98)

Species: rat Sex: male
Strain: no data
Route of administration: inhalation
Exposure period: no data
Frequency of treatment: 4 h/d
Post exposure period: no data specified
Doses: 216-270 ppm
Control Group: no data specified

NOAEL: 270 ppm

Test substance: other TS: benzyl alcohol, purity not noted

Remark: No. of animals: 6.
Result: Subacute exposure to male rats for 4 h periods produced no clinical or pathologic signs of toxicity.
14-FEB-2002 (40)

Species: rat Sex: male/female
Strain: no data
Route of administration: gavage
Exposure period: 3 w
Frequency of treatment: 6 d/w
Post exposure period: no
Doses: 50, 150, 500 mg/kg

Control Group: yes

Test substance: other TS: benzyl alcohol, purity not noted

Remark: No. of animals: 5/sex/dose.

Result: The compound was administered in propylene glycol. Increases in weight were the same in all groups, and there were no pathological effects on blood or organs.

14-FEB-2002

(38)

Species: mouse

Sex: no data

Strain: no data

Route of administration: gavage

Exposure period: 8 d

Frequency of treatment: daily

Post exposure period: no data specified

Doses: 325, 645, 1300, 2595 mg/kg/d

Control Group: no data specified

Test substance: other TS: benzyl alcohol, purity not noted

Remark: No. of animals: no data.

Result: Decreased muscle coordination, a "hunched" appearance, depression, and fur changes were reported in mice given 645 mg/kg but not in those receiving 325 mg/kg or below. At 1300 mg/kg, animals additionally suffered breathing difficulties,

discharge from the eyes, and various CNS effects, and death occurred on day 1 in all mice given 2595 mg/kg.

14-FEB-2002

(100)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test

System of testing: S. typhimurium TA 98, TA 100, TA 1535, TA 1537

Concentration: up to 6666 ug/ml

Cytotoxic Concentration: >= 3333 ug/plate

Metabolic activation: with and without

Result: negative

Method: other: similar to OECD Guide-line 471; protocol according to Haworth, et.al. (1983)

Year: 1983

GLP: yes
 Test substance: other TS: technical grade benzyl alcohol
 (purity = 99%)

Method: Separate trials were done using metabolic
 activation with Aroclor 1254-induced S9 from
 male Syrian hamster liver and
 male Sprague-Dawley rat liver.

Reliability: (1) valid without restriction
 GLP guideline study

Flag: Critical study for SIDS endpoint
 14-FEB-2002 (98)

Type: other: Point-mutation
 System of testing: E. coli
 Metabolic activation: with and without
 Result: negative

Test substance: other TS: benzyl alcohol, purity not noted

Flag: Critical study for SIDS endpoint
 14-FEB-2002 (101) (102)

Type: Cytogenetic assay
 System of testing: CHO cells
 Concentration: up to 5000 ug/ml

Cytotoxic Concentration: none noted
 Metabolic activation: without
 Result: negative

Method: other: similar to OECD 473; Galloway S.M. et
 al., Environ. Mutagen. 7, 1-52 (1985)
 Year: 1989
 GLP: yes

Test substance: other TS: technical grade benzyl alcohol
 (purity = 99%)

Result: No significant increase in chromosome
 aberrations was observed after exposure to
 benzyl alcohol in the absence of S9.

Reliability: (1) valid without restriction
 GLP guideline study

Flag: Critical study for SIDS endpoint
 14-FEB-2002 (103) (98) (104)

Type: Cytogenetic assay
 System of testing: CHO cells
 Concentration: up to 5000 ug/ml
 Cytotoxic Concentration: none noted

Metabolic activation: with
Result: positive

Method: other: similar to OECD 473; according to
Galloway S.M. et al. Environm. Mutagen.7, 1-52
(1985)
Year: 1989
GLP: no data
Test substance: other TS: technical grade benzyl alcohol
(purity = 99%)

Result: A significant increase in chromosome
aberrations was observed after exposure to
benzyl alcohol in the presence of S9.

Reliability: (1) valid without restriction
Similar to Guideline study

Flag: Critical study for SIDS endpoint
14-FEB-2002 (103) (98) (104)

Type: Cytogenetic assay
System of testing: CHO cells
Concentration: 16 -5000 ug/ml
Cytotoxic Concentration: none noted
Metabolic activation: with and without
Result: equivocal

Method: other: similar to guideline study
Year: 1989
GLP: yes
Test substance: other TS: technical grade benzyl alcohol
(purity = 99%)

Result: Sister chromatid exchange (SCE) an equivocal
response with and without metabolic
activation.

Reliability: (1) valid without restriction
Similar to Guideline study

Flag: Critical study for SIDS endpoint
14-FEB-2002 (98)

Type: Bacillus subtilis recombination assay
System of testing: B. subtilis M 45, H 17
Result: positive

Remark: limited data
Flag: Critical study for SIDS endpoint
12-FEB-2002 (105)

Type: Mouse lymphoma assay
System of testing: L5178Y cells
Concentration: up to 5000 ug/ml
Cytotoxic Concentration: >/= 3500 ug/ml
Metabolic activation: with and without

Method: other: similar to OECD 476; according to Myhr G.
et al., Prog. Mutat. Res. 5, 555-586 (1985)
GLP: yes
Test substance: other TS: technical grade benzyl alcohol
(purity = 99%)

Result: Benzyl alcohol induced an increase in
trifluorothymidine-resistant cells in the
absence, but not in the presence of, S9
activation. The effect was associated with
toxicity.

Reliability: (1) valid without restriction
GLP guideline study

Flag: Critical study for SIDS endpoint

14-FEB-2002 (98)

Type: other: transformation assay
System of testing: BALB/c-3T3 cells
Concentration: 5 to 20 mM
Cytotoxic Concentration: The cytotoxic response (millimolar
LD50) = 17.9.
Metabolic activation: without
Result: positive

Method: other: Matthews E.J., J. Tissue Culture Methods
10, 157-164 (1986), Matthewy E.J. et al.,
Environm. Health Perspect. 101
[Suppl 2], 319-345 (1993)
Year: 1993
GLP: no data
Test substance: other TS: Supplied by Radian Corp. (Houston,
TX); purity not noted

Method: The A31-1-13 clone of BALB/c-3T3 cells was used
to evaluated the transforming potential of
numerous chemicals including benzyl alcohol.
Each transformation assay contained a
standard clonal survival assay, a co-culture
clonal survival assay, and a transformation
assay.
For each test, chemical-induced transformation
was detected using 18-20 vessels per dose
seeded with 3.2x10(e4) cells/vessel.

Remark:

Result:

Each dose was applied to cell cultures for 48 hrs. days 2-4, using standard procedures.
A total of 3 to 6 test chemicals were included in each transformation experiment and each was tested at four treatment doses in at least two independent trials.
The doses covered a range of cytotoxicity responses of approximately 10-100% relative cloning efficiency.
Each test chemical in each experiment was evaluated as sufficiently positive (statistically significant at two or more doses), limited activity (statistically significant at one dose at 99% conf. or two at 95% conf.), sufficiently negative (no statistically significant responses), or limited negative (no cytotoxicity or abnormal positive control). The number of type I-III transformed foci were identified microscopically considering their various different phenotypic properties.
REFERENCES:
Matthews E.J., J. Tissue Culture Methods 10, 157-164 (1986),
Matthews E.J. et al., Environm. Health Perspect. 101 [Suppl 2], 319-345 (1993)
Benzyl alcohol (BA) was tested as a coded sample.
The author noted that BA can be oxidized by air and may have been altered during the treatment period. They state that BA was noncytotoxic to BALB/c-3T3 cells and that the statistical sensitivities for trial 1 and 2 were 2 and 38/110, respectively. BA was evaluated as active in this assay with actual and estimated rank t-statistics both 1.95.
For the purpose of this study benzyl alcohol (BA) was grouped as a noncytotoxic, nonmutagenic, noncarcinogenic chemical. Notations for BA were: reacts with acid, air, acid chlorides and is temperature sensitive. BA's potential to be oxidized by air was noted as a potential confounding factor. It had limited activity in the first test and Was sufficiently positive in the second. It, therefore, was given the overall evaluation of active in the transformation assay. The cytotoxic response (millimolar LD50) 17.9.
In trial 1 BA concentrations ranged 5 to 20mM

	with an increase in transformation only noted at the 10mM concentration (85% coculture clonal survival). RESULT: 7.36foci/vessel - rank order 2 (p<=0.001) -limited active mean t-statistic 2.11 In trial 2 BA concentrations ranged 5 to 20mM with an increase in transformation noted at the 10mM concentration (95% coculture clonal survival;p<=0.001) and 15mM concentration (84% coculture clonal survival;p<= 0.01 to 0.05).
	Fewer foci were observed in the second trial. RESULT: 0.609 foci/vessel - rank order 38 - sufficient positive mean t-statistic 1.79 The positive control B(a)P performed well. The number of foci/vessel for the neg control was 7.36 and 0.609 in Trials 1 and 2, respectively.
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment
Flag:	Critical study for SIDS endpoint
14-FEB-2002	(106)
Type:	Ames test
System of testing:	S. typhimurium TA 98, TA 100, TA 1535, TA 1537
Metabolic activation:	with and without
Result:	negative
Reliability:	(2) valid with restrictions Meets generally accepted scientific standards, well documented and acceptable for assessment
14-FEB-2002	(107)
Type:	Ames test
System of testing:	S. typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538
Metabolic activation:	without
Result:	negative
16-JAN-2001	(108)
Type:	Ames test
System of testing:	S. typhimurium TA 98, TA 100, TA 1535, TA 1537

Metabolic activation:	with and without	
Result:	negative	
Remark:	Rat and hamster liver S-9 mix.	
16-JAN-2001		(109) (104)
Type:	Ames test	
System of testing:	S. typhimurium TA 98, TA 100	
Metabolic activation:	without	
Result:	negative	
16-JAN-2001		(110)
Type:	Ames test	
System of testing:	S. typhimurium TA 92, TA 94, TA 98, TA 100, TA 1535, TA 1537	
Metabolic activation:	with	
Result:	negative	
16-JAN-2001		(111)
Type:	Ames test	
System of testing:	S. typhimurium TA 98, TA 1535	
Metabolic activation:	no data	
Result:	negative	
16-JAN-2001		(112)
Type:	other: Point-mutation	
System of testing:	E. coli WP2 uvrA	
Metabolic activation:	no data	
Result:	negative	
16-JAN-2001		(105)
Type:	Bacillus subtilis recombination assay	
System of testing:	B. subtilis M 45, H 17	
Result:	positive	
16-JAN-2001		(113)
Type:	other: Point-mutation	
System of testing:	E. coli WP2 uvrA	
Metabolic activation:	without	
Result:	negative	
16-JAN-2001		(113)
Type:	Cytogenetic assay	

System of testing: CHL cells

Result: negative

16-JAN-2001 (111) (114)

Type: Mouse lymphoma assay
System of testing: L5178Y tk+/tk- cells
Metabolic activation: with and without
Result: ambiguous

16-JAN-2001 (115) (116) (104)
Type:

Ames test
System of testing: S. typhimurium TA 100
Metabolic activation: without
Result: negative

16-JAN-2001 (117)

Type: Sister chromatid exchange assay
System of testing: CHO cells
Metabolic activation: with and without
Result: positive

16-JAN-2001 (104)

Type: other: DNA Double Strand Breaks
System of testing: rat hepatocytes
Concentration: 0, 1, 3, 10 mM in 1 % DMSO
Metabolic activation: no data
Result: ambiguous

Method: other: in vitro alkaline elution assay
Year: 1994
GLP: no data
Test substance: no data

Remark: Positive only in the highest dose.
16-JAN-2001 (118) (119)

5.6 Genetic Toxicity 'in Vivo'

Type: Micronucleus assay
Species: mouse Sex: male
Strain: other: ddY strain, obtained from Shizuoka
Agricultural Cooperative Association for
Laboratory Animals, Shizuoka, Japan

Route of admin.: i.p.
Exposure period: 24 h
Doses: 50, 100, 200 mg/kg
Result: negative

Method: OECD Guide-line 474 "Genetic Toxicology:
Micronucleus Test"
Year: 1983
GLP: no data
Test substance: other TS: benzyl alcohol, purity not noted

Remark: No. of animals: 6/dose.
Result: There was no indication of micronucleus
induction at any dose tested.

	1 Dose (mg/kg)	MNPCE (%)	PCE (%)
Mortality			
0	0.23 +/-0.18	48.8 +/-6.2	0/6
50	0.23 +/-0.15	55.5 +/-4.0	0/6
100	0.27 +/-0.12	51.8 +/-9.5	0/6
200	0.12 +/-0.10	48.7 +/-5.2	0/6
(4 doses)			
100	0.20 +/-0.14	63.1 +/-4.1	0/6
Mitomycin C			
2.0	2.63 +/-0.32*	43.8 +/-1.1	0/6

MNPCE = Micronucleated polychromatic erythrocyte
PCE = polychromatic erythrocyte
* = (P < 0.01)

Reliability: (1) valid without restriction
Guideline study

Flag: Critical study for SIDS endpoint
14-FEB-2002 (120)

Type: other: replicative DNA synthesis
Species: rat Sex: male
Strain: Fischer 344
Route of admin.: gavage
Exposure period: once
Doses: 0, 300, 600 mg/kg bw
Result: negative

Method: other: according to Uno Y. et al., Toxicol.
Lett. 63, 191-199, 201-209 (1992)
Year: 1994
GLP: no data
Test substance: no data

Result: Benzyl alcohol did not induce replicative DNA synthesis in rat hepatocytes following oral treatment.

Flag: Critical study for SIDS endpoint (121)
14-FEB-2002

Type: other: replicative DNA synthesis
Species: mouse Sex: male
Strain: B6C3F1
Route of admin.: gavage
Exposure period: once
Doses: 0, 400, 800 mg/kg bw
Result: negative

Method: other: according to Uno Y. et al.,
Toxicol.Lett.63,191-199,201-209 (1992),
Year: 1995
GLP: no data
Test substance: no data

Result: Benzyl alcohol did not induce replicative DNA synthesis in mice hepatocytes following oral treatment.

Flag: Critical study for SIDS endpoint (122)
23-MAR-2001

Type: Drosophila SLRL test
Species: Drosophila melanogaster Sex: male
Strain: other: Canton S
Route of admin.: drinking water
Exposure period: 72 hrs
Doses: 0, 5000 (unit not given) in 5 % succrose solution

Method: other
Year: 1994
GLP: no data
Test substance: other TS: purity: 99.8 %

Result: no evidence for mutagenicity

19-JAN-2001 (123)

Type: Drosophila SLRL test
Species: Drosophila melanogaster Sex: male
Strain: other: Canton S
Route of admin.: i.p.
Exposure period: once
Doses: 0, 8000 (unit not given)

Method: other
Year: 1994
GLP: no data
Test substance: other TS: purity.99.8 %

Result: no evidence for mutagenicity
19-JAN-2001 (123)

5.7 Carcinogenicity

Species: rat Sex: male/female
Strain: other: F344/N
Route of administration: gavage
Exposure period: 103 w
Frequency of treatment: 5 d/w
Post exposure period: no
Doses: 200, 400 mg/kg/d
Result: negative
Control Group: yes

Method: OECD Guide-line 451 "Carcinogenicity Studies"
Year: 1981
GLP: yes
Test substance: other TS: technical grade benzyl alcohol
(purity =99%)

Method: Benzyl alcohol was administered in corn oil by gavage to groups of 50 Fischer 344/N rats of each sex at a dose of 0, 200, or 400 mg/kg bw per day on five days a week for 103 weeks. The rats were observed twice daily, and body weights were recorded weekly for the first 12 weeks and once a month thereafter. Gross necropsy was performed on all animals; and 49 tissues and organs, including brain, kidney, pancreas,

and skeletal muscle, from all female rats and from male rats in the vehicle control and high-dose groups and those in the other groups that died before 22 months or which had gross lesions were examined histologically.

Remark: Biochemistry and hematolgy studies were not performed.

Result: The mean body weights of treated and control animals were comparable throughout the study. No compound-related clinical signs were observed, although a sialodacryoadenitis viral infection

was widespread among the study animals in the third month. The survival of treated females was significantly lower than that of vehicle controls: 70% of controls, 34% of low-dose females, and 34% of high-dose females; this was due to a much higher incidence of accidental deaths related to the gavage process.

Survival among the male rats was comparable in all groups: 56% of controls, 54% at the low dose, and 48% at the high dose.

Cataracts and retinal atrophy were observed at increased incidences in rats at the high dose.

The authors attributed this effect to the proximity of this group of animals to fluorescent light for most of the study. An increased incidence of hyperplasia of the forestomach epithelium was seen (not statistically significant) in male rats: control, 0/48; low dose, 0/19; high dose, 4/50.

Haemorrhage and foreign material in the respiratory tract seen in treated rats that died before the end of the study were suggested by the authors to have been the result of either direct deposition of material into the lung during gavage 'accidents' or the anaesthetic properties of benzyl alcohol resulting in reflux of gavage material and aspiration into the lungs. No pancreatic acinar-cell adenomas were reported, and no other effects of treatment were seen at gross necropsy or histopathological examination.

Reliability: (1) valid without restriction

Flag: GLP guideline study
14-FEB-2002 Critical study for SIDS endpoint

(124) (98)

Species:	mouse	Sex: male/female
Strain:	B6C3F1	
Route of administration:	gavage	
Exposure period:	103 w	
Frequency of treatment:	5 d/w	
Post exposure period:	no	
Doses:	100, 200 mg/kg/d	
Result:	negative	
Control Group:	yes	

Method: OECD Guide-line 451 "Carcinogenicity Studies"
Year: 1981
GLP: yes
Test substance: other TS: technical grade benzyl alcohol
(purity =99%)

Method: Benzyl alcohol (purity, 99%) was given to groups of 50 B6C3F1 mice of each sex, eight to nine weeks of age, at a dose of 0, 100, or 200 mg/kg bw per day in corn oil by gavage on five days a week for 103 weeks. The doses were selected on the basis of those found to induce neurotoxic effects (lethargy and staggering) in short-term studies. The mice were observed twice daily, and their body weights were recorded weekly for the first 12 weeks and once a month thereafter. Gross necropsy was performed on all animals, and 50 tissues and organs, including brain, liver, kidney, and stomach, from all vehicle controls, animals at the high dose, and animals at the other doses that died before 22 months or had gross lesions were examined histologically.

Remark: Biochemistry and hematology studies were not performed.

Result: The mean body weights of treated and control mice were comparable throughout the study. The survival of control females was significantly lower than that of animals at the high dose after week 74, but no other differences in

survival were seen: 68% of control, 66% of low-dose, and 70% of high-dose males; and 50% of control, 62% of low-dose, and 72% of high-dose females.

No significant treatment-related effects were noted at gross necropsy or histopathological examination. No increase was seen in the incidence of hepatocellular or forestomach neoplasia.

Reliability: (1) valid without restriction

GLP guideline study

Flag: Critical study for SIDS endpoint

14-FEB-2002

(124) (98)

Species: mouse

Sex: male

Strain: B6C3F1

Route of administration: i.p.

Exposure period: 22 d

Frequency of treatment: once on day 1, 8, 15, 22
Post exposure period: up to 1 a
Doses: 3.75 umol (total dose) in trioctanoin
Control Group: yes

Remark: 35 mice received injections prior to weaning.
The mice were weaned at 4 weeks of age. All
surviving mice were killed at 12 months for
enumeration of hepatomas.

Result: Benzyl alcohol had no detectable activity for
the initiation of hepatic tumors on
administration to male mice prior to weaning.

19-JAN-2001

(125)

5.8.1 Toxicity to Fertility

Type: other: 2 year gavage study
Species: rat
Sex: male/female
Strain: Fischer 344
Route of administration: gavage
Exposure Period: 103 weeks
Frequency of treatment: 5d/w
Duration of test: 103 weeks
Doses: 200, 400 mg/kg/d
Control Group: yes
NOAEL Parental: 400 ml/kg bw

Method: other: OECD 451

Year: 1981

GLP: yes

Test substance: other TS: technical grade benzyl alcohol
(purity = 99%)

Remark: Benzyl alcohol was administered in corn oil.

Result: No evidence of compound related effects in the
testes or ovaries of treated rats.
Changes noted in general in the reproductive
system were inconsequential.

Reliability: (1) valid without restriction
GLP guideline study

Flag: Critical study for SIDS endpoint

14-FEB-2002

(98)

Type: other: 2 year gavage study
Species: mouse
Sex: male/female
Strain: B6C3F1
Route of administration: gavage

Exposure Period: 103 weeks
 Frequency of treatment: 5 d/w
 Duration of test: 103 weeks
 Doses: 100, 200 mg/kg/d
 Control Group: yes
 NOAEL Parental: 200 ml/kg bw

Method: other: OECD 451
 GLP: yes
 Test substance: other TS: technical grade benzyl alcohol
 (purity =99%)

Remark: Benzyl alcohol was administered in corn oil.
 Result: No evidence of compound related effects in the
 testes or ovaries of treated mice.
 Changes noted in general in the reproductive
 system were inconsequential.

Reliability: (1) valid without restriction
 GLP guideline study

Flag: Critical study for SIDS endpoint
 14-FEB-2002 (98)

Type: other: 4 generation study
 Species: rat
 Strain: no data
 Route of administration: oral feed
 Exposure Period: generation 1 and 2: lifelong;
 generation 3: 16 weeks;
 generation 4: until breeding

Frequency of treatment: continuously in diet
 Doses: 0.5 or 1 % in diet (approx. 375 or
 750 mg/kg/day)

Control Group: yes
 NOAEL Parental: >= 750 ml/kg bw
 NOAEL F1 Offspring: >= 750 ml/kg bw
 NOAEL F2 Offspring: >= 750 ml/kg bw

Test substance: other TS: benzoic acid

Remark: See IUCLID data set on benzoic acid
 (CAS# 65-85-0). Benzyl alcohol will rapidly be
 metabolized to benzaldehyde and so to benzoic
 acid.
 Therefore the data of benzoic acid can also be
 supportive to state that benzyl alcohol is not
 a reproductive (fertility and developmental)
 toxicant.

Result: No effects on fertility, lactation, growth and
 survival or the incidence of foetal
 malformations were observed in a 4 generation

reproduction study with rats (20 m and 20 f)
exposed to 0.5% and 1.0% benzoic acid in the
diet.
Flag: Critical study for SIDS endpoint (126)
06-JUN-2001

Type: Fertility
Species: rat
Sex: female
Route of administration: oral unspecified
Exposure Period: 32 weeks
Frequency of treatment: every second day
Premating Exposure Period
female: 75 days
Duration of test: 32 weeks
Doses: 5 mg/kg
NOAEL Parental: 5 mg/kg bw

Test substance: other TS: benzaldehyde

Remark: Benzyl alcohol will rapidly be metabolized to
benzaldehyde and so to benzoic acid.
Therefore the data of benzaldehyde can also be
supportive to state that benzyl alcohol is not
a reproductive (fertility and developmental)
toxin.

Result: No treatment related effects noted.
Flag: Critical study for SIDS endpoint
16-JAN-2001 (127) (128)

5.8.2 Developmental Toxicity/Teratogenicity

Species: mouse Sex:
female
Strain: CD-1
Route of administration: gavage
Exposure period: day 7-14 of gestation
Frequency of treatment: daily
Duration of test: until 3 days after pregnancy
Doses: 750 mg/kg bw/day
Control Group: yes
LOAEL Maternal Toxicity : 750 mg/kg bw
LOAEL Fetotoxicity : 750 mg/kg bw

GLP: no data
Test substance: other TS: benzyl alcohol, purity not noted

Method: Benzyl alcohol dissolved in distilled water was
administered by gavage at a dose of 750 mg/kg
bw per day to 50 mice on days 7-14 of

Result:

gestation; evidence of copulation was considered the first day of gestation. A control group of 50 animals received distilled water only. All animals were allowed to deliver their litters and nurse their pups for three days, at which time necropsies were performed. Maternal body-weight gain and mortality, mating, gestation, numbers of live and dead pups per litter, total litter weight on days 1 and 2 post partum, litter weight change between days 1 and 3 post partum, and pup survival on days 1 and 3 post partum were recorded.

During the treatment period, 18 deaths were reported, all

of which were attributed to treatment; a further death was reported on day 15 of gestation, the day after treatment was terminated. Clinical signs of toxicity, including hunched posture, tremors, inactivity, prostration, hypothermia, ataxia, dyspnoea, swollen or cyanotic abdomen, and piloerection, were reported in up to 20 mice during treatment. Piloerection was also reported in some animals up to day 3 post partum, but no other clinical signs were seen after the period of administration. No differences were observed in the mating or gestation indices, the total number of resorptions, the mean length of gestation, or the number of live pups per litter between treated and control groups. Maternal body weight, measured on days 4 and 7 of gestation, was not significantly different from control values; however, statistically significant reductions were reported on day 18 of gestation ($P < 0.001$) and on day 3 post partum ($P < 0.05$). Maternal body-weight gain during days 7-18 of gestation was significantly lower than that of controls ($P < 0.001$). Significant reductions in pup body weight were reported, including a lower mean pup weight per litter on days 1 ($P < 0.01$) and 3 post partum ($P < 0.001$), a mean litter weight change between day 1 and day 3 post partum ($P < 0.05$), and a mean pup weight change between days 1 and 3 post partum ($P < 0.001$). No differences in pup survival

Conclusion: were observed by day 3 post partum.
The authors concluded that benzyl alcohol may be a reproductive hazard, apparently on the basis of the reductions in pup body weights, an effect that was observed in conjunction with maternal toxicity evidenced by increased mortality, reduced body weights, and clinical toxicity during the period of administration. As effects were seen on the dams and fetuses at the only dose used in this study, there was no NOAEL. The LOAEL was 750 mg/kg bw per day.

Reliability: (2) valid with restrictions
Meets generally accepted scientific standards, well documented and acceptable for assessment

Flag: Critical study for SIDS endpoint
14-FEB-2002 (129) (130) (131)

Species: mouse Sex: female

Route of administration: gavage

Exposure period: days 6-15 of gestation

Frequency of treatment: daily

Duration of test: until day 3 post partum

Doses: 550 mg/kg bw

Control Group: yes, concurrent vehicle

NOAEL Maternal Toxicity: 550 mg/kg bw

NOAEL Teratogenicity: 550 mg/kg bw

GLP: no data

Test substance: other TS: benzyl alcohol; purity not noted

Method: 50 female mice were given benzyl alcohol at 550 mg/kg bw per day by gavage on days 6-15 of gestation; a further 50 mice received the corn oil vehicle. All dams were allowed to deliver naturally, and pups and dams were observed until day 3 post partum, when the experiment was terminated. Body weight, clinical observations, and mortality were recorded daily throughout treatment and up to day 3 post partum.

Remark: abstract only

Result: Mortality was not significantly increased in animals given benzyl alcohol over that in the control group.
One treated mouse showing languid behaviour, laboured breathing, and a rough coat died, but no other deaths or clinical signs were

reported. Maternal body weight and body-weight gain during treatment and up to day 3 post partum were virtually identical for treated and control animals. All other parameters examined, including gestation index, average number of live pups per litter, and postnatal survival and pup body weight on days 0 and 3 post partum, were not significantly different from the control values.

Conclusion: The authors concluded that, at the predicted LD10, benzyl alcohol had no significant effects on the development of CD-1 mice. The NOAEL was 550 mg/kg bw per day.

Reliability: (2) valid with restrictions

Flag: Critical study for SIDS endpoint

14-FEB-2002 (132) (133)

Species: rat Sex: male/female

Strain: no data

Route of administration: oral feed

Exposure period: generation 1 and 2: lifelong; generation 3: 16 weeks; generation 4: until breeding

Frequency of treatment: continuously in diet

Duration of test: 4 generations

Doses: 0.5 or 1% in diet (approx. 375 or 750 mg/kg/day)

Control Group: yes

NOAEL Maternal Toxicity: 750 mg/kg bw

NOAEL Teratogenicity: 750 mg/kg bw

Method: other

GLP: no data

Test substance: other TS: benzoic acid

Remark: See IUCLID data set on benzoic acid (CAS# 65-85-0). Benzyl alcohol will rapidly be metabolized to benzaldehyde and so to benzoic acid. Therefore the data of benzoic acid can also be supportive to state that benzyl alcohol is not a reproductive (fertility and development) toxicant.

Result: No effects on the dams or on the growth and development of the offspring were seen when groups of 10 rats were fed diets containing up to 1% benzoic acid during pregnancy and lactation.

Flag: Critical study for SIDS endpoint
06-JUN-2001 (126)

Species: other: chicken embryo Sex:
Route of administration: other
Exposure period: 11 to 18 d

Frequency of treatment: 1 injection before incubation or on
different d after incubation

Doses: 0.01-0.02 ml/egg = 10-20 mg/egg

Control Group: yes

Remark: Injections of benzyl alcohol into the yolks of
fertile eggs, either before incubation, or
from the 1. through the 7. d after the
beginning of their incubation give rise to
meningoceles, limb deformities, beak defects
such as, arched upper beaks, localized blebs
and generalized edema.

no post observation
30-JAN-2001 (134)

5.8.3 Toxicity to Reproduction, Other Studies

5.9 Specific Investigations

5.10 Exposure Experience

Remark: Benzyl alcohol poisoning can cause the
gasping syndrome in neonates. The infants
had a typical course of gradual neurologic
deterioration, severe metabolic acidosis,
the striking onset of gasping respirations,
thrombocytopenia, hepatic and renal failure,
hypotension, cardiovascular collapse and
death. In every infant, unmetabolized
benzyl alcohol was identified in the urine.

19-JAN-2001 (135) (136) (137) (138) (139) (140) (141) (142)

Remark: Local anaesthesia occurred when neat benzyl
alcohol was applied to the (presumably
uncovered) skin or when 1 % aqueous
solution was injected intradermally.

Source: Bayer AG Leverkusen
20-AUG-1992 (37)

Remark: A methylprednisolone sodium succinate formulation, containing 18 mg / dose of benzyl alcohol, was well tolerated in human volunteers after i.v. injection. No important drug-related side effects were encountered.

Source: Bayer AG Leverkusen (143)
20-AUG-1992

Remark: Cases of allergic contact dermatitis, and even systemic hypersensitivity have been reported in humans.

Source: Bayer AG Leverkusen
15-JUL-1993 (144) (145) (146) (147) (148) (149) (150) (151) (152) (153) (154) (155)

Remark: No contact allergy could be detected in humans treated with a 10 % formulation of benzyl alcohol (no other data).

Source: Bayer AG Leverkusen (156)
20-AUG-1992

Remark: Premature neonates may receive multiple drugs in the neonatal intensive care unit, some of which may contain benzyl alcohol. As there may be no safe lower dose of benzyl alcohol in these patients, it would seem prudent to avoid the use of multiple dose vials containing benzyl alcohol whenever alternatives exist.

Source: Bayer AG Leverkusen (157)
20-AUG-1992

Remark: It also seems prudent to avoid the use of products containing benzyl alcohol to pregnant patients within whom the benzyl alcohol molecule, given its small size, presumably crosses the placental barrier into immature fetal tissues as readily as it crosses the blood-brain barrier.

Source: Bayer AG Leverkusen (158)
22-MAR-1993

Remark: high levels of benzyl alcohol (5-500 ug/10 ml plasma) were found in uremic patients on hemodialysis; benzyl alcohol was not detected in normal controls.

Source: Bayer AG Leverkusen (159)
24-FEB-1998

Remark: In 2 long-term double blind studies on humans comparing benzyl alcohol , placebo and Catalin in the topical treatment of progressive cataract, rapid (2-3 weeks treatment) reversal of incipient cataract was obtained accompanied by a marked improvement of vision and by a significantly lower percentage of eyes requirering surgery after 22 months of treatment with benzyl alcohol than with placebo and Catalin.

Source: Bayer AG Leverkusen (160)
24-FEB-1998

Remark: Study on healthy adult voluteers: Benzyl alcohol is itself an effective anesthetic and can reduce the pain of injection for lidocain without adversely affecting its anesthetic properties.

Source: Bayer AG Leverkusen (161)
24-FEB-1998

Remark: Benzyl alkohol is commonly used as a preservative in many injectable drugs and solutions. A number of neonatal deaths and severe respiratory and metabolic complications in low-birth-weight premature infants have been associated with the use of this agent.

Source: Bayer AG Leverkusen (162) (163) (164) (165) (166) (167) (168)
26-FEB-1998

5.11 Additional Remarks

Type: Metabolism

Remark: Humans, rabbits and rats readily oxidize benzyl alcohol to benzoic acid, which, after conjugation with glycine, is rapidly eliminated as hippuric acid in the urine.

19-JAN-2001 (169) (170) (171) (172) (173) (174) (175)

Type: other

Remark: Bacillus subtilis spore rec-assay can be used as a simple screening test taking the place of animal methods for detection of the allergenicity.

Source: Bayer AG Leverkusen (176)
24-FEB-1998

Type: other

Remark: yeast test: according to the author an alternative to the contemporary mode of acute toxicity testing. In the test, the increase in the cell count after treatment in relationship to the increase in cell count of untreated cells is measured and expressed as "medium inhibitory concentration = IC 50 " : benzyl alcohol IC 50 = 277 mg/l

Source: Bayer AG Leverkusen (177) (178)
24-FEB-1998

Type: other

Remark: Benzylalkohol differentially altered the specific activity of subcellular rat epididymal and testicular aldehyde dehydrogenase activity as well as hepatic aldehyde dehydrogenase activity.

Source: Bayer AG Leverkusen (179) (180) (181) (182)
24-FEB-1998

Type: other

Remark: different concentrations of benzyl alcohol (1,2,5,10 % v/v) in sesame oil were subcutaneously injected to rats. only the 1 % benzyl alcohol produced an insignificant increase in skin fold thickness.

Source: Bayer AG Leverkusen (183)
24-FEB-1998

Type: other

Remark: In vitro, benzyl alcohol relaxes airway smooth muscle, probably through the decrease in intracellular Ca^{2+} release by inhibiting agonist-mediated phosphatidylinositol turnover.

Source: Bayer AG Leverkusen

24 -FEB-1998 (184)

Remark: Aseptic meningitis has been observed following intrathecal administration of radiopharmaceuticals that contain benzyl-alcohol as a preservative. Cisterna magna injections of benzylalcohol in concentrations as high as 10 times that normally used did not produce meningitis in adult or immature dogs. With 9 % benzyl alcohol, transient respiratory arrest was observed in adult dogs and death was observed in immature dogs; 7 % and 4.5 % benzyl alcohol produced clonic seizures in puppies.

Source: Bayer AG Leverkusen

15 -JUL-1993 (185)

Remark: Injection of benzyl alcohol (700-900 mg/kg, i.p.) caused rapid immobilization of mice. The mice were immobilized within 2 min. and remained unresponsive (no righting reflex, no wink reflex, and no leg reflex) for about 30 min. The immobilizing effect was accompanied by a marked hyperglycemia. Tracer studies indicated that the hyperglycemic effect may have resulted from increased gluconeogenesis.

Source: Bayer AG Leverkusen

27 -MAY-1993 (186)

Remark: Benzyl alcohol used as a stabilizer for antibiotics of aminoglycosid structure is the substance responsible for the displacement of bilirubin from albumin. The free, unbound, unconjugated bilirubin tends to diffuse into the lipid of the brain of young Gunn rats with resultant kernicterus.

Source: Bayer AG Leverkusen

15-JUL-1993

(187)

Remark: Duodenal and jejunal brush border membrane vesicle integrity was studied after in vitro treatment of rabbit tissue with benzylalcohol. The effect of the alcohol on gastric parietal cell apical and microsomal membrane vesicle integrity was also studied. Exposure of vesicles to the alcohol caused concentration dependent decreases in enclosed volume. All concentrations tested reduced the enclosed volume of both gastric apical membrane vesicles and gastric microsomes. The alcohol induced disruption of the vesicle membranes appears to result from a fluidising effect. The main effect of the raised fluidity is to increase membrane fragility.

Source: Bayer AG Leverkusen

15-JUL-1993

(188) (189)

Remark: Benzyl alcohol as a fragrance ingredient used in cosmetic and other products is lipophilic and therefore has the potential to be readily absorbed through skin. The percutaneous absorption was determined in vivo in rhesus monkeys. Absorption through occluded skin was high (56-80 %) in 24 h. No correlation was seen between skin penetration and the octanol-water partition coefficient. Under unoccluded conditions skin penetration was reduced (32 %), because of evaporation of the compound.

Source: Bayer AG Leverkusen

27-MAY-1993

(190)

Remark: After i.v. injection in mice, benzyl alcohol was found to inhibit TBPS binding and to stimulate GABA receptor mediated Cl influx into brain vesicles.

Source: Bayer AG Leverkusen

27-MAY-1993

(191)

Remark: Benzyl alcohol can cause hemolysis of human and rabbit erythrocytes in the presence of 0.9 % NaCl.

Source: Bayer AG Leverkusen

15-JUL-1993

(192) (193)

Remark: Benzyl alcohol produced up to 6-fold increases in cAMP concentrations in purified human peripheral blood lymphocytes. Significant but less marked augmentation of cAMP was observed in human platelets, human granulocytes, and rabbit alveolar macrophages. The mechanism of the alcohol-induced cAMP accumulation is probably secondary to membrane perturbation and consequent activation of adenylate cyclase.

Source: Bayer AG Leverkusen

15-JUL-1993

(194)

Remark: Uncoupled sonic submitochondrial particles from beef heart and rat liver were studied for mitochondrial electron transport. Benzyl alcohol was found to inhibit each of the segments of the electron transport chain assayed. NADH oxidase and NADH-cytochrome c oxidoreductase required the lowest concentration for inhibition, and cytochrome c oxidase required the highest concentration. Beef heart submitochondrial particles are less sensitive to inhibition than are rat liver particles.

Source: Bayer AG Leverkusen

27-MAY-1993

(195)

Remark: Lactated Ringer's solution containing 1.5 % benzyl alcohol can cause severe symptoms of toxicity in cats including hyperesthesia leading to depression, coma, and finally death. In the cat, only hippuric acid is formed, as this species lacks adequate glucuronic acid conjugation capacity, resulting in a decreased rate of metabolism.

Source: This results in an accumulation of benzoic acid. Benzoic acid has been shown to be extremely toxic to cats, causing clinical signs similar to those observed.
Bayer AG Leverkusen

15-JUL-1993 (196)

Remark: 50 mM benzyl alcohol fluidized proximal brush-border membranes prepared from human small intestine and increased p-nitrophenyl-phosphatase activity in this membrane. This agent also shifted the phase transition temperature of the membrane and breakpoint temperature of this enzymatic activity.

Source: Bayer AG Leverkusen

15-JUL-1993 (197)

Remark: Microscopic examination revealed local nerve degeneration when 5 % benzyl alcohol was injected into the side of a cat's face. At 10 % local anaesthesia was produced.

Source: Bayer AG Leverkusen

27-MAY-1993 (198)

Remark: Benzyl alcohol displays a pronounced antiarrhythmic-anti- fibrillatory effect, when injected i.v. into dogs and rats with spontaneous or drug-induced arrhythmias. Mechanisms which might be responsible for the antiarrhythmic effect: lengthening of the effective refractory period, local and general anaesthetic effects, changes of osmolality. The i.v. injection of benzyl-alcohol in high doses, produces intravascular haemolysis.

Source: Bayer AG Leverkusen

27-MAY-1993 (199)

Remark: The length of the oestrus cycle was reduced when 0.52-2.1 d (1-4 mg/kg bw) benzyl alcohol was injected into the uterus of each of 48 cows.

Source: Bayer AG Leverkusen

27-MAY-1993 (200)

Remark: The in vitro effect of local anesthetic benzyl alcohol was studied using isolated cells from rat stomach. Lower concentrations of the alcohol increased the basal aminopyrine accumulation and potentiated the secretory response of parietal cells to histamine and dbcAMP.
At higher concentrations the alcohol progressively inhibited both the basal 14-C-aminopyrine accumulation and that stimulated by histamine, dbcAMP or carbachol. While a low concentration increased gastric microsomal (H-K)-ATPase activity, higher concentrations inhibited enzyme activity to about 80 % of those activities found in resting parietal cells.

Source: Bayer AG Leverkusen

15-JUL-1993 (201)

Remark: Benzyl alcohol is a fairly efficient anesthetic for intact mucous membranes, greatly surpassing procain. Its action is not as lasting as that of cocain. It appears that 1 % does not produce satisfactory anesthesia of the tongue, even after 10 min.

contact.

Source: Bayer AG Leverkusen

27-MAY-1993 (202)

Remark: Benzyl alcohol in non-toxic concentrations was found to markedly reduce the hemoglobin minor/hemoglobin major ratio and to moderately reduce the total hemoglobin induced by DMSO or HMBA in mouse erythroleukemia (MEL) cells, while only slightly decreasing the ratio induced by hemin or butyrate.

Source: Bayer AG Leverkusen

27-MAY-1993 (203)

Remark: It was demonstrated that benzyl alcohol, a neutral local anesthetic, inhibits the uptake and degradation of lowdensity lipoprotein and endocytosis of transferrin receptors of guinea-pig leukemic B lymphocytes. This inhibition is very rapid, concentration dependant and reversible by simple washing. Membrane fluidity of the living cells is also modified.

Source: Bayer AG Leverkusen

27-MAY-1993 (204)

Remark: The tissue culture lethal dose (TCLD50) in mouse embryo cells was found to be 0.002 mg/ml.

Source: Bayer AG Leverkusen

27-MAY-1993 (205)

Remark: Benzyl alcohol is more toxic to infant jaundiced (jj) than to non-jaundiced (Jj) Gunn rats. Before excretion as hippuric acid, benzyl alcohol is metabolized to benzoic acid, a potent competitor for bilirubin-albumin binding sites. These pathways are immature in newborns. Therefore the kernicterus in jj pups is probably due to increased levels of unbound bilirubin.

Source: Bayer AG Leverkusen

27-MAY-1993 (206)

Remark: The plasma half-life of benzyl alcohol administered as a 2.5 % solution in saline was found to be approximately 1.5 h in dogs injected i.v. at doses of 52 and 105 mg/kg.

Source: Bayer AG Leverkusen

27-MAY-1993 (47)

Remark: Larger percentages of benzyl alcohol doses were found in urine as benzoic acid in preterm babies, while less hippuric acid appeared in their urine than in term newborns.

-
- Source: These results indicate that hippuric acid formation is deficient in preterm neonates.
Bayer AG Leverkusen
- 27-MAY-1993 (207)
- Remark: In vitro studies of human liver alcohol dehydrogenase (ADH) variants revealed that benzyl alcohol is slowly metabolized by beta-2-ADH. Working with this solvent might lead to toxic effects; these could be particularly prominent in individuals possessing the beta-2-ADH if they have a lower capacity to eliminate them, or they could be particularly prominent in those with beta-1-ADH if they quickly convert them into toxic aldehydes.
- Source: Bayer AG Leverkusen
- 27-MAY-1993 (208)
- Remark: Perfusing the anterior chamber of enucleated rabbit eyes with 1.18 % benzyl alcohol, the corneal endothelial cells changed the appearance and the corneas began to swell.
- Source: Bayer AG Leverkusen
- 24-AUG-1993 (209)
- Remark: The invitro effects of benzyl alcohol and benzaldehyde on subcellular rat liver NAD-dependant alcohol and aldehyde dehydrogenase were studied as a function of gender. These effects were compared with those of the primary substrates ethanol and acetaldehyde. the results suggest metabolic competitions between benzyl alcohol and ethyl alcohol for catalysis by alcohol dehydrogenase.
- Source: Bayer AG Leverkusen
- 03-MAR-1998 (210)
- Remark: Acute intravenous toxicity of benzyl alcohol was determined in CD2F1 (0.05-0.2 ml/kg bw), B6D2F1 (0.05-0.4 ml/kg) and C57BL/6 mice.

The lowest dose was a safe dose and the highest one was the dose causing mortality in no more than half the animals of each group. Clinical signs were convulsion, dyspnea and reduced mortality in all strains for 24 hours. The slight decrease in body weight in the first week following treatment returned to normal in the second week.

Source: Bayer AG Leverkusen

03-MAR-1998

(211)

6.1 Analytical Methods

6.2 Detection and Identification

7.1 Function

7.2 Effects on Organisms to be Controlled

7.3 Organisms to be Protected

7.4 User

7.5 Resistance

8.1 Methods Handling and Storing**8.2 Fire Guidance****8.3 Emergency Measures****8.4 Possib. of Rendering Subst. Harmless****8.5 Waste Management****8.6 Side-effects Detection****8.7 Substance Registered as Dangerous for Ground Water****8.8 Reactivity Towards Container Material**

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10.1 End Point Summary**10.2 Hazard Summary****10.3 Risk Assessment**

PRIVILEGED AND CONFIDENTIAL

**LITERATURE SEARCH AND REVIEW TOBACCO INGREDIENTS USED BY
MANUFACTURERS IN THE PRODUCTION OF CIGARETTES**

**FINAL REPORT: 2005
For 2004 list of ingredients**

**Donald E. Gardner PhD, F.ATS
Susan C. Gardner PhD
Inhalation Toxicology Associates
Savannah, GA**

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75	Urea	00057-13-6	56
76	Sodium carbonate	00497-19-8	57
77	Fructose	00057-48-7	57
78	Davana oil	08016-03-3	57
79	Lime oil	68916-84-7	57
80	Ethyl 2-methylbutyrate	07452-79-1	57
81	Peppermint oil and absolute and peppermint oil terpeneless	08006-90-4	58
82	Spearmint oil	08008-79-5	58
83	Orange oil and extract (sweet, distilled, terpeneless, and sour/bitter orange oils)	08008-57-9 68606-94-0 68916-04-1	58
84	Molasses extract	08052-35-5	58
85	Coriander extract, seed, and oil	08008-52-4 84775-50-8	58
86	Ethyl vanillin	00121-32-4	58
87	L-Menthone	14073-97-3	58
88	Vanillin	00121-33-5	59
89	Chamomile flower oil, extract and absolute	08002-66-2 08015-92-7	59
	Standard Ingredients		60
90	Acetanisole	00100-06-1	61
91	Acetic acid	00064-19-7	61
92	Acetoin	00513-86-0	61
93	Acetophenone	00098-86-2	61
94	Acetylpyrazine (2-)	22047-25-2	61

95	3-Acetylpyridine (beta-Acetylpyridine)	00350-03-8	61
96	2-Acetylthiazole	24295-03-2	61
97	DL-Alanine, L-Alanine	00302-72-7 00056-41-7	61
98	Alfalfa extract	84082-36-0	61
99	Allyl hexanoate	00123-68-2	62
100	Ammonium alginate	09005-34-9	62
101	Ammonium hydroxide	01336-21-6	62
102	Ammonium phosphate dibasic (Diammonium phosphate)	07783-28-0	62
103	Amyl alcohol	00071-41-0	62
104	Amyl butyrate	00540-18-1	62
105	Amyl formate	00638-49-3	62
106	Amyl octanoate	00638-25-5	62
107	alpha-Amylcinnamaldehyde	00122-40-7	62
108	trans-Anethole	04180-23-8 00104-46-1	63
109	Angelica root extract and oil	84775-41-7	64
110	Anise star oil	08007-70-3	64
111	Anisyl acetate	00104-21-2	64
112	Apple juice concentrate, essence and extract	85251-63-4	64
113	L-Arginine	00074-79-3	64
114	Ascorbic acid	00050-81-7	64
115	L-Aspartic acid	00056-84-8	65
116	Balsam peru and oil	08007-00-9	65
117	Beeswax resinoid and absolute	08006-40-4 08012-89-3	65
118	Beet juice concentrate	89957-90-4	66
119	Benzaldehyde	00100-52-7	66
120	Benzaldehyde glyceryl acetal	01319-88-6	66
121	Benzoic acid	00065-85-0	66
122	Benzoin, resin, resinoid, tincture, gum and absolute	09000-05-9 84012-39-5 09000-72-0	67
123	Benzyl alcohol	00100-51-6	67
124	Benzyl benzoate	00120-51-4	69
125	Benzyl cinnamate (Propenic acid, 3-phenyl, phenylmethyl ester,2-)	00103-41-3	70
126	Benzyl Phenylacetate	00102-16-9	71
127	Benzyl Propionate	00122-63-4	71
128	Bornyl acetate	00076-49-3	71
129	1,3-Butanediol	00107-88-0	71

130	2, 3-Butanedione (Diacetyl)	00431-03-8	71
131	Butanoic acid, 3-methyl-, 4-methylphenyl ester (para-Tolyl 3-methylbutyrate) (p-Tolyl isovalerate)	55066-56-3	71
132	Butter, butter esters, and butter oil	91745-88-9 97926-23-3	71
133	Butyl acetate	00123-86-4	71
134	Butyl alcohol (1-Butanol)	00071-36-3	72
135	Butyl butyryl lactate (butoxy-1-methyl-2-oxoethyl ester butanoic acid, 2-)	07492-70-8	73
136	n-Butyl isovalerate	00109-19-3	73
137	3-Butylidenephthalide	00551-08-6	73
138	Butyric acid	00107-92-6	74
139	Caprylic/Capric triglyceride	65381-09-1	74
140	Caramel and caramel color	08028-89-5	74
141	Carbon	07440-44-0	74
142	Carbon dioxide	00124-38-9	74
143	Cardamom oleoresin, oil, extract, seed oil, and powder	08000-66-6 96507-91-4	76
144	Carob bean gum, absolute and extract	09000-40-2 84961-45-5	77
145	beta-Carotene	07235-40-7	77
146	Carrot oil, seed	08015-88-1	84
147	4-Carvomenthenol	00562-74-3	84
148	beta-Caryophyllene	00087-44-5	84
149	beta-Caryophyllene oxide	01139-30-6	84
150	Cassia bark, buds, oils, and extract	08007-80-5 84961-46-6	84
151	Castoreum, liquid, extract, tincture and absolute	08023-83-4	84
152	Celery seed oil	89997-35-3	84
153	Cellulose and Cellulose fiber	65996-61-4 09004-34-6	84
154	Chamomile flower oil, extract and absolute	08002-66-2 08015-92-7	84
155	Chicory extract	68650-43-1	85
156	Chocolate and chocolate liquor		85
157	1,8-Cineole (Eucalyptol)	00470-82-6	85
158	Cinnamaldehyde	00104-55-2	85
159	Cinnamon bark, buds, leaf, oil, and extract	08015-91-6 08007-80-5	87

160	Cinnamyl acetate	00103-54-8	87
161	Cinnamyl alcohol	00104-54-1	87
162	Cinnamyl cinnamate	00122-69-0	88
163	Citral	05392-40-5	88
164	Citric acid	00077-92-9	92
165	Citronella oil	08000-29-1	92
166	Citronellol	00106-22-9	93
167	Clary sage oil and extract	08016-63-5	93
168	Cocoa, cocoa shells, extract, distillate, powder, alkalized, absolute and tincture	08002-31-1 84649-99-0 68916-17-6 95009-22-6	93
169	Coconut oil	08001-31-8	93
170	Coffee and coffee solid extract	08001-67-0 68916-18-7 84650-00-0	93
171	Cognac white and green oil	08016-21-5	93
172	Coriander extract, seed, and oil	08008-52-4 84775-50-8	93
173	Corn starch	09005-25-8	93
174	beta-Damascone	23726-92-3 23726-91-2	94
175	Davana oil	08016-03-3	94
176	Decanal	00112-31-2	94
177	delta-Decalactone	00705-86-2	94
178	gamma-Decalactone	00706-14-9	94
179	Decanoic acid	00334-48-5	94
180	Diacetyl	00431-03-8	94
181	Diethyl malonate	00105-53-3	94
182	2,3-Diethylpyrazine	15707-24-1	94
183	2,6-Dimethoxyphenol	00091-10-1	95
184	Dimethyl benzyl carbinyl butyrate (alpha, alpha-Dimethylphenethyl butyrate)	10094-34-5	95
185	Dimethyl sulfide	00108-50-9	95
186	3,4-Dimethyl-1,2-cyclopentadione	13494-06-9	95
187	3,7-Dimethyl-1,3,6-octatriene	13877-91-3	95
188	4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one (3-Hydroxy-4,5-dimethyl-2(5h)furanone)	28664-35-9	95
189	2, 5-Dimethyl-4-hydroxy-3(2h)-furanone (4-Hydroxy-2,5-dimethyl-3(2h)furanone)	03658-77-3	95
190	3,7-Dimethyl-6-octenoic acid	00502-47-6	96

	(Citronellic acid)		
191	alpha,para-Dimethylbenzyl alcohol	00536-50-5	96
192	2,3-Dimethylpyrazine	05910-89-4	95
193	2,5-Dimethylpyrazine	00123-32-0	96
194	Dodecahydro-3A,6,6,9A-tetramethylnaphtho (2,1-B)furan (1,5,5,9-Tetramethyl-13-oxatricyclo(8.3.0.0(4,9))Tridecane)	03738-00-9 06790-58-5	96
195	delta-Dodecalactone	00713-95-1	97
196	gamma-Dodecalactone	02305-05-7	97
197	Ethyl acetate	00141-78-6	97
198	Ethyl alcohol, including SDA-4	00064-17-5	98
199	Ethyl benzoate	00093-89-0	98
200	Ethyl butyrate	00105-54-4	98
201	Ethyl cinnamate (Propenic acid,3-phenyl-,ethyl ester,2-)	00103-36-6	98
202	Ethyl decanoate	00110-38-3	98
203	4-Ethyl guaiacol (4-Ethyl-2-methoxy-phenol)	02785-89-9	98
204	Ethyl heptanoate	00106-30-9	98
205	Ethyl hexanoate (Ethyl caproate)	00123-66-0	98
206	Ethyl isovalerate	00108-64-5	99
207	Ethyl lactate	00097-64-3	99
208	Ethyl laurate	00106-33-2	99
209	Ethyl levulinate	00539-88-8	99
210	Ethyl maltol	04940-11-8	99
211	Ethyl 2-methylbutyrate	07452-79-1	99
212	Ethyl methyl phenylglycidate	00077-83-8	99
213	Ethyl myristate	00124-06-1	99
214	Ethyl nonanoate	00123-29-5	99
215	Ethyl octadecanoate	00111-61-5	100
216	Ethyl octanoate	00106-32-1	100
217	Ethyl oleate	00111-62-6	100
	Ingredients used in mixture studies tobacco smoke studies		100
	Relevant reviews & interesting papers		107

INTRODUCTION

In a continuing effort to improve the safety evaluation of ingredients added to tobacco, this literature review program identifies and reviews relevant abstracts and documents for information regarding potential health effects of a large number of ingredients.

This review is intended to provide an appropriate means for the continuing safety assessment of the ingredients added to tobacco. This is not intended to be a summary of all available data on a particular ingredient; rather, the aim and scope of this review is on providing the sponsors with an overview of available data regarding issues that can play a role in establishing their safe use. Results from this review can aid in (1) prioritizing for additional toxicology testing and for mechanistic studies, (2) facilitating the evaluation of any proposed modifications to cigarettes, and (3) allowing data exchange between the sponsors and the panel members.

A list of 217 ingredients was provided by Covington and Burling as representing the high-priority chemicals. These ingredients are divided into four categories:

- 1). New ingredients (8). For these ingredients Inhalation Toxicology Associates (ITA) searched the databases for all citations entered into the database since 1965.
- 2). Major ingredients (45). These are ingredients having a maximum use level (MUL) of 500 ppm or greater. For this category ITA searched the databases for relevant citations between the dates of the last search to 2004.
- 3). High MUL ingredients (36). This category includes ingredients whose MUL has increased by a factor of 10 or more from the prior year. For these high MUL ingredients ITA searched the databases for relevant citations between the dates of the last search to 2004.
- 4). Standard ingredients (128) as identified by Covington and Burling. For this category ITA searched the databases for relevant citations between the dates of the last search to 2004.

The first stage involves the collection of relevant data, including the results of *in vivo* and *in vitro* studies. The second stage involves the assessment of these data to determine the acceptability of the study and relevance of the results to the substance as a tobacco ingredient. To meet these objectives, ITA searches the databases using chemical abstract numbers for relevant citations during the dates corresponding to the category in which they are listed. ITA primarily used the American Chemical Society's Chemical Abstract Services and Dialog Database to search for information about ingredients of interest.

A series of databases were used to search for relevant national and international studies. If in the judgment of ITA, the search for a particular ingredient in any of these databases was not expected to produce relevant information, ITA was authorized to omit

the search of such database(s). ITA was also authorized to modify the literature search strategies in order to better meet the needs of the sponsors.

After the sponsors/panel members have had an opportunity to examine this 2004 report and they believe the goals and objectives of this project would benefit by including some “other” sources, ITA would be most willing to expand our coverage to seek out additional publications/reports for any specific ingredient they determine needs more coverage. If it is decided that “other” sources should be added to our list of databases in future years, we would be most pleased to add these sources to our list of databases searched. During 2004, a total of 9427 titles were retrieved of which 461 were identified as potentially relevant and their abstracts were collected and reviewed by ITA. Using the data from these abstracts, a total of 134 full text copies of relevant documents were retrieved by ITA for a more in-depth review.

As in previous years, it is appropriate to establish some generally accepted and recognized criteria that can be used in assessing the toxicological risk of ingredients in a relatively efficient manner. These guidelines are intended to expedite the safety assessment of ingredients added to tobacco. While the material examined was extensive, most of the toxicological testing of ingredients was not designed to evaluate the health hazards of ingredients in cigarette smoke, but instead focused on the hazards associated with exposure to either the pure substances or as additives in some other medium, such as food. This adds to the complexity of trying to interpret and extrapolate this data for assessing and predicting human health risk associated with exposure to those ingredients found in cigarettes. Although many of these studies were not designed to evaluate tobacco additives, the results have to be considered since they aid in providing a complete picture of the database for these chemicals.

From the large number of studies encountered, it was practical to summarize only the most specific and relevant observations. However, situations that have become controversial are dealt with in more detail. While it was appropriate that ITA considered all data and make decisions about the validity and usefulness of these data, certain research areas received lower priority and may have been excluded from further examination. Examples would be studies involving 1) the use of such ingredients in the treatment of a variety of diseases, 2) new methodologies for measurement, 3) studies addressing potential anti-microbial or pesticidal activity, 4) effects reported on plants and lower animal systems and 5) publications not in English. Even with these exclusions, ITA has provided the sponsors with a vast amount of information. Good decisions are most likely to result from integration of all available data, including those demonstrating adverse effects as well as well-designed studies indicting no effects. This was done to provide the sponsors with a broad base of published literature, and they can select from these studies the most relevant information useful in meeting their unique needs. For each ingredient where there was relevant scientific data addressing the safe use of ingredients in cigarette products, these studies are discussed below. All of the titles and abstracts retrieved have been retained, and hard copies of the most relevant papers are available upon request.

In our professional judgment, based on the literature reviewed during this time period, no information has been generated which indicates that the use of the ingredients evaluated in this review presents a hazard to the health of the consumer at the level being used, so far as can be judged by the scientific evidence available.

Thank you for providing ITA the opportunity to review this subject matter and to express an opinion regarding the health effects of these ingredients. We are available to provide further clarification or discussion if you have questions.

**Donald E. Gardner PhD., Fellow ATS,
National Associate of the National Academy of Science
Susan C. Gardner PhD.
DATE: February 15, 2006
Inhalation Toxicology Associates, Inc.**

INGREDIENTS REVIEW

CATEGORY: NEW INGREDIENTS

PARA-TOLUALDEHYDE CAS: 104-87-0

Number of relevant papers: 7

GENERAL COMMENTS ON PAPERS LISTED BELOW:

The first five papers listed below provide a broad view of biological activity for a large number (239 to 464) of individual tobacco smoke constituents using an array of short-term assays. The general conclusion reached was that tobacco smoke contains a number of substances that inhibit cell growth using Ascites sarcoma cells, inhibits noradrenaline, stimulated oxidative metabolism in isolated brown fat cells, damages plasma membrane of cultured human lung fibroblasts and may be mutagenic in the Ames test. Although not directly applicable to the human exposure situation, these assays provide information on possible mechanisms involved in the interaction of specific smoke constituents and cell function.

1. Effects of tobacco smoke compounds on the ciliary activity of the embryo chicken trachea in vitro.

Pettersson B, Curvall M, Enzell CR.

Toxicology. 1982;23(1):41-55.

ABSTRACT: The ciliotoxicity of 316 individual compounds representative of the gaseous and semivolatile phases of tobacco smoke has been investigated using chicken tracheal organ cultures. When examined at 5 mM concentration and measuring the time to complete ciliostasis, 36% of the compounds were found to cause ciliostasis within 15 min, while about 50% had no visible effect on the ciliary activity during a 60-min exposure. The majority of the ciliotoxic compounds were either alkylated phenylethers, benzonitriles, benzaldehydes, phenols, benzenes, naphthalenes and indoles, or alpha, beta-unsaturated ketones and aldehydes or C6-C10 aliphatic alcohols, aldehydes, acids and nitriles. Most of the compounds classified as benzoic acids, esters, polyaromatic hydrocarbons, amines and N-heterocycles, except indoles, were found to be inactive.

COMMENTS: Comments are provided above.

2. Effects of tobacco smoke compounds on the noradrenaline induced oxidative metabolism in isolated brown fat cells.

Pettersson B, Curvall M, Enzell CR.

Toxicology. 1980;18(1):1-15.

ABSTRACT: The effect on cell metabolism of 320 individual smoke components have been investigated by measuring their inhibition of noradrenaline induced respiration in isolated hamster brown fat cells. The compounds are representative of the gaseous and semivolatile phases of tobacco smoke. The strongest inhibitors were found within the groups of aliphatic alcohols, aldehydes and acids, of alkylated phenols and indoles and of alpha, beta-unsaturated aliphatic aldehydes and ketones. Some of the aliphatic aldehydes and acids significantly increased the basal respiration of the cells, probably by acting as substrates and/or uncoupling of mitochondrial respiratory control.

COMMENTS: Comments are provided above.

3. Effect of tobacco smoke compounds on the plasma membrane of cultured human lung fibroblasts

Thelestam M, Curvall M, Enzell CR.
Toxicology. 1980;15(3):203-17.

ABSTRACT: The ability of compounds derived from tobacco and tobacco smoke to increase the permeability of the membranes of human lung fibroblasts has been studied by measuring the release of an intracellular marker after short term exposure. Of the 464 compounds tested, about 25% gave rise to severe membrane damage. The most active compounds, when divided according to functionality, were found within the groups of amines, strong acids and alkylated phenols, whereas nitriles and polycyclic aromatic hydrocarbons were found completely inactive. A pronounced effect of the chain length on the activity was observed for the aliphatic alcohols, aldehydes and acids, and all monocyclic aromatic compounds but benzonitriles and benzoic acids showed an increase in activity with increasing alkylsubstitution. It is concluded that tobacco smoke contains a number of membrane damaging substances. These membrane active compounds could not only cause direct toxic reactions but also potentiate the toxic effect by promoting the cell membrane penetration of other toxic substances in tobacco smoke.

COMMENTS: Comments are provided above.

4. Screening of tobacco smoke constituents for mutagenicity using the Ames' test

Florin I, Rutberg L, Curvall M, Enzell CR.
Toxicology. 1980;15(3):219-232.

ABSTRACT: To clarify the mutagenic activity of individual smoke components, 239 compounds, representative of the gaseous and semivolatile phases of tobacco smoke, were assayed for mutagenicity towards 4 histidine-requiring mutants of *Salmonella typhimurium* (TA 98, TA 100, TA 1535 and TA 1537). All compounds were tested qualitatively both with and without metabolic activation using a liver fraction (S-9) from Aroclor 1254 or methylcholanthrene induced rats. Without S-9, only 2,3-dimethylindole and 2,3,5-trimethylindole showed mutagenic activity that was not enhanced by the

metabolic activation system. 2,6-Diaminotoluene and coronene, which like the above compounds are not documented carcinogens were found to be mutagenic for strain TA 98 with S-9. Mutagenic activity was also observed for the previously known mutagens benz[a]pyrene, chrysene, benz[a]-anthracene, perylene and beta-naphthylamine, on exposure to strains TA 98 and/or TA 100 with S-9.

COMMENTS: Comments are provided above.

5. Effects of tobacco and tobacco smoke constituents on cell multiplication in vitro - CA

Pilotti A, Ancker K, Arrhenius E, Enzell C.

Toxicology. 1975 Sep;5(1):49-62.

ABSTRACT: Ascites sarcoma BP8 cells, cultured in suspension in vitro were used as a general toxicity test system for tobacco and tobacco smoke constituents. Some 250 compounds, representative of these materials, were examined by exposing cells to different concentrations of these constituents and measuring the inhibition of culture growth, which was related to corresponding effects encountered for positive standards. When employing the present cell toxicity test system possible effects of factors such as penetration, distribution and microsomal metabolism of the compounds studied, are not taken into account. The most active constituents were found to be unsaturated aldehydes and ketones, phenols and indoles. The good correlation observed between functional groups and toxicity permits, within the range of functionalities studied, prediction of the toxicity for a compound of known structure.

COMMENTS: Comments are provided above.

6. AMES SALMONELLA/MAMMALIAN MICROSOME MUTAGENICITY TEST AND REVERSE MUTATION ASSAY - E. COLI WP2 UVRA A (STANDARD PLATE TEST AND PREINCUBATION TEST) (OCT. 19, 1988)

Source: EPA/OTS; Doc #86-920000590

ABSTRACT: P-Tolualdehyde (CAS # 104-87-0) was evaluated for mutagenicity in the Ames test (strains TA1535, TA100, TA1537, TA98) with and without metabolic activation (S-9 mix) and in the Escherichia coli (WP2 uvrA) reverse mutation assay at a dose range of 20 ug - 5000 ug/plate in the standard plate test (SPT) and 4 ug - 2500 ug/plate in the preincubation test (PIT). No bacteriotoxic effect was observed with E. coli. Bacteriotoxicity was detected in all Salmonella strains detected at 2500 ug/plate (PIT) and at 5000 ug/plate (SPT). The test substance was determined to be non-mutagenetic.

COMMENTS: P-Tolualdehyde was determined to be non-mutagenic in the Ames test.

7. Naturally occurring carbonyl compounds are mutagens in Salmonella tester strain TA 104

**Marnett LJ, Hurd HK, Hollstein MC, Levin DE, Esterbauer H, Ames BN.
Mutat Res. 1985 Jan-Feb;148(1-2):25-34.**

ABSTRACT: Strains of *Salmonella typhimurium* that carry a nonsense mutation at the site of reversion detect a variety of naturally occurring and synthetic carbonyl compounds as direct-acting mutagens. TA104 is reverted efficiently by formaldehyde, alpha, beta-unsaturated aldehydes (enals), and dicarbonyl compounds, such as diacetyl and glutaraldehyde. This strain is much more sensitive to carbonyl mutagenesis than is TA100, a strain previously reported to detect aldehydes as mutagens, or any other characterized strains of *Salmonella*. Long-chain enals are very toxic to TA104, but addition of a reduced glutathione chase following an incubation period decreases this toxicity, thus enabling the detection of 4-hydroxy-pentenal, a homolog of the lipid peroxidation product, 4-hydroxy-nonenal, as a mutagen. This is the first report of the mutagenicity of a hydroxy-enal, a class of enals produced by lipid peroxidation. Testing conducted with strains that carry the nonsense mutation in different repair backgrounds indicates that the presence of pKM101 and the deletion of the *uvrB* gene facilitate the detection of enals and dicarbonyls, but not malondialdehyde, as mutagens. Since carbonyl compounds are widely distributed in foods, are generated during cellular metabolism, and are present in body fluids, they may make a significant contribution to the risk of human cancer.

COMMENTS: Additional comments not necessary, abstract satisfactory.

CITRONELLOL CAS: 106-22-9

Number of relevant papers: 3

1. Effects of fragrance inhalation on sympathetic activity in normal adults

**Haze S, Sakai K, Gozu Y.
Jpn J Pharmacol. 2002 Nov;90(3):247-53.**

ABSTRACT: We investigated the effects of fragrance inhalation on sympathetic activity in normal adult subjects using both power spectral analysis of blood pressure fluctuations and measurement of plasma catecholamine levels. Fragrance inhalation of essential oils, such as 19 Effects of fragrance inhalation on sympathetic activity in normal adults

COMMENTS: This study demonstrated that inhalation of fragrances can stimulate or depress sympathetic activity in human volunteers. While citronellol was not tested, it was identified as being present (27.7%) in rose oil that was tested. Inhaled rose oil significantly inhibited sympathetic activity and decreased adrenaline levels. The authors

suggest that citronellol might be involved in the modulation of sympathetic activity in normal adults.

2. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters

Fukayama MY, Easterday OD, Serafino PA, Renskers KJ, North-Root H, Schrankel KR.

Toxicol Lett. 1999 Dec 20;111(1-2):175-87.

ABSTRACT: Users of consumer products are invariably and intentionally exposed to complex mixtures in such products. With finished fragrance products, these mixtures may represent 100 or more fragrance raw materials (FRMs). The objective of the described studies was to evaluate the safety of finished fragrance products via the inhalation route. In total, the finished products contained approximately 100 FRMs at concentrations of 1% or greater. Major FRMs evaluated included benzyl acetate, coumarin, hydroxycitronellal, musk ketone, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-2-benzopyran (HHCB) and phenyl ethyl alcohol. Groups of rats or hamsters were exposed by inhalation (whole body) to the mixtures at 5, 9 or 50 mg/m³ for 4 h per day, 5 days per week for 6 or 13 weeks. For each of the fragrance products, the doses used generally represented a ten- to 100-fold exaggeration of levels expected to be achieved during typical use by consumers. With one exception, the fragrances were aerosolized prior to introduction into the inhalation chamber. The exception product was formulated with a propellant, packaged in a pressurized container and expelled with an automated actuator. In all studies, chamber concentrations of fragrance were monitored. Particle sizes ranged from 0.5 to 7.5 microm, depending on the study. Subchronic exposure to all fragrance mixtures resulted in no toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters. No gross pathological or histopathological findings related to test material exposures were observed. These studies support the conclusions that the fragrance mixtures would not pose a hazard to product users based on repeated and exaggerated inhalation exposures of animals.

COMMENTS: Rats and hamsters were exposed by inhalation to a complex mixture of fragrances. The exposure levels were 10 to 100 fold greater than one would expect to be encountered by humans using such fragrances. None of the fragrances produced signs of toxicity following exposures up to 13 weeks. No histopathological abnormalities were reported in trachea or lungs. The results are consistent with those earlier studies of Gaworski 1998 that exposed rats to mixtures of flavor ingredients in a subchronic smoke inhalation study.

3. Fragrance compounds and essential oils with sedative effects upon inhalation

Buchbauer G, Jirovetz L, Jager W, Plank C, Dietrich H.

J Pharm Sci. 1993 Jun;82(6):660-4.

ABSTRACT: Fragrance compounds and essential oils with sedative effects influence the motility of mice in inhalation studies under standardized conditions. A significant drop in the motility of mice was registered following exposure to these fragrances. The same results were achieved when the mice were artificially induced into overagitation by intraperitoneal application of caffeine and subsequently subjected to inhalation of fragrance compounds and essential oils. These results proved the sedative effects of these fragrances via inhalative exposure in low concentrations. Blood samples were taken from the mice after a 1-h inhalation period. Chromatographic and spectroscopic methods were used to detect and characterize the actual effective compounds after solid-phase extraction. Serum concentrations of 42 different substances, including fragrance compounds, were found in low ranges (ng/mL serum). The results contribute to the correct interpretation of the term aromatherapy (i.e., a stimulating or sedative effect on the behaviour of individuals only upon inhalation of fragrance compounds).

COMMENTS: A one-hour inhalation of citronellol showed a significant sedative effect in over-agitated (caffeine-treated) mice but not with animals without prior caffeine induction. These sedative effects were observed at low blood concentrations (2.0 ng/mL). Substances that produce such an effective may interact with lipids of cell membranes in the cortex thus indicating a direct pharmacological interaction of fragrance molecules with bodily tissue.

ETHYL HEPTANOATE

CAS: 106-30-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ISOAMYL FORMATE

CAS: 110-45-2

Number of relevant papers: 2

1. Mutagenic and antimutagenic activities of flavoring agents used in foodstuffs -

Yoo, Y.S. (1986)

Osaka-shi Igakkai Zasshi [J. Osaka City Medical Center], 34(3-4), 267-288

ABSTRACT: N/A

COMMENTS: This article was in Japanese and was not translated. Briefly, these investigators tested for genotoxicity in 33 synthetic flavorings used in foodstuffs. Isoamyl formate had little or no toxic effect and was considered to be negative in the assay system.

2. Primary mutagenicity screening of food additives currently used in Japan –

**Ishidate, M; Sofuni, T; Yoshikawa, K;
Food Chem Toxicol 22:623-636.**

ABSTRACT: Salmonella/microsome tests (Ames tests) and chromosomal aberration tests *in vitro* using a Chinese hamster fibroblast cell line were carried out on 190 synthetic food additives and 52 food additives derived from natural sources, all of which are currently used in Japan. Fourteen out of 200 tested in the Ames assay showed positive effects and 54 out of 242 were positive in the chromosome test. Three additives (erythorbic acid, chlorine dioxide and beet red) were positive only in the Ames test, although their mutagenic potentials were relatively weak, while 43 additives were positive only in the chromosome test. Eleven additives (calcium hypochlorite, cinnamic aldehyde, L-cysteine monohydrochloride, Food Green No. 3 (Fast Green FCF), hydrogen peroxide, potassium bromate, sodium chlorite, sodium hypochlorite, sodium nitrite, cacao pigment and caramel) were positive in both the Ames test and the chromosome test. The usefulness of such primary screening tests combining two different genetic end-points, gene mutation and chromosomal aberration, and some correlation between mutagenicity and carcinogenicity of food additives are discussed.

COMMENTS: These investigators did primary screening of over 200 food additives using both the Ames test and chromosomal aberration tests. Only a few (11) were positive in both tests. More additives were positive in the chromosome test than the Ames test indicating that chromosomal aberrations can be induced by a wider range of additives than the Ames test, and suggesting that this may indicate not only initiators of carcinogenesis but also promoters. It should be recognized that data from such short term *in vitro* tests needs further *in vivo* testing to predict carcinogenicity. The correlation between carcinogenicity and mutagenicity of additives are discussed.

HEXYL ACETATE

CAS: 142-92-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

PECTIN

CAS: 9000-69-5

Number of relevant papers: 3

1. Pectin and cashew nut allergy: Cross-reacting allergens?

**Rasanen L, Mäkinen-Kiljunen S, Harvima RJ.
Allergy. 1998 Jun;53(6):626-8.**

ABSTRACT: N/A

COMMENTS: This case report indicates that exposure to pectin may cause sneezing, rhinitis, conjunctivitis and contact urticaria. Occupational sensitization with rhinitis and asthma from pectin has been previously identified. In this study, blood basophil histamine release and serum IgE were positive.

2. Occupational asthma caused by pectin inhalation during the manufacture of jam

AJ Cohen, MS Forse and SM Tarlo
Chest, Vol 103, 309-311, Copyright © 1993

ABSTRACT: We report a case of pectin-induced occupational asthma in a 35-year-old man. His job involved mixing powdered pectin into a fruit puree during the manufacture of jam. Within minutes of adding pectin, he developed coryza, rhinorrhea, coughing, and wheezing. His symptoms cleared during weekends while away from work and improved with the use of a protective facemask at work. Peak flow rates were significantly lower while at work compared with those at home, and a prick skin test with the pectin powder was positive. We conclude that pectin should be added to the list of the substances known to induce occupational asthma.

COMMENTS: This is another case report describing a pectin-induced occupational asthma. The individual exhibited positive skin testing to pectin. The authors suggested that pectin should be considered to be an allergen that causes occupational asthma.

3. Bacterial mutagenicity testing of 49 food ingredients gives very few positive results.

Prival MJ, Simmon VF, Mortelmans KE.
Mutat Res. 1991 Aug; 260(4):321-9.

ABSTRACT: 49 substances permitted for use in food in the United States was tested for mutagenicity in the Ames Salmonella typhimurium assay and in Escherichia coli strain WP2. Four of these substances caused increases in revertant counts in S. typhimurium. Two of these four (papain and pepsin) were found to contain histidine, and therefore the results of the tests on these two substances could not be taken as demonstrating mutagenicity. The other two substances causing increases in revertant counts (hydrogen peroxide and potassium nitrite) were mutagenic. The results on one chemical, beta-carotene, were evaluated as inconclusive or questionable. The remaining 44 substances were nonmutagenic in the test systems used. It is concluded that, for those generally physiologically innocuous chemicals tested, there are very few 'false positives' in the bacterial test systems used.

COMMENTS: The Salmonella Ames test and E coli mutagenicity assays were used to evaluate the mutagenicity of a number of food ingredients. Pectin gave no evidence of

mutagenicity. In these studies, the frequency of positive results was much lower than in many other previous studies. The authors believe that this is due to the fact that the chemicals tested were almost all nontoxic to mammals and to bacteria even at relatively high doses.

CORN STARCH **9005-25-8**

Number of relevant papers: 2

1. Inhaled cornstarch glove powder increases latex-induced airway hyper-sensitivity in guinea-pigs

Barbara J.; Santais M.-C.1; Levy D.A.2; Ruff F.1; Leynadier F.
Clinical & Experimental Allergy 34 (6): 978-983

ABSTRACT: Summary Background. Breathing is one of the most important modes of sensitization to natural rubber latex (NRL) for health-care workers, a group most at risk. Cornstarch powder (CSp) from medical powdered NRL gloves is known to be an allergen carrier, and sensitization to NRL can occur by inhaling airborne particles from such gloves. Objective: The aim of this study was to demonstrate, using an experimental model, which CSp may act as an adjuvant in NRL-induced airway hyper-responsiveness. Methods: Guinea-pigs were exposed to aerosolized NRL-contaminated CSp or to NRL in saline solution for 1 h every day for 2 weeks. The control groups were exposed either to CSp or to saline alone. An additional group of guinea-pigs was exposed to aerosolized ovalbumin (OVA) in saline. Three weeks after the last exposure, specific bronchial challenges were performed. In addition, Specific IgG and IgG1 in sera and thromboxane (Tx) B2 levels in bronchoalveolar lavage fluid (BALF) were measured. Results: The NRL challenge caused significant bronchospasm in the animals that had been exposed to NRL compared with those in the control groups ($P < 0.02$). Guinea-pigs exposed to OVA also demonstrated a significant bronchospasm after OVA challenge ($P < 0.001$). The guinea-pigs that had inhaled NRL-contaminated CSp had a significantly higher bronchoconstriction level than those that had inhaled NRL alone ($P < 0.02$). Specific IgG and IgG1 were undetectable in sera from all groups, whereas significant amounts of TxB2 ($P < 0.001$) were found in the lungs of the guinea-pigs exposed to NRL or OVA. Conclusion: Inhaling CSp increases the airway response to NRL. The fact that specific IgG and IgG1 were not detected might be the result of an immune response limited to the airways. This finding is supported by a significant increase of TxB2 level in the BALF of sensitized guinea-pigs.

COMMENTS: These authors previously reported that corn starch acts as an immunoadjuvant in guinea pigs that were previously sensitized to rubber latex by the IP route. This experimental model was used to determine if corn starch potentiates immunotoxicity of rubber latex through inhaling latex adsorbed onto corn starch. While the direct relevance of this study was to the health care workers who become sensitive to

rubber latex, it also indicates that breathing corn starch may induce hypersensitivity and may act as an adjuvant, resulting in increased airway responsiveness.

2. Bronchial provocation testing in the diagnosis of occupational asthma due to latex surgical gloves –

G Pisati, A Baruffini, F Bernabeo, and R Stanizzi
Eur Respir J 1994; 7: 332-336

ABSTRACT: In sensitized subjects, provocation tests to latex may induce severe systemic reactions and even anaphylactic shock. It is probable that part of the risk is due to the difficulty in grading the stimulating dose and in starting from very low levels of exposure. To identify the aetiological agent of work-related asthma in four nurses with previous allergic contact urticaria to latex surgical gloves dusted with cornstarch powder, we performed a specific bronchial provocation test study, based on exposure on three different days to nonpowdered latex surgical glove extract, powdered latex surgical glove extract and cornstarch powder extract, respectively. Extracts were nebulized in increasing concentrations in a 7 m³ challenge room, in the absence of the patients. The initial extract concentration was a tenfold dilution of the predetermined skin test end-point in the individual undergoing challenge, and the highest concentration was the undiluted extract. After exposure, the patients' forced expiratory volume in one second (FEV₁) was monitored for 2 h. If FEV₁ decreased by at least 15%, the next scheduled exposure was not carried out and FEV₁ was monitored over a period of 24 h. Whereas nebulization of cornstarch powder extract caused no bronchial reaction in the patients, nebulization of nonpowdered latex surgical glove extract induced immediate bronchoconstriction in two subjects as an undiluted solution, and nebulization of powdered latex surgical glove extract induced immediate bronchoconstriction in all subjects at the 1:10 dilution. No systemic reaction was elicited by the bronchial provocation challenges. Our results demonstrate that airborne powder from latex gloves can be an inhalative occupational hazard. Latex, absorbed by the cornstarch powder and then airborne when gloves were handled, was the causative agent of the respiratory events in our patients. The standardized method that we used minimizes the risk of eliciting systemic reactions when performing specific bronchial provocation tests to latex.

COMMENTS: This paper describes the use of skin tests and specific bronchial challenge to determine the causative agent of asthma in four hospital nurses. The nurses were experimentally exposed to cornstarch powder alone and in combination with latex glove extract. The skin tests with powdered latex surgical gloves extract gave a positive reaction from the 1:100 dilution, whereas pure cornstarch powder did not induce any reaction. The results of the bronchial provocation test similarly demonstrated that latex was the causative agent of asthma in these patients, since bronchoconstriction was observed after the challenges with powdered and unpowdered glove extract, but not after the cornstarch powder extract alone.

L-MENTHONE
14073-97-3

Number of relevant papers: 1

1. Inhibition of Human Liver Microsomal (S)-Nicotine Oxidation by (-)-Menthol and Analogues

MacDougall JM, Fandrick K, Zhang X, Serafin SV, and Cashman JR
Chem Res Toxicol 16: 988-993

ABSTRACT: Menthol is a widely used flavoring ingredient present in mouthwash, foods, toothpaste, and cigarettes; yet, the pharmacological effects of menthol have not been widely studied. Mentholated cigarette smoking may increase the risk for lung cancer. Many African American smokers smoke mentholated cigarettes, and African Americans have a significantly higher incidence of lung cancer as compared with whites. There may be a relationship between the incidence of lung cancer and the type of cigarette smoked because the use of mentholated cigarettes by white smokers is significantly less and the incidence of lung cancer is less. The mechanism whereby (-)-menthol could increase the health risk of smoking is not known. The results of our in vitro studies herein show that (-)-menthol and synthetic congeners inhibit the microsomal oxidation of nicotine to cotinine and the P450 2A6-mediated 7-hydroxylation of coumarin. Replacement of the alcohol oxygen atom of menthol with other heteroatoms increased the potency of P450 2A6 inhibition. Thus, the K(i) value of (-)-menthol for inhibition of microsomal nicotine oxidation was 69.7 micro M but neomenthyl thiol possesses a K(i) value of 13.8 micro M. Menthylamine inhibited nicotine oxidation with a K(i) value of 49.8 micro M, but its hydroxylamine derivative gave an IC(50) value of 2.2 micro M. A series of 16 menthol derivatives and putative metabolites were procured or chemically synthesized and tested as inhibitors of P450 2A6. While highly potent inhibition of P450 2A6 was not observed for the menthol analogues examined, it is nevertheless possible that smoking mentholated cigarettes leads to inhibition of nicotine metabolism and allows the smoker to achieve a certain elevated dose of nicotine each day. This may be another example of self-medication to obtain the desired effect of nicotine.

COMMENTS: Abstract summary of the paper is adequate.

CATEGORY: HIGH MUL'S INGREDIENTS**ACETIC ACID
CAS: 64-19-7**

Number of relevant papers: 2

**1. On the deposition of volatiles and semivolatiles from cigarette smoke aerosols:
Relative rates of transfer of nicotine and ammonia from particles to the gas phase**

Seeman Jeffrey I; Lipowicz Peter J; Piade Jean-Jacques; Poget Laurent; Sanders Edward B; Snyder James P; Trowbridge Clarence G
Chemical Research in Toxicology , Volume: 17 , Number: 8 , Page: 1020-1037

ABSTRACT: The hypothesis that elevated levels of ammonia-releasing compounds in tobacco and ammonia in mainstream (MS) smoke increase the rate and amount of nicotine evaporation from the particles of MS smoke aerosol was examined by kinetic modeling and experiments with MS cigarette smoke. Computational simulation of a kinetic mechanism describing volatile loss of nicotine, ammonia, and acetic acid from an aqueous solution was used to compute the time-dependent concentration of all species in the model. Because of the high volatility of ammonia relative to that of nicotine, variation over a wide range of initial ammonia concentration had no significant effect upon the rate of loss of nicotine from the model system. The effects of a variation in the volatile loss rate constant for ammonia and for the acid were examined. The simulations show that ammonia is lost from the model solution at a greater rate than nicotine and acid, and the loss of volatile acid has a significant role in the rate and amount of nicotine loss. Simulations with a model system undergoing a continuous steady addition of ammonia showed that high rates of ammonia addition could significantly increase the rate of nicotine volatile loss from the model solution. A series of smoking experiments was performed using blended cigarettes connected to a denuder tube. Deposition of smoke constituents can occur directly from the gas phase and by the deposition of smoke aerosol particles themselves. As nicotine exists >99% in the particle phase of MS smoke, in the absence of particle deposition, denuder tube deposition of nicotine occurs via the evaporation-deposition pathway. Solanesol, a nonvolatile tobacco and smoke terpene, was used to quantify the amount of particle deposition onto the denuder tube. The amount of ammonia deposited on the denuder tube was an order of magnitude greater than that of nicotine, showing that ammonia evaporates from the MS smoke particles much faster than does nicotine. The experimental results were supported and explained by the aqueous model simulations. Included in these experiments are cigarettes that differ in their MS smoke ammonia content by a factor of ca. five. However, an increased amount of MS smoke ammonia does not increase the rate of nicotine loss from the particles. The combined results support the conclusion that ammonia in mainstream smoke has little effect, if any, upon the rate and amount of nicotine evaporation from MS smoke particles.

COMMENTS: A computation model using chemical kinetics was employed to exam the role of volatile acids (acetic acid or formic acid) and bases (ammonia) in nicotine evaporation from smoke aerosol particles. Experimental results and model simulations indicate that ammonia and acetate evaporate from particles far faster than nicotine. Ammonia in mainstream smoke aerosol has little effect on nicotine loss in smoke particles. Increasing acid volatility increased the rate and amount of nicotine and ammonia loss. Formic acid caused a similar but slower effect than acetic acid. This paper is relevant to the effects of acetic acid as an ingredient in cigarette smoke in that it describes the theoretical effect of acetic acid on nicotine and ammonia volatility.

2. Physician diagnosed asthma, respiratory symptoms, and associations with workplace tasks among radiographers in Ontario, Canada

G M Liss, S M Tarlo, J Doherty, J Purdham, J Greene, L McCaskell, M Kerr
Occup Environ Med 2003; 60:254–261.

ABSTRACT: Background: Medical radiation technologists (MRTs) or radiographers have potential exposure to chemicals including sensitizers and irritants such as glutaraldehyde, formaldehyde, sulphur dioxide, and acetic acid. Aims: To determine the prevalence of asthma and work related respiratory symptoms among MRTs compared with physiotherapists, and to identify work related factors in the darkroom environment that are associated with these outcomes. Methods: As part of a two component study, we undertook a questionnaire mail survey of the members of the professional associations of MRTs and physiotherapists in Ontario, Canada, to ascertain the prevalence of physician diagnosed asthma, and the prevalence in the past 12 months of three or more of the nine respiratory symptoms (previously validated by Venables et al to be sensitive and specific for the presence of self reported asthma). Information on exposure factors during the past 12 months, such as ventilation conditions, processor leaks, cleanup activities, and use of personal protective equipment was also collected. Results: The survey response rate was 63.9% among MRTs and 63.1% among physiotherapists. Most analyses were confined to 1110 MRTs and 1523 physiotherapists who never smoked. The prevalence of new onset asthma (since starting in the profession) was greater among never smoking MRTs than physiotherapists (6.4% v 3.95%), and this differed across gender: it was 30% greater among females but fivefold greater among males. Compared with physiotherapists, the prevalence of reporting three or more respiratory symptoms, two or more work related, and three or more work related respiratory symptoms in the past 12 months was more frequent among MRTs, with odds ratios (ORs) (and 95% confidence intervals) adjusted for age, gender, and childhood asthma, of 1.9 (1.5 to 2.3), 3.7 (2.6 to 5.3), and 3.2 (2.0 to 5.0), respectively. Analyses examining latex glove use indicated that this was not likely to account for these differences. Among MRTs, respiratory symptoms were associated with a number of workplace and exposure factors likely to generate aerosol or chemical exposures such as processors not having local ventilation, adjusted OR 2.0 (1.4 to 3.0); leaking processor in which clean up was delayed, 2.4 (1.6 to 3.5); floor drain clogged, 2.0 (1.2 to 3.2); freeing a film jam, 2.9 (1.8 to 4.8); unblocking a blocked processor drain, 2.4 (1.6 to 3.7); and cleaning up processor chemical spill, 2.8 (1.9 to 4.2). These outcomes were not associated with routine tasks unlikely to generate exposures, such as working

outside primary workplace, loading film into processor, routine cleaning of processors, or removing processed film. Males reported that they carried out a number of tasks potentially associated with irritant exposures more frequently than females, consistent with the marked increase in risk for new onset asthma. Conclusions: These findings suggest an increase of work related asthma and respiratory symptoms shown to denote asthma among MRTs, which is consistent with previous surveys. The mechanism is not known but appears to be linked with workplace factors and may involve a role for irritant exposures.

COMMENTS: This study described a higher prevalence of asthma and work-related respiratory symptoms among medical radiation technologists as compared to other workers (physiotherapists) and attempted to identify environmental factors associated with these outcomes. Medical radiation technologists are exposed to acetic acid and other chemicals during the processing of films, however, the causative agent(s) in these work-related respiratory symptoms is currently unknown.

BENZALDEHYDE
CAS: 100-52-7

Number of relevant papers: 2

1. The GreenScreen genotoxicity assay: a screening validation programme -

Cahill PA, Knight AW, Billinton N, Barker MG, Walsh L, Keenan PO, Williams CV, Tweats DJ, Walmsley RM.
Mutagenesis. 2004 Mar;19(2):105-19

ABSTRACT: A yeast (*Saccharomyces cerevisiae*) DNA repair reporter assay termed the GreenScreen assay (GSA) is described. This is a novel, cost-effective genotoxicity screen, developed to provide a pre-regulatory screening assay for use by the pharmaceutical industry and in other applications where significant numbers of compounds need to be tested. It provides a higher throughput and a lower compound consumption than existing eukaryotic genotoxicity assays and is sensitive to a broad spectrum of mutagens and, importantly, clastogens. We describe a simple, robust assay protocol and a validation study. The end-point of the test reflects the typically eukaryotic chromosomes and DNA metabolizing enzymes of yeast. The capacity for metabolic activation (MA) in yeast is limited compared with the mammalian liver or its extracts, but the assay does detect a subset of compounds that would require MA in existing genotoxicity tests. The GSA detects a different spectrum of compounds to bacterial genotoxicity assays and thus, together with an in silico structure-activity relationship (SAR) screen, and possibly a high throughput bacterial screen, would provide an effective preview of the regulatory battery of genotoxicity tests.

COMMENTS: This paper describes a genotoxicity assay that measures a different end-point (DNA repair induction) using a different type of cell (yeast) than the Ames test. The

authors used this yeast assay to test over 100 compounds. In this assay benzaldehyde was positive for genotoxicity.

2. Effects of garage employment and tobacco smoking on breathing-zone concentrations of carbonyl compounds.

Zhang L; Chung FL; Boccia L; Colosimo S; Liu WL; Zhang JF.
AIHA Journal 64(3): 388-393, 2003. (26 refs.)

ABSTRACT: Exposure to carbonyl compounds may cause adverse health effects. The present study examined whether working in a garage and smoking can significantly affect personal "daily" exposure to a number of important carbonyl compounds. The study was carried out on 37 subjects including 22 garage workers (9 smokers and 13 nonsmokers) and 15 nongarage workers or so-called controls (4 smokers and 11 nonsmokers). Daily exposure was estimated using 48-hour integrated measurement of breathing-zone concentrations. The measurement involved the use of a passive carbonyl sampler and high performance liquid chromatography/fluorescence analysis technique. Each subject was measured for up to three measurement sessions. A wide range of breathing-zone concentrations (unit: microgram per cubic meter) was observed for each of the following carbonyls: formaldehyde (14.1-80.1); acetaldehyde (8.41-80.3); acetone (0.65-1096); acrolein (<0.14-3.71); propionaldehyde (1.08-14.6); crotonaldehyde (<0.13-2.80); benzaldehyde (1.79-9.91); and hexaldehyde (0.122-22.4). Statistical significance of smoking effects and working in a garage effects were assessed using SAS mixed models. The results show that the garage workers had significantly higher levels of formaldehyde and acetaldehyde than the controls, and that the smokers had significantly higher levels of acetaldehyde, propionaldehyde, and hexaldehyde, than the nonsmokers ($P < .10$). Garage employment and smoking appeared to increase breathing-zone concentrations of crotonaldehyde. In general, within-subject variations were smaller than between-subject variations on 48-hour averaged breathing-zone concentrations of carbonyl compounds.

COMMENTS: While the primary focus of this study was to determine exposure to 8 carbonyl compounds commonly found in a garage environment, they also examined the added effect of smoking on breathing zone concentration of these carbonyl compounds. While all carbonyls tested are known to be present in tobacco smoke, only acetaldehyde, propionaldehyde and hexaldehyde were found to be higher in the workers' breathing zone area of smokers as compared to nonsmokers.

BUTYRIC ACID
CAS: 107-92-6

Number of relevant papers: 1

1. Oncogenic Ras promotes butyrate-induced apoptosis through inhibition of gelsolin expression

Lidija Klampfer, Jie Huang, Takehiko Sasazuki, Senji Shirasawa, and Leonard Augenlicht

J. Biol. Chem., Vol. 279, Issue 35, 36680-36688

ABSTRACT: Activation of Ras promotes oncogenesis by altering a multiple of cellular processes, such as cell cycle progression, differentiation, and apoptosis. Oncogenic Ras can either promote or inhibit apoptosis, depending on the cell type and the nature of the apoptotic stimuli. The response of normal and transformed colonic epithelial cells to the short chain fatty acid butyrate, a physiological regulator of epithelial cell maturation, is also divergent: normal epithelial cells proliferate, and transformed cells undergo apoptosis in response to butyrate. To investigate the role of k-ras mutations in butyrate-induced apoptosis, we utilized HCT116 cells, which harbor an oncogenic k-ras mutation and two isogenic clones with targeted inactivation of the mutant k-ras allele, Hkh2, and Hke-3. We demonstrated that the targeted deletion of the mutant k-ras allele is sufficient to protect epithelial cells from butyrate-induced apoptosis. Consistent with this, we showed that apigenin, a dietary flavonoid that has been shown to inhibit Ras signaling and to reverse transformation of cancer cell lines, prevented butyrate-induced apoptosis in HCT116 cells. To investigate the mechanism whereby activated k-ras sensitizes colonic cells to butyrate, we performed a genome-wide analysis of Ras target genes in the isogenic cell lines HCT116, Hkh2, and Hke-3. The gene exhibiting the greatest down-regulation by the activating k-ras mutation was gelsolin, an actin-binding protein whose expression is frequently reduced or absent in colorectal cancer cell lines and primary tumors. We demonstrated that silencing of gelsolin expression by small interfering RNA sensitized cells to butyrate-induced apoptosis through amplification of the activation of caspase-9 and caspase-7. These data therefore demonstrate that gelsolin protects cells from butyrate-induced apoptosis and suggest that Ras promotes apoptosis, at least in part, through its ability to down-regulate the expression of gelsolin.

COMMENTS: This paper indicates a possible butyrate effect, but is not directly relevant to inhaled ingredient.

CAPRYLIC/CAPRIC TRIGLYCERIDE
CAS: 65381-09-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BETA-CARYOPHYLLENE OXIDE**CAS: 1139-30-6**

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

GAMMA-DECALACTONE**CAS: 706-14-9**

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2,5-DIMETHYLPYRAZINE**123-32-0**

Number of relevant papers: 3

1. Pyrazine derivatives in cigarette smoke inhibit hamster oviductal functioning –**Karen Riveles, Ryan Roza, Janet Arey and Prue Talbot****Reproductive Biology and Endocrinology 2004, 2:23 doi:10.1186/1477-7827-2-23**

ABSTRACT: Our past studies have shown that cigarette smoke inhibits oviductal functioning in vivo and in vitro. The goals in this study were to identify pyrazine derivatives in cigarette smoke solutions that inhibit ciliary beat frequency, oocyte pickup rate, and infundibular smooth muscle contraction in the hamster oviduct and to determine their lowest observable adverse effect levels (LOAELs) using in vitro bioassays.

Methods: MS smoke solutions were fractionated using solid phase extraction cartridges and the fractions were both tested on the hamster oviduct in vitro and analyzed by gas chromatography-mass spectrometry to identify individual pyrazine derivatives. Commercial pyrazine standards were purchased, assayed for purity, and tested in dose-response studies on hamster oviducts. The LOAEL and efficacy were determined for each compound in the in vitro bioassays. Statistical significance was determined using the Student's t-Test where $p < 0.05$. Results: The LOAELs for the most inhibitory pyrazine derivatives in the ciliary beat frequency, oocyte pickup rate, and infundibular smooth muscle contraction assays were as follows: for pyrazine (1 picomolar, 10 picomolar, and 1 nanomolar); for 2-methylpyrazine (1 picomolar, 10 picomolar, and 10 picomolar); and for 2-ethylpyrazine (1 picomolar, 10 picomolar, and 1 picomolar). Six of the seven pyrazine derivatives tested (pyrazine, 2-methylpyrazine, 2-ethylpyrazine, 2-methoxy-3-methylpyrazine, 2,5-dimethylpyrazine, and 2,3,5-trimethylpyrazine) were inhibitory in picomolar or nanomolar doses in all three bioassays, while the seventh derivative, 2,6-dimethylpyrazine, had LOAELs in the nanomolar to micromolar range.

Conclusion: This work shows that very low doses of pyrazines significantly inhibit proper oviductal functioning, raising questions regarding the safety of these compounds in cigarettes and other consumer products.

COMMENTS: An *in vitro* assay was used to study the effects of pyrazine and its derivatives in cigarette smoke on hamster oviductal functioning. Pyrazine derivatives had

equal or greater effects than pyrazine and inhibited ciliary beating, oocyte pickup rate and smooth muscle contraction in hamster oviductal explants at nanomolar and picomolar doses. Oocyte pickup rate was the most sensitive parameter, and derivatives with single ethyl or methyl substitutions were among the most inhibitory. The LOAEL of 2,5-dimethylpyrazine was equal to pyrazine for oocyte pickup rate (10^{-11} M) and smooth muscle contraction (10^{-9} M), but 10,000 times greater for ciliary beat frequency (10^{-8} M). For all three measurements, 2,5-dimethylpyrazine was more potent than 2,6-dimethylpyrazine. The authors suggested that these data concur with results reported from *in vivo* hamster studies and epidemiological studies that show increased risk of ectopic pregnancies and spontaneous abortions in female smokers. The authors recommend that further toxicological testing of pyrazines be conducted.

2. The influence of cigarette moisture to the chemistry of particulate phase smoke of a common commercial cigarette -

Q. Zha and S.C. Moldoveanu

**Beiträge zur Tabakforschung International/Contributions to Tobacco Research
Volume 21(3):184-191**

ABSTRACT: This study presents the results on the influence of cigarette moisture content to the chemical composition of particulate phase smoke. Seventy-five selected compounds were monitored for the comparison of particulate phase smoke of a commercial full-flavored (FF) cigarette with three different moisture contents at 7.8%, 14.5% and 20.4%, respectively. It was demonstrated that the smoke of a dry cigarette is richer in lower molecular mass compounds than a regular cigarette. On the other hand, the smoke of a moist cigarette is richer in higher molecular mass compounds than a regular cigarette. To maximize the influence of cigarette moisture to the chemical composition, a separate set of measurements were done using only the first three puffs of smoke. The accumulation of moisture in the tobacco column of a burning cigarette may influence the smoke composition, as generated during burning. The differences between dry, regular and moist cigarettes were more obvious for the first three puffs.

COMMENTS: While this is not a health effect study, the results are interesting. These investigators compared the chemical composition of cigarette smoke from cigarettes with three moisture levels (dry-8.3%, regular-11.6%, and moist- 12.9%). The first three puffs showed the greatest differences. The nicotine content and total particulate matter (TPM) was reduced with increasing moisture. The data would indicate that the dry cigarette had a higher percentage of semi-volatile compounds in TPM. The data presents additional evidence that the cigarette moisture content significantly affects the chemistry of the particulate phase of smoke. Of the 75 compounds tested, the more volatile compounds were more affected than the less volatile compounds. Compared to the first three puffs, the particulate phase of smoke from the entire cigarette was less sensitive to the moisture content.

3. Identification of compounds in cigarette smoke that inhibit hamster oviductal functioning. -

K. Riveles, R. Roza and P. Talbot. Cell Biology & Neuroscience, UC Riverside, Riverside, CA. Poster SETAC Utah 2003.

ABSTRACT: Our past studies have shown that chemicals in cigarette smoke inhibit oviductal functioning in vivo and in vitro. The purposes of this study were to identify the individual toxicants in cigarette smoke solutions that inhibit oocyte pickup rate, ciliary beat frequency, and infundibular smooth muscle contraction and to determine their effective doses using in vitro bioassays. Solid phase extraction and gas chromatography-mass spectrometry were used to identify individual chemicals in the mainstream and sidestream cigarette smoke solutions that were active in the above assays. Pyridines, pyrazines, indoles, quinolines, and phenols were identified in the solutions of mainstream and sidestream cigarette smoke. Commercially available standards of the identified compounds were purchased, assayed for purity, and tested in dose-response studies on hamster oviducts. The lowest observable adverse effect level and efficacy were determined for each compound using the oocyte pickup rate, ciliary beat frequency, and infundibular muscle contraction assays. Previously, we have shown that several pyridine compounds including 2-methylpyridine, 4-methylpyridine, 2-ethylpyridine, 3-ethylpyridine, and 4-vinylpyridine were inhibitory at picomolar concentrations in all three bioassays. Further studies have shown that compounds in the pyrazine group: 2-methylpyrazine, ethylpyrazine, 2-methoxy-3-methylpyrazine, 2, 5-dimethylpyrazine, and 2, 3, 5-trimethylpyrazine, were inhibitory in pico or nanomolar doses. Both quinoline and isoquinoline were inhibitory in picomolar doses. 5-Methylindole showed inhibition in the nanomolar range. Indole, which is found in large quantities relative to other compounds in the smoke, showed inhibition at 10-15M. The phenolic compounds were not as inhibitory as the other classes of compounds in the bioassays, although hydroquinone and 4-ethylphenol were inhibitory at nanomolar doses. This work is important because it shows that very low doses of cigarette smoke components significantly inhibit proper oviductal functioning raising questions regarding the safety of these compounds.

COMMENTS: POSTER PRESENTATION -- Paper N/A

ETHYL BUTYRATE

CAS: 105-54-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL DECANOATE

CAS: 110-38-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL HEXANOATE

CAS: 123-66-0

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL ISOVALERATE

CAS: 108-64-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL LACTATE

CAS: 97-64-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL LAURATE

CAS: 106-33-2

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL MYRISTATE

CAS: 124-06-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL OCTANOATE

CAS: 106-32-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL PHENYLACETATE

CAS: 101-97-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2-ETHYL-3,(5 OR 6)-DIMETHYLPYRAZINE

CAS: 27043-05-6

CAS: 13925-07-0

CAS: 13360-65-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

5-ETHYL-3-HYDROXY-4-METHYL-2(5H)-FURANONE

CAS: 698-10-2

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

HEXYL PHENYLACETATE

CAS: 5421-17-0

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ISOAMYL ACETATE

CAS: 123-92-2

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ISOBUTYL CINNAMATE

CAS: 122-67-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ISOBUTYL PHENYLACETATE

CAS: 102-13-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

**ALPHA-ISOBUTYLPHENETHYL ALCOHOL (BENZYL ISOBUTYL
CARBINOL) (BENZENEETHANOL, ALPHA- (2-METHYLPROPYL)-)**

CAS: 7779-78-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ISOBUTYRIC ACID

CAS: 79-31-2

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2-,5-, OR 6-METHOXY-3-METHYLPYRAZINE

CAS: 2847-30-5

Number of relevant papers: 1

1. Pyrazine derivatives in cigarette smoke inhibit hamster oviductal functioning

Karen Riveles, Ryan Roza , Janet Arey and Prue Talbot

Reproductive Biology and Endocrinology 2004, 2:23 doi:10.1186/1477-7827-2-23

ABSTRACT: Our past studies have shown that cigarette smoke inhibits oviductal functioning in vivo and in vitro. The goals in this study were to identify pyrazine derivatives in cigarette smoke solutions that inhibit ciliary beat frequency, oocyte pickup rate, and infundibular smooth muscle contraction in the hamster oviduct and to determine their lowest observable adverse effect levels (LOAELs) using in vitro bioassays. Methods: MS smoke solutions were fractionated using solid phase extraction cartridges and the fractions were both tested on the hamster oviduct in vitro and analyzed by gas chromatography-mass spectrometry to identify individual pyrazine derivatives. Commercial pyrazine standards were purchased, assayed for purity, and tested in dose-response studies on hamster oviducts. The LOAEL and efficacy were determined for each compound in the in vitro bioassays. Statistical significance was determined using the Student's t-Test where $p < 0.05$. Results: The LOAELs for the most inhibitory pyrazine derivatives in the ciliary beat frequency, oocyte pickup rate, and infundibular smooth muscle contraction assays were as follows: for pyrazine (1 picomolar, 10 picomolar, and 1 nanomolar); for 2-methylpyrazine (1 picomolar, 10 picomolar, and 10 picomolar); and for 2-ethylpyrazine (1 picomolar, 10 picomolar, and 1 picomolar). Six of the seven pyrazine derivatives tested (pyrazine, 2-methylpyrazine, 2-ethylpyrazine, 2-methoxy-3-methylpyrazine, 2,5-dimethylpyrazine, and 2,3,5-trimethylpyrazine) were inhibitory in picomolar or nanomolar doses in all three bioassays, while the seventh derivative, 2,6-dimethylpyrazine, had LOAELs in the nanomolar to micromolar range.

COMMENTS: An *in vitro* assay was used to study the effects of pyrazine and its derivatives in cigarette smoke on hamster oviductal functioning. Pyrazine derivatives had equal or greater effects than pyrazine and inhibited ciliary beating, oocyte pickup rate and smooth muscle contraction in hamster oviductal explants at nanomolar and picomolar doses. Oocyte pickup rate was the most sensitive parameter, and derivatives with single ethyl or methyl substitutions were among the most inhibitory. The 2-methoxy-3-methylpyrazine LOAELs for oocyte pickup rate (10^{-12} M) and muscle contraction assays (10^{-12} M) were the lowest of all pyrazines tested. The LOAEL for ciliary beat frequency (10^{-9} M) was similar to the trimethyl substituted pyrazines. The authors suggested that these data concur with results reported from in vivo hamster studies and epidemiological studies that show increased risk of ectopic pregnancies and spontaneous abortions in female smokers. The authors recommend that further toxicological testing of pyrazines be conducted.

2-METHYLHEPTANOIC ACID CAS: 1188-02-9

Number of relevant papers: 1

1. Evaluation of certain food additives and contaminants -

**Sixty-first report of the Joint FAO/WHO Expert Committee on
Food Additives
WHO Technical Report Series 922, 2004 Geneva**

ABSTRACT: This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives, with a view to recommending acceptable daily intakes (ADIs) and to prepare specifications for the identity and purity of food additives. The first part of the report contains a general discussion of the principles governing the toxicological evaluation of food additives (including flavouring agents) and contaminants, assessments of intake, and the establishment and revision of specifications for food additives. A summary follows of the Committee's evaluations of toxicological and intake data on various specific food additives (a-amylase from *Bacillus licheniformis* containing a genetically engineered a-amylase gene from *B. licheniformis*, annatto extracts, curcumin, diacetyl and fatty acid esters of glycerol, D-tagatose, laccase from *Myceliophthora thermophila* expressed in *Aspergillus oryzae*, mixed xylanase, b-glucanase enzyme preparation produced by a strain of *Hemicolla insolens*, neotame, polyvinyl alcohol, quillaia extracts and xylanase from *Thermomyces lanuginosus* expressed in *Fusarium venenatum*), flavouring agents, a nutritional source of iron (ferrous glycinate, processed with citric acid), a disinfectant for drinking-water (sodium dichloroisocyanurate) and contaminants (cadmium and methylmercury). Annexed to the report are tables summarizing the Committee's recommendations for ADIs of the food additives, recommendations on the flavouring agents considered, and tolerable intakes of the contaminants considered, changes in the status of specifications and further information requested or desired.

COMMENTS: The abstract describes this report well.

2-METHYLPYRAZINE CAS: 109-08-0

Number of relevant papers: 3

1. Pyrazine derivatives in cigarette smoke inhibit hamster oviductal functioning –

Karen Riveles , Ryan Roza , Janet Arey and Prue Talbot

Reproductive Biology and Endocrinology 2004, 2:23 doi:10.1186/1477-7827-2-23

ABSTRACT: Our past studies have shown that cigarette smoke inhibits oviductal functioning in vivo and in vitro. The goals in this study were to identify pyrazine derivatives in cigarette smoke solutions that inhibit ciliary beat frequency, oocyte pickup rate, and infundibular smooth muscle contraction in the hamster oviduct and to determine their lowest observable adverse effect levels (LOAELs) using in vitro bioassays.

Methods : MS smoke solutions were fractionated using solid phase extraction cartridges and the fractions were both tested on the hamster oviduct in vitro and analyzed by gas chromatography-mass spectrometry to identify individual pyrazine derivatives. Commercial pyrazine standards were purchased, assayed for purity, and tested in dose-response studies on hamster oviducts. The LOAEL and efficacy were determined for each compound in the in vitro bioassays. Statistical significance was determined using

the Student's t-Test where $p < 0.05$. Results: The LOAELs for the most inhibitory pyrazine derivatives in the ciliary beat frequency, oocyte pickup rate, and infundibular smooth muscle contraction assays were as follows: for pyrazine (1 picomolar, 10 picomolar, and 1 nanomolar); for 2-methylpyrazine (1 picomolar, 10 picomolar, and 10 picomolar); and for 2-ethylpyrazine (1 picomolar, 10 picomolar, and 1 picomolar). Six of the seven pyrazine derivatives tested (pyrazine, 2-methylpyrazine, 2-ethylpyrazine, 2-methoxy-3-methylpyrazine, 2,5-dimethylpyrazine, and 2,3,5-trimethylpyrazine) were inhibitory in picomolar or nanomolar doses in all three bioassays, while the seventh derivative, 2,6-dimethylpyrazine, had LOAELs in the nanomolar to micromolar range. Conclusion: This work shows that very low doses of pyrazines significantly inhibit proper oviductal functioning, raising questions regarding the safety of these compounds in cigarettes and other consumer products.

COMMENTS: An *in vitro* assay was used to study the effects of pyrazine and its derivatives in cigarette smoke on hamster oviductal functioning. Pyrazine derivatives had equal or greater effects than pyrazine and inhibited ciliary beating, oocyte pickup rate and smooth muscle contraction in hamster oviductal explants at nanomolar and picomolar doses. Oocyte pickup rate was the most sensitive parameter, and derivatives with single ethyl or methyl substitutions were among the most inhibitory. 2-methylpyrazine was one of the most potent derivatives in this study, causing effects at concentrations as low as 10^{-12} M. The authors suggested that these data concur with results reported from *in vivo* hamster studies and epidemiological studies that show increased risk of ectopic pregnancies and spontaneous abortions in female smokers. The authors recommend that further toxicological testing of pyrazines be conducted.

2. The influence of cigarette moisture to the chemistry of particulate phase smoke of a common commercial cigarette

Q. Zha and S.C. Moldoveanu

**Beiträge zur Tabakforschung International/Contributions to Tobacco Research
Volume 21(3):184-191**

ABSTRACT: This study presents the results on the influence of cigarette moisture content to the chemical composition of particulate phase smoke. Seventy-five selected compounds were monitored for the comparison of particulate phase smoke of a commercial full-flavored (FF) cigarette with three different moisture contents at 7.8%, 14.5% and 20.4%, respectively. It was demonstrated that the smoke of a dry cigarette is richer in lower molecular mass compounds than a regular cigarette. On the other hand, the smoke of a moist cigarette is richer in higher molecular mass compounds than a regular cigarette. To maximize the influence of cigarette moisture to the chemical composition, a separate set of measurements were done using only the first three puffs of smoke. The accumulation of moisture in the tobacco column of a burning cigarette may influence the smoke composition, as generated during burning. The differences between dry, regular and moist cigarettes were more obvious for the first three puffs.

COMMENTS; While this is not a health effect study, the results are interesting. These investigators compared the chemical composition of cigarette smoke from cigarettes with three moisture levels (dry-8.3%, regular-11.6%, and moist- 12.9%). The first three puffs showed the greatest differences. The nicotine content and total particulate matter (TPM) were reduced with increasing moisture. The data would indicate that the dry cigarette had a higher percentage of semi-volatile compounds in TPM. The data presents additional evidence that the cigarette moisture content significantly affects the chemistry of the particulate phase of smoke. Of the 75 compounds tested the more volatile compounds were more affected than the less volatile compounds. Compared to the first three puffs, the particulate phase of smoke from the entire cigarette was less sensitive to the moisture content.

3. Growth and Angiogenesis Are Inhibited in Vivo in Developing Tissues by Pyrazine and Its Derivatives –

Goar Melkonian, Holly Lautenschlager, Melinda Wu, Yuhuan Wang, Cathy Tong, Karen Riveles, P. Talbot.

Toxicological Sciences Volume 75, Number 2 Pp. 393-401

ABSTRACT: Sidestream cigarette smoke solution was previously screened to identify the groups of chemicals in smoke that inhibit growth and angiogenesis in the chick chorioallantoic membrane (CAM). Pyrazine and several pyrazine derivatives were identified as a major chemical group in this screen. In the current study, purified pyrazine and six pyrazine derivatives identified in the screen were tested in dose response experiments to measure their effects on CAM growth, embryo growth, and angiogenesis. Chemicals or control medium were placed on CAMs in ovo on day 5 of development, and results were evaluated on day 6. Of the chemicals tested, pyrazine was the most potent and inhibited both CAM and embryo growth at picomolar doses. 2-ethylpyrazine and 2,3, dimethylpyrazine were inhibitory at nanomolar doses. Inhibition of growth by pyrazine was correlated with inhibition of DNA synthesis. The pattern of blood vessel development in CAMs was disturbed by micromolar doses of pyrazine and 2,3,-dimethylpyrazine. Migration of mesodermal blood vessels to the ectoderm of CAMs and their subsequent differentiation into the capillary plexus was impaired by nanomolar doses of pyrazine. In summary, these data show that pyrazine and some of its derivatives inhibit growth and certain process important in angiogenesis at very low doses. Since pyrazine and some of its derivatives are considered safe food additives, further toxicological testing of pyrazine, in particular on developing tissues, should be done to fully evaluate its safety as a consumer product additive.

COMMENTS: These authors previously presented data that both mainstream and sidestream smoke inhibit growth and angiogenesis in chick chorioallantoic membrane (CAM). The CAM is important to the chick since it serves as the respiratory organ for gaseous exchange until hatching. These studies are an extension of their earlier work in hope to identify the compound responsible for this effect. The data show that pyrazine in sidestream smoke can inhibit CAM, embryo growth and impair angiogenesis in nano and picomolar doses. Of the pyrazines tested, 2-methylpyrazine significantly inhibited CAM

growth at 5×10^{-5} M but did not significantly affect embryo growth at any dose tested. The authors state that the implications of this data to human reproduction are not known.

GAMMA-OCTALACTONE

CAS: 104-50-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2,3-PENTANEDIONE

CAS: 600-14-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2-PHENETHYL ACETATE

CAS: 103-45-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

PHENYLACETALDEHYDE

CAS: 122-78-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

SODIUM BICARBONATE

CAS: 144-55-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

SUCROSE OCTAACETATE

CAS: 126-14-7

Number of relevant papers: 1

1. The Contribution of Taste Bud Populations to Bitter Avoidance in Mouse Strains Differentially Sensitive to Sucrose Octa-acetate and Quinine -

St John SJ, Boughter JD Jr.

Chem Senses. 2004 Nov;29(9):775-87.

ABSTRACT: Mice of the SWR/J (SW) strain avoid orally delivered sucrose octa-acetate (SOA), whereas the mice of the C3HeB/FeJ (C3) strain are insensitive to SOA. Mice of both strains and of a congenic strain (C3.SW) that shares more than 99% of the C3 genome, were tested in a taste-salient brief-access taste test for responses to SOA and quinine hydrochloride, before and after transection of the glossopharyngeal or chorda tympani nerve, or sham surgery. Prior to surgery, congenic SOA tasters (C3.SW(T)) were

phenotypically identical to the SW strain in avoidance of SOA, but showed a greater reduction in sensitivity after nerve transection. For quinine avoidance, which is thought to be a polygenic trait, SW mice showed the greatest sensitivity to quinine, C3 the least and C3.SW(T) mice were different from both parental strains, showing intermediate sensitivity. Nerve transections had only a moderate effect on quinine sensitivity, suggesting that both anterior and posterior taste bud fields contribute to behavioral quinine avoidance. These findings are discussed with regard to the distribution in the oral cavity of putative taste receptors for quinine and SOA and the peripheral organization of bitter taste.

COMMENTS: This paper has to do with taste receptors. Although it is not directly health-related, it may be of interest.

2,3,5,6-TETRAMETHYLPYRAZINE

CAS: 1124-11-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

TRIETHYL CITRATE

CAS: 77-93-0

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

4-(2,6,6-TRIMETHYLCYCLOHEX-1-ENYL)BUT-2-EN-4-ONE (BETA-DAMASCONE)

CAS: 23726-91-2

CAS: 35044-68-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CATEGORY: MAJOR INGREDIENTS

GLYCEROL

CAS: 56-81-5

Number of relevant papers: 1

1. Glycerol transfer in cigarette mainstream smoke

C.Liu.

Beitrag Tabakforschung Int., 21 (2004) No. 2, pp.111-116.

ABSTRACT: Experiments have been conducted to examine the effect of different levels of blend cigarette at 36 for a 11.4 blend glycerol. For cigarettes with different designs the glycerol in NFDPM may also depend on the glycerol loading per unit rod length. The tobacco rod filtration did not change significantly within the glycerol range investigated and hence plays a relatively minor role. Significant glycerol condensation ahead of the burning coal after a puff was measured. This condensation may have implications on glycerol levels in the sidestream smoke during inter-puff smouldering.

COMMENTS: While this is not a health effect study, it does present data on the transfer of glycerol into MSS when added at higher levels than normally used as a humectant. The author's conclusion was that 1. mainstream glycerol yield increased with the blend glycerol levels, 2. the tobacco rod filtration was not significantly altered by glycerol levels, and 3. significant glycerol condensation was found ahead of the burning coal after a puff. Unfortunately, the levels of glycerol in the sidestream smoke, butt and filter were not measured.

CARBON
CAS: 7440-44-0

Number of relevant papers: 4

GENERAL COMMENTS ON CARBON AND GRAPHITE

There are numerous inhalation studies on the health effects associated with exposure to a variety of carbonaceous materials including activated carbon, graphite, coal dust, lamp black, soot, and diesel emissions. Some of these materials appear to cause tumors in rats when inhaled chronically at high concentrations. Such reports may be of interest since carbon-based particles can have absorbed onto them a variety of organic compounds including polycyclic aromatic hydrocarbons, nitroaromatic compounds, and heterocyclic compounds. The presence of similar organic substances in smoke may also be absorbed onto any carbon particles which theoretically could act synergistically or additively producing a greater response as compared to single components. Such effects may be involved in tumor development and DNA damage through both the chemical and particulate-mediated cytotoxicity responses. Particle size has a significant role in the toxicity of inhaled particles. For example, studies would indicate that ultrafine particles (<0.1 μm diameter) produce significantly greater inflammatory response than do fine particles per given mass. Such inhaled particles can lead to production of a number of mediators such as reactive oxygen and nitrogen species, cytokines, growth factors and other substances that might mediate tissue injury and contribute to the pathogenesis of pulmonary disease. Studies also suggest an excess risk of esophageal cancer, particularly squamous cell carcinomas, with exposure to carbon black combined with acid aerosols. However, the level of exposure in many of these studies was several orders of magnitude higher than one would expect from cigarette smoke inhalation.

It is important to differentiate between the types of carbon-based particles. Carbon black, for example, is manufactured under controlled conditions, while the soot-types of carbon contain numerous unwanted byproducts from the combustion of carbon-based materials. Often the terms carbon black and soot are used interchangeably. However, they are physically and chemically distinct. Soots have a much greater percentage of ash and more organic compounds can be extracted from particle surfaces.

Increases in human cancers have been attributed to high exposure to carbon black dust during working conditions and it has been classified as a possible lung and bladder carcinogen by IRAC. Carbon black is mutagenic in the Ames assay. Inhaled carbon black has also been shown to be carcinogenic in rat bioassays. For example, exposure for 24 mo to carbon black 16 hr/day, for 5 days/wk at a concentration of 2.5 or 6.5 mg/m³ produced malignant and benign lung tumors. In such cases, clearance was impaired and particles accumulated progressively. There is evidence that lung overloading is a requisite for induction of lung tumors in this animal model. A similar range of tumor phenotypes has been reported in the lungs of rats exposed to high concentrations of diesel exhaust and coal dust. Intratracheal instillation of carbon black resulted in a dose-response neutrophil inflammation. Bronchoalveolar lavage cell population was associated with increased mutation rates in alveolar type II epithelial cells. Subchronic inhalation studies in rats did not show increases in mutation frequency at a concentration of 1.1 mg/m³, and lung clearance was not impaired at that level of exposure.

1. Inhaled particles and lung cancer, part B: Paradigms and risk assessment -

Borm PJ, Schins RP, Albrecht C.

Int J Cancer. 2004 May 20;110(1):3-14.

ABSTRACT: Poorly soluble particles of low toxicity (PSP), such as CB, TiO₂ and coal mine dust, have been demonstrated to cause lung cancer in rodents, being most pronounced in rats. Adequate epidemiologic studies do not clearly indicate increased lung cancer rates in humans exposed to such particles. This has caused controversial positions in regulatory decisions on PSP on different levels. The present review discusses the current paradigms in rodent particle carcinogenicity, i.e., (i) role of particle overload and of persistent inflammation and (ii) fibrosis as an intermediate step in particle-induced lung cancer with regard to human risk assessment. Fibrosis, which is usually considered a precursor of lung cancer in humans, was not related to lung tumors in an animal study using 6 different particles, each at 3 dosages. Lung tumors after both inhalation and intratracheal instillation of PSP are related to particle surface dose, which forwards hazard assessment at surface-based nonoverload concentrations and a standard setting using surface as an exposure metric. The scarce data available on humans do not support the overload concept but suggest a role for persistent lung inflammation. Differences in antioxidant protection between different rodent species correlate with susceptibility to PSP-induced carcinogenicity and support the need for detailed studies on antioxidant response in humans. Apart from such bridging studies, further focus is also needed on surface chemistry and modifications in relation to their adverse biologic effects.

COMMENTS: This manuscript reviews the possible mechanism of action associated with particle-induced lung carcinogenesis. These authors attempt to explain why a number of poorly soluble particles (PSP), such as carbon black and graphite, has been shown to be carcinogenic in the rat and may or may not be carcinogenic in humans. The extrapolation of these rodent studies to humans is difficult because of lack of knowledge regarding antioxidant response, the significance of inflammation in the process of genotoxicity and proliferations in the human lung. The authors state that all inhaled particles are likely to induce tumors in the rat model if the particles are inhaled or instilled at sufficiently high doses and highly durable. While carbon has been identified as a possible carcinogen by IRAC, based on these rodent studies, these authors state that this action may be premature and needs further consideration.

2. Pulmonary and systemic effects of short-term inhalation exposure to ultrafine carbon black particles -

Gilmour PS, Ziesenis A, Morrison ER, Vickers MA, Drost EM, Ford I, Karg E, Mossa C, Schroeppe A, Ferron GA, Heyder J, Greaves M, MacNee W, Donaldson K.

Toxicol Appl Pharmacol. 2004 Feb 15;195(1):35-44.

ABSTRACT: While environmental particles are associated with mortality and morbidity related to pulmonary and cardiovascular (CV) disease, the mechanisms involved in CV health effects are not known. Changes in systemic clotting factors have been associated with pulmonary inflammation. We hypothesized that inhaled ultrafine particles result in an inflammatory response which may stimulate systemic clotting factor release. Adult male Wistar rats were exposed to either fine or ultrafine carbon black (CB) for 7 h. The attained total suspended particle concentrations were 1.66 mg/m³ for ultrafine CB and 1.40 mg/m³ for fine CB. Particle concentration of ultrafine particles was more than 10 times greater than that of fine particles and the count median aerodynamic diameter averaged 114 nm for the ultrafine and 268 nm for the fine carbon particles. Data were collected immediately, 16 and 48 h following exposure. Only ultrafine CB caused an increase in total bronchoalveolar lavage (BAL) leukocytes, whereas both fine (2-fold) and ultrafine (4-fold) carbon particles caused an increase in BAL neutrophils at 16 h postexposure. Exposure to the ultrafine, but not fine, carbon was also associated with significant increases in the total numbers of blood leukocytes. Plasma fibrinogen, factor VII and von Willebrand factor (vWF) were unaffected by particle treatments as was plasma Trolox equivalent antioxidant status (TEAC). Macrophage inflammatory protein-2 mRNA was significantly increased in BAL cells 48 h following exposure to ultrafine CB. The data show that there is a small but consistent significant proinflammatory effect of this exposure to ultrafine particles that is greater than the effect of the same exposure to fine CB.

COMMENTS: In this study, rats were exposed by inhalation to approximately 1.5 mg/m³ fine and ultrafine carbon black (CB) particles. Following a single 7-h exposure, the bronchoalveolar lavage (BAL) inflammatory profile was assessed at 0, 16, and 48 hours post-exposure. A total deposition of 3.9 µg particle mass in the deep lung was estimated.

The results indicate that particle size is an important determinant of pulmonary responses to CB, since exposure to ultrafine CB particles was associated with effects not seen following fine CB particle exposure. An increase in BAL cells in rats exposed to ultrafine CB was observed as well as an increase in the number of neutrophils (PMNs) in the BAL fluid and an increase in blood leukocytes. No effects on blood coagulation factors or plasma antioxidant capacity were observed. These findings are consistent with previous studies of acute human exposure to concentrated ambient particles, with the exception that an increase in blood fibrinogen was observed in humans. The authors note that difference in findings between the two studies may be due to species differences or the more heterogeneous nature of ambient particles used in the human studies.

3. Immunological biomarkers in salt miners exposed to salt dust, diesel exhaust and nitrogen oxides -

Backe E, Lotz G, Tittelbach U, Plitzko S, Gierke E, Schneider WD.
Int Arch Occup Environ Health. 2004 Jun;77(5):319-27. Epub 2004 Jun 12

ABSTRACT: Air pollutants can affect lung function and also the immune system. In a study about lung function of salt miners in relation to the complex exposure in a salt mine, we also analysed selected immunological parameters and inflammation markers in the blood of miners. Effect of salt dust, diesel exhaust, nitrogen oxides (NO_x) and smoking on the biomarkers was analysed. **METHODS:** Blood was drawn from 286 salt miners, and the soluble intercellular adhesion molecule-1 (s-ICAM), monocyte chemotactic protein (MCP-1) and clara cell protein (CC16) were analysed by an immunoassay, blood profile was done and lymphocyte subpopulations (CD3, CD3/CD4, CD3/CD8, CD19, NK-cells, CD3/HLA-DR) were determined by flow cytometry. Salt dust was measured by two-step gravimetry (personal sampling). Diesel exhaust was measured as elemental carbon concentration by coulometry. NO_x were determined by an electrochemical cell method. Differences between non-smokers, former smokers and active smokers were analysed by analysis of variance. Linear regression analysis to describe exposure-response relationships was done with regard to confounding factors [smoking, inflammatory diseases, time of blood drawing, respiratory infection and body-mass index (BMI)]. **RESULTS:** Significant differences between non-smokers and active smokers were found for most of the leukocyte types (e.g. granulocytes $P = 0.000$, lymphocytes $P = 0.002$, T-cells $P = 0.033$) and for some soluble parameters (ICAM $P = 0.000$, IgM $P = 0.007$, IgE $P = 0.035$). Increasing numbers of total lymphocytes, T-cells and HLA-DR positive T-cells in relation to exposure were found by linear regression analysis (e.g. for inhalable dust:total lymphocytes $P = 0.011$, T-cells $P = 0.061$, HLA-DR positive T-cells $P = 0.007$). **CONCLUSION.** Comparison of immunological markers in non-smokers and active smokers confirms leukocytosis and inflammation following tobacco consumption. The combined exposure of salt dust, diesel exhaust and NO_x seems to influence the immune system. Together, the results suggest that the analysis of leukocytes and their subsets can complete other investigations (lung function, questionnaire) to monitor exposure-response relationships in occupational studies investigating the effect of inhaled substances. Longitudinal studies will be necessary to

determine the predictive value of the immunological changes. Copyright 2004 Springer-Verlag

COMMENTS: Immunological parameters and inflammation markers were assessed in salt mine workers exposed to complex mixtures of salt dust, nitrogen oxides and diesel exhaust. These same markers were also evaluated in relation to tobacco smoke exposure. Exposure –dependent increases in lymphocytes, T-cells and activated T-cells indicated an effect on the immune system, however, it was not possible to distinguish between the contributions of the different exposure types. The effect of exposure to these mixtures was confounded by smoking and body-mass index which contributed to alterations in the number of immunocompetent cells. Differences between smokers and nonsmokers included increases in immune cells and some soluble markers in blood. Lymphocytes and T-cells were positively correlated with the number of cigarettes smoked per day. Extrapolation of the findings of this study to the health effects of carbon as an ingredient in cigarettes is difficult.

4. Ultrafine particle deposition in subjects with asthma -

David C. Chalupa, Paul E. Morrow, Günter Oberdörster, Mark J. Utell, and Mark W. Frampton

Environmental Health Perspectives Volume 112, Number 8, June 2004

Abstract: Ambient air particles in the ultrafine size range (diameter < 100 nm) may contribute to the health effects of particulate matter. However, there are few data on ultrafine particle deposition during spontaneous breathing, and none in people with asthma. Sixteen subjects with mild to moderate asthma were exposed for 2 hr, by mouthpiece, to ultrafine carbon particles with a count median diameter (CMD) of 23 nm and a geometric standard deviation of 1.6. Deposition was measured during spontaneous breathing at rest (minute ventilation, 13.3 ± 2.0 L/min) and exercise (minute ventilation, 41.9 ± 9.0 L/min). The mean $\pm$ SD fractional deposition was 0.76 ± 0.05 by particle number and 0.69 ± 0.07 by particle mass concentration. The number deposition fraction increased as particle size decreased, reaching 0.84 ± 0.03 for the smallest particles (midpoint CMD = 8.7 nm). No differences between sexes were observed. The deposition fraction increased during exercise to 0.86 ± 0.04 and 0.79 ± 0.05 by particle number and mass concentration, respectively, and reached 0.93 ± 0.02 for the smallest particles. Experimental deposition data exceeded model predictions during exercise. The deposition at rest was greater in these subjects with asthma than in previously studied healthy subjects (0.76 ± 0.05 vs. 0.65 ± 0.10 , $p < 0.001$). The efficient respiratory deposition of ultrafine particles increases further in subjects with asthma. Key words: air pollution, asthma, deposition, dosimetry, inhalation, ultrafine particles. Environ Health Perspect 112:879-882 (2004). doi:10.1289/ehp.6851 available via <http://dx.doi.org/> [Online 2 March 2004]

COMMENTS: This study focused on ultrafine particle (UFP) deposition in individuals with asthma. The hypothesis was if lung dose of UFP are higher for individuals with asthma, than the health risk might also increase. Previous studies have shown that

individuals with chronic obstructive pulmonary disease have enhanced deposition of both fine and ultrafines. These results indicated that when both increased deposition fraction and minute ventilation were considered, the total number of carbon particles retained in the lung was 74% greater in subjects with asthma than healthy subjects which may make them more susceptible to respiratory disease.

GRAPHITE
CAS: 7782-42-5

Number of relevant papers: 2

1. Translocation of inhaled ultrafine particles to the brain -

Oberdorster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, Cox C.
Inhal Toxicol. 2004 Jun;16(6-7):437-45.

ABSTRACT: Ultrafine particles (UFP, particles <100 nm) are ubiquitous in ambient urban and indoor air from multiple sources and may contribute to adverse respiratory and cardiovascular effects of PM (Particulate Matter). Depending on their particle size, inhaled UFP are efficiently deposited in nasal, tracheobronchial and alveolar regions due to diffusion. Our previous rat studies have shown that UFP can translocate to interstitial sites in the respiratory tract as well as to extrapulmonary organs such as liver within 4-24 hrs. post-exposure. There were also indications that the olfactory bulb of the brain was targeted. Our objective in this follow-up study, therefore, was to determine whether translocation of inhaled ultrafine solid particles to regions of the brain takes place, hypothesizing that UFP depositing on the olfactory mucosa of the nasal region will translocate along the olfactory nerve into the olfactory bulb. This should result in significant increases in that region on the days following the exposure as opposed to other areas of the CNS. We generated ultrafine elemental ¹³C particles (CMD = 36 nm; GSD = 1.66) from ¹³C graphite rods by electric spark discharge in an argon atmosphere at a concentration of 160 µg/m³. Rats were exposed for 6 hrs. and lungs, cerebrum, cerebellum and olfactory bulbs were removed 1,3,5 and 7 days after exposure. ¹³C concentrations were determined by isotope ratio mass spectroscopy and compared to background ¹³C levels of sham-exposed controls (day 0). The background corrected pulmonary ¹³C added as ultrafine ¹³C particles on day 1 post-exposure was 1.34 µg/lung. Lung ¹³C concentration decreased from 1.39 µg/g (day 1) to 0.59 µg/g by 7 days post-exposure. There was a significant and persistent increase in added ¹³C in the olfactory bulb of 0.35 µg/g on day 1 which increased to 0.43 µg/g by day 7. Day 1 ¹³C concentrations of cerebrum and cerebellum were also significantly increased but the increase was inconsistent, significant only on one additional day of the post-exposure period, possibly reflecting translocation across the blood-brain barrier in certain brain regions. The increases in olfactory bulbs are consistent with earlier studies in non-human primates and rodents which demonstrated that intranasally-instilled solid UFP translocate along axons of the olfactory nerve into the CNS. We conclude from our study that the CNS can be targeted by airborne solid ultrafine particles and that the most likely

mechanism is from deposits on the olfactory mucosa of the nasopharyngeal region of the respiratory tract and subsequent translocation via the olfactory nerve. Depending on particle size, >50% of inhaled UFP can be depositing in the nasopharyngeal region during nasal breathing. Preliminary estimates from the present results show that ~20% of the UFP deposited on the olfactory mucosa of the rat can be translocated to the olfactory bulb. Such neuronal translocation constitutes an additional not generally recognized clearance pathway for inhaled solid UFP, whose significance for humans, however, still needs to be established. It could provide a portal of entry into the CNS for solid UFP, circumventing the tight blood-brain barrier. Whether this translocation of inhaled UFP can cause CNS effects needs to be determined in future studies.

COMMENTS: These authors report that they found significant and continuous increases of ultrafine particles in the olfactory bulb throughout a 7 day inhalation exposure. These results suggest that inhaled ultrafine carbon particles are translocated to the CNS. This provides evidence of a direct portal of entry for ultrafines into the CNS. Such evidence could indicate potential long term effects and accumulation of such particles to other regions of the CNS.

2. Inhaled particles and lung cancer, part B: Paradigms and risk assessment -

Borm PJ, Schins RP, Albrecht C.
Int J Cancer. 2004 May 20;110(1):3-14.

ABSTRACT: Poorly soluble particles of low toxicity (PSP), such as CB, TiO₂ and coal mine dust, have been demonstrated to cause lung cancer in rodents, being most pronounced in rats. Adequate epidemiologic studies do not clearly indicate increased lung cancer rates in humans exposed to such particles. This has caused controversial positions in regulatory decisions on PSP on different levels. The present review discusses the current paradigms in rodent particle carcinogenicity, i.e., (i) role of particle overload and of persistent inflammation and (ii) fibrosis as an intermediate step in particle-induced lung cancer with regard to human risk assessment. Fibrosis, which is usually considered a precursor of lung cancer in humans, was not related to lung tumors in an animal study using 6 different particles, each at 3 dosages. Lung tumors after both inhalation and intratracheal instillation of PSP are related to particle surface dose, which forwards hazard assessment at surface-based nonoverload concentrations and a standard setting using surface as an exposure metric. The scarce data available on humans do not support the overload concept but suggest a role for persistent lung inflammation. Differences in antioxidant protection between different rodent species correlate with susceptibility to PSP-induced carcinogenicity and support the need for detailed studies on antioxidant response in humans. Apart from such bridging studies, further focus is also needed on surface chemistry and modifications in relation to their adverse biologic effects.

COMMENTS: See General Comments for this paper under the Carbon Ingredient listing.

INVERT SUGAR

CAS: 8013-17-0

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

MAPLE SYRUP

CAS: 8029-81-0

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

HIGH FRUCTOSE CORN SYRUP

8029-43-4

977042-84-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CORN SYRUP

8029-43-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CELLULOSE AND CELLULOSE FIBER

65996-61-4

09004-34-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

SUCROSE

CAS: 57-50-1

Number of relevant papers: 3

1. Sucrose and IQ induced mutations in rat colon by independent mechanism

Hansen M, Hald MT, Autrup H, Vogel U, Bornholdt J, Moller P, Molck AM, Lindecrona R, Poulsen HE, Wallin H, Loft S, Dragsted LO. Mutat Res. 2004 Oct 4;554(1-2):279-86.

ABSTRACT: Sucrose-rich diets have repeatedly been observed to have co-carcinogenic actions in colon and liver of rats and to increase the number of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) induced aberrant crypt foci in rat colon. To investigate a possible interaction between sucrose and IQ on the genotoxicity in rat liver and colon, we gave Big Blue ratsTM a diet containing sucrose (0%, 3.45% or 13.4% w/w) and/or IQ (70 ppm) for a period of 3 weeks. Sucrose and IQ increased the mutation frequency in the colon. The effect of combined treatments with IQ and sucrose on the mutation frequencies was additive indicating that sucrose and IQ act independently. This

was supported by the mutation spectra where sucrose expands the background mutations in the colon, whereas IQ, in other studies, more specifically has induced G:C → T:A transversions. In the liver IQ increased the mutation frequency, whereas addition of sucrose reduced the effect of IQ in a dose-dependent manner. The level of bulky DNA adducts in liver and colon was increased in animals exposed to either sucrose or IQ. In animals exposed to IQ, addition of sucrose had marginal effects on the level of bulky DNA adducts. Markers of oxidative damage and DNA repair were generally unaffected by the treatments. In conclusion, sucrose and IQ in the diet induced mutations in the colon by independent mechanisms, whereas an interaction was observed in liver leading to a decrease in mutations by the combined treatment.

COMMENTS: The interaction between high doses (3.4 and 13.45%) of sucrose and 2-amino-3-methylimidazo [4,5-f]quinoline (IQ) which is a strong hepatic carcinogen in non-human primates) were assessed in rats using 3-week dietary exposures. The authors state that this study confirms previous reports of the mutagenic effects of sucrose in the rat colon. In the liver, they report a decrease in mutation frequencies with increased levels of sucrose however, the level of DNA adducts was increased by sucrose in both the colon and liver, possibly indicating that other factors may be influencing the mutagenic effects.

2. Assessment of the performance of the Ames II assay: a collaborative study with 19 coded compounds –

Fluckiger-Isler S, Baumeister M, Braun K, Gervais V, Hasler-Nguyen N, Reimann R, Van Gompel J, Wunderlich HG, Engelhardt G.
Mutat Res. 2004 Mar 14;558(1-2):181-97.

ABSTRACT: Nineteen coded chemicals were tested in an international collaborative study for their mutagenic activity. The assay system employed was the Ames II Mutagenicity Assay, using the tester strains TA98 and TAMix (TA7001–7006). The test compounds were selected from a published study with a large data set from the standard Ames plate-incorporation test. The following test compounds including matched pairs were investigated: cyclophosphamide, 2-naphthylamine, benzo(a)pyrene, pyrene, 2-acetylaminofluorene, 4,4'-methylene-bis(2-chloroaniline), 9,10-dimethylanthracene, anthracene, 4-nitroquinoline-N-oxide, diphenylnitrosamine, urethane, isopropyl-N(3-chlorophenyl)carbamate, benzidine, 3,3',5,5'-tetramethylbenzidine, azoxybenzene, 3-aminotriazole, diethylstilbestrol, sucrose and methionine. The results of both assay systems were compared, and the inter-laboratory consistency of the Ames II test was assessed. Of the eight mutagens selected, six were correctly identified with the Ames II assay by all laboratories, one compound was judged positive by five of six investigators and one by four of six laboratories. All seven non-mutagenic samples were consistently negative in the Ames II assay. Of the four chemicals that gave inconsistent results in the traditional Ames test, three were uniformly classified as either positive or negative in the present study, whereas one compound gave equivocal results. A comparison of the test outcome of the different investigators resulted in an inter-laboratory consistency of 89.5%. Owing to the high concordance between the two test systems, and the low inter-

laboratory variability in the Ames II assay results, the Ames II is an effective screening alternative to the standard Ames test, requiring less test material and labor.

COMMENTS: While there are studies reporting mutagenic effects of sucrose, this study examined 19 coded compounds and came to the conclusion that sucrose was consistently negative in the Ames II assay. The Ames II assay is a liquid microtiter modification of the traditional Ames test and is considered to be a suitable alternative to the standard type Ames plate method.

3. Sucrose consumption enhances the analgesic effects of cigarette smoking in male and female smokers

Kanarek RB, Carrington C.

Psychopharmacology (Berl). 2004 Apr;173(1-2):57-63. Epub 2004 Jan 14.

ABSTRACT: Abstract Rationale: Nicotine has analgesic actions in experimental animals and humans. Moreover, the analgesic properties of nicotine in experimental animals are increased by intake of sweet-tasting nutritive fluids. It is important to determine if the effects of diet on nicotine-induced analgesia are limited to experimental animals, or if these effects can be translated from the laboratory to clinical research situations. Objective: This study investigated whether intake of a sweet-tasting sucrose solution would enhance the pain relieving actions of nicotine, administered in the form of cigarette smoking, in male and female college-aged students. The effects of smoking and sucrose intake on mood were also examined. Method: Using the cold pressor test, pain thresholds and pain tolerance were determined in 24 male and 25 female smokers. Each participant was tested 4 times. On 2 of the test days, participants drank a sucrose-containing beverage, and on 2 of the days, drank water. Twenty-five minutes later, participants either smoked a cigarette or did not smoke. Participants were tested 5 min later for their responses on the cold pressor test. To determine if mood was altered by smoking or sucrose intake, the Profile of Mood Scale was administered immediately preceding and following experimental manipulations. Results: Cold threshold and cold tolerance were greater when participants were allowed to smoke than when they were not allowed to smoke. While men and women responded in a similar manner to the experimental manipulations, men displayed significantly greater cold threshold and cold tolerance than women. Sucrose consumption augmented the effects of smoking on cold threshold, but not on cold tolerance. Men reported feeling significantly more vigorous and less angry, and women reported feeling significantly less tense after they had smoked than when they had not smoked. Sucrose consumption did not alter self reports of mood in either men or women. Conclusion: These findings suggest that sucrose augments the analgesic properties of nicotine in humans, as well as in experimental animals, and suggest that diet could serve as an adjunct in the control of pain.

COMMENTS: This study was designed to investigate the interactions between sucrose intake and smoking on pain sensitivity and mood in humans. Cigarette smoking led to increases in pain threshold and tolerance. Sucrose intake (28.5 g, achieved by drinking a sucrose-containing beverage) increased pain threshold when combined with smoking, but

not alone. Sucrose intake also did not affect self-reported mood. The authors speculate that both sucrose and nicotine may alter central cholinergic neurons. The relevance of this study to sucrose as an ingredient in cigarettes is minor because of the differences in sucrose exposure concentration and route of exposure.

PROPYLENE GLYCOL

57-55-6

Number of relevant papers: 2

1. NTP-CERHR Expert Panel report on the reproductive and developmental toxicity of propylene glycol -

Center for the Evaluation of Risks to Human Reproduction
Reproductive Toxicology Volume 18, Issue 4 , June 2004, Pages 533-579

Abstract: The National Toxicology Program (NTP) and the National Institute of Environmental Health Sciences (NIEHS) established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June 1998. The purpose of the Center is to provide timely, unbiased, scientifically sound evaluations of human and experimental evidence for adverse effects on reproduction, including development, caused by agents to which humans may be exposed. Propylene glycol was selected for evaluation by the CERHR based on its high production and widespread public exposure due to its use as an antifreeze and de-icing agent, as well as its use in paints, coatings, foods, drugs, and cosmetics. This evaluation results from the efforts of a nine-member panel of government and non-government scientists that culminated in a public expert panel meeting held February 11–13, 2003. This report has been reviewed by CERHR staff scientists and by members of the Ethylene Glycol/Propylene Glycol Expert Panel. Copies have been provided to the CERHR Core Committee, which is made up of representatives of NTP-participating agencies. This report is a product of the expert panel and is intended to (1) interpret the strength of scientific evidence that propylene glycol is a reproductive or developmental toxicant based on data from in vitro, animal, or human studies, (2) assess the extent of human exposures to include exposures of the general public, occupational groups, and other sub-populations, (3) provide objective and scientifically thorough assessments of the scientific evidence that adverse reproductive/developmental health effects may be associated with such exposures, and (4) identify knowledge gaps to help establish research and testing priorities to reduce uncertainties and increase confidence in future assessments of risk. The Expert Panel Report on Propylene Glycol will be a central part of the subsequent NTP CERHR Monograph. The monograph will include the NTP CERHR Brief, the expert panel report, and all public comments on the expert panel report. The NTP CERHR Monograph will be made publicly available and transmitted to appropriate health and regulatory agencies.

COMMENTS: This paper provides a thorough review of the use, exposure, metabolism and toxicity of propylene glycol. The panel estimates 25 million pounds (2.9% of the

total consumption) of propylene glycol was used as tobacco humectant in 1999. American Industrial Hygiene Association Workplace Environmental Exposure Level guide of 50 ppm total exposure and 10mg/m³ inhalation aerosol exposure have been determined. Propylene glycol has a short half life and very low systemic toxicity, is not mutagenic, nor developmentally toxic. Although human inhalation exposures were considered within this review (in situations such as actors exposed to theatrical fog), studies have not included propylene glycol as an ingredient in cigarettes and data available on inhalation exposure in animals are inconclusive. The panel concluded “the current estimated exposures to propylene glycol are of negligible concern for reproductive or developmental toxicity in humans.” Potentially sensitive subpopulations include patients with impaired liver or kidney function.

2. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters

Fukayama MY, Easterday OD, Serafino PA, Renskers KJ, North-Root H, Schrankel KR.

Toxicol Lett. 1999 Dec 20;111(1-2):175-87.

ABSTRACT: Users of consumer products are invariably and intentionally exposed to complex mixtures in such products. With finished fragrance products, these mixtures may represent 100 or more fragrance raw materials (FRMs). The objective of the described studies was to evaluate the safety of finished fragrance products via the inhalation route. In total, the finished products contained approximately 100 FRMs at concentrations of 1% or greater. Major FRMs evaluated included benzyl acetate, coumarin, hydroxycitronellal, musk ketone, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-2-benzopyran (HHCB) and phenyl ethyl alcohol. Groups of rats or hamsters were exposed by inhalation (whole body) to the mixtures at 5, 9 or 50 mg/m³ for 4 h per day, 5 days per week for 6 or 13 weeks. For each of the fragrance products, the doses used generally represented a ten- to 100-fold exaggeration of levels expected to be achieved during typical use by consumers. With one exception, the fragrances were aerosolized prior to introduction into the inhalation chamber. The exception product was formulated with a propellant, packaged in a pressurized container and expelled with an automated actuator. In all studies, chamber concentrations of fragrance were monitored. Particle sizes ranged from 0.5 to 7.5 microm, depending on the study. Subchronic exposure to all fragrance mixtures resulted in no toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters. No gross pathological or histopathological findings related to test material exposures were observed. These studies support the conclusions that the fragrance mixtures would not pose a hazard to product users based on repeated and exaggerated inhalation exposures of animals.

COMMENTS: This paper presents results from subchronic inhalation studies of fragrance mixtures in rats and hamsters. Eight fragrance products were tested, which include approximately 200 components, including propylene glycol (13 – 52 µg/m³). No toxicological effects were identified from exposure to the fragrance mixtures at levels 10- to 100 fold greater than expected human exposure conditions for 6 or 13 weeks. The

results are consistent with those earlier studies of Gaworski 1998 that exposed rats to mixtures of flavor ingredients in a subchronic smoke inhalation study.

BROWN SUGAR
CAS: 57-50-1
 SEE MAJOR INGREDIENTS

HONEY
CAS: 8028-66-8
 NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

MENTHOL AND L-MENTHOL
CAS: 89-78-1

Number of relevant papers: 6

1. On the biological properties of fragrance compounds and essential oils - UBER BIOLOGISCHE WIRKUNGEN VON DUFTSTOFFEN UND ATHERISCHEN OLEN -

Buchbauer G.

Wien Med Wochenschr. 2004 Nov;154(21-22):539-47.

ABSTRACT: In the present review the physiological and/or pharmacological properties of essential oils and of single fragrance compounds are discussed. Essential oils are known and have been used since ancient times as natural medicines. As natural products essential oils are dependent on climate and their composition varies according to conditions of soil, to solar irradiation, to harvest time, to production methods, to storage conditions and similar facts which are discussed in chapter 2 of this review. The next chapters deal with the therapeutic use of essential oils in treating diseases, disorders or ailments of the nervous system, against cancer and as penetration enhancers. For space-saving reasons, however, the manifold antimicrobial and antifungal properties of these natural products have been left out. In the last chapter, the pros and cons in the use of essential oils in therapy are also discussed.

COMMENTS: This article is in German and was not translated.

2. Mentholated cigarette smoking inhibits nicotine metabolism

Neal L. Benowitz, Brenda Herrera, and Peyton Jacob, III

Journal of Pharmacology And Experimental Therapeutics 310:1208-1215, 2004

ABSTRACT: Smoking mentholated cigarettes has been suggested to convey a greater cancer risk compared with smoking nonmentholated cigarettes. Two of the possible mechanisms by which mentholated cigarette smoking could increase risk are by increasing systemic exposure to tobacco smoke toxins and by affecting the metabolism of nicotine or tobacco smoke carcinogens. To examine these possibilities, we performed a crossover study in 14 healthy smokers, one-half of whom were African-Americans and one-half whites. Subjects were randomly assigned to smoke mentholated or nonmentholated cigarettes for 1 week, then to cross over to the other type of cigarettes for another week. Subjects were confined to a Clinical Research Center for 3 days of each week, during which time blood levels of nicotine and carbon monoxide were measured throughout the day and an intravenous infusion of deuterium-labeled nicotine and cotinine was administered to determine the rate and pathways of nicotine metabolism. The systemic intake of nicotine and carbon monoxide was, on average, not affected by mentholation of cigarettes. Mentholated cigarette smoking did significantly inhibit the metabolism of nicotine (clearance: 1289 versus 1431 ml/min, two sided, $p = 0.02$). Inhibition of nicotine metabolism occurred both by slower oxidative metabolism to cotinine and by slower glucuronide conjugation. Our data do not support the hypothesis that mentholated cigarette smoking results in a greater absorption of tobacco smoke toxins. Our finding of impaired metabolism of nicotine while mentholated cigarette smoking suggests that mentholated cigarette smoking enhances systemic nicotine exposure.

COMMENTS: This is an expansion of previous research where the authors have shown that African-Americans metabolize nicotine to its metabolite, cotinine, differently as compared to whites. The authors report that when the number of cigarettes smoked per day is controlled, and the cigarettes smoked are in machine-determined yield as well as nicotine content, there is no difference in systemic nicotine and CO intake from smoking mentholated cigarettes compared to nonmentholated cigarettes. The results did not indicate that menthol accelerates nicotine metabolism, thus excluding the possibility that a more rapid metabolism of nicotine might explain a greater risk of intake of smoke and thus a greater carcinogenic risk.

3. Epidemiology of menthol cigarette use -

Giovino GA, Sidney S, Gfroerer JC, O'Malley PM, Allen JA, Richter PA, Cummings KM.

Nicotine Tob Res. 2004 Feb;6 Suppl 1:S67-81.

ABSTRACT: Approximately one-fourth of all cigarettes sold in the United States are mentholated. An understanding of the consequences, patterns, and correlates of menthol cigarette use can guide the development and implementation of strategies to reduce smoking prevalence and smoking-attributable morbidity and mortality. This paper summarizes the literature on the health effects of mentholated cigarettes and describes various patterns of use as indicated by consumption and survey data from the United States and other nations. The epidemiological literature on menthol cigarettes and cancer risk is inconclusive regarding whether these cigarettes confer a risk for cancer above that

of nonmentholated varieties. Available data indicate that mentholated cigarettes are at least as dangerous as their nonmentholated counterparts. In addition, because mentholation improves the taste of cigarettes for a substantial segment of the smoking population and appears to mask disease symptoms, this additive may facilitate initiation or inhibit quitting. Menthol market share is high in the Philippines (60%), Cameroon (35%-40%), Hong Kong (26%), the United States (26%), and Singapore (22%). Newport has become the leading menthol brand in the United States. Surveys from four nations indicate that menthol use among adult smokers is more common among females than males. Among U.S. smokers, 68.9% of Blacks, 29.2% of Hispanics, and 22.4% of Whites reported smoking a mentholated variety. Research is needed to better explain factors that may influence menthol preference, such as marketing, risk perceptions, brand formulation, and taste preferences. Such research would guide the development of potentially more effective programs and policies.

COMMENTS: This paper summarizes the literature on the health effects of mentholated cigarettes and describes various patterns of use as indicated by consumption and survey data from the United States and other nations. The epidemiological literature on menthol cigarettes and cancer risk is inconclusive regarding whether these cigarettes confer a risk for cancer above that of nonmentholated varieties.

4. Adolescent menthol smokers: Will they be a harder target for cessation? -

Eric T. Moolchan

Nicotine & Tobacco Research Volume 6, Supplement 1 (February 2004) S93-S95

ABSTRACT: Menthol smoking may influence the development of tobacco addiction and related health consequences, yet limited data on menthol smoking by youth are available. We assessed usual brand menthol preference by Baltimore-area teenage smokers applying to a smoking cessation study between September 1999 and December 2002. Of a biethnic (Black and White) sample of 593 youths (mean age~15.5; 1.4 years, 51% female, 45% African American), the overwhelming majority (93%) were menthol smokers. Menthol preference rates were highest among African American girls and lowest among White boys. Overall, a statistically significant association was found between ethnicity and menthol preference, χ^2 (df~1)~19.4, p ~.001. This association also was observed separately for girls, χ^2 (df~1)~9.21, p ~.0024, and for boys, χ^2 (df~1)~9.59, p ~.0020. Menthol smoking did not vary with age in either ethnic group. These findings of overwhelming menthol preference in a treatment-seeking sample of adolescents warrant further research on the developmental trajectory, cessation, and health-related impact of menthol smoking by youth.

COMMENTS: This study compared the prevalence of menthol preference of Baltimore adolescents of different genders and ethnicities. The study found an overwhelming preference for menthol cigarettes (93%) in teenagers participating in this study. Both ethnicity and gender were significant factors associated with menthol preference. Menthol preference rates were highest in African Americans and females, and lowest in

white males. The findings of this paper were not relevant to the health effects of menthol as an ingredient in cigarettes.

5. Menthol pharmacology and its potential impact on cigarette smoking behavior -

Karen Ahijevych, Bridgette E. Garrett

Nicotine & Tobacco Research Volume 6, Supplement 1 (February 2004) S17–S28

ABSTRACT: Menthol is the only tobacco additive promoted and advertised by the tobacco industry. Although a considerable body of research has examined the effects of menthol when it is administered alone and unburned, the effects of menthol when burned in cigarette smoke are more complex because it is administered in a matrix of more than 4,000 substances. Therefore, it is difficult to isolate potential pharmacological and toxic effects of menthol when it is administered in a smoke mixture. Menthol properties include cooling and local anesthesia, as well as effects on drug absorption and metabolism, bronchodilation and respiration changes, and electrophysiology. Subjective effects of smoothness and less harshness have been identified as reasons for menthol cigarette smoking, but findings have been inconclusive regarding the effect of menthol on carbon monoxide exposure and smoking topography parameters. Gaps in the research literature and future research areas include the following: (a) What is the role of menthol in tobacco reinforcement and addiction? (b) In the absence of nicotine, is menthol reinforcing? (c) Are the pharmacological and physiological effects of menthol mediated by a menthol-specific receptor or some other central nervous system-mediated action? (d) What are the influences of menthol and menthol metabolism on the metabolic activation and detoxification of carcinogens in tobacco smoke? and (e) Do differences exist in cigarette smoking topography in relation to the interaction of ethnicity, gender, and menthol cigarette preference? Answers to these questions will help to elucidate the function of menthol in cigarettes and its impact on smoking behavior.

COMMENTS: These authors reviewed the current knowledge regarding the impact associated with smoking mentholated cigarettes. In this review, the authors attempted to extrapolate the actions of menthol as a nontobacco additive to its potential pharmacological and physiological effects in cigarettes. The authors provided their response to a number of questions that were related to addiction. CNS mediated effects, interaction with race, sex and cigarette preference were all addressed.

6. Percutaneous penetration enhancers in cigarette mainstream smoke -

Smith CJ, Perfetti TA, Garg R, Martin P, Hansch C.

Food Chem Toxicol. 2004 Jan;42(1):9-15.

ABSTRACT: Percutaneous penetration enhancers (PPEs) are chemicals used to enhance the transdermal delivery of drugs. Fifty-eight of the approximately 150 PPEs used for the transdermal delivery of drugs have been reported in cigarette mainstream smoke (MS). MS is a complex aerosol of minute liquid droplets (termed the particulate phase) suspended within a mixture of gases (CO(2), CO, NO(x), etc.) and semi-volatile

compounds. The gases and many of the semi-volatiles are termed the vapor phase. Twenty-nine of the 58 PPEs have been identified in MS vapor phase, 15 in the particulate phase and 14 in both the vapor and particulate phases. There is a tendency for MS PPEs to be hydrophobic, with 40 of the 58 compounds (69%) being either hydrophobic or strongly hydrophobic, and only 24% being hydrophilic. Many of the 4800 known constituents of MS are hydrophilic and would not be expected to readily cross cell membranes or penetrate tissue when delivered as single compounds. The in vivo effect on biological activity of the juxtaposition within the cigarette smoke aerosol of the large number of hydrophilic constituents with the 58 PPEs is currently unknown. As an initial step in understanding this potential complex interaction, the 58 PPEs in MS have been identified and a number of molecular parameters related to the ability to penetrate tissue have been calculated, including MS concentration, measured and calculated base ten logarithm of the octanol-water partition coefficient (Mlog P and Clog P), molecular volume (MgVol) and calculated molar refractivity (CMR).

COMMENTS: Percutaneous penetration enhancers (PPEs) are used by pharmaceutical industry to enhance delivery of drugs that are poorly absorbed. This paper identifies 58 PPEs, including menthol, found in cigarette mainstream smoke and calculates molecular parameters related to the ability to penetrate tissues for each. The authors concluded that the interaction of PPEs in cigarette mainstream smoke with constituents of smoke aerosol cannot be accurately predicted at this time and warrants the study whole cigarette smoke rather than MS fractions.

POTASSIUM CARBONATE

CAS: 584-08-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

RUM AND RUM EXTRACT

CAS: 90604-30-1

CAS: 977089-45-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

COCOA, COCOA SHELLS, EXTRACT, DISTILLATE, POWDER, ALKALIZED, ABSOLUTE AND TINCTURE

CAS: 08002-31-1

CAS: 84649-99-0

CAS: 68916-17-6

CAS: 95009-22-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

GUAR GUM**CAS: 9000-30-0**

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

PRUNE JUICE AND CONCENTRATE**CAS: 90082-87-4**

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL ALCOHOL, INCLUDING SDA-4**CAS: 64-17-5**

Number of relevant papers: 7

GENERAL COMMENTS:

There are numerous papers on a wide range of health effects of drinking alcohol and/or smoking cigarettes. Exposure to one or both substances is a risk factor for possible colon and gastric cancers, abortions, diseases of the mouth and throat, gastro-reflux disease, olfactory ability etc. Few papers are available that examines the effects of inhaled ethanol. While most of the reports did not involve inhalation of the test substance, they all addressed possible synergy between tobacco and alcohol consumption. All of these studies used high levels of EtOH exposure to produce the reported effects. These studies did not mimic the route of exposure nor concentration of EtOH that would be associated with this ingredient used in cigarettes.

1. Pathology of the olfactory epithelium: Smoking and ethanol exposure -

Vent J, Robinson AM, Gentry-Nielsen MJ, Conley DB, Hallworth R, Leopold DA, Kern RC.

Laryngoscope. 2004 Aug;114(8):1383-8.

ABSTRACT: To investigate the effects of tobacco smoke on the olfactory epithelium. Cigarette smoking has been associated with hyposmia; however, the pathophysiology is poorly understood. The sense of smell is mediated by olfactory sensory neurons (OSNs) exposed to the nasal airway, rendering them vulnerable to environmental injury and death. As a consequence, a baseline level of apoptotic OSN death has been demonstrated even in the absence of obvious disease. Dead OSNs are replaced by the mitosis and maturation of progenitors to maintain sufficient numbers of neurons into adult life. Disruption of this balance has been suggested as a common cause for clinical smell loss. This current study will evaluate the effects of tobacco smoke on the olfactory mucosa, with emphasis on changes in the degree of OSN apoptosis. **STUDY DESIGN:** A rat model was used to assess the olfactory epithelium after exposure to tobacco smoke. **METHODS:** Rats were exposed to tobacco smoke alone (for 12 weeks), smoke plus dietary ethanol (for the final 5 weeks), or to neither (control). Immunohistochemical analysis of the olfactory epithelium was performed using an antibody to the active form

of caspase-3. Positive staining for this form of the caspase-3 enzyme indicates a cell undergoing apoptotic proteolysis. **RESULTS:** Control rats demonstrated a low baseline level of caspase-3 activity in the olfactory epithelium. In contrast, tobacco smoke exposure triggered a dramatic increase in the degree of OSN apoptosis that affected all stages of the neuronal lineage. **CONCLUSIONS:** These results support the following hypothesis: smell loss in smokers is triggered by increased OSN death, which eventually overwhelms the regenerative capacity of the epithelium.

COMMENTS: This study assessed the degree of olfactory sensory neuron (OSN) apoptosis in rats exposed to tobacco smoke with and without ethanol. The report indicates that apoptosis, as demonstrated by caspase-3 activation, is significant after exposure but there was no additional or synergistic effect on caspase-3 activity with ethanol ingestion. This study has little relevance to ethyl alcohol added to cigarette smoke but the authors suggest that increased apoptotic death of OSNs caused by sinusitis and aging, overwhelms the regenerative capacity of the epithelium mediating clinical olfactory loss.

2. A 2-year follow-up study of cigarette smoking and risk of dementia -

D. Juan, D. H. D. Zhou, J. Li, J. Y. J. Wang, C. Gao and M. Chen
European Journal of Neurology Volume 11 Issue 4 Page 277 - April 2004

ABSTRACT: The report focused on investigating the relationship between cigarette smoking and dementia in elderly people through prospective studies. We did a 2-year follow-up study of elderly people. A total of 2820 participants aged 60 years old and over from six communities of Chongqing agreed to take part. Dementia was diagnosed with MMSE (Mini-Mental State Examination) and DSM-III-R (Diagnostic and Statistical Manual of Mental Disorders). Participants were classified as never smokers, past smokers, and current smokers. During follow-up, we recorded incident cases of dementia. The association of smoking and dementia was investigated using proportional hazards regression analysis. A total of 121 incident cases of dementia were detected, of which 84 (69%) were Alzheimer's disease, 17 (14%) were vascular dementia, and 21 (17%) were other dementia. Compared with never smokers, current smokers had an increased risk of Alzheimer's disease (RR = 2.72; 95% CI = 1.63–5.42) and vascular dementia (RR = 1.98; 95% CI = 1.53–3.12) adjusting for age, sex, education, blood pressure, and alcohol intake. Compared with light smokers, the adjusted risk of Alzheimer's disease was significantly increased among smokers with a medium level of exposure (RR = 2.56; 95% CI = 1.65–5.52), with an even higher risk of Alzheimer's disease in the heavy smoking group (RR = 3.03; 95% CI = 1.25–4.02). Smoking was associated with the risk of dementia. This study suggests that both smoking status and amount is associated with dementia.

COMMENTS: This paper describes a follow up to a previous study of the relationship between cigarette smoking and cognitive impairment among elderly people in China. Current smoking increased the risk of dementia even after adjusting for other risk factors such as age, sex, education, blood pressure and alcohol intake. However, the risk of

Alzheimer's disease and other forms of dementia was not associated with past smoking amount. The results of this study were not relevant to the health effects of ethyl alcohol as an ingredient in cigarettes.

3. Risk factors for oral and pharyngeal cancer in young adults

Rodriguez T, Altieri A, Chatenoud L, Gallus S, Bosetti C, Negri E, Franceschi S, Levi F, Talamini R, La Vecchia C.
Oral Oncol. 2004 Feb;40(2):207-13.

ABSTRACT: Mortality from oral cancer has been rising in the young in several areas of the world until the early 1990s. We analyzed data from two case-control studies from Italy and Switzerland including 137 cases of oral and pharyngeal cancer below age 46 and 298 hospital controls. The multivariate odds ratios (OR) were 20.7 for heavy smokers and 4.9 for heavy drinkers. The combination of high tobacco and alcohol consumption led to an OR of over 48. Body mass index (OR=0.28, for the highest tertile), high consumption of coffee (OR=0.25), fresh vegetables (OR=0.39), fruit (OR=0.73) and beta-carotene (OR=0.48) were inversely related to risk. Tobacco accounted for 77% of all cancer cases in this population, alcohol for 52%, low vegetable consumption for 52%, and the combination of the three factors for 85%.

COMMENTS: The authors examined the data from two large case-control studies of oral and pharyngeal cancer. This report is not relevant to inhaled ethanol since the authors' conclusions are based on use of very high levels of alcohol and an exposure route that did not mimic inhalation. However, the authors' statements regarding the risk for oral/pharyngeal cancers and smoking may be of interest. Heavy consumption of both tobacco smoke and alcohol may result in an over 48-fold increase in health risk in young people. This tobacco-related risk substantially declines within a few years and was not substantially elevated after 5 years of stopping smoking.

4. Desensitization of PKA-stimulated ciliary beat frequency in an ethanol-fed rat model of cigarette smoke exposure -

Wyatt TA, Gentry-Nielsen MJ, Pavlik JA, Sisson JH.
Alcohol Clin Exp Res. 2004 Jul;28(7):998-1004

ABSTRACT: Our previous studies have shown that the ciliary beat frequency (CBF) of cultured ciliated airway epithelial cells exposed to chronic ethanol fails to increase in response to beta-agonist stimulation. This loss of the ciliary "flight response" correlates with an ethanol-mediated desensitization of adenosine 3':5'-cyclic monophosphate-dependent protein kinase (PKA), a known regulatory component of CBF stimulation. We hypothesized that a similar ethanol-mediated desensitization of CBF would occur in vivo. **METHODS:** Sprague Dawley rats were fed a liquid diet containing various concentrations of ethanol for 1 or 5 weeks. Half were exposed to cigarette smoke for 12 weeks and half were sham exposed. Animals were killed and tracheal epithelial cells analyzed for CBF and PKA activity. **RESULTS:** Baseline CBF (approximately 6 Hz) was

unchanged in tracheal epithelial cells of rats consuming diets containing 0-36% ethanol for 5 weeks. Isoproterenol stimulated CBF to 12 to 13 Hz in the tracheal epithelial cells of control rats not administered ethanol. However, isoproterenol stimulation of CBF was blunted to 7.5 Hz in rats eating a 26% ethanol diet, and there was no stimulation of CBF in rats fed a diet containing 36% ethanol. Similarly, isoproterenol stimulated a 2- to 3-fold increase in PKA activity in control rats, but this PKA response to isoproterenol was blunted in rats fed increasing concentrations of ethanol. No isoproterenol-stimulated PKA response was observed in rats fed 36% ethanol. No ethanol-induced changes in cyclic guanosine monophosphate-dependent protein kinase or protein kinase C were observed in the rats' tracheal epithelial cells. Cigarette smoke exposure slightly elevated baseline CBF and lowered the ethanol consumption level for isoproterenol-desensitization of CBF and PKA activation to 16%. No isoproterenol desensitization was observed after 1 week of alcohol feeding. Furthermore, 36% ethanol-feeding for 1 week stimulated rat tracheal CBF and PKA. **CONCLUSION:** These data demonstrate that *in vivo* administration of ethanol to rats results in decreased ciliary beating and the desensitization of PKA. This suggests a mechanism for mucociliary clearance dysfunction in alcoholics.

COMMENTS: These authors used a rat model to study the combined effects of smoking and ingestion of EtOH to examine the role that smoking has in alcohol-related lung disease. Chronic EtOH use results in desensitization of B-agonist stimulated ciliary beat frequency (CBF), both *in vivo* and *in vitro*, but short term exposure to EtOH does not. Combining cigarette smoke exposure with ethanol further decreases CBF. It is interesting that smoke exposure alone elevated CBF.

5. Percutaneous penetration enhancers in cigarette mainstream smoke.

Smith CJ, Perfetti TA, Garg R, Martin P, Hansch C.
Food Chem Toxicol. 2004 Jan;42(1):9-15.

ABSTRACT: Percutaneous penetration enhancers (PPEs) are chemicals used to enhance the transdermal delivery of drugs. Fifty-eight of the approximately 150 PPEs used for the transdermal delivery of drugs have been reported in cigarette mainstream smoke (MS). MS is a complex aerosol of minute liquid droplets (termed the particulate phase) suspended within a mixture of gases (CO(2), CO, NO(x), etc.) and semi-volatile compounds. The gases and many of the semi-volatiles are termed the vapor phase. Twenty-nine of the 58 PPEs have been identified in MS vapor phase, 15 in the particulate phase and 14 in both the vapor and particulate phases. There is a tendency for MS PPEs to be hydrophobic, with 40 of the 58 compounds (69%) being either hydrophobic or strongly hydrophobic, and only 24% being hydrophilic. Many of the 4800 known constituents of MS are hydrophilic and would not be expected to readily cross cell membranes or penetrate tissue when delivered as single compounds. The *in vivo* effect on biological activity of the juxtaposition within the cigarette smoke aerosol of the large number of hydrophilic constituents with the 58 PPEs is currently unknown. As an initial step in understanding this potential complex interaction, the 58 PPEs in MS have been identified and a number of molecular parameters related to the ability to penetrate tissue have been calculated, including MS concentration, measured and calculated base ten

logarithm of the octanol-water partition coefficient (Mlog P and Clog P), molecular volume (MgVol) and calculated molar refractivity (CMR).

COMMENTS: Percutaneous penetration enhancers (PPEs) are used by pharmaceutical industry to enhance delivery of drugs that are poorly absorbed. This paper identifies 58 PPEs, including ethanol, found in cigarette mainstream smoke. The molecular parameters related to the ability to penetrate tissues were calculated for each. The authors conclude that the interaction of PPEs in cigarette mainstream smoke with constituents of smoke aerosol cannot be accurately predicted at this time and warrants the study of whole cigarette smoke rather than MS fractions.

6. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters

Fukayama MY, Easterday OD, Serafino PA, Renskers KJ, North-Root H, Schrankel KR.

Toxicol Lett. 1999 Dec 20;111(1-2):175-87.

ABSTRACT: Users of consumer products are invariably and intentionally exposed to complex mixtures in such products. With finished fragrance products, these mixtures may represent 100 or more fragrance raw materials (FRMs). The objective of the described studies was to evaluate the safety of finished fragrance products via the inhalation route. In total, the finished products contained approximately 100 FRMs at concentrations of 1% or greater. Major FRMs evaluated included benzyl acetate, coumarin, hydroxycitronellal, musk ketone, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-2-benzopyran (HHCB) and phenyl ethyl alcohol. Groups of rats or hamsters were exposed by inhalation (whole body) to the mixtures at 5, 9 or 50 mg/m³ for 4 h per day, 5 days per week for 6 or 13 weeks. For each of the fragrance products, the doses used generally represented a ten- to 100-fold exaggeration of levels expected to be achieved during typical use by consumers. With one exception, the fragrances were aerosolized prior to introduction into the inhalation chamber. The exception product was formulated with a propellant, packaged in a pressurized container and expelled with an automated actuator. In all studies, chamber concentrations of fragrance were monitored. Particle sizes ranged from 0.5 to 7.5 microm, depending on the study. Subchronic exposure to all fragrance mixtures resulted in no toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters. No gross pathological or histopathological findings related to test material exposures were observed. These studies support the conclusions that the fragrance mixtures would not pose a hazard to product users based on repeated and exaggerated inhalation exposures of animals.

COMMENTS: This paper presents results from subchronic inhalation studies of fragrance mixtures in rats and hamsters. Eight fragrance products were tested, which include approximately 200 components, including ethyl alcohol (126 µg/m³). No toxicological effects were identified from exposure to the fragrance mixtures at levels 10- to 100 fold greater than expected human exposure conditions for 6 or 13 weeks. The

results are consistent with those earlier studies of Gaworski 1998 that exposed rats to mixtures of flavor ingredients in a subchronic smoke inhalation study.

7. In utero exposure to tobacco and alcohol modifies neurobehavioral development in mice offspring: consideration a role of oxidative stress

Li Y, Wang H, Li JF.

Pharmacol Res 2004; 49: 467-473

ABSTRACT: Objective: To determine whether in utero tobacco and alcohol exposure induces long-term neurobehavioral alterations and whether oxidative stress/damage is a possible causal factor. Methods: Gravid mice were subjected to tobacco smoking and alcohol consumption. Their offspring were subsequently evaluated in developmental and behavioral tests. Antioxidative enzymes and erythrocyte membrane fluidity of adult offspring were measured. Results: The intrauterine tobacco and alcohol exposure has resulted in significant reduced postnatal body and organ weights accompanied by reduced gestational body weight gain in their mothers. Such exposure also induced remarkable developmental delay in neonatal reflexes and notable behavioral deficit in adulthood, namely reduced motive coordination and locomotor activity as well as impaired learning and memory abilities. Furthermore, the formation of malondialdehyde (MDA) increased significantly whereas the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), catalase (Cat) and glutathione S-transferases (GST) decreased in the cerebral cortex and liver of prenatal intoxicated offspring. The embryonic intoxication also markedly reduced erythrocyte membrane fluidity in offspring. Conclusion: Our study shows the long-term neurotoxicity associated with prenatal tobacco and alcohol exposure, and suggests that the deleterious outcome may be in relation to increased free radicals formation and oxidative stress.

COMMENTS: Pregnant mice were exposed to cigarette smoke and wine in order to examine the prenatal effects of the combined substances. Significant reductions in body weight and delayed neurobehavioral development were observed in the pups of the treated mice. The effects appeared to be long-lasting and related to reductions in the enzyme-mediated antioxidant system. However, this paper was not directly relevant to the health effects of ethyl alcohol as an ingredient in cigarettes.

LICORICE ROOT, FLUID EXTRACT AND POWDER

CAS: 68916-91-6

CAS: 08008-94-4

CAS: 97676-23-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMMONIUM PHOSPHATE DIBASIC (DIAMMONIUM PHOSPHATE)
CAS: 7783-28-0

Number of relevant papers: 1

1. The effect of tobacco blend additives on the retention of nicotine and solanesol in the human respiratory tract and on subsequent plasma nicotine concentrations during cigarette smoking -

Alan K. Armitage, Michael Dixon,* Barrie E. Frost, Derek C. Mariner,* and Neil M. Sinclair
Chem. Res. Toxicol., 17 (4), 537 -544

ABSTRACT: The influence of the tobacco additives diammonium hydrogen phosphate (DAP) and urea on the delivery and respiratory tract retention of nicotine and solanesol and on the uptake of nicotine into venous blood was investigated in 10 smokers under mouth-hold and 75 and 500 mL inhalation conditions. Three cigarettes with identical physical specifications were produced from a common lamina tobacco blend. The control cigarette contained nonammoniated reconstituted tobacco sheet (RTS), whereas DAP and other ammonia compounds were added to the RTS of the second cigarette. Urea was added to the tobacco of the third cigarette. The presence of DAP or urea in the test cigarettes did not significantly influence solanesol retention within the mouth during the mouth-hold condition. Nicotine retention within the mouth during the mouth-hold condition was, however, significantly higher for the DAP cigarette ($64.3 \pm 10.5\%$) than for the urea ($53.3 \pm 11.3\%$) or control cigarette ($46.3 \pm 8.6\%$), but this did not result in an increase in nicotine uptake into venous blood. Solanesol retentions during the 75 and 500 mL inhalation volume conditions and nicotine retentions during the 75 mL inhalation volume condition were not significantly different for the three cigarette types. Although the nicotine retention approached 100% with each cigarette type during the 500 mL inhalation condition, the nicotine retention for the urea-treated cigarette ($99.6 \pm 0.2\%$) was marginally, but statistically, significant, higher than for the control ($99.1 \pm 0.5\%$) and DAP-treated cigarettes ($98.8 \pm 0.6\%$). There were no statistically significant differences between the indices of nicotine uptake into venous blood for the three cigarette types in any of the inhalation conditions.

COMMENTS: It has been postulated that certain ammonium compounds when used as a tobacco additive can increase smoke pH thus increasing the transfer of nicotine from tobacco to the smoke and increasing the “addictiveness” of nicotine. This study assesses the retention of nicotine in the respiratory tract and its uptake into the blood system under controlled inhalation conditions. These results do not indicate that the addition of diammonium hydrogen phosphate or urea resulted in an enhanced uptake of nicotine from the respiratory tract into the systemic circulation during smoking. The authors found that most of the nicotine inhaled in cigarette smoke is absorbed irrespective of the

pH and that the pH does not affect bioavailability but instead influences the perceived strength of the cigarette.

AMMONIUM ALGINATE

CAS: 9005-34-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CHOCOLATE AND CHOCOLATE LIQUOR

MAJOR

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

LACTIC ACID

CAS: 50-21-5

CAS: 598-82-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

PLUM JUICE, CONCENTRATE AND EXTRACT

CAS: 90082-87-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CAROB BEAN GUM, ABSOLUTE AND EXTRACT

CAS: 9000-40-2

CAS: 84961-45-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

FIG JUICE CONCENTRATE AND EXTRACT

CAS: 90028-74-3

CAS: 68916-52-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

SORBITOL

CAS: 50-70-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMMONIUM HYDROXIDE

CAS: 1336-21-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

GLUCOSE/ DEXTROSE**CAS: 50-99-7****CAS: 492-62-6**

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

UREA**CAS: 57-13-6**

Number of relevant papers: 1

1. The effect of tobacco blend additives on the retention of nicotine and solanesol in the human respiratory tract and on subsequent plasma nicotine concentrations during cigarette smoking -**Alan K. Armitage, Michael Dixon,* Barrie E. Frost, Derek C. Mariner,* and Neil M. Sinclair****Chem. Res. Toxicol., 17 (4), 537 -544**

ABSTRACT: The influence of the tobacco additives diammonium hydrogen phosphate (DAP) and urea on the delivery and respiratory tract retention of nicotine and solanesol and on the uptake of nicotine into venous blood was investigated in 10 smokers under mouth-hold and 75 and 500 mL inhalation conditions. Three cigarettes with identical physical specifications were produced from a common lamina tobacco blend. The control cigarette contained nonammoniated reconstituted tobacco sheet (RTS), whereas DAP and other ammonia compounds were added to the RTS of the second cigarette. Urea was added to the tobacco of the third cigarette. The presence of DAP or urea in the test cigarettes did not significantly influence solanesol retention within the mouth during the mouth-hold condition. Nicotine retention within the mouth during the mouth-hold condition was, however, significantly higher for the DAP cigarette ($64.3 \pm 10.5\%$) than for the urea ($53.3 \pm 11.3\%$) or control cigarette ($46.3 \pm 8.6\%$), but this did not result in an increase in nicotine uptake into venous blood. Solanesol retentions during the 75 and 500 mL inhalation volume conditions and nicotine retentions during the 75 mL inhalation volume condition were not significantly different for the three cigarette types. Although the nicotine retention approached 100% with each cigarette type during the 500 mL inhalation condition, the nicotine retention for the urea-treated cigarette ($99.6 \pm 0.2\%$) was marginally, but statistically, significant, higher than for the control ($99.1 \pm 0.5\%$) and DAP-treated cigarettes ($98.8 \pm 0.6\%$). There were no statistically significant differences between the indices of nicotine uptake into venous blood for the three cigarette types in any of the inhalation conditions.

COMMENTS: It has been postulated that certain ammonium compounds when used as a tobacco additive can increase smoke pH thus increasing the transfer of nicotine from tobacco to the smoke and increasing the “addictiveness” of nicotine. This study assesses the retention of nicotine in the respiratory tract and its uptake into the blood system under controlled inhalation conditions. These results do not indicate that the addition of

diammonium hydrogen phosphate or urea resulted in an enhanced uptake of nicotine from the respiratory tract into the systemic circulation during smoking. The authors found that most of the nicotine inhaled in cigarette smoke is absorbed irrespective of the pH and that the pH does not affect bioavailability but instead influences the perceived strength of the cigarette.

SODIUM CARBONATE
CAS: 497-19-8

Number of relevant papers: 1

1. Cancer incidence in textile manufacturing workers in Australia

Fritschi L, Lakhani R, Nadon L.
J Occup Health 2004 Nov;46(6):493-6.

ABSTRACT: N/A

COMMENTS: The study was designed to assess the associated of incidence of cancer with the likely exposure to individual chemicals in textile manufacturing workers. There were no significant increases in relative risk of cancer associated with any of the 32 substances assessed, including sodium carbonate, which had a relative risk of 1.55.

FRUCTOSE
CAS: 57-48-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DAVANA OIL
CAS: 8016-03-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

LIME OIL
CAS: 68916-84-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL 2-METHYLBUTYRATE
CAS: 7452-79-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

PEPPERMINT OIL AND ABSOLUTE AND PEPPERMINT OIL TERPENELESS

CAS: 8006-90-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

SPEARMINT OIL

CAS: 8008-79-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

**ORANGE OIL AND EXTRACT (SWEET, DISTILLED, TERPENELESS, AND
SOUR/BITTER ORANGE OILS)**

CAS: 8008-57-9

CAS: 68606-94-0

CAS: 68916-04-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

MOLASSES EXTRACT

CAS: 8052-35-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CORIANDER EXTRACT, SEED, AND OIL

CAS: 8008-52-4

CAS: 84775-50-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL VANILLIN

CAS: 121-32-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

L-MENTHONE

CAS: 14073-97-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

VANILLIN
CAS: 121-33-5

Number of relevant papers: 1

1. Mutagens and Sensitizers-An Unequal Relationship? -

A. M. Wolfreys A1 and D. A. Basketter A1

Journal of Toxicology: Cutaneous and Ocular Toxicology Volume 23, Number 3 / 2004 197 – 205.

ABSTRACT: For some years, those involved with the safety assessment of chemicals have in one way or another considered the degree to which data on either skin sensitization potential or on carcinogenicity may inform them on the other endpoint for a particular substance. In this work, we have taken a pragmatic perspective on the question and assessed mutagens, rather than carcinogens, and sensitizers as this better reflects the potential for biological macromolecule interaction. A dataset of 100 substances, the majority of which have come under scrutiny for one reason or another during our own toxicology investigations, was interrogated. We focused on the extent to which results from the primary screen for skin sensitization correlated with the results from the two *in vitro* tests used as a screen for mutagenicity, namely the bacterial mutation assay and the *in vitro* chromosome aberration assay. Although there was some concordance between the two endpoints, as standalone methods, neither predicted the other particularly accurately, with 32% showing disagreement. It is probable that there are several critical elements missing from this top level assessment, not least an appreciation of which substances are positive in mutagenicity tests via non genotoxic mechanisms which could seriously impair such a correlation between results from the two different endpoints.

COMMENTS: This paper discusses the relationship between skin sensitizers and carcinogens. Previous data indicate that chemicals that induced allergic contact dermatitis had a 50% chance of being a rodent carcinogen. To investigate this hypothesis the authors examined *in vitro* mutagenicity screening data on 100 chemicals and compared the results with information on skin sensitization potential of these substances. In these comparisons about one-third of the chemicals that were positive in the mutagenicity screen would not be classified as skin sensitizer. Vanillin was mutagenic but was not a skin sensitizer. The author's conclusion was that neither endpoint is a reliable indicator of the other.

CHAMOMILE FLOWER OIL, EXTRACT AND ABSOLUTE

CAS: 8002-66-2

CAS: 8015-92-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CATEGORY: STANDARD INGREDIENTS**1. Percutaneous penetration enhancers in cigarette mainstream smoke -**

Smith CJ, Perfetti TA, Garg R, Martin P, Hansch C.
Food Chem Toxicol. 2004 Jan;42(1):9-15.

This paper examines a number of standard ingredients including:

BENZYL ALCOHOL 100-51-6
 1,3-BUTANEDIOL 107-88-0
 BUTYL ACETATE 123-86-4
 CARBON DIOXIDE 124-38-9
 ETHYL ACETATE 141-78-6
 DECANOIC ACID 334-48-5
 BUTYL ALCOHOL (1-BUTANOL) 71-36-3

ABSTRACT: Percutaneous penetration enhancers (PPEs) are chemicals used to enhance the transdermal delivery of drugs. Fifty-eight of the approximately 150 PPEs used for the transdermal delivery of drugs have been reported in cigarette mainstream smoke (MS). MS is a complex aerosol of minute liquid droplets (termed the particulate phase) suspended within a mixture of gases (CO₂, CO, NO_x, etc.) and semi-volatile compounds. The gases and many of the semi-volatiles are termed the vapor phase. Twenty-nine of the 58 PPEs have been identified in MS vapor phase, 15 in the particulate phase and 14 in both the vapor and particulate phases. There is a tendency for MS PPEs to be hydrophobic, with 40 of the 58 compounds (69%) being either hydrophobic or strongly hydrophobic, and only 24% being hydrophilic. Many of the 4800 known constituents of MS are hydrophilic and would not be expected to readily cross cell membranes or penetrate tissue when delivered as single compounds. The in vivo effect on biological activity of the juxtaposition within the cigarette smoke aerosol of the large number of hydrophilic constituents with the 58 PPEs is currently unknown. As an initial step in understanding this potential complex interaction, the 58 PPEs in MS have been identified and a number of molecular parameters related to the ability to penetrate tissue have been calculated, including MS concentration, measured and calculated base ten logarithm of the octanol-water partition coefficient (Mlog P and Clog P), molecular volume (MgVol) and calculated molar refractivity (CMR).

COMMENTS: Percutaneous penetration enhancers (PPEs) are used by pharmaceutical industry to enhance delivery of drugs that are poorly absorbed. This paper identifies 58 PPEs found in cigarette mainstream smoke and calculates molecular parameters related to the ability to penetrate tissues for each. The authors concluded that the interaction of PPEs in cigarette mainstream smoke with constituents of smoke aerosol cannot be accurately predicted at this time and warrants the study of whole cigarette smoke rather than MS fractions.

ACETANISOLE

CAS: 100-06-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ACETIC ACID

CAS: 64-19-7

SEE HIGH MUL'S INGREDIENTS

ACETOIN

CAS: 513-86-0

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ACETOPHENONE

CAS:98-86-2

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ACETYLPYRAZINE (2-)

CAS: 22047-25-2

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

3-ACETYLPYRIDINE (BETA-ACETYLPYRIDINE)

CAS: 350-03-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2-ACETYLTIAZOLE

CAS: 24295-03-2

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DL-ALANINE, L-ALANINE

CAS: 302-72-7

CAS: 56-41-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ALFALFA EXTRACT

CAS: 84082-36-0

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ALLYL HEXANOATE

CAS: 123-68-2

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMMONIUM ALGINATE

CAS: 9005-34-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMMONIUM HYDROXIDE

CAS: 1336-21-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMMONIUM PHOSPHATE DIBASIC (DIAMMONIUM PHOSPHATE)

CAS: 7783-28-0

SEE MAJOR INGREDIENTS

AMYL ALCOHOL

CAS: 71-41-0

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMYL BUTYRATE

CAS: 540-18-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMYL FORMATE

CAS: 638-49-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

AMYL OCTANOATE

CAS: 638-25-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ALPHA-AMYLCINNAMALDEHYDE

CAS: 122-40-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

TRANS-ANETHOLE**CAS: 4180-23-8****CAS: 104-46-1**

Number of relevant papers: 1

1. Cytotoxic and xenoestrogenic effects via biotransformation of trans-anethole on isolated rat hepatocytes and cultured MCF-7 human breast cancer cells -**Nakagawa Y, Suzuki T.****Biochem Pharmacol. 2003 Jul 1;66(1):63-73.**

ABSTRACT: The metabolism and action of trans-anethole (anethole) and the estrogen-like activity of the compound and its metabolites were studied in freshly isolated rat hepatocytes and cultured MCF-7 human breast cancer cells, respectively. The incubation of hepatocytes with anethole (0.25–2.0 mM) caused a concentration- and time-dependent cell death accompanied by losses of cellular ATP and adenine nucleotide pools. Anethole at a weakly toxic level (0.5 mM) was metabolized to 4-methoxycinnamic acid (4MCA), 4-hydroxy-1-propenylbenzene (4OHPB), and the monosulfate conjugate of 4OHPB; the levels of 4OHPB sulfate and 4MCA reached approximately 20 and 200 mM within 2 hr, respectively, whereas that of free unconjugated 4OHPB was less than approximately 0.5 mM. At a moderately toxic concentration (1.0 mM), unconjugated 4OHPB reached approximately 10 mM, followed by abrupt loss of 30-phosphoadenosine 50-phosphosulphate (PAPS). Based on cell viability and adenine nucleotide levels, 4OHPB was more toxic than anethole and 4MCA. The addition of 2,6-dichloro-4-nitrophenol (50 mM), an inhibitor of sulfotransferase, enhanced the anethole-induced cytotoxicity associated with losses of ATP, PAPS, and 4OHPB sulfate, and symmetrically increased the unconjugated 4OHPB concentration. 4OHPB as well as diethylstilbestrol (DES) and bisphenol A (BPA), which are known xenoestrogenic compounds, competitively displaced 17 β -estradiol bound to the estrogen receptor α in a concentration-dependent manner; IC₅₀ values of these compounds were approximately 1×10^{-5} , 1×10^{-8} and 5×10^{-5} M, respectively. 4OHPB also caused a concentration (10^{-8} to 10^{-6} M)-dependent proliferation of MCF-7 cells, whereas neither anethole nor 4MCA (10^{-9} to 10^{-5} M) affected cell proliferation. However, at higher concentrations ($>10^{-4}$ M), 4OHPB rather than anethole and 4MCA was cytotoxic. These results suggest that the biotransformation of anethole induces a cytotoxic effect at higher concentrations in rat hepatocytes and an estrogenic effect at lower concentrations in MCF-7 cells based on the concentrations of the hydroxylated intermediate, 4OHPB.

COMMENTS: The toxicity of trans-anethole and its metabolites were measured in rat hepatocytes and MCF-7 cells. Concentration-dependent and time-dependent cytotoxicity was observed in rat hepatocytes at anethole exposures ranging from 0.25 – 2. mM. The hydroxylated metabolite, 4-hydroxy-1-propenylbenzene (4OHPB) and not the parent compound, induced cytotoxic and estrogenic effects. Treatment with 4OHPB resulted in decreased cell viability and loss of intracellular levels of ATP and total adenine

nucleotide pools in hepatocytes. Estrogenic activity of 4OHPB was observed based on a proliferative assay of estrogen-responsive human breast cancer cells and a concentration-dependent displacement of 17 β -estradiol bound to ER α . This study suggests that anethole may become cytotoxic and estrogenic via biotransformation and highlights the importance of using *in vivo* experiments to assess anethole toxicity.

ANGELICA ROOT EXTRACT AND OIL

CAS: 84775-41-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ANISE STAR OIL

CAS: 8007-70-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ANISYL ACETATE

CAS: 104-21-2

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

APPLE JUICE CONCENTRATE, ESSENCE AND EXTRACT

CAS: 85251-63-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

L-ARGININE

CAS: 74-79-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ASCORBIC ACID

CAS: 50-81-7

Number of relevant papers: 1

1. Cigarette smoke effects on salivary antioxidants and oral cancer - Novel concepts

Rafael M. Nagler MD DMD PhD and Abraham Z. Reznick PhD

Isr Med Assoc J 2004 Nov;6:691-4

ABSTRACT: Oral squamous cell carcinoma is the most common malignancy of the head and neck, with a worldwide incidence of over 300,000 new cases annually [1]. The disease is characterized by a high rate of morbidity and mortality (about 50%) [1 $\pm$ 4]. The major inducer of oral SCC is exposure to tobacco, considered to be responsible for

50±90% of cases worldwide [5±7]. The incidence of oral SCC in cigarette smokers is four to seven times higher than in non-smokers; when alcohol is also consumed this incidence is even higher. Moreover, compared with non-smokers, the higher cigarette smoke-related risk for oral SCC is manifested by a reduction in the mean age of development of the disease by 15 years [8,9]. The "field cancerization" concept is the currently accepted explanation for the carcinogenic effect of cigarette smoke on oral mucosa [10]. According to this theory, there is a constant and direct attack of various cigarette smoke reagents on the oral epithelial cells, which gradually accumulate and cause a step-wise malignant transformation. It has been suggested that free radicals, reactive oxygen species and reactive nitrogen species in the inhaled cigarette smoke induce this gradually evolving process, initially expressed by dysplastic lesions of the mucosa, are then trans-formed into in situ carcinoma lesions and eventually result in full-blown infiltrating and metastasizing oral SCC. Further credence for the suggested role of free radicals in the pathogenesis of evolving oral SCC is found in a recent study [11] demonstrating that ROS, such as hydroxyl radical, are formed in the human oral cavity during areca quid chewing, and that the activity might cause oxidative DNA damage to the surrounding tissues. In this respect the salivary anticarcinogenic capacity, which has only recently been recognized, may be based on its antioxidant system.

COMMENTS: Aspects of the salivary defense system are discussed including antioxidant enzymes (peroxidase and superoxide dismutase) and molecules such as uric acid and ascorbic acid. Cigarette smoke has been shown to reduce activity of salivary antioxidant enzymes, but not antioxidant molecules. Salivary peroxidase activity was not affected by exposure to purified aldehydes, nicotine or ascorbic acid, but appeared to be affected by hydrogen cyanide exposure. The enzyme activity returned to pre-smoking levels after 30 minutes, presumably due to the secretion of new saliva into the oral cavity.

L-ASPARTIC ACID

CAS: 56-84-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BALSAM PERU AND OIL

CAS: 8007-00-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BEESWAX RESINOID AND ABSOLUTE

CAS: 8006-40-4

CAS: 8012-89-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BEET JUICE CONCENTRATE

CAS: 89957-90-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BENZALDEHYDE

CAS: 100-52-7

SEE HIGH MUL'S INGREDIENTS

BENZALDEHYDE GLYCERYL ACETAL

CAS: 1319-88-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BENZOIC ACID

CAS: 65-85-0

Number of relevant papers: 1

1. Controversies in toxicology assessing food additive toxicity using a cell model -

**Stefanidou M; Alevisopoulos G; Chatziioannou A; Koutselinis
Veterinary and Human Toxicology, 2003 , 45/2 (103-105)**

ABSTRACT: Food additives are widely used for technological purposes and their presence is often substantial daily diet. They have also been accused for various toxic reactions in humans. The toxicity of the food color tartrazine, the preservatives sodium nitrate and sodium benzoate, and the antioxidant BHT, was studied using the protozoan *Tetrahymena pyriformis* as a toxicological model. The 4 food additives were added to *Tetrahymena* cultures and DNA content of the protozoan nuclei measured by an image analysis system. These food additives caused a statistically significant increase in DNA content suggesting stimulation of the mitotic process. This system may contribute to the investigation of the cellular action of food additives, since mitogenic stimuli substantially alter susceptibility to chemical carcinogenesis. (32 References)

COMMENTS: These investigators tested the cytotoxic effect of 4 food additives, including sodium benzoate using a protozoan assay. Sodium benzoate activity is dependent on the concentration of undissociated benzoic acid. Some individuals exhibit allergy to benzoates. All four of the additives produced significant increase in DNA synthesis in protozoa macronucleus. The authors suggest that when this effect occurs, other cell activities are also depressed such as phagocytosis.

BENZOIN, RESIN, RESINOID, TINCTURE, GUM AND ABSOLUTE

CAS: 9000-05-9

CAS: 84012-39-5

CAS: 9000-72-0

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BENZYL ALCOHOL

CAS: 100-51-6

Number of relevant papers: 3

1. Anti-estrogenic activity of fifty chemicals evaluated by in vitro assays

**Joohee Jung , Kunie Ishida and Tsutomu Nishihara ,
Life Sciences Volume 74, Issue 25 , 7 May 2004, Pages 3065-3074**

ABSTRACT: We examined the anti-estrogenic activity of 50 chemicals by the yeast two-hybrid assay and detected the activity of hexachlorophene, pentachlorophenol, and vitamin K3 (menadione), in that order. These chemicals were also observed to inhibit the transcriptional activity of 17 β -estradiol in a reporter gene assay system using MCF-7 cells, estrogen receptor-positive breast cancer cells, and to bind directly to estrogen receptor α in a competitive binding assay system, although the order of the activity was slightly different among the 3 assays. These findings suggested that three of fifty chemicals could inhibit estrogen activity by competitive binding with 17 β -estradiol to the estrogen receptor.

COMMENTS: The inhibitory effect of various chemicals against 17 β -estradiol was assessed using the yeast two-hybrid assay. Fifty chemicals, including benzyl alcohol were tested in a range from 10^{-3} to 10^{-9} M. Only three chemicals showed inhibition of estrogenic activity. No anti-estrogenic activity was reported for benzyl alcohol within the range of concentrations tested.

2. Neurologic issues with solvents

**Rutchik JS, Wittman RI.
Clin Occup Environ Med. 2004 Nov;4(4):621-56, v-vi.**

ABSTRACT: Organic solvents are a chemical class of compounds that are used routinely in commercial industries. They possess a low molecular weight, share a similar structure, lipophilicity, and volatility, and they exist in liquid at room temperature. They may be grouped further into aliphatic compounds that exist in chain form, such as n-hexane, and aromatic compounds that exist in a 6-carbon ring form, such as benzene or xylene. Aliphatics and aromatics may contain a substituted halogen element and may be referred to as halogenated hydrocarbons, such as perchloroethylene, trichloroethylene,

and carbon tetrachloride. Alcohols, ketones, glycols, esters, ethers, aldehydes, and pyridines exist due to substitutions for a hydrogen group.

COMMENTS: This is a well-documented review of neurologic effects from exposure to a variety of solvents. The only discussion focusing on benzyl alcohol was that it was shown to block neuronal action potentials reversibly *in vitro* and exposure of rat nerve roots results in scattered demyelination and axonal degeneration.

3. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters

Fukayama MY, Easterday OD, Serafino PA, Renskers KJ, North-Root H, Schrankel KR.

Toxicol Lett. 1999 Dec 20;111(1-2):175-87.

ABSTRACT: Users of consumer products are invariably and intentionally exposed to complex mixtures in such products. With finished fragrance products, these mixtures may represent 100 or more fragrance raw materials (FRMs). The objective of the described studies was to evaluate the safety of finished fragrance products via the inhalation route. In total, the finished products contained approximately 100 FRMs at concentrations of 1% or greater. Major FRMs evaluated included benzyl acetate, coumarin, hydroxycitronellal, musk ketone, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-2-benzopyran (HHCB) and phenyl ethyl alcohol. Groups of rats or hamsters were exposed by inhalation (whole body) to the mixtures at 5, 9 or 50 mg/m³ for 4 h per day, 5 days per week for 6 or 13 weeks. For each of the fragrance products, the doses used generally represented a ten- to 100-fold exaggeration of levels expected to be achieved during typical use by consumers. With one exception, the fragrances were aerosolized prior to introduction into the inhalation chamber. The exception product was formulated with a propellant, packaged in a pressurized container and expelled with an automated actuator. In all studies, chamber concentrations of fragrance were monitored. Particle sizes ranged from 0.5 to 7.5 microm, depending on the study. Subchronic exposure to all fragrance mixtures resulted in no toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters. No gross pathological or histopathological findings related to test material exposures were observed. These studies support the conclusions that the fragrance mixtures would not pose a hazard to product users based on repeated and exaggerated inhalation exposures of animals.

COMMENTS: This paper presents results from subchronic inhalation studies of fragrance mixtures in rats and hamsters. Eight fragrance products were tested, which include approximately 200 components, including benzyl alcohol (52 µg/m³). No toxicological effects were identified from exposure to the fragrance mixtures at levels 10- to 100 fold greater than expected human exposure conditions for 6 or 13 weeks. The results are consistent with those of earlier studies of Gaworski 1998 that exposed rats to mixtures of flavor ingredients in a subchronic smoke inhalation study.

BENZYL BENZOATE
CAS: 120-51-4

Number of relevant papers: 2

1. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters

Fukayama MY, Easterday OD, Serafino PA, Renskers KJ, North-Root H, Schrankel KR.

Toxicol Lett. 1999 Dec 20;111(1-2):175-87.

ABSTRACT: Users of consumer products are invariably and intentionally exposed to complex mixtures in such products. With finished fragrance products, these mixtures may represent 100 or more fragrance raw materials (FRMs). The objective of the described studies was to evaluate the safety of finished fragrance products via the inhalation route. In total, the finished products contained approximately 100 FRMs at concentrations of 1% or greater. Major FRMs evaluated included benzyl acetate, coumarin, hydroxycitronellal, musk ketone, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-2-benzopyran (HHCB) and phenyl ethyl alcohol. Groups of rats or hamsters were exposed by inhalation (whole body) to the mixtures at 5, 9 or 50 mg/m³ for 4 h per day, 5 days per week for 6 or 13 weeks. For each of the fragrance products, the doses used generally represented a ten- to 100-fold exaggeration of levels expected to be achieved during typical use by consumers. With one exception, the fragrances were aerosolized prior to introduction into the inhalation chamber. The exception product was formulated with a propellant, packaged in a pressurized container and expelled with an automated actuator. In all studies, chamber concentrations of fragrance were monitored. Particle sizes ranged from 0.5 to 7.5 microm, depending on the study. Subchronic exposure to all fragrance mixtures resulted in no toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters. No gross pathological or histopathological findings related to test material exposures were observed. These studies support the conclusions that the fragrance mixtures would not pose a hazard to product users based on repeated and exaggerated inhalation exposures of animals.

COMMENTS: This paper presents results from subchronic inhalation studies of fragrance mixtures in rats and hamsters. Eight fragrance products were tested, which include approximately 200 components, including benzyl benzoate (3 - 694 µg/m³). No toxicological effects were identified from exposure to the fragrance mixtures at levels 10- to 100 fold greater than expected human exposure conditions for 6 or 13 weeks. The results are consistent with those of earlier studies of Gaworski 1998 that exposed rats to mixtures of flavor ingredients in a subchronic smoke inhalation study.

2. Inhibitory effects of the essential oil from SuHeXiang Wan on the central nervous system after inhalation -

Koo BS, Lee SI, Ha JH, and Lee DU

Biological & Pharmaceutical Bulletin Vol. 27 (2004), No. 4, 515-519.

ABSTRACT: The present study was performed to evaluate the central nervous system inhibitory effects of the essential oil from SuHeXiang Wan (Storax pill), a prescription usually used for treating epilepsy in traditional Chinese medicine, on fragrance inhalation (aroma therapy). Preinhalation of the fragrance oil markedly delayed the appearance of pentylenetetrazole-induced convulsion, but showed weak activities on picrotoxin- and strychnine-induced convulsions, which implies this drug may inhibit the convulsion by GABAergic neuromodulation. This essential oil inhibited the binding of [3H]Ro15-1788, a selective antagonist for the benzodiazepine receptor and also the binding of [3H]flunitrazepam, a selective agonist for the receptor, in the presence of g-aminobutyric acid (GABA) and NaCl, showing a positive GABA shift, which suggested the strong possibility of the agonistic activity of the essential oil to the GABA/benzodiazepine receptor complex in rat cerebral cortices. Furthermore, inhalation inhibited the activity of GABA transaminase as the inhalation period was lengthened. The GABA level was significantly increased and glutamate content was significantly decreased in mouse brain by preinhalation of the essential oil. The above results suggest that the anticonvulsive effect of this essential oil can also originate from the enhancement of GABA level in the mouse brain, because convulsion depends partially on GABA concentration which can be properly preserved by inhibiting GABA transaminase. Fragrance inhalation progressively prolonged the pentobarbital-induced sleeping time as inhalation time was lengthened and inhibited brain lipid peroxidation, to which the anticonvulsive action is attributed; this also supported the above results, confirming the inhibitory effects of the essential oil of SuHeXiang Wan on the CNS via the GABAergic system.

COMMENTS: Fragrance inhalation of essential oils which make up Chinese medicinal prescriptions was shown to possess anticonvulsive and sedative properties in mouse experiments. Anticonvulsive effect of the essential oils was attributed to enhanced GABA levels and decreased lipid peroxidation in mouse brain. Benzyl benzoate was one of 10 compounds detected in the essential oils, and accounted for only 5.4% of the content of the mixture. Therefore, the relevance of this study to the health effects of benzyl benzoate as an ingredient in cigarettes is minimal.

**BENZYL CINNAMATE (PROPENIC ACID, 3-PHENYL, PHENYLMETHYL
ESTER,2-)**

CAS: 103-41-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BENZYL PHENYLACETATE

CAS: 102-16-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BENZYL PROPIONATE

CAS: 122-63-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BORNYL ACETATE

CAS: 76-49-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

1,3-BUTANEDIOL

CAS: 107-88-0

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2, 3-BUTANEDIONE (DIACETYL)

CAS: 431-03-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

**BUTANOIC ACID, 3-METHYL-, 4-METHYLPHENYL ESTER (PARA-TOLYL
3-METHYLBUTYRATE) (P-TOLYL ISOVALERATE)**

CAS: 55066-56-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BUTTER, BUTTER ESTERS, AND BUTTER OIL

CAS: 91745-88-9

CAS: 97926-23-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BUTYL ACETATE

CAS: 123-86-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BUTYL ALCOHOL (1-BUTANOL)
CAS: 71-36-3

Number of relevant papers: 1

1. Chemically induced renal tubule tumors in the laboratory rat and mouse: Review of the NCI/NTP database and categorization of renal carcinogens based on mechanistic information

Edward Lock; Gordon Hard

Critical Reviews in Toxicology, Volume 34, Number 3, May-June 2004, pp. 211-299(89)

Abstract: The incidence of renal tubule carcinogenesis in male and female rats or mice with 69 chemicals from the 513 bioassays conducted to date by the NCI/NTP has been collated, the chemicals categorized, and the relationship between carcinogenesis and renal tubule hyperplasia and exacerbation of the spontaneous, age-related rodent disease chronic progressive nephropathy (CPN) examined. Where information on mechanism or mode of action exists, the chemicals have been categorized based on their ability to directly or indirectly interact with renal DNA, or on their activity via epigenetic pathways involving either direct or indirect cytotoxicity with regenerative hyperplasia, or exacerbation of CPN. Nine chemicals were identified as directly interacting with DNA, with six of these producing renal tubule tumors at high incidence in rats of both sexes, and in some cases also in mice. Ochratoxin A was the most potent compound in this group, producing a high tumor incidence at very low doses, often with metastasis. Three chemicals were discussed in the context of indirect DNA damage mediated by an oxidative free radical mechanism, one of these being from the NTP database. A third category included four chemicals that had the potential to cause DNA damage following conjugation with glutathione and subsequent enzymatic activation to a reactive species, usually a thiol-containing entity. Two chemicals were allocated into the category involving a direct cytotoxic action on the renal tubule followed by sustained compensatory cell proliferation, while nine were included in a group where the cell loss and sustained increase in renal tubule cell turnover were dependent on lysosomal accumulation of the male rat-specific protein, 2-globulin. In a sixth category, morphologic evidence on two chemicals indicated that the renal tumors were a consequence of exacerbated CPN. For the remaining chemicals, there were no pertinent data enabling assignment to a mechanistic category. Accordingly, these chemicals, acting through an as yet unknown mechanism, were grouped as either being associated with an enhancement of CPN mechanism, were grouped as either being associated with an enhancement of CPN (category 7, 16 chemicals), or not associated with enhanced CPN (category 8, 4 chemicals). A ninth category dealt with 11 chemicals that were regarded as producing increases in renal tubule tumors that did not reach statistical significance. A 10th category discussed 6 chemicals that induced renal tumors in mice but not in rats, plus 8 chemicals that produced a low incidence of renal tubule tumors in mice that did not reach statistical significance. As more mechanistic data are generated, some chemicals will inevitably be placed in different groups, particularly those from categories

7 and 8. A large number of chemicals in the series exacerbated CPN, but those in category 7 especially may be candidates for inclusion in category 6 when further information is gleaned from the relevant NTP studies. Also, new data on specific chemicals will probably expand category 5 as cytotoxicity and cell regeneration are identified as obligatory steps in renal carcinogenesis in more cases. Additional confirmatory outcomes arising from this review are that metastases from renal tubule tumors, while encountered with chemicals causing DNA damage, are rare with those acting through an epigenetic pathway, with the exception being fumonisin B1; that male rats and mice are generally more susceptible than female rats and mice to chemical induction of renal tubule tumors; and that a background of atypical tubule hyperplasia is a useful indicator reflecting a chemically associated renal tubule tumor response. With respect to renal tubule tumors and human risk assessment, chemicals in categories 1 and 2, and possibly 3, would currently be judged by linear default methods; chemicals in category 4 (and probably some in category 3) as exhibiting a threshold of activity warranting the benchmark approach; and those in categories 5 and 6 as representing mechanisms that have no relevance for extrapolation to humans.

COMMENTS: This paper provides a review of 69 chemicals tested in the National Cancer Institute / National Toxicology Program (NCI/NTP) carcinogenicity bioassay database including butyl alcohol. The selected chemicals are those that have shown an association with renal tubule tumors in rat and/or mouse. Butyl alcohol was placed in category 5, considered “chemicals inducing renal tumors via indirect cytotoxicity and sustained tubule cell regeneration associated with $\alpha_2\mu$ -globulin accumulation.” Chemicals placed in this category have a nongenotoxic mechanism that has no relevance for extrapolation to renal tumors in humans. However, data on butyl alcohol exposure in drinking water to female rats demonstrate a dose-related increase in the severity of chronic progressive nephropathy, and an increased incidence of thyroid gland follicular cell hyperplasia and adenomas in mice. This review was focused towards oral exposures and did not address inhalation exposure of butyl alcohol.

**BUTYL BUTYRYL LACTATE (BUTOXY-1-METHYL-2-OXOETHYL ESTER
BUTANOIC ACID, 2-)**

CAS: 7492-70-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

N-BUTYL ISOVALERATE

CAS: 109-19-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

3-BUTYLIDENEPHTHALIDE

CAS: 551-08-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BUTYRIC ACID

CAS: 107-92-6

SEE HIGH MUL'S INGREDIENTS

CAPRYLIC/CAPRIC TRIGLYCERIDE

CAS: 65381-09-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CARAMEL AND CARAMEL COLOR

CAS: 8028-89-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CARBON

CAS: 7440-44-0

SEE MAJOR INGREDIENTS

CARBON DIOXIDE

CAS: 124-38-9

Number of relevant papers: 3

1. CO₂ induced acute respiratory acidosis and brain tissue intracellular pH: A SUP31P NMR study in swine -

Martoft L.1; Stødtkilde-Jørgensen H.2; Forslid A.3; Pedersen H.D.1; Jørgensen P.F.1

Laboratory Animals, Volume 37, Number 3, 1 July 2003, pp. 241-248(8)

ABSTRACT: High concentration carbon dioxide (CO₂) is used to promote pre-slaughter anaesthesia in swine and poultry, as well as short-lasting surgical anaesthesia and euthanasia in laboratory animals. Questions related to animal welfare have been raised, as CO₂ anaesthesia does not set in momentarily. Carbon dioxide promotes anaesthesia by lowering the intracellular pH in the brain cells, but the dynamics of the changes in response to a high concentration of CO₂ is not known. Based on ³¹P NMR spectroscopy, we describe CO₂-induced changes in intracellular pH in the brains of live pigs inhaling 90% CO₂ in ambient air for a period of 60 s, and compare the results to changes in arterial blood pH, PCO₂, O₂ saturation and HCO₃⁻ concentration. The intracellular pH paralleled the arterial pH and PCO₂ during inhalation of CO₂; and it is suggested that the acute reaction to CO₂ inhalation mainly reflects respiratory acidosis, and not metabolic regulation as for example transmembrane fluxes of H₂⁺=HCO₃⁻. The intracellular pH decreased to approximately 6.7 within the 60 s inhalation period, and the situation was metabolically reversible after the end of CO₂ inhalation. The fast decrease in intracellular

pH supports the conclusion that high concentration CO₂ leads to anaesthesia soon after the start of inhalation.

COMMENTS: The objective of this study was to assess the acute response of intracellular pH changes in brain of pigs induced by inhalation of 90% CO₂ in ambient air for a period of 60 seconds and to relate these changes to arterial blood. Intracellular pH decreased from the start of CO₂ inhalation period at a higher pace than that observed in arterial pH, and reached levels (6.7) lower than that observed in arterial pH. Reversal to pre-exposure conditions of intracellular pH was also rapid. The authors predict that the levels might have returned more slowly if the pigs had been allowed to respire freely due to CO₂ induced neuronal depression, which would slow the exhalation of CO₂. The objective of this work was to resolve questions related to animal welfare following the high concentrations of carbon dioxide used to promote pre-slaughter anaesthesia in livestock. Because of the high concentrations of CO₂ used in this study, the extrapolation to the effects of CO₂ exposure from cigarette smoke is difficult.

2. TOXICOLOGICAL EVALUATION OF HONEY AS AN INGREDIENT ADDED TO CIGARETTE TOBACCO

Mari S. Stavanja, Paul H. Ayres, Daniel R. Meckley, Betsy R. Bombick, Deborah H. Pence, Michael F. Borgerding, Michael J. Morton, Arnold T. Mosberg, James E. Swauger

Journal of Toxicology and Environmental Health, Part A, 66:1453–1473, 2003

ABSTRACT: A tiered testing strategy has been developed to evaluate the potential for new ingredients, tobacco processes, and technological developments to increase or reduce the biological activity that results from burning tobacco. In the manufacture of cigarettes, honey is used as a casing ingredient to impart both aroma and taste. The primary objective of this document is to summarize and interpret chemical and toxicological studies that have been conducted to evaluate the potential impact of honey on the biological activity of either mainstream cigarette smoke or cigarette smoke condensate. As part of ongoing stewardship efforts, cigarettes produced with honey (5% wet weight) as an alternative to invert sugar in tobacco casing material were subjected to extensive evaluation. Principal components of this evaluation were a determination of selected mainstream smoke constituent yields, Ames assay, sister chromatid exchange assay in Chinese hamster ovary cells, a 30-wk dermal tumor promotion evaluation of cigarette smoke condensate in SENCAR mice, and a 13-wk inhalation study of cigarette smoke in Sprague-Dawley rats. Comparative analytical evaluations demonstrated that the substitution of honey for invert sugar as a casing material in cigarettes had no significant impact on mainstream smoke chemistry. In addition, in vitro and in vivo studies demonstrated that cigarettes containing tobacco cased with honey had comparable biological activity to cigarettes containing invert sugar. Collectively, these data demonstrate that the use of honey as an alternative casing material in the manufacture of cigarettes does not alter the potential toxicity of cigarette smoke condensate (CSC) or cigarette smoke; therefore the use of honey as an ingredient added to cigarette tobacco is acceptable from a toxicological perspective.

COMMENTS: This paper compares the use of honey in place of invert sugar as casing material in cigarettes. No differences were observed in carbon dioxide measured in the mainstream smoke chemistry between the two cigarettes (mean = 41 - 42.2 mg/cig). No differences in toxicological endpoints were observed between the reference cigarette and those including honey. This paper has minor relevance to assessing the effects of carbon dioxide as an ingredient in cigarettes, but does conclude that the substitution of honey for invert sugar as a casing material does not significantly alter smoke chemistry.

3. Acute carbon dioxide exposure in healthy adults: evaluation of a novel means of investigating the stress response -

Kaye J.1; Buchanan F.2; Kendrick A.2; Johnson P.1; Lowry C.1; Bailey J.3; Nutt D.3; Lightman S.1 Source: Journal of Neuroendocrinology, Volume 16, Number 3, March 2004, pp. 256-264(9)

ABSTRACT: Acute hypercapnia was studied to assess its potential as a noninvasive and simple test for evoking neuroendocrine, cardiovascular and psychological responses to stress in man. A single breath of four concentrations of carbon dioxide, 5%, 25%, 35%, and 50% was administered to nine healthy volunteers in a randomized, single-blind fashion. Although no adverse effects occurred, most subjects were unable to take a full inspired vital capacity breath of 50%. In response to the remaining exposures, subjective and somatic symptoms of anxiety increased in a dose-dependent manner. Unlike 5% and 25% CO₂, 35% stimulated significant adrenocorticotrophic hormone and noradrenaline release at 2 min. and cortisol and prolactin release at 15 mins. following inhalation. This same dose also provoked a significant bradycardia that was followed by an acute pressor response. No significant habituation of psychological, hypothalamic-pituitary-adrenal (HPA) or cardiovascular responses following 35% CO₂ was seen when this dose was repeated after 1 week. A single breath of 35% CO₂ safely and reliably produced sympathetic and HPA axis activation and should prove a useful addition to currently available laboratory tests of the human stress response.

COMMENTS: While the aim of this study was to evaluate the stress response to acute CO₂, the data does indicate that the response to hypercapria in normal individuals is dose-dependent and anxiety produced is transient. Exposure to 35% CO₂ stimulated the release of cortisol, adrenocorticotrophic prolactin and noradrenaline hormone but not at concentrations of 5% or 25%. A single breath of 35% CO₂ also produced a marked systolic response that was preceded by a significant and persistent bradycardia. The lower doses did not have significant effect on cardiovascular parameters or catecholamine release.

CARDAMOM OLEORESIN, OIL, EXTRACT, SEED OIL, AND POWDER

CAS: 8000-66-6

CAS: 96507-91-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CAROB BEAN GUM, ABSOLUTE AND EXTRACT

CAS: 9000-40-2

CAS: 84961-45-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BETA-CAROTENE

CAS: 7235-40-7

Number of relevant papers: 8

1. Bacterial Mutagenicity testing of 49 food ingredients gives very few positive results.

PRIVAL M J,; SIMMON V F; MORTELMANS K E
GENETIC TOXICOLOGY BRANCH, FOOD DRUG ADMINISTRATION, 200 C
STREET SW, WASHINGTON, DC 20204, USA USA
Mutation Research , Volume: 260 , Number: 4 , Page: 321-330 , 1991

ABSTRACT: 49 substances permitted for use in food in the United States were tested for mutagenicity in the Ames Salmonella typhimurium assay and in Escherichia coli strain WP2. Four of these substances caused increases in revertant counts in S. typhimurium. Two of these four (papain and pepsin) were found to contain histidine, and therefore the results of the tests on these two substances could not be taken as demonstrating mutagenicity. The other two substances causing increases in revertant counts (hydrogen peroxide and potassium nitrite) were mutagenic. The results on one chemical, .beta.-carotene, were evaluated as inconclusive or questionable. The remaining 44 substances were nonmutagenic in the test systems used. It is concluded that, for those generally physiologically innocuous chemicals tested, there are very few 'false positives' in the bacterial test systems used.

COMMENTS: The Salmonella Ames test and E coli mutagenicity assays were used to evaluate the mutagenicity of a number of food ingredients. β -carotene did not give a significant and reproducible increase in mutant counts and thus β -carotene is classified as questionable or inconclusive rather than a nonmutagen. β -carotene is an insoluble chemical and the Ames test is not considered to be suitable for testing insoluble substances.

2. beta-Carotene exacerbates DNA oxidative damage and modifies p53-related pathways of cell proliferation and apoptosis in cultured cells exposed to tobacco smoke condensate

Palozza P, Serini S, Di Nicuolo F, Boninsegna A, Torsello A, Maggiano N, Ranelletti FO, Wolf FI, Calviello G, Cittadini A.
Carcinogenesis. 2004 Aug;25(8):1315-25. Epub 2004 Apr 8.

ABSTRACT: Human intervention trials have suggested that supplemental b-carotene resulted in more cancer in smokers, whereas it was protective in non-smokers. However, the mechanisms underlying these effects are still unknown. The aim of this study was to evaluate the effects of an association of cigarette smoke condensate (tar) and b-carotene on DNA oxidative damage and molecular pathways involved in cell cycle progression and apoptosis in cultured cells. In RAT-1 fibroblasts, tar caused increased levels of 8-hydroxyl-20-deoxyguanosine (8-OHdG) and this effect was enhanced by the concomitant presence of b-carotene (0.5--4.0 mM) in a dose- and time-dependent manner. In contrast, b-carotene alone did not significantly modify it. Fibroblasts treated with tar alone decreased their cell growth with respect to control cells through an arrest of cell cycle progression in the G0/G1 phase and an induction of apoptosis. These effects were accompanied by an increased expression of p53, p21 and Bax and by a decreased expression of cyclin D1. In contrast, fibroblasts treated with tar and b-carotene, after an initial arrest of cell growth at 12 h, re-entered in cell cycle and were unable to undergo apoptosis at 36 h. Concomitantly, their p53 expression, after an increase at 12 h, progressively returned at basal levels at 36 h by a mechanism independent of Mdm2. Such a decrease was followed by a decrease in p21 and Bax expression and by an increase in cyclin D1 expression. Moreover, the presence of the carotenoid remarkably enhanced cyclooxygenase-2 expression induced by tar. During tar treatment, a depletion of b-carotene was observed in fibroblasts. The effects of tar and b-carotene on 8-OHdG levels, cell growth and apoptosis were also observed in Mv1Lu lung, MCF-7 mammary, Hep-2 larynx and LS-174 colon cancer cells. This study supports the evidence for potential detrimental effects of an association between b-carotene and cigarette smoke condensate.

COMMENTS: This study explores a new mechanism for carcinogenic association between β -carotene and cigarette smoke using cultured cells exposed to a combination of β -carotene and tar. Together, β -carotene and tar caused significant increases in oxidative DNA damage over either alone. These effects were both dose- and time- dependent and were observed over a range of β -carotene concentrations from 0.75 - 4 μ M, which corresponds to the concentrations in serum of subjects receiving supplements in clinical trials. Exposure to these substances together resulted in increased cell growth using RAT-1 fibroblasts and a clonogenic assay. Similar results were observed when tested with a variety of human tumor cell lines. The authors conclude that pro oxidant action of β -carotene exacerbates DNA oxidative damage caused by cigarette smoke and induce changes in p53-related pathways. At low concentrations, β -carotene increased DNA resistance to oxidative damage.

3. Effect of alpha-tocopherol and beta-carotene supplementation on coronary heart disease during the 6-year post-trial follow-up in the ATBC study. - 2004 -

Tornwall ME, Virtamo J, Korhonen PA, Virtanen MJ, Taylor PR, Albanes D, Huttunen JK.

Eur Heart J. 2004 Jul;25(13):1171-8.

ABSTRACT: Aims To evaluate the 6-year post-trial effects of a-tocopherol and b-carotene supplementation on coronary heart disease (CHD) in the a-tocopherol, b-carotene cancer prevention (ATBC) study. Methods and results 29 133 male smokers, aged 50–69 years were randomised to receive a-tocopherol 50 mg, or b-carotene 20 mg, or both, or placebo daily for 5–8 years. At the beginning of the post-trial follow-up, 23 144 men were still at risk for a first-ever major coronary event (MCE), and 1255 men with pre-trial history of myocardial infarction (MI) were at risk for MCE. Post-trial risk for MCE (n ¼ 2059) was 0.95 (95% confidence interval 0.87–1.04) among a-tocopherol recipients compared with non-recipients, and 1.14 (1.04–1.24) among b-carotene recipients compared with non-recipients. The risk for non-fatal MI (n ¼ 993) was 0.96 (0.85–1.09) and 1.16 (1.03–1.32), and for fatal CHD (n ¼ 1066) 0.94 (0.83–1.06) and 1.11 (0.99–1.25), respectively. Among men with pre-trial MI no effects were observed in post-trial risk of MCE (n ¼ 257). Conclusion b-Carotene seemed to increase the post-trial risk of first-ever non-fatal MI but there is no plausible mechanism to support it. Our findings do not advocate the use of a-tocopherol or b-carotene supplements in prevention of CHD among male smokers.

COMMENTS: Research continues to accumulate to attempt to uncover the underlying mechanism of action of β -carotene toxicity. High doses have been shown to increase risk of lung cancer among smokers. β -carotene has been suggested as a singlet oxygen quencher. These investigators report on post-trial effects of β -carotene on major coronary events such as non-fatal MI and fatal CHD. These studies indicate that β -carotene possibly increases the post-trial risk of first ever non-fatal myocardial infarction but they failed to suggest a possible mechanism to explain this effect.

4. The enigma of beta-carotene in carcinogenesis: What can be learned from animal studies. -

Robert M. Russell

The American Society for Nutritional Sciences J. Nutr. 134:262S-268S, January 2004

ABSTRACT: β -carotene and other carotenoids have been thought to have anti-cancer activity, either because of antioxidant activity or because of their ability to be converted to vitamin A. Nevertheless, two large scale intervention studies in humans using high doses of β -carotene found that β -carotene supplementation resulted in more lung cancer rather than less lung cancer among smoking and asbestos exposed populations. Studies conducted in the ferret have elucidated molecular mechanisms behind this observation, in that high-dose β -carotene and smoke exposure in these animals leads to squamous metaplasia, a pre-cancerous lesion in the lung. High dose β -carotene in the smoke exposed animals was found to give rise to a number of transient oxidative metabolites, which include P450 enzymes that result in the destruction of retinoic acid, and diminished retinoid signaling, and enhanced cell proliferation. In addition, eccentric cleavage β -carotene metabolites facilitate the binding of smoke derived carcinogens to DNA. In other ferret studies low dose β -carotene smoke exposure provided mild protection against squamous metaplasia. Thus, it appears that the explanation of the

apparent paradoxical effects of β -carotene on lung cancer is related to dose. The metabolism and breakdown of natural products should be thoroughly investigated in animal models before embarking on large scale intervention trials, particularly when using unusually high doses that greatly exceed normal dietary levels.

COMMENTS: The study used ferrets as an animal model to assess the effects of β -carotene in smoke-exposed animals. Localized proliferation of alveolar cells and alveolar macrophages with keratinized squamous epithelium was observed in animals given high dose β -carotene (equivalent to 30 mg/d in humans), and the most severe responses (focal proliferation of alveolar cells, squamous metaplasia, and alveolar wall destruction) were observed in those exposed to both beta carotene and smoke. Cell proliferation was observed in both groups, but highest in the lung tissue of ferrets exposed to both β -carotene and smoke. Retinoic acid levels were lower in both smoke-exposed and β -carotene- treated groups as compared to controls. Using *in vitro* experiments, the authors demonstrated that lower β -carotene levels in animals exposed to smoke were due to enhanced molecular breakdown. The authors propose a mechanism by which β -carotene breakdown products might induce P450 enzyme activity resulting in the destruction of retinoic acid, and subsequent diminished retinoid signaling. The interference of this signaling pathway results in enhanced cell proliferation in ferret lung tissue. Oxidative products of β -carotene also facilitate binding of benzo[a]pyrene metabolites to DNA. However, these effects appear to occur at high β -carotene doses only, and not associated with low doses (equivalent to 6 mg in humans).

5. beta-Carotene: A cancer chemopreventive agent or a co-carcinogen?

Paolini M, Abdel-Rahman SZ, Sapone A, Pedulli GF, Perocco P, Cantelli-Forti G, Legator MS.
Mutat Res. 2003 Jun;543(3):195-200.

ABSTRACT: Evidence from both epidemiological and experimental observations have fueled the belief that the high consumption of fruits and vegetables rich in carotenoids may help prevent cancer and heart disease in humans. Because of its well-documented antioxidant and antigenotoxic properties, the carotenoid β -carotene (β CT) gained most of the attention in the early 1980s and became one of the most extensively studied cancer chemopreventive agents in population-based trials supported by the National Cancer Institute. However, the results of three randomized lung cancer chemoprevention trials on β CT supplementation unexpectedly contradicted the large body of epidemiological evidence relating to the potential benefits of dietary carotenoids. Not only did β CT show no benefit, it was associated with significant increases in lung cancer incidence, cardiovascular diseases, and total mortality. These findings aroused widespread scientific debate that is still ongoing. It also raised the suspicion that β CT may even possess co-carcinogenic properties. In this review, we summarize the current data on the co-carcinogenic properties of β CT that is attributed to its role in the induction of carcinogen metabolizing enzymes and the over-generation of oxidative stress. The data presented provide convincing evidence of the harmful properties of this compound if given alone to smokers, or to individuals exposed to environmental carcinogens, as a micronutrient

supplement. This has now been directly verified in a medium-term cancer transformation bioassay. In the context of public health policies, while the benefits of a diet rich in a variety of fruits and vegetables should continue to be emphasized, the data presented here point to the need for consideration of the possible detrimental effects of certain isolated dietary supplements, before mass cancer chemoprevention clinical trials are conducted on human subjects. This is especially important for genetically predisposed individuals who are environmentally or occupationally exposed to mutagens and carcinogens, such as those found in tobacco smoke and in industrial settings.

COMMENTS: This document provides a review of the literature related to the protective and carcinogenic actions of β -carotene. Although β -carotene is known to act as an antioxidant, it can also behave as a pro-oxidant at high oxygen pressure. The author described that β -carotene itself does not exert cell transforming activity, but enhances the bioactivity and carcinogenicity of other compounds (i.e. benzo[a]pyrene) either through an induction of metabolizing enzymes (CYP) or generation of oxidative stress. These effects were observed at realistic concentrations observed in clinical trials using β -carotene as a dietary supplement.

6. In vitro investigations into the interaction of beta-carotene with DNA: evidence for the role of carbon-centered free radicals -

Jos C. S. Kleinjans 1*, Marcel H. M. van Herwijnen 1, Jan M. S. van Maanen 1, Lou M. Maas 1, Theo M. C. M. de Kok 1, Harald J. J. Moonen 1, and Jacob J. Briedé 1

Carcinogenesis Advance Access

ABSTRACT: Supplementation by β -carotene has unexpectedly appeared to increase lung cancer risk among smokers. In order to explain this, it has been suggested that at high serum levels of β -carotene, prooxidant characteristics of β -carotene may become manifest, yielding reactive oxygen species (ROS) and inducing oxidative DNA damage. It has further been hypothesized that cigarette smoke carcinogens such as benzo(a)pyrene (B[a]P) and/or B[a]P metabolites, may directly react with β -carotene; furthermore, β -carotene oxidation products may have a role in the bioactivation of B[a]P analogous to the peroxide-shunt pathway of cytochrome P-450 supported by cumene hydroperoxide. The aim of this study was to assess the effects of β -carotene on the formation of B[a]P-DNA adducts and oxidative DNA damage in vitro in isolated DNA, applying as metabolizing systems rat liver and lung metabolizing fractions, and lung metabolizing fractions from smoking and non-smoking humans. We established that β -carotene in the presence of various metabolizing systems was not able to induce oxidative DNA damage (8-oxo-dG), although β -carotene is capable of generating ROS spontaneously in the absence of metabolizing fractions. Also, we could not find an effect of β -carotene on DNA adduct formation induced by B[a]P upon metabolic activation. We could however provide evidence of the occurrence of a carbon-centered β -carotene radical which was found to be able to interact with B[a]P, and to intercalate with DNA.

COMMENTS: This study assessed the *in vitro* effects of β -carotene concentrations comparable with serum levels obtained during human intervention trials. No induction of oxidative DNA damage or benzo(a)pyrene-DNA adduct formation was associated with β -carotene exposure in the presence of various metabolizing systems. However, the authors suggest that a carbon-centered β -carotene radical may be capable of interacting with DNA and contribute to the mutagenic effects of DNA adducts formed by carcinogens. They conclude that a complex interaction including β -carotene cancer-promoting and anti-carcinogenic properties may exist *in vivo* and requires further research.

7. Neoplastic and antineoplastic effects of beta-carotene on colorectal adenoma recurrence: Results of a randomized trial. -

Baron JA, Cole BF, Mott L, Haile R, Grau M, Church TR, Beck GJ, Greenberg ER. Journal of the National Cancer Institute. Vol. 95, No. 10. May 21, 2003

ABSTRACT: In two large, randomized prevention trials, supplementation with β -carotene increased the risk of lung cancer. Subjects in these studies were predominantly cigarette smokers, and the adverse effects were concentrated among those who also drank alcohol. Although β -carotene supplementation appeared not to increase the risk of cancer generally, it is not clear if smoking and/or alcohol use alters the effect of β -carotene on carcinogenesis at sites outside the lung. **Methods:** We studied the effect of β -carotene supplementation on colorectal adenoma recurrence among subjects in a multicenter double-blind, placebo-controlled clinical trial of antioxidants for the prevention of colorectal adenomas. A total of 864 subjects who had had an adenoma removed and were polyp-free were randomly assigned (in a factorial design) to receive β -carotene (25 mg or placebo) and/or vitamins C and E in combination (1000 mg and 400 mg, respectively, or placebo), and were followed with colonoscopy for adenoma recurrence 1 year and 4 years after the qualifying endoscopy. A total of 707 subjects had two followup examinations and provided smoking and alcohol use data. Adjusted multivariate risk ratios (RRs) and 95% confidence intervals (CIs) were used to assess the effects of β -carotene on adenoma recurrence. **Results:** Among subjects who neither smoked cigarettes nor drank alcohol, β -carotene was associated with a marked decrease in the risk of one or more recurrent adenomas (RR = 0.56, 95% CI = 0.35 to 0.89), but β -carotene supplementation conferred a modest increase in the risk of recurrence among those who smoked (RR = 1.36, 95% CI = 0.70 to 2.62) or drank (RR = 1.13, 95% CI = 0.89 to 1.43). For participants who smoked cigarettes and also drank more than one alcoholic drink per day, β -carotene doubled the risk of adenoma recurrence (RR = 2.07, 95% CI = 1.39 to 3.08; P for difference from nonsmoker/nondrinker RR < .001). **Conclusion:** Alcohol intake and cigarettesmoking appear to modify the effect of β -carotene supplementation on the risk of colorectal adenoma recurrence.

COMMENTS: Evidence indicates that cigarette smoking plays a role in carcinogenic effects seen with β -carotene supplementation. However, the increase in lung cancer incidence was also associated with alcohol consumption, leading to the hypothesis that alcohol intake modifies the effect of β -carotene to increase lung cancer risk. In this clinical trial, β -carotene supplementation was beneficial (anti-neoplastic) in subjects who

did not smoke or drink but the proneoplastic risk increased (doubled) among those who smoke and drank alcohol. The authors suggest that smoking and use of alcohol modifies the effects of β -carotene on the risk of colorectal cancers.

8. Exposing ferrets to cigarette smoke and a pharmacological dose of beta-carotene supplementation enhance in vitro retinoic acid catabolism in lungs via induction of cytochrome P450 enzymes. -

Liu C, Russell RM, Wang XD.
J Nutr. 2003 Jan;133(1):173-9.

ABSTRACT: In our previous studies, we found lower levels of retinoic acid (RA) in the lungs of ferrets exposed to cigarette smoke and/or a pharmacological dose of β -carotene. To determine whether this is involved in excessive catabolism of RA via cytochrome P450 (CYP) induction, we carried out in vitro incubations of RA with the lung microsomal fractions of ferrets with or without CYP inhibitors and antibodies against CYP. The polar metabolites (4-oxo-RA and 18-hydroxy-RA) of RA metabolism after the incubation were analyzed by HPLC. Expressions of CYP (1A1, 1A2, 2E1 and 3A1) were examined using Western blot analysis. Incubation of various concentrations of RA with the lung microsomal fraction from ferrets exposed to cigarette smoke, a pharmacological dose of β -carotene or their combination dose-dependently increased the levels of 4-oxo-RA and 18-hydroxy-RA compared with that of the control ferrets. At all RA concentrations, this increase was the greatest in lung tissue from the combined treatment group. Furthermore, this enhanced RA catabolism was substantially (80%) inhibited by nonspecific CYP inhibitors (disulfiram and liarozole), but was partially (50%) inhibited by resveratrol (CYP1A1 inhibitor), -naphthoflavone (CYP1A2 inhibitor) and antibodies against CYP1A1 and CYP1A2. Cigarette smoke exposure and/or pharmacological doses of β -carotene increased levels of CYP1A1 and 1A2 by three- to sixfold but not levels of 2E1 and 3A1 in ferret lung tissue. These findings suggest that low levels of RA in the lung of ferrets exposed to cigarette smoke and/or pharmacological doses of β -carotene may be caused by the enhanced RA catabolism via induction of CYP, CYP1A1 and CYP1A2 in particular, which provides a possible explanation for enhanced lung carcinogenesis seen with pharmacological doses of β -carotene supplementation in cigarette smokers.

COMMENTS: Earlier studies by this group reported that ferrets exposed to cigarette smoke and fed β -carotene, had increased molecular markers of cellular proliferation and histopathological changes in lung tissue. This study examined induction of cytochrome p450 enzymes (CYP) in ferret lung by smoke exposure and pharmacological doses (equivalent to human dose of 30 mg/d) of β -carotene. CYP1A1 and CYP1A2 were markedly higher in lung tissue of ferrets exposed to smoke, β -carotene, or both as compared to controls. The authors also established links between CYP induction and retinoic acid catabolism by cigarettes and/or β -carotene. Because of the action of retinoic acid on blocking squamous metaplasia in bronchial epithelium, the authors suggest that reduced retinoic acid levels may contribute to lung carcinogenesis, in addition to the bioactivation of carcinogens due to induced cytochrome p450 enzymes.

CARROT OIL, SEED

CAS: 8015-88-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

4-CARVOMENTHENOL

CAS: 562-74-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BETA-CARYOPHYLLENE

CAS: 87-44-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

BETA-CARYOPHYLLENE OXIDE

CAS: 1139-30-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CASSIA BARK, BUDS, OILS, AND EXTRACT

CAS: 8007-80-5

CAS: 84961-46-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CASTOREUM, LIQUID, EXTRACT, TINCTURE AND ABSOLUTE

CAS: 8023-83-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CELERY SEED OIL

CAS: 89997-35-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CELLULOSE AND CELLULOSE FIBER

CAS: 65996-61-4

CAS: 9004-34-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CHAMOMILE FLOWER OIL, EXTRACT AND ABSOLUTE

CAS: 8002-66-2

CAS: 8015-92-7

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CHICORY EXTRACT

CAS: 68650-43-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CHOCOLATE AND CHOCOLATE LIQUOR

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

1,8-CINEOLE (EUCALYPTOL)

CAS: 470-82-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CINNAMALDEHYDE

CAS: 104-55-2

Number of relevant papers: 2

1. Structure-Activity Relationships for the Mutagenicity and Carcinogenicity of Simple and alpha-beta Unsaturated Aldehydes - 2003 - EMBASE® - US\$2.94

**Benigni R, Passerini L, Rodomonte A.
Environ Mol Mutagen. 2003;42(3):136-43.**

ABSTRACT: Aldehydes are important industrial compounds that are used for the synthesis of chemicals and pharmaceuticals and as solvents, food additives, and disinfectants. Because of their reactivity, aldehydes are able to interact with electron-rich biological macromolecules and adverse health effects have been reported, including general toxicity, allergenic reactions, mutagenicity, and carcinogenicity. The cost, time, and number of animals necessary to adequately screen these chemicals places serious limitations on the number of aldehydes whose health potential can be studied and points to the need of using alternative methods for assessing, at least in a preliminary way, the risks associated with the use of aldehydes. A method of choice is the study of quantitative structure-activity relationships (QSARs). In the present work, we present QSAR models for the mutagenicity and carcinogenicity of simple aldehydes and α - β unsaturated aldehydes. The models point to the role of electrophilicity, bulkiness, and hydrophobicity in the genotoxic activity of the aldehydes and lend themselves to the prediction of the activity of other untested chemicals of the same class.

COMMENTS: Although cinnamaldehyde and citral were found to be inactive in the NTP bioassay, there are several aldehydes that are suspected genotoxic carcinogens. These authors used QSAR analysis to determine toxicity of these two compounds based on molecular structure properties of these chemicals. Using their model, citral was described as extremely weak (well below the potency range of mutagens) and cinnamaldehyde was described as very weak.

2. Toxicology and carcinogenesis studies of microencapsulated trans-cinnamaldehyde in rats and mice -

Hooth MJ, Sills RC, Burka LT, Haseman JK, Witt KL, Orzech DP, Fuciarelli AF, Graves SW, Johnson JD, Bucher JR.
Food Chem Toxicol. 2004 Nov;42(11):1757-68.

ABSTRACT: trans-Cinnamaldehyde is a widely used natural ingredient that is added to foods and cosmetics as a flavoring and fragrance agent. Male and female F344/N rats and B6C3F1 mice were exposed to microencapsulated trans-cinnamaldehyde in the feed for three months or two years. All studies included untreated and vehicle control groups. In the three-month studies, rats and mice were given diets containing 4100, 8200, 16,500, or 33,000 ppm trans-cinnamaldehyde. In rats, feed consumption was reduced in all exposed groups. In mice, feed consumption was reduced in the highest dose groups. Body weights of all treated males were less than controls. Body weights were reduced in female rats exposed to 16,500 or 33,000 ppm and female mice exposed to 8200 ppm or greater. All rats survived to the end of the study but some male mice in the highest dose groups died due to inanition from unpalatability of the dosed feed. The incidence of squamous epithelial hyperplasia of the forestomach was significantly increased in rats exposed to 8200 ppm or greater and female mice exposed to 33,000 ppm. In mice, the incidence of olfactory epithelial degeneration of the nasal cavity was significantly increased in males and females exposed to 16,500 ppm and females exposed to 33,000 ppm. In the two-year studies, rats and mice were exposed to 1000, 2100, or 4100 ppm trans-cinnamaldehyde. Body weights were reduced in mice exposed to 2100 ppm and in rats and mice exposed to 4100 ppm. In rats, hippuric acid excretion was dose proportional indicating that absorption, metabolism, and excretion were not saturated. No neoplasms were attributed to trans-cinnamaldehyde in rats or mice. Squamous cell papillomas and carcinomas of the forestomach were observed in male and female mice but the incidences were within the NTP historical control range and were not considered to be related to trans-cinnamaldehyde exposure.

COMMENTS: Although the oral route of exposure was used in these studies, the results described are of interest. The authors selected to test and characterize the toxicity of microencapsulated trans-cinnamaldehyde because of its structural similarity to cinnamyl anthranilate and 3,4,5-trimethoxy-cinnamaldehyde, two known rodent carcinogens. In a 3-month study, both rats and mice were exposed to concentrations ranging from 4000 to 33,000 ppm. A 2 years study exposed the test animals to concentrations of 1000, 2100, 4100 ppm. As expected the forestomach was the target organ for both species. There was a significant increase in hyperplasia in both rats and mice and in mice, olfactory epithelial degeneration was reported of the nasal cavity. In the 2-year study, no neoplasms were observed but olfactory epithelial pigmentation was reported in mice.

CINNAMON BARK, BUDS, LEAF, OIL, AND EXTRACT

CAS: 8015-91-6

CAS: 8007-80-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CINNAMYL ACETATE

CAS: 103-54-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CINNAMYL ALCOHOL

CAS: 104-54-1

Number of relevant papers: 2

1. Toxicology databases and the concept of thresholds of toxicological concern as used by the JECFA for the safety evaluation of flavouring agents

Renwick AG.

Toxicol Lett. 2004 Apr 1;149(1-3):223-34.

ABSTRACT: Since 1996 the FAO/WHO Joint Expert Committee on Food Additives (JECFA) has evaluated the safety of 1259 flavouring substances, based on a decision tree that incorporates a series of thresholds of toxicological concern. Safety conclusions are based on the predicted consequences of metabolism and whether the estimated intake is above or below a threshold of toxicological concern that is relevant to that compound. Compounds are allocated to one of three structural classes, and the intake compared with a threshold of toxicological concern derived using data from chronic and sub-chronic toxicity studies on compounds in the same structural class. If the substance is predicted to be metabolised to innocuous products there is no safety concern if the intake is below the threshold, but suitable toxicity data on the compound or structural analogues are required if the intake exceeds the threshold. If the substance is not predicted to be metabolized to innocuous products, and the intake is below the appropriate threshold, safety evaluation is based on data on the compound or structural analogues. An additional threshold of 1.5 µg per day, derived from doses of investigated chemicals giving a calculated cancer risk of one in a million, is applied when appropriate toxicity data are not available.

COMMENTS: This paper addresses the concept of “threshold of toxicity” as it relates to safety assessments of flavoring agents. The decision-making process for safety evaluation is reviewed, including chemical structural class allocation, consideration of predicted metabolism, estimated intake (per capita) and a comparison of the intake with the threshold of toxicological concern. Substances structurally related to menthol were included in a summary of the application of the procedure and all 14 compounds were classified as “no safety concern”. However, this assessment is directed towards additives in food and does not attempt to address inhalation exposures.

2. The FEMA GRAS assessment of cinnamyl derivatives used flavor ingredients

Adams, T.B., Cohen, S.M., Doull, J., Feron, V.J., Goodman, J.I., Marnett, L.J., Munro, I.C., Portoghese, P.S., Smith, R.L., Waddell, W.J., and Wagner, B.M. (2004) Food and Chemical Toxicology, 42, 157-185.

ABSTRACT: This publication is the seventh in a series of safety evaluations performed by the Expert Panel of the Flavor and Extract Manufacturers Association (FEMA). In 1993, the Panel initiated a comprehensive program to re-evaluate the safety of more than 1700 GRAS flavoring substances under conditions of intended use. Elements that are fundamental to the safety evaluation of flavor ingredients include exposure, structural analogy, metabolism, pharmacokinetics and toxicology. Flavor ingredients are evaluated individually and in the context of the available scientific information on the group of structurally related substances. Scientific data relevant to the safety evaluation of the use of cinnamyl derivatives as flavoring ingredients is evaluated.

COMMENT: This panel evaluated the safety of cinnamyl derivatives used as flavor ingredients. These compounds were reaffirmed as GRAS. Acute oral LD50 in mice and rats indicated a low level of toxicity. Reproductive/developmental studies with this compound indicted no observed effects. This panel did report that this compound was found to have inhibitory effects on platelet function. Increase inhibition of platelet aggregation correlated with increase lipophilicity of the test substance.

CINNAMYL CINNAMATE

CAS: 122-69-0

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CITRAL

CAS: 5392-40-5

Number of relevant papers: 5

1. Structure-Activity Relationships for the Mutagenicity and Carcinogenicity of Simple and alpha-beta Unsaturated Aldehydes -

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Environ Mol Mutagen. 2003;42(3):136-43.**

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and number of animals necessary to adequately screen these chemicals places serious limitations on the number of aldehydes whose health potential can be studied and points to the need of using alternative methods for assessing, at least in a preliminary way, the risks associated with the use of aldehydes. A method of choice is the study of quantitative structure–activity relationships (QSARs). In the present work, we present QSAR models for the mutagenicity and carcinogenicity of simple aldehydes and unsaturated aldehydes. The models point to the role of electrophilicity, bulkiness, and hydrophobicity in the genotoxic activity of the aldehydes and lend themselves to the prediction of the activity of other untested chemicals of the same class.

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2. Toxicology and carcinogenesis studies of microencapsulated Citral in rats and mice -

Ress NB, Hailey JR, Maronpot RR, Bucher JR, Travlos GS, Haserman JK, Orzech DP, Johnson JD, Hejmancik MR.

Toxicological Sciences. 71, 198-206, 2003

ABSTRACT: Citral, a widely used natural ingredient, is added to foods and cosmetics as a flavoring and fragrance agent. Male and female F344/N rats and B6C3F1 mice were exposed to microencapsulated citral in the feed for 14 weeks or two years. All studies included untreated and vehicle control groups. In the 14-week studies, rats and mice were given diets containing 3900, 7800, 15,600, or 31,300 ppm citral. In rats, food consumption was reduced in the two highest dose groups. In mice an apparent increase in food consumption was observed, but was due to mice scattering the feed. Body weights of all treated animals were less than controls. All rats and four male mice were killed moribund in the high dose groups. In rats, forestomach and kidney lesions were observed. At the higher doses, lesions observed in the bone marrow, testes, and thymus in rats and in the ovary in mice were considered related to inanition and resultant moribundity. In the two-year studies, rats were exposed to 1000, 2000, or 4000 ppm citral. Body weights were reduced in the 4000 ppm rats. Mice were exposed to 500, 1000, or 2000 ppm citral. Body weights in the 1000 and 2000 ppm groups were reduced. No neoplasms were attributed to citral in rats or mice. Malignant lymphoma occurred with a positive trend and was significantly greater than controls in female mice in the 2000 ppm group. However, the incidences were within the NTP historical control range and could not be clearly related to citral administration.

COMMENTS: Citral was administered through the diet of rats and mice and evaluated for toxicity and carcinogenicity. Exposures were conducted for 14 weeks and 2 years with maximum concentrations in the diet of 31,300 ppm and 4000 ppm, respectively. The

minimum daily doses in the 2-year study were more than 10 times greater than the average daily intake in humans. Palatability issues resulted in decreased food consumption and lower weight gain in both species. Transient treatment-related hematological and serum biochemical effects were noted in rats, but were consistent with physiological responses related to decreased food and water consumption. Nephropathy with renal tubule granular casts was observed in treated male rats from the 14-week treatment, but no citral-related kidney neoplasms were observed in the 2-year study. In mice, there was an increase in the incidences of malignant lymphoma in the highest treatment groups during the 2-year study, but this incidence was low and within the historical range of control female mice fed similar diets. Extrapolation of the findings of this study to the effects of citral as an ingredient in cigarettes is difficult because of the route of exposure (diet) and the high concentrations of citral used in this study which were far above the expected exposure through cigarette smoke.

3. Classification of Diverse Organic Compounds That Induce Chromosomal Aberrations in Chinese Hamster Cells -

**McElroy NR, Thompson ED, Jurs PC.
J Chem Inf Comput Sci. 2003 Nov-Dec;43(6):2111-9.**

ABSTRACT: A data set of 297 diverse organic compounds that cause varying degrees of chromosomal aberrations in Chinese hamster lung cells is examined. Responses of an assay are categorized as clastogenic (>10% aberrant cells) and nonclastogenic (<5% aberrant cells). Each of the compounds is represented by calculated structural descriptors that encode topological, geometric, electronic, and polar surface features. A genetic algorithm (GA) employing a k-nearest neighbor (kNN) fitness evaluator is used to iteratively search a reduced descriptor space to find small, information-rich subsets of descriptors that maximize the classification rates for clastogenic and nonclastogenic responses. To further improve modeling, a similarity measure using atom-pair descriptors is employed to create more homogeneous data subsets. Three different data sets are examined. Results for a set of 297 compounds using the GA-kNN method were 86.5% and 80.0% correct classification in the training set and prediction set, respectively. Results for a subset of 279 compounds in model 2 are 85.7% and 85.7% for the training and prediction sets, respectively. Results for a subset of 182 compounds in model 3 are 91.5% and 94.4% for the training and prediction sets, respectively. Creating smaller, more topologically similar data sets result in improved classification rates.

COMMENTS: Predictive classification models were designed that link molecular structure of 297 organic compounds to their genotoxic potential, as determined by chromosomal aberration assays using Chinese hamster lung cells. The predictive ability of the models was examined using external data sets. Citral was predicted correctly to be nonclastogenic, defined as inducing fewer than 5% aberrant cells. The relevance of this study to citral as an ingredient in cigarette smoke is minimal except for the potential of such predictive models to be applied to effects assessments for smoke components.

4. Analysis of thresholds for carcinogenicity. -

William J. Waddell ,

Toxicology Letters Volume 149, Issues 1-3 , 1 April 2004, Pages 415-419

Proceedings of EUROTOX 2003. The XLI European Congress of Toxicology. Science for Safety

ABSTRACT: Re-evaluations of large prominent studies, e.g. the ED01 study and N-nitrosodiethylamine, unequivocally have demonstrated that thresholds exist for carcinogenicity when the dose-response curves for animal studies done at high doses are calculated according to fundamental principles of chemistry. This requires dose to be on a logarithmic scale and percent tumors on a linear scale. Fifteen compounds approved by the Flavor and Extract Manufacturers Association (FEMA) expert panel as Generally Recognized As Safe (GRAS) have been reported to be carcinogenic in rodent studies. The thresholds for tumors of these flavors were at least several orders of magnitude greater than the estimated daily dose of these flavoring agents to individuals in the United States. Similarly, comparisons of thresholds of carcinogenicity of chemicals and drugs to which humans are exposed with their exposure levels suggest that experimental animals are more sensitive to carcinogenicity than humans. The animal studies should be viewed as providing evidence for the safety of these flavors and other compounds at current levels of human exposure.

COMMENTS: This author has published extensively, presenting good evidence for thresholds of carcinogenicity of flavors. This paper examines the threshold for 6 compounds, providing estimates of the current level of exposure and a safety factor for each chemical. For citral the minimum safety ratio of 407 was suggested. The authors suggest that the actual safety ratios are probably greater.

5. Safety evaluations of food chemicals by "COMPACT" 1. A study of some acyclic terpenes

Lewis DF, Ioannides C, Walker R, Parke DV.

Food Chem Toxicol. 1994 Nov;32(11):1053-9.

ABSTRACT: A group of 19 acyclic terpenes have been evaluated for potential toxicity/carcinogenicity by molecular orbital determinations of their spatial and electronic parameters, and hence prediction of their metabolic activation or detoxication by the cytochrome P-450 (CYP) superfamily of mixed-function oxidase enzymes. Previous studies have characterized the spatial dimensions of the CYP1A1, 1A2 and 2E1 enzymes, which are known to activate mutagens and carcinogens and to be involved in other mechanisms of toxicity. None of the terpenes was found to have shape or electronic parameters appropriate for metabolic activation by CYP1A1 or 1A2, and hence they are unlikely to be carcinogenic or mutagenic. Furthermore, none of these chemicals had spatial parameters critical for substrates of CYP2E, and they are therefore unlikely to induce the formation of reactive oxygen species (ROS) or to initiate or promote malignancy or toxicity by mechanisms involving ROS. However, citral, and others of

these terpenes are known to undergo metabolism to carboxylic acids that may induce CYP4, and are therefore possible inducers of hepatic peroxisomal proliferation at high dosage, which may have implications for possible hepatotoxicity.

COMMENTS: Abstract sufficient, no additional comments needed.

CITRIC ACID
CAS: 77-92-9

Number of relevant papers: 1

1. Cough reflex induced by microinjection of citric acid into the larynx of guinea pigs: New coughing model. -

Tanaka M, Maruyama K.
J Pharmacol Sci. 2003 Dec;93(4):465-70.

ABSTRACT: We developed a new coughing model that evoked coughs by microinjection of citric acid into the larynx in unanesthetized unrestrained guinea pigs; additionally, we recorded synchronous sounds and waveforms of coughing utilizing built-in microphones and a whole body plethysmograph. The coughing model was able to distinguish a coughing response from other expiratory responses, such as an expiratory reflex or a sigh, by examining the waveform of the expiratory response and the existence of sound. It was not necessary to distinguish a cough from a sneeze, since the administration site was restricted to the larynx. Microinjection of 0.4 M citric acid, total of 20 μ l (10 times, 2 μ l at 30-s intervals), induced coughs (27.03 $\pm$ 4.03 coughs in 10-min observation) that were stable and independent of the inhalation volume. In the inhalation studies, animals were exposed to citric acid only once, because the number of coughs remarkably decreased with repeated administration at intervals of 24 h (tachyphylaxis). However our coughing model was able to repeatedly challenge the microinjection of citric acid at an interval of 24 h. These results indicated that this coughing model was highly sensitive and correctly assessed the cough response.

COMMENTS: Using unanesthetized, unrestrained guinea pigs, these authors demonstrated that microinjection of citric acid stimulated both the larynx and the bifurcation of the trachea, inducing cough and bronchoconstriction.

CITRONELLA OIL
CAS: 8000-29-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CITRONELLOL

CAS: 106-22-9

SEE NEW INGREDIENTS

CLARY SAGE OIL AND EXTRACT

CAS: 8016-63-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

**COCOA, COCOA SHELLS, EXTRACT, DISTILLATE, POWDER, ALKALIZED,
ABSOLUTE AND TINCTURE**

CAS: 8002-31-1

CAS: 84649-99-0

CAS: 68916-17-6

CAS: 95009-22-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

COCONUT OIL

CAS: 8001-31-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

COFFEE AND COFFEE SOLID EXTRACT

CAS: 8001-67-0

CAS: 68916-18-7

CAS: 84650-00-0

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

COGNAC WHITE AND GREEN OIL

CAS: 8016-21-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CORIANDER EXTRACT, SEED, AND OIL

CAS: 8008-52-4

CAS: 84775-50-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

CORN STARCH

CAS: 9005-25-8

SEE NEW INGREDIENTS

BETA-DAMASCONE

CAS: 23726-92-3

CAS: 23726-91-2

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DAVANA OIL

CAS: 8016-03-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DECANAL

CAS: 112-31-2

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DELTA-DECALACTONE

CAS: 705-86-2

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

GAMMA-DECALACTONE

CAS: 706-14-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DECANOIC ACID

CAS: 334-48-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DIACETYL

CAS: 431-03-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DIETHYL MALONATE

CAS: 105-53-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2,3-DIETHYLPYRAZINE

CAS: 15707-24-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2,6-DIMETHOXYPHENOL

CAS: 91-10-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DIMETHYL BENZYL CARBINYL BUTYRATE (ALPHA, ALPHA-DIMETHYLPHENETHYL BUTYRATE)

CAS: 10094-34-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DIMETHYL SULFIDE

CAS: 18-50-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

3,4-DIMETHYL-1,2-CYCLOPENTADIONE

CAS: 13494-06-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

3,7-DIMETHYL-1,3,6-OCTATRIENE

CAS: 13877-91-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

4,5-DIMETHYL-3-HYDROXY-2,5-DIHYDROFURAN-2-ONE (3-HYDROXY-4,5-DIMETHYL-2(5H)FURANONE)

CAS: 28664-35-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

**2, 5-DIMETHYL-4-HYDROXY-3(2H)-FURANONE
(4-HYDROXY-2,5-DIMETHYL-3(2H)FURANONE) 3658-77-3**

and

**2,3-DIMETHYLPYRAZINE 5910-89-4
STANDARD**

Number of relevant papers: 1

1. The influence of cigarette moisture to the chemistry of particulate phase smoke of a common commercial cigarette –

Zha, Q; Moldoveanu S C, (Reprint)

***Beitraege zur Tabakforschung International* , Volume: 21 , Number: 3 , Page: 184-191, October 2004 , 2004**

ABSTRACT: This study presents the results on the influence of cigarette moisture content to the chemical composition of particulate phase smoke. Seventy-five selected compounds were monitored for the comparison of particulate phase smoke of a commercial full-flavored (FF) cigarette with three different moisture contents at 7.8%, 14.5% and 20.4%, respectively. It was demonstrated that the smoke of a dry cigarette is richer in lower molecular mass compounds than a regular cigarette. On the other hand, the smoke of a moist cigarette is richer in higher molecular mass compounds than a regular cigarette. To maximize the influence of cigarette moisture to the chemical composition, a separate set of measurements were done using only the first three puffs of smoke. The accumulation of moisture in the tobacco column of a burning cigarette may influence the smoke composition, as generated during burning. The differences between dry, regular and moist cigarettes were more obvious for the first three puffs.

COMMENTS: While this is not a health effect study, the results are interesting. These investigators compared the chemical composition of cigarette smoke from cigarettes with three moisture levels (dry-8.3%, regular-11.6%, and moist- 12.9%). The first three puffs showed the greatest differences. The nicotine content and total particulate matter (TPM) was reduced with increasing moisture. The data would indicate that the dry cigarette had higher percentage of semi-volatile compounds in TPM. The data presents additional evidence that the moisture content in cigarette significantly affects the chemistry of the particulate phase of smoke. Of the 75 compounds tested the more volatile compounds were more affected than the less volatile compounds. Compared to the first three puffs, the particulate phase of smoke from the entire cigarette was less sensitive to the moisture content.

3,7-DIMETHYL-6-OCTENOIC ACID (CITRONELLIC ACID)

CAS: 502-47-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ALPHA, PARA-DIMETHYLBENZYL ALCOHOL

CAS: 536-50-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

2,5-DIMETHYLPYRAZINE

CAS: 123-32-0

SEE HIGH MUL'S INGREDIENTS

**DODECAHYDRO-3A,6,6,9A-TETRAMETHYLNAPHTHO (2,1-B)FURAN
(1,5,5,9-TETRAMETHYL-13-OXATRICYCLO(8.3.0.0(4,9))TRIDECANE)**

CAS: 3738-00-9

CAS: 6790-58-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

DELTA-DODECALACTONE**CAS: 713-95-1**

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

GAMMA-DODECALACTONE**CAS: 2305-05-7**

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL ACETATE**CAS: 141-78-6**

Number of relevant papers: 1

1. Subchronic inhalation neurotoxicity studies of ethyl acetate in rats. -**Christoph GR, Hansen JF, Leung HW.
Neurotoxicology. 2003 Dec;24(6):861-74.**

ABSTRACT: Rats were exposed to 0, 350, 750 or 1500 ppm of ethyl acetate by inhalation for 6 h per day, 5 days per week for 13 weeks. Functional observational battery (FOB) and motor activity tests occurred on non-exposure days during weeks 4, 8 and 13, after which tissues were microscopically examined for neuropathology. A subset of rats was monitored during a 4-week recovery period. Exposure to 750 and 1500 ppm, diminished behavioral responses to unexpected auditory stimuli during the exposure session and appeared to be an acute sedative effect. There were no signs of acute intoxication 30 min after exposure sessions ended. Rats exposed to 750 and 1500 ppm had reduced body weight, body weight gain, feed consumption, and feed efficiency, which fully or partially recovered within 4 weeks. Reductions in body weight gain and feed efficiency were observed in male rats exposed to 350 ppm. The principal behavioral effect of subchronic exposure was reduced motor activity in the 1500 ppm females, an effect that was not present after the 4-week recovery period. All other FOB and motor activity parameters were unaffected, and no pathology was observed in nervous system tissues. Operant sessions were conducted in another set of male rats preconditioned to a stable operant baseline under a multiple fixed ratio–fixed interval (FR–FI) schedule of food reinforcement. FR response rate, FR post-reinforcement pause duration, and the pattern of FI responding were not affected during or after the exposure series. In contrast, within-group FI rate for the treatment groups increased over time whereas those of the controls decreased. A historical control group, however, also showed a similar pattern of increase, indicating that these changes did not clearly represent a treatment related effect. Results from these studies indicate a LOEL of 350 ppm for systemic toxicity based on the decreased body weight gain in male rats, and a LOEL of 1500 ppm for neurotoxicity based on the transient reduction in motor activity in female rats. In conclusion, there was no evidence that subchronic exposure up to 1500 ppm ethyl acetate produced any enduring neurotoxic effects in rats.

COMMENTS: A large number of behavioral and neuropathological endpoints were measured by these investigators (37 functional observational battery tests, 2 motor activity and 5 operant tests). These studies suggest a LOEL of 350 ppm for decrease in body weight and a 1,500 ppm for reduction in motor activity. Even at this high concentration the authors reported no persistent adverse effect.

ETHYL ALCOHOL, INCLUDING SDA-4

CAS: 64-17-5

SEE MAJOR INGREDIENTS CATEGORY

ETHYL BENZOATE

CAS: 93-89-0

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL BUTYRATE

CAS: 105-54-4

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL CINNAMATE (PROPENIC ACID,3-PHENYL-,ETHYL ESTER,2-)

CAS: 103-36-6

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL DECANOATE

CAS: 110-38-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

4-ETHYL GUAIACOL (4-ETHYL-2-METHOXY-PHENOL)

CAS: 2785-89-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL HEPTANOATE

CAS: 106-30-9

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL HEXANOATE (ETHYL CAPROATE)

CAS: 123-66-0

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL ISOVALERATE

CAS: 108-64-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL LACTATE

CAS: 97-64-3

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL LAURATE

CAS: 106-33-2

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL LEVULINATE

CAS: 539-88-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL MALTOL

CAS: 4940-11-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL 2-METHYLBUTYRATE

CAS: 7452-79-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL METHYL PHENYLGLYCIDATE

CAS: 77-83-8

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL MYRISTATE

CAS: 124-06-1

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL NONANOATE

CAS: 123-29-5

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL OCTADECANOATE**CAS: 111-61-5**

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL OCTANOATE**CAS: 106-32-1**

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

ETHYL OLEATE**CAS: 111-62-6**

NO RELEVANT INFORMATION PUBLISHED FOR THIS INGREDIENT

INGREDIENTS USED IN MIXTURE STUDIES TOBACCO SMOKE STUDIES**GENERAL COMMENTS:**

In addition to studies that looked at individual ingredients, there were a few studies specifically designed to evaluate the potential effects of a large number of ingredients commonly added to cigarettes. The studies were unique in that all ingredients were tested by adding them in groups to a single research cigarette. The strength of this research is that these studies were specifically designed to determine (1) the effect of pyrolysis on the toxicity of the ingredients, (2) if new toxic substances were produced, (3) if the mixture of ingredients acts in a synergistic manner that might increase the toxicity of the inhaled smoke and (4) if these substances produced any identifiable new target organ toxicity not associated with cigarette smoke from cigarettes without the added ingredients. Well-established *in vitro* tests were included to identify mutagenicity (Ames test), cytotoxicity (neutral red uptake assay), carcinogenicity (two-stage mouse dermal assay), as well as a 90-day rat inhalation study. To complement these studies the chemical composition of the mainstream smoke from cigarettes with and without the added ingredients were also determined. These studies indicated that the addition of these chemicals, even at exaggerated levels, did not increase the bacterial mutagenicity, cytotoxicity nor the pathological response to the inhaled cigarettes with ingredients as compared to control cigarettes. When the results were compared to reference cigarettes without ingredients, the tests would indicate that the presence of these ingredients did not alter the biological activity. This model system represents a realistic model capable of detecting potential interactions among ingredient pyrolysis products together with various constituents known to be present in cigarette smoke. A number of the ingredients being reviewed in this report were included in these mixture studies. A list of these ingredients can be found below.

These extensive reviews would indicate that no chemical nor biological evidence has been presented to support the claim that ingredients added to cigarettes modifies the chemistry or biology activity of inhaled tobacco smoke.

The following ingredients were tested as a mixture added to cigarettes. Relevant mixture and review papers are listed below:

CITRONELLOL	CAS: 106-22-9
PARA-TOLUALDEHYDE	CAS: 104-87-0
ETHYL HEPTANOATE	CAS: 106-30-9
ISOAMYL FORMATE	CAS: 110-45-2
HEXYL ACETATE	CAS: 142-92-7
PECTIN	CAS: 9000-69-7
CORN STARCH	CAS: 9005-25-8
L-MENTHONE	CAS: 14073-97-3
ACETIC ACID	CAS: 64-19-7
ENZALDEHYDE	CAS: 100-52-7
BUTRIC ACID	CAS: 107-92-6
BETA-CARYOPHLENE OXIDE	CAS: 1139-30-6
GAMMA-DECALACTONE	CAS: 706-14-9
2,5-DECALACTONE	CAS: 123-32-0
ETHYL BUTYRATE	CAS: 105-54-4
ETHYL DECANOATE	CAS: 110-38-3
ETHYL HEXANOATE	CAS: 123-66-0
ETHYL ISOVALERATE	CAS: 108-64-5
ETHYL LACTATE	CAS: 97-64-3
ETHYL LAURATE	CAS: 106-33-2
ETHYL MYRISTATE	CAS: 124-06-1
ETHYL OCTANOATE	CAS: 106-32-1
ETHYL PHENYLACETATE	CAS: 101-97-3
5-ETHYL-3-HYDROXY-4METHYL-2(5H)-FURANONE	CAS: 698-10-2
ISOAMYL ACETATE	CAS: 123-92-2
ISOBUTYL CINNAMATE	CAS: 122-67-8
ISOBUTYL PHENYLACETATE	CAS: 102-13-6
ISOBUTYRIC ACID	CAS: 79-31-2
2-METHYLPYRAZINE	CAS: 109-08-0
GAMMA-OCTALACTONE	CAS: 104-50-7
2,3-PENTANEDIONE	CAS: 600-14-7
2-PHENETHYL ACETATE	CAS: 103-45-7
PHENYLACETALDEHYDE	CAS: 122-78-1
SODIUMBICARBONATE	CAS: 144-55-8
2,3,5,6-TETRAMETHYLPYRAZINE	CAS: 1124-11-4
TRIETHYL CITRATE 77-93-0	
4-(2,6,6-TRIMETHYLCYCLOHEX-1-ENY) BUT-2-4- ONE	
(BETA-DAMASCONE)	CAS: 23726-91-2; 35044-68-9
GLYCEROL	CAS: 56-81-5
INVERTED SUGAR	CAS: 8013-17-0
CELLULOSE AND CELLULOSE	

FIBER	CAS: 65996-61-4; 9004-34-6
PROPYLENE GLYCOL	CAS: 57-55-6
METHOL AND L-MENTHOL	CAS: 89-78-1; 216-51-5
ETHYL ALCOHOL, INCLUDING SDA-4	CAS: 64-17-5
CHOCHOLATE AND CHOCOLATE LIQUOR	CAS: N/A
LACTIC ACID	CAS: 50-21-5; 598-82-3
SORBITOL	CAS: 50-70-4
AMMONIUM HYDROXIDE	CAS: 1336-21-6
GLUCOSE/DEXTROSE	CAS: 50-99-7; 492-62-6
SODIUM CARBONATE	CAS: 497-19-8
ETHYL 2-METHYLBUTYRATE	CAS: 7452-79-1
VANILLIN	CAS: 121-33-5

Each of the papers listed below, except for papers 7, 8, 9, and 10, has been reviewed and evaluated in previous review documents and will not be repeated here. The new papers have extensive abstracts fully defining the goals and conclusions reached by the authors.

1. Evaluation of the potential effects of ingredients added to cigarettes. Part 1: Cigarette design, testing approach, and review of results.

Food and Chemical Toxicology. Volume 40, Issue 1, pp. 77-91, January, 2002

E.L. Carmines et al

2. Evaluation of the potential effects of ingredients added to cigarettes. Part 2: Chemical composition of mainstream smoke.

AUTHORS: K. Rustemeiera, R. Stabberta, H.-J. Haussmanna, E. Roemera, E.L. Carmines.

SOURCE: Food and Chemical Toxicology. Vol. 40, Issue 1, pp. 93-104, January, 2002

3. Evaluation of the potential effects of ingredients added to cigarettes. Part 3: In vitro genotoxicity and cytotoxicity.

AUTHORS: E. Roemera, F.J. Tewesa, T.J. Meisgena, D.J. Veltela, E.L. Carmines.

SOURCE. Food and Chemical Toxicology. Vol. 40, Issue 1, pp. 105-111, January, 2002

4. Evaluation of the potential effects of ingredients added to cigarettes. Part 4: Subchronic inhalation toxicity.

AUTHORS: P.M. Vanscheeuwijcka, A. Teredesaib, P.M. Terpstra, J. Verbeeck, P. Kuhl, B. Gerstenberg, S. Gebel, E.L. Carmines.

PUBLICATION SOURCE: Food and Chemical Toxicology, Volume 40, Issue 1, pp. 113-131, January, 2002

5. Toxicologic evaluation of flavor ingredients added to cigarette tobacco: skin painting bioassay of cigarette smoke condensate in SENCAR mice.

AUTHORS:C. L. Gaworski, J. D. Hecka, M. B. Bennetta and M. L. Wenk.

PUBLICATION SOURCE. Toxicology. Volume 139, Issues 1-2, 29 November 1999, Pages 1-17

6. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters.

AUTHORS: Fukayama Mark Y(a); Easterday Otho D; Serafino Patricia A; Renskers Kevin J ; North-Root Helen; Schrankel Kenneth R

SOURCE: Toxicology Letters 111(1-2 p 175-187 Dec. 20, 1999

7. The pyrolysis of tobacco ingredients -

Baker, R.R.;Bishop, L.J.

Journal of Analytical and Applied Pyrolysis, Volume 71, Issue 1, 1 March 2004, Pages 223-311

ABSTRACT: Relationships between tobacco components and smoke products are complex and often difficult to unravel. Pyrolysis experiments have commonly been used to establish such relationships. However, unless they are performed under dynamic conditions that are relevant to those that occur during tobacco burning, results can be obtained which have little resemblance to those obtained during cigarette smoking. The relevance of pyrolysis experiments to the behaviour of tobacco ingredients in a burning cigarette is considered. Based on the temperature, heating rate, oxygen levels and gas flow conditions that occur inside the burning zone of a cigarette, together with a review of relevant pyrolysis and smoking experiments, a set of pyrolysis conditions has been developed that approximates those occurring in the pyrolysis region of the burning cigarette. The conditions include heating the sample at 30 °C s⁻¹ from 300 to 900 °C under a flow of 9% oxygen in nitrogen. Experiments on the pyrolytic behaviour of eleven relatively volatile substances under these conditions give results that are in good agreement with results from thirteen published studies in which cigarettes incorporating labelled versions of the substances were smoked. Subsequently, 291 single-compound tobacco ingredients have been pyrolysed under this set of conditions, most of which are relatively volatile. This enables the behaviour of these ingredients in a burning cigarette to be estimated in terms of intact transfer to mainstream smoke versus pyrolytic decomposition. It is predicted that almost a third of the substances would transfer to mainstream smoke at least 99% intact, and almost two-thirds would transfer 95% intact. Where pyrolytic decomposition does occur, the products are listed together with an estimate of the levels in smoke that would arise from the ingredient.

8. The effect of tobacco ingredients on smoke chemistry. Part I: Flavourings and additives

Baker RR; da Silva JRP; Smith G

Food and Chemical Toxicology 42(Supplement S): S3-S37, 2004. (34 refs.)

ABSTRACT: The effects of 450 tobacco ingredients added to tobacco on the forty-four "Hoffmann analytes" in mainstream cigarette smoke have been determined. These analytes are believed by regulatory authorities in the USA and Canada to be relevant to smoking related diseases. They are based on lists published by D. Hoffmann and co-workers of the American Health Foundation in New York. The ingredients comprised 431 flavours, 1 flavour/solvent, 1 solvent, 7 preservatives, 5 binders, 2 humectants, 2 process aids and 1 filler. The cigarettes containing mixtures of the ingredients were smoked using the standard ISO smoking machine conditions. The levels of the "Hoffmann analytes" in the smoke from the test cigarettes containing the ingredient mixture were compared to those from control cigarettes without the ingredients. In practice, flavouring ingredients are typically added to tobacco that also contains casing ingredients and reconstituted tobacco materials. In order to keep the tobacco mixtures as authentic as possible, three comparisons have been made in this study. These are: (a) control cigarette containing a typical US blended, cased tobacco incorporating reconstituted tobacco versus test cigarettes that had flavouring ingredients added to this tobacco; (b) control cigarette containing tobacco only versus test cigarettes with the tobacco cased and incorporating flavourings; (c) control cigarette containing tobacco only versus test cigarette incorporating additives made in an experimental sheet material. The significances of differences between the test and control cigarettes were determined using both the variability of the data on the specific occasion of the measurement, and also taking into account the long-term variability of the analytical measurements over the one-year period in which analyses were determined in the present study. This long-term variability was determined by measuring the levels of the 44 "Hoffmann analytes" in a reference cigarette on many occasions over the one-year period of this study. The ingredients were added to the experimental cigarettes at or above the maximum levels used commercially by British American Tobacco. The effect of the ingredient mixtures on total particulate matter and carbon monoxide levels in smoke was not significantly different to the control in most cases, and was never more than 10% with any ingredient mixture. It was found that, in most cases, the mixtures of flavouring ingredients (generally added in parts per million levels) had no statistically significant effect on the analyte smoke yields relative to the control cigarette. Occasionally with some of the mixtures, both increases and decreases were observed for some smoke analyte levels relative to the control cigarette. These differences were generally up to about 15% with the mixtures containing flavouring ingredients. The significance of many of the differences was not present when the long-term variability of the analytical methodology was taken into account. For the test cigarettes with ingredient mixtures containing casing ingredients, there were again no significant changes in smoke analyte levels in most cases. Those changes that were observed are as follows. Decreases in smoke levels were observed with some ingredient mixtures for most of the tobacco specific nitrosamines (up

to 24%), NO_x, most of the phenols (up to 34%), benzo[a]pyrene, and some of the aromatic amines and miscellaneous organic compounds on the "Hoffmann list". Increases were observed for some test cigarettes in smoke ammonia, HCN, formaldehyde and lead levels (up to 24%). The significance of the ammonia and lead increases was not present when the long-term variability of the analytical methodology was taken into account. The yields of some carbonyl compounds in smoke were increased in one comparison with an additives mixture containing cellulosic components; in particular, formaldehyde was increased by 68%. This was the largest single change seen in any smoke analyte level in this study. These carbonyls are produced from the pyrolysis of cellulosic and other polysaccharide materials, present in the additives mixture. With this test cigarette, all tobacco specific nitrosamines, phenols, semi-volatile bases, NO, and some aromatic amines and miscellaneous organic compounds on the "Hoffmann list" were decreased, by up to 22%. The significance of many of these differences remained even when the long-term variability of the analytical methodology was taken into account. The levels of all other "Hoffmann analytes" in the smoke were not significantly different to those of the control cigarette. With the exception of the determinations of "tar", nicotine and carbon monoxide, there are currently no internationally recognised standard methods for measurement of the other "Hoffmann analytes". Each laboratory uses its own methods and there are large laboratory-to-laboratory variations, as well as variations over time in a given laboratory. Therefore, it is important that in any comparison of smoke analytes amongst different cigarettes, all the analytes should be measured in the same laboratory and at the same time. This was the case in the present study and all the methods have been validated internally.

9. The effect of tobacco ingredients on smoke chemistry. Part II: Casing ingredients

Baker RR, Pereira da Silva JR, Smith G.
Food Chem Toxicol. 2004;42 Suppl:S39-52.

This is the second part of a study in which the effects of adding a range of ingredients to tobacco on the chemistry of cigarette mainstream smoke are assessed. The examination of smoke chemistry has concentrated on those constituents in smoke that regulatory authorities in the USA and Canada believe to be relevant to smoking-related diseases. In this part of the study the effects of 29 casing ingredients and three humectants have been assessed at the maximum levels typically used on cigarettes by British American Tobacco. This brings the total number of ingredients assessed in Parts I and II of this study to 482. The casing ingredients were added at levels of up to 68 mg on the cigarettes. Their effects on smoke constituents were generally larger than the effects of flavouring ingredients, which were added at parts per million levels. Many of the casing ingredient mixtures either had no statistically significant effect on the level of the analytes investigated in smoke relative to a control cigarette, or they produced decreases of up to 44% in some cases. Those analytes that were increased in smoke are highlighted in this paper. The largest increases were for formaldehyde levels, up to 26 microg (73%) in one case, observed from casing mixtures containing sugar. This is most likely due to the generation of formaldehyde by pyrolysis of sugars. Occasional small increases were also observed for other analytes. However, the statistical significance of many of these

increases was not present when the long-term variability of the analytical method was taken into account. The significance and possible reasons for the increases are discussed.

10. An overview of the effects of tobacco ingredients on smoke chemistry and toxicity -

**Baker RR, Massey ED, Smith G.
Food Chem Toxicol. 2004;42 Suppl:S53-83.**

ABSTRACT: This paper presents an overview of a series of studies designed to assess the influence of 482 tobacco ingredients on cigarette smoke chemistry and toxicity. The studies are: pyrolysis of the ingredients; influence of the ingredients on smoke constituents believed by regulatory authorities to be relevant to smoking-related diseases ("Hoffmann analytes"); influence of the ingredients on in vitro genotoxicity and cytotoxicity of smoke particulate matter; and influence of the ingredients on the inhalation toxicity of smoke. The present paper brings the salient features of these studies together. A pyrolysis technique has been developed which, as far as practicably possible, mimics the combustion conditions inside a burning cigarette. The results from 291 single-substance ingredients indicate that almost a third would transfer out of the cigarette burning zone at least 99% intact (i.e. less than 1% pyrolysis), and almost two thirds would transfer at least 95% intact. Of the ingredients that underwent some degree of pyrolysis, a few "Hoffmann analytes" were detected amongst the pyrolysis products of 19 ingredients. Taking into account maximum use levels, their maximum pyrolysis levels were generally small and often insignificant compared to the levels typically present in smoke. Possible exceptions were acetaldehyde and benzene from the pyrolysis of malic acid. However, subsequent smoke chemistry studies indicated that the maximum levels predicted from pyrolysis of this involatile substance were overestimated, suggesting that malic acid does not undergo complete pyrolysis in the burning cigarette and/or generates acetaldehyde and benzene at similar rates to that of tobacco on a per weight basis. When added to tobacco, many of the ingredient mixtures produced no significant effect on the levels of many of the "Hoffmann analytes" in smoke, while some produced increases or decreases relative to the relevant control cigarettes. The study has concentrated on the increases. Many of the differences were found to be not significant when the long-term variability of the analytical methodology was taken into account. However, even taking this into account, the smoke formaldehyde levels in two of the test cigarettes were significantly increased relative to their controls, by up to 26 microg (73%). These increases are likely to be due to the pyrolysis of sugars, cellulose and other polysaccharide materials. The activity of smoke particulate matter from cigarettes containing tobacco ingredients has been determined with three in vitro bioassays, two for genotoxicity and one for cytotoxicity. These were the Ames test, the mammalian cell micronucleus assay, and the neutral red uptake cytotoxicity assay. Within the sensitivity and specificity of these bioassays, the specific activity of the cigarette smoke particulate matter was not changed by the addition of ingredients to the cigarette. Three 90-day sub-chronic inhalation studies have been undertaken and histopathological and histomorphometric assessments made within the respiratory tracts of animals exposed to smoke from cigarettes containing the various ingredient mixtures and their control

cigarettes. The response due to tobacco smoke exposure was not distinguishable between the test and control cigarettes, indicating that the presence of the ingredients had made no discernable differences to the type and severity of the treatment-related changes.

RELEVANT REVIEWS & INTERESTING PAPERS

1. Evaluation of certain food additives and contaminants -

Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives WHO Technical Report Series 922, 2004 Geneva

GENERAL COMMENT: In this document, examples of additives that were reviewed include citric acid, 2 methylheptanoic, citral, citronellol and much more.

ABSTRACT: This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives, with a view to recommending acceptable daily intakes (ADIs) and to prepare specifications for the identity and purity of food additives. The first part of the report contains a general discussion of the principles governing the toxicological evaluation of food additives (including flavouring agents) and contaminants, assessments of intake, and the establishment and revision of specifications for food additives. A summary follows of the Committee's evaluations of toxicological and intake data on various specific food additives (a-amylase from *Bacillus licheniformis* containing a genetically engineered a-amylase gene from *B. licheniformis*, annatto extracts, curcumin, diacetyl and fatty acid esters of glycerol, D-tagatose, laccase from *Myceliophthora thermophila* expressed in *Aspergillus oryzae*, mixed xylanase, b-glucanase enzyme preparation produced by a strain of *Humicola insolens*, neotame, polyvinyl alcohol, quillaia extracts and xylanase from *Thermomyces lanuginosus* expressed in *Fusarium venenatum*), flavouring agents, a nutritional source of iron (ferrous glycinate, processed with citric acid), a disinfectant for drinking-water (sodium dichloroisocyanurate) and contaminants (cadmium and methylmercury). Annexed to the report are tables summarizing the Committee's recommendations for ADIs of the food additives, recommendations on the flavouring agents considered, and tolerable intakes of the contaminants considered, changes in the status of specifications and further information requested or desired.

COMMENTS: This is a massive report that one needs to be aware of since this Committee had access to documents called Technical Data Sheets, which were prepared using new or existing food additives and which had not been published because the detailed information on manufacturing processes described therein could be commercially sensitive. These documents, however, also contain valuable information, which was not made public, on chemical and technological approaches.

The Committee recognized the need for a working definition of the term “flavouring agent” and recommended that such a definition be agreed at a future meeting. At its present meeting, the Committee noted that a range of regulatory definitions of “flavouring” and similar terms exist in different countries and concluded that any definition would need to be elaborated in an international forum, such as the Codex Alimentarius Commission. The Committee reiterated the criteria that need to be met for an individual flavouring agent to be evaluated by the existing Procedure for the Safety Evaluation of Flavouring Agents:

- The substance should be chemically defined, such that at least 95% of the commercially used material consists either of the named chemical, or of the named chemical and identified secondary constituents. The substance is added to food for flavouring purposes, including the generation of active flavouring substances during storage or processing of the food.
- There is a valid estimate of current exposure to the named substance and, if appropriate, its breakdown or reaction products.

Some substances that have a use as flavouring agents may have been evaluated previously by the Committee in relation to other food additive functions. The use of such a substance, or its breakdown or reaction products, as a flavouring agent is included in the relevant, previously-established ADI.

2. Human functional neuroimaging in nicotine and tobacco research: Basics, background, and beyond - 2004 –

F. Joseph McClernon and David G. Gilbert

Nicotine & Tobacco Research Volume 6 : 941 - 959

ABSTRACT: Modern functional neuroimaging techniques allow nicotine and tobacco researchers to investigate the neurobiological basis of addiction in humans. We introduce the methods and measures of the following neuroimaging techniques: Electroencephalography and event-related cortical potentials, positron emission tomography, and functional magnetic resonance imaging. We outline strengths and limitations across modalities and describe new and emerging technologies. We provide summaries of recent neuroimaging findings in the field of nicotine and tobacco research for neurochemistry, smoking and nicotine administration, craving and cue-reactivity, cognitive and affective information processing, and tobacco withdrawal. We address limitations of studies to date and identify opportunities for future research.

3. Effect of exposure to secondhand smoke on markers of inflammation: the ATTICA study –

Demosthenes B. Panagiotakos PhDa, , , Christos Pitsavos MD, PhDa, Christina Chrysohou MD, PhDa, John Skoumas MDa, Constadina Masoura MDa, Pavlos Toutouzias MD, PhDa and Christodoulos Stefanadis MD, PhDa

The American Journal of Medicine Volume 116, Issue 3 , 1 February 2004, Pages 145-150

ABSTRACT: We sought to investigate the effect of secondhand smoke exposure on inflammatory markers related to cardiovascular disease. Methods. During 2001 to 2002,

we randomly selected a stratified (age-sex) sample of adults without clinical evidence of cardiovascular disease. Exposure to secondhand smoke (>30 minutes per day and ≥ 1 day per week) was recorded. Multivariate regression analysis was used to evaluate the effects of exposure to secondhand smoke on levels of C-reactive protein, fibrinogen, homocysteine, and oxidized low-density lipoprotein (LDL) cholesterol, and on white blood cell count. Results. One hundred and thirty-seven (38%) of the 357 men who had never smoked and 211 (33%) of the 638 never-smoking women reported current exposure to secondhand smoke. Compared with those who were not exposed to secondhand smoke, those exposed more than 3 days per week had higher white blood cell counts (by 600 cells per μL ; $P = 0.02$), as well as higher levels of C-reactive protein (by 0.08 mg/dL; $P = 0.03$), homocysteine (by 0.4 $\mu\text{mol/L}$; $P = 0.002$), fibrinogen (by 5.2 mg/dL; $P = 0.4$), and oxidized LDL cholesterol (by 3.3 mg/dL; $P = 0.03$), after adjusting for several potential confounders. Conclusion: Our findings suggest another pathophysiological mechanism by which exposure to secondhand smoke is associated with the development of atherosclerosis.

4. Influence of smoking and sinus on the prevalence and incidence of type 2 diabetes amongst men: the northern Sweden MONICA study

M. Eliasson^{1,2}, K. Asplund², S. Nasic² & B. Rodu³

Journal of Internal Medicine Volume 256 Issue 2 Page 101 - August 2004

ABSTRACT: To explore the effect of smoking and smokeless tobacco, 'snus', on the risk of type 2 diabetes. Design. Population-based cross-sectional and prospective follow-up study in northern Sweden. Subjects. A total of 3384 men, aged 25–74 years, who participated in the MONICA study in 1986, 1990, 1994 or 1999, 1170 of whom had an oral glucose tolerance test. In 1999, 1757 men from previous cohorts returned for re-examination. Main outcome measures. We compared the prevalence of type 2 diabetes or pathological glucose tolerance (PGT) amongst tobacco users to that of nonusers at entry into the study and at follow-up, using odds ratios. Results. Compared with never users, the ageadjusted risk of prevalent clinically diagnosed diabetes for ever smokers was 1.88 (CI 1.17–3.0) and for smokers 1.74 (0.94–3.2). Corresponding odds ratios for snus users were 1.34 (0.65–2.7) and 1.18 (0.48–2.9). We found no increased risk of prevalent PGT in snus users or smokers. Former smokers and snus users had an insignificantly increased risk for PGT. Compared with nonusers, the age-adjusted risk of developing clinically diagnosed diabetes during follow-up was 4.63 (1.37–16) in consistent exclusive smokers, 3.20 (1.16–8.8) in ex-smokers and no cases in consistent snus users. The risk of PGT during follow-up was not increased in consistent tobacco users but evident, although not statistically significant, in those who quit snus during the follow-up period, 1.85 (0.60–5.7). Adjustment for physical activity and alcohol consumption did not change the major findings. Conclusions. The risk of diabetes for snus users was not significantly increased. Smoking was associated with prevalent and incident cases of diabetes. Ex-tobacco users tended towards more PGT.

COMMENTS: This paper describes an epidemiological study comparing the effects of smoking and smokeless tobacco use on type 2 diabetes. The study confirmed previous

findings that smoking is a risk factor for type 2 diabetes, but did not find a similar association with the use of smokeless tobacco.

5. Chemically induced renal tubule tumors in the laboratory rat and mouse: Review of the NCI/NTP database and categorization of renal carcinogens based on mechanistic information

Edward Lock; Gordon Hard

Critical Reviews in Toxicology, Volume 34, Number 3, May-June 2004, pp. 211-299(89)

Abstract: The incidence of renal tubule carcinogenesis in male and female rats or mice with 69 chemicals from the 513 bioassays conducted to date by the NCI/NTP has been collated, the chemicals categorized, and the relationship between carcinogenesis and renal tubule hyperplasia and exacerbation of the spontaneous, age-related rodent disease chronic progressive nephropathy (CPN) examined. Where information on mechanism or mode of action exists, the chemicals have been categorized based on their ability to directly or indirectly interact with renal DNA, or on their activity via epigenetic pathways involving either direct or indirect cytotoxicity with regenerative hyperplasia, or exacerbation of CPN. Nine chemicals were identified as directly interacting with DNA, with six of these producing renal tubule tumors at high incidence in rats of both sexes, and in some cases also in mice. Ochratoxin A was the most potent compound in this group, producing a high tumor incidence at very low doses, often with metastasis. Three chemicals were discussed in the context of indirect DNA damage mediated by an oxidative free radical mechanism, one of these being from the NTP database. A third category included four chemicals that had the potential to cause DNA damage following conjugation with glutathione and subsequent enzymatic activation to a reactive species, usually a thiol-containing entity. Two chemicals were allocated into the category involving a direct cytotoxic action on the renal tubule followed by sustained compensatory cell proliferation, while nine were included in a group where the cell loss and sustained increase in renal tubule cell turnover were dependent on lysosomal accumulation of the male rat-specific protein, 2-globulin. In a sixth category, morphologic evidence on two chemicals indicated that the renal tumors were a consequence of exacerbated CPN. For the remaining chemicals, there were no pertinent data enabling assignment to a mechanistic category. Accordingly, these chemicals, acting through an as yet unknown mechanism, were grouped as either being associated with an enhancement of CPN mechanism, were grouped as either being associated with an enhancement of CPN (category 7, 16 chemicals), or not associated with enhanced CPN (category 8, 4 chemicals). A ninth category dealt with 11 chemicals that were regarded as producing increases in renal tubule tumors that did not reach statistical significance. A 10th category discussed 6 chemicals that induced renal tumors in mice but not in rats, plus 8 chemicals that produced a low incidence of renal tubule tumors in mice that did not reach statistical significance. As more mechanistic data are generated, some chemicals will inevitably be placed in different groups, particularly those from categories 7 and 8. A large number of chemicals in the series exacerbated CPN, but those in

category 7 especially may be candidates for inclusion in category 6 when further information is gleaned from the relevant NTP studies. Also, new data on specific chemicals will probably expand category 5 as cytotoxicity and cell regeneration are identified as obligatory steps in renal carcinogenesis in more cases. Additional confirmatory outcomes arising from this review are that metastases from renal tubule tumors, while encountered with chemicals causing DNA damage, are rare with those acting through an epigenetic pathway, with the exception being fumonisin B1; that male rats and mice are generally more susceptible than female rats and mice to chemical induction of renal tubule tumors; and that a background of atypical tubule hyperplasia is a useful indicator reflecting a chemically associated renal tubule tumor response. With respect to renal tubule tumors and human risk assessment, chemicals in categories 1 and 2, and possibly 3, would currently be judged by linear default methods; chemicals in category 4 (and probably some in category 3) as exhibiting a threshold of activity warranting the benchmark approach; and those in categories 5 and 6 as representing mechanisms that have no relevance for extrapolation to humans.

COMMENTS: This paper provides a review of 69 chemicals tested in the National Cancer Institute / National Toxicology Program (NCI/NTP) carcinogenicity bioassay database. The selected chemicals are those that have shown an association with renal tubule tumors in rat and/or mouse, and was focused on oral exposures.

6. Cigarette smoking exacerbates chronic alcohol-induced brain damage: A preliminary metabolite imaging study -

Durazzo TC, Gazdzinski S, Banys P, Meyerhoff DJ.
Alcohol Clin Exp Res. 2004 Dec;28(12):1849-60.

ABSTRACT: Cigarette smoking is common among alcohol-dependent individuals. Nevertheless, previous research has typically not accounted for the potential independent or compounding effects of cigarette smoking on alcohol-induced brain injury and neurocognition. **METHODS:** Twenty-four 1-week-abstinent recovering alcoholics (RAs; 14 smokers and 10 nonsmokers) in treatment and 26 light-drinking controls (7 smokers and 19 nonsmokers) were compared on measures of common brain metabolites in gray matter and white matter of the major lobes, basal ganglia, midbrain, and cerebellar vermis, obtained via multislice short-echo time proton magnetic resonance spectroscopic imaging. Smoking and nonsmoking RAs were also contrasted on measures of neurocognitive functioning, as well as laboratory markers of drinking severity and nutritional status. **RESULTS:** Chronic alcohol dependence, independent of smoking, was associated with lower concentrations of frontal N-acetylaspartate (NAA) and frontal choline-containing compounds, as well as lower parietal and thalamic choline. Smoking RAs had lower NAA concentrations in frontal white matter and midbrain and lower midbrain choline than nonsmoking RAs. A four-group analysis of covariance also demonstrated that chronic cigarette smoking was associated with lower midbrain NAA and choline and with lower vermian choline. In smoking RAs, heavier drinking was associated with heavier smoking, which correlated with numerous subcortical metabolite abnormalities. The 1-week-abstinent smoking and nonsmoking RAs did not differ

significantly on a brief neurocognitive battery. In smoking RAs, lower cerebellar vermis NAA was associated with poorer visuomotor scanning speed and incidental learning, and in nonsmoking RAs lower vermis NAA was related to poorer visuospatial learning and memory. **CONCLUSIONS:** These human in vivo proton magnetic resonance spectroscopic imaging findings indicate that chronic cigarette smoking exacerbates chronic alcohol-induced neuronal injury and cell membrane damage in the frontal lobes of RAs and has independent adverse effects on neuronal viability and cell membranes in the midbrain and on cell membranes of the cerebellar vermis. Higher smoking levels are associated with metabolite concentrations in select subcortical structures. Greater consideration of the potential effects of comorbid cigarette smoking on alcohol-induced brain damage and other diseases affecting the central nervous system is warranted.

7. Alert for an epidemic of oral cancer due to use of the betel quid substitutes gutkha and pan masala: A review of agents and causative mechanisms

Urmila Nair, Helmut Bartsch and Jagadeesan Nair
Mutagenesis vol. 19 no. 4 pp. 251-262, July 2004

ABSTRACT: In south-east Asia, Taiwan and Papua New Guinea, smoking, alcohol consumption and chewing of betel quid with or without tobacco or areca nut with or without tobacco are the predominant causes of oral cancer. In most areas, betel quid consists of a mixture of areca nut, slaked lime, catechu and several condiments according to taste, wrapped in a betel leaf. Almost all habitual chewers use tobacco with or without the betel quid. In the last few decades, small, attractive and inexpensive sachets of betel quid substitutes have become widely available. Aggressively advertised and marketed, often claimed to be safer products, they are consumed by the very young and old alike, particularly in India, but also among migrant populations from these areas world wide. The product is basically a flavoured and sweetened dry mixture of areca nut, catechu and slaked lime with tobacco (gutkha) or without tobacco (pan masala). These products have been strongly implicated in the recent increase in the incidence of oral submucous @brosis, especially in the very young, even after a short period of use. This precancerous lesion, which has a high rate of malignant transformation, is extremely debilitating and has no known cure. The use of tobacco with lime, betel quid with tobacco, betel quid without tobacco and areca nut have been classi@d as carcinogenic to humans. As gutkha and pan masala are mixtures of several of these ingredients, their carcinogenic affect can be surmised. We review evidence that strongly supports causative mechanisms for genotoxicity and carcinogenicity of these substitute products. Although some recent curbs have been put on the manufacture and sale of these products, urgent action is needed to permanently ban gutkha and pan masala, together with the other established oral cancer-causing tobacco products. Further, education to reduce or eliminate home-made preparations needs to be accelerated.

COMMENTS: Well-marketed and conveniently packaged commercial preparations containing chewing tobacco with various combinations of lime, betel quid and areca nut have popularized the use of these products in Asia. The authors summarize available

evidence of the carcinogenic potential of these mixtures, and suggest a ban on products such as gutkha and pan masala.

8. Alcohol, acetaldehyde, and digestive tract cancer

**SALASPURO, M. Alcohol, acetaldehyde, and digestive tract cancer. In: Nutrition and alcohol, pp. 393-411. Boca Raton, CRC Press, 2004.
Book Chapter**

ABSTRACT N/A

COMMENTS: This monograph reviews the health issues associated with use of alcohol and states that cancer risk is dose-dependent and alcohol and smoking is synergistic, producing a greater effect together than either alone. Moderate smoking without drinking and moderate drinking without smoking had a slight or negative effect on esophageal cancer risk. But simultaneous exposure to the same moderate amounts increased risk 12 to 19-fold in men and women respectively.

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

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

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

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

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

MATERIALS AND METHODS

Cigarette Design

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

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

TABLE 1
Blend composition of prototype cigarettes

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

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

Cigarette Performance

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

In Vitro Study Design

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

TABLE 2
Ingredients added to test cigarettes in study 1

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

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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

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

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

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

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

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

^aChemical Abstract Service registry number.

^bThe Flavor and Extract Manufacturers Association reference number.

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

^dCouncil of Europe reference number.

Inhalation Toxicity Study Design

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

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

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

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

Smoke Generation and Exposure System

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

TABLE 3
Ingredients added to study 2 test cigarettes

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

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

^aChemical Abstract Service registry number.

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

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

^dCouncil of Europe reference number.

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

Exposure Atmosphere Characterization

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

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

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

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

Animals and Animal Care

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

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

Body Weight and Clinical Observations

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

Respiratory Function Measurements

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

Carboxyhemoglobin and Plasma Nicotine Determinations

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

Clinical Pathology

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

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

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

Necropsy and Tissue Collection

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

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

Histopathology

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

Evaluation of Cell Proliferation Rates of Respiratory-Tract Tissues

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

Statistical Methods

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

RESULTS

Cigarette Performance

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

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

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

Note. Cig, cigarette.

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

In Vitro Mutagenicity Assays

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

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

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

Note. Cig, cigarette.

Exposure Atmosphere Characterization

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

Body Weights and Clinical Observations

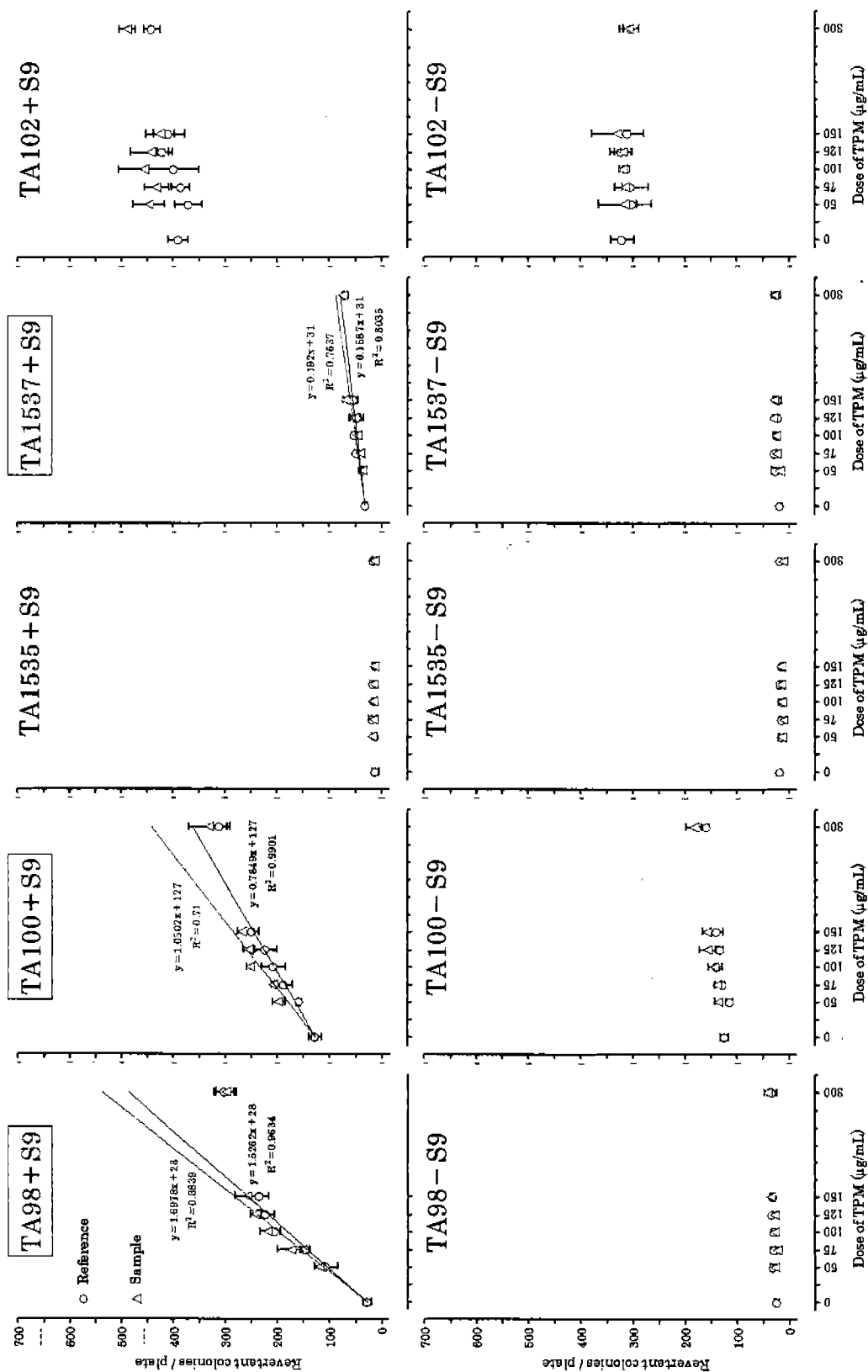
No significant mortality occurred in either study. Exposure-related adverse clinical signs were absent. Clinical observations noted were minor in consequence and low in incidence.

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

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

Organ Weights

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



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

FIG. 1. Ames assay results, study 1 cigarettes.

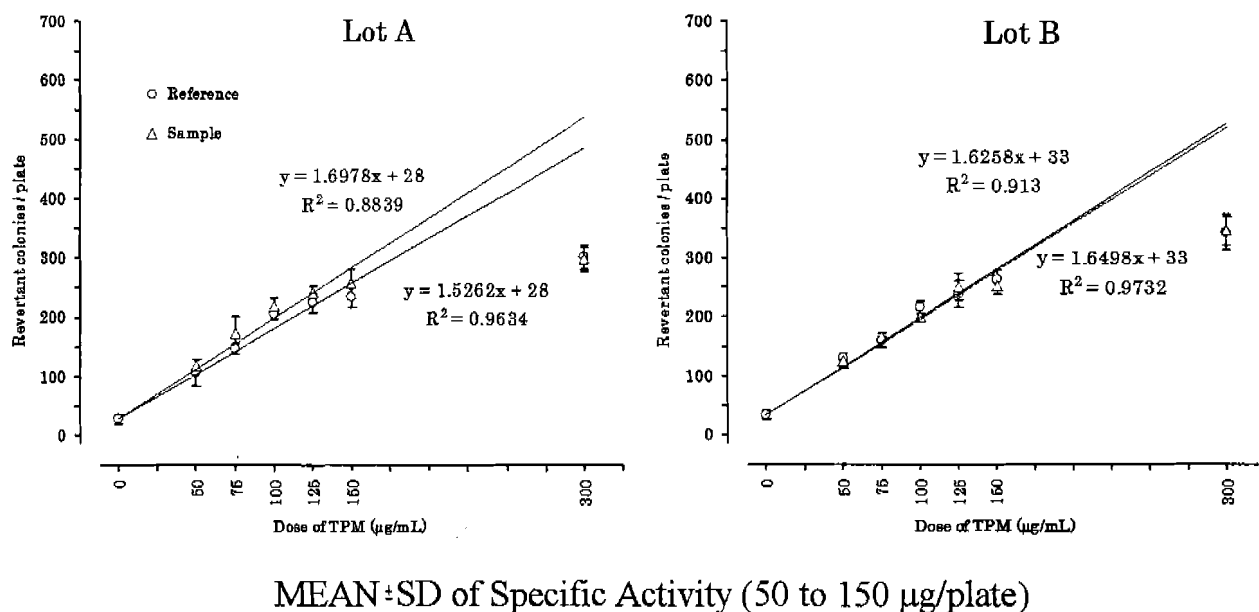


FIG. 2. Ames assay results, study 1 with TA98 metabolic activation.

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

Respiratory Physiology

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

Clinical Pathology

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

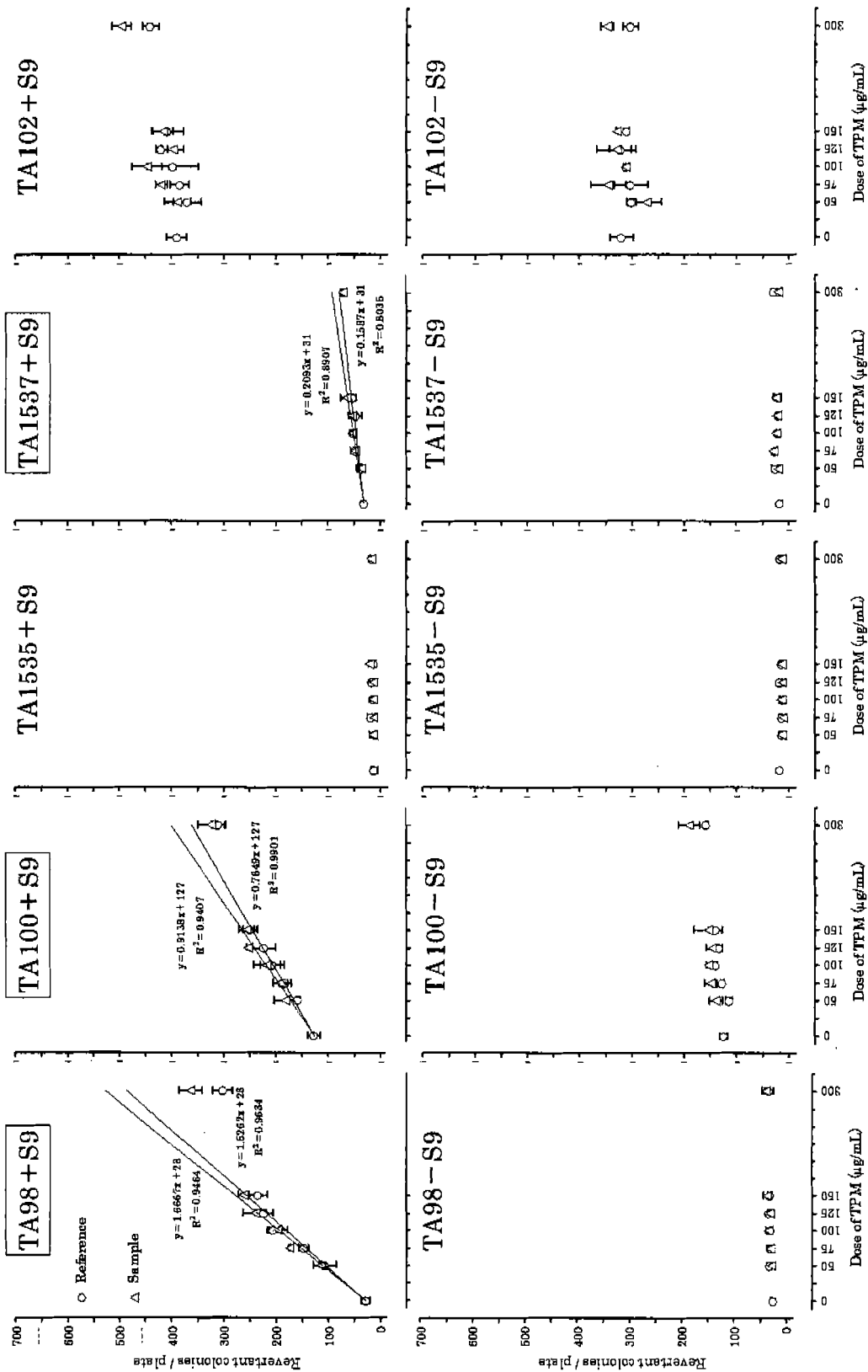
comparable age. There were also statistically significant differences in several hematology and clinical chemistry parameters between groups exposed to similar concentrations of test and reference cigarette smoke. These differences are not considered to be of toxicologic significance, nor were they exposure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

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

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

Pathology

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



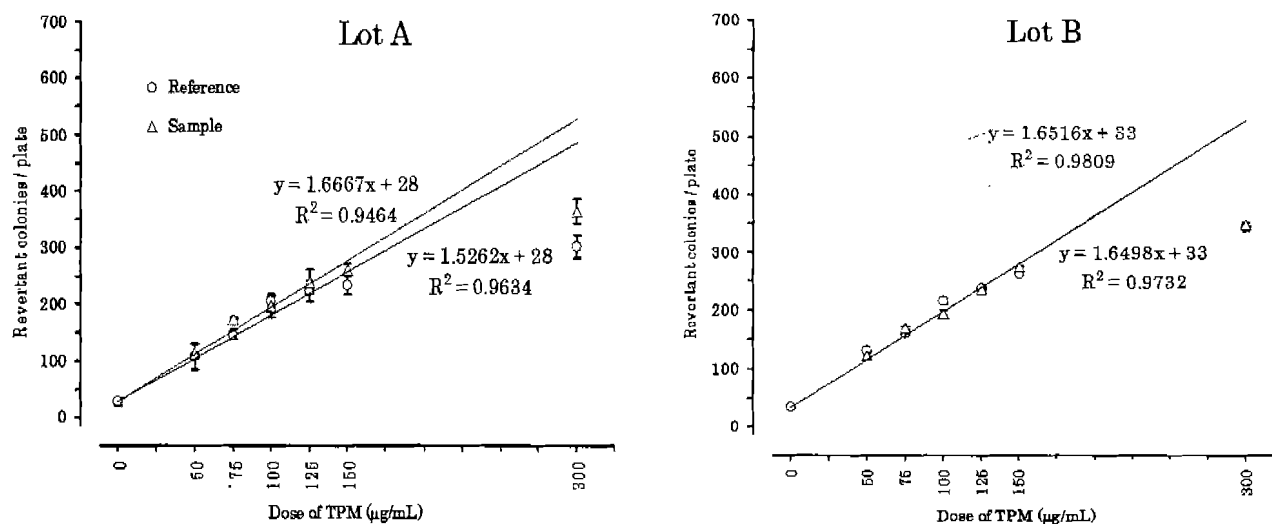
N=2. Only the first lot (Lot A) is indicated in this figure.
The second lot (Lot B) showed the same tendency as the first lot.

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

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

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

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



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

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

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

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

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

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

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

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

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

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

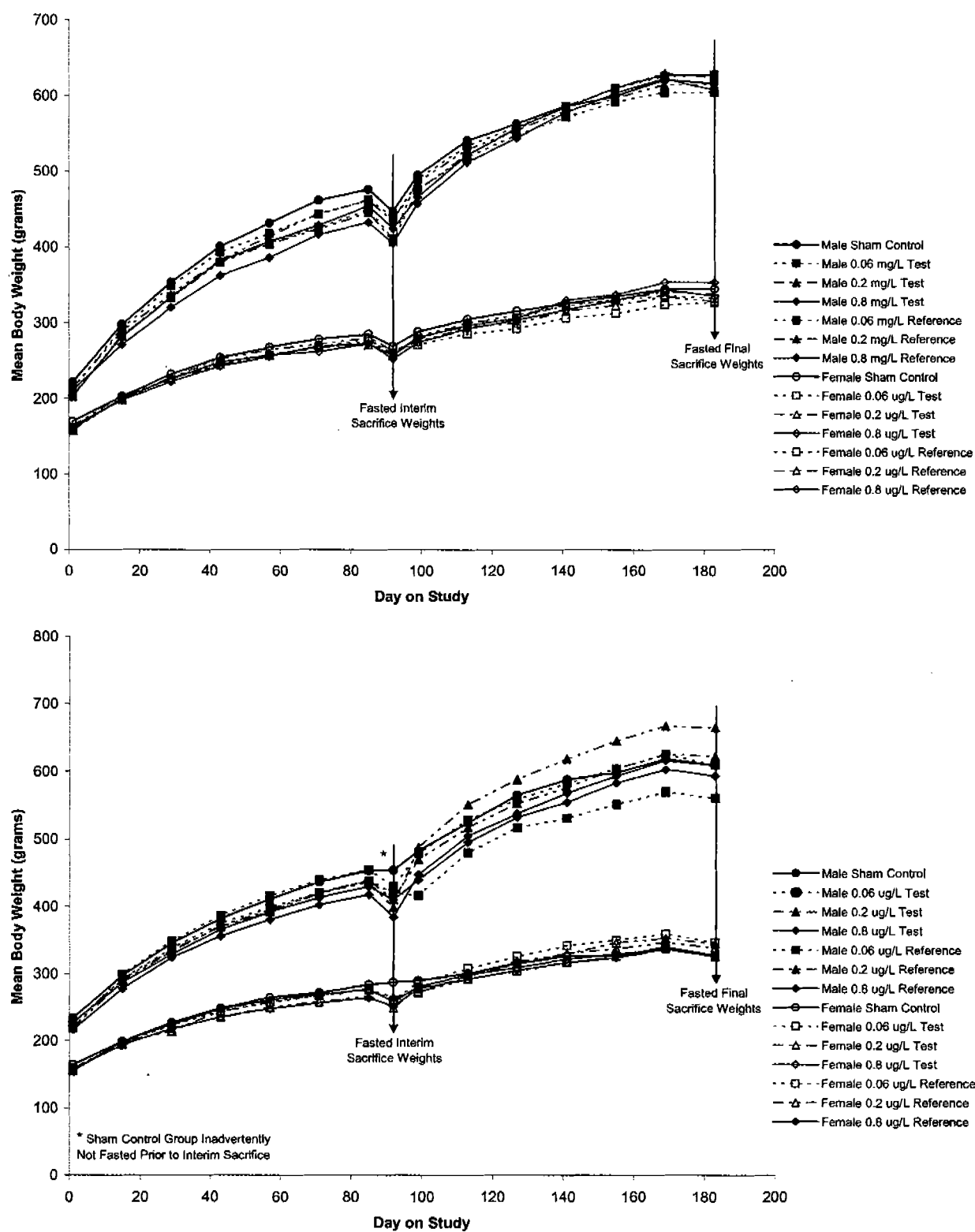


FIG. 5. Body weights, study 1 (top) and study 2 (bottom).

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

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

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

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

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

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

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

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

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

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

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

^aNumber of tissues or animals examined.

^bNumber of diagnoses made.

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

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

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

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

^aNumber of tissues or animals examined.

^bNumber of diagnoses made.

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

Evaluation of Cell Proliferation Rates

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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Scientific Committee on Consumer Safety

SCCS

OPINION
on
Fragrance allergens in cosmetic products

The SCCS adopted this opinion at its 15th plenary meeting

of 26-27 June 2012

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Summary

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

1. Background

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

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

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

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

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

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

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

Furthermore, two fragrances (natural mixtures) were added

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

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

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

2. Terms of reference

- 1. Does the SCCS still consider that the fragrance allergens currently listed in Annex III, entries 67-92, for labelling purposes represent those fragrance ingredients that the consumer needs to be made aware of when present in cosmetic products?*
- 2. Can the SCCS establish any threshold for their safe use based on the available scientific data?*
- 3. Can the SCCS identify substances where processes (e.g. metabolism, oxidation and hydrolysis) may lead to cross-reactivity and new allergens which are relevant for the protection of the consumer?*

3. Introduction

Fragrance ingredients

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

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

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

4. Clinical aspects of contact allergy to fragrance ingredients

4.1. Spectrum of reactions

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

4.1.1. Allergic contact dermatitis

Mechanism

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

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

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

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

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

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

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

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

Overview of clinical features

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

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

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

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

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

Hands

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

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

Axillae

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

Face

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

4.1.2. Irritant reactions (including contact urticaria)

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

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

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

4.1.3. Pigmentary anomalies

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

4.1.4. Photo-reactions

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

4.1.5. General/respiratory

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

4.2. Patch testing

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

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

4.3. Epidemiology of fragrance allergy

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

A fragrance formula may consist of ten to 300 or more different ingredients. The CosIng database lists 2587 ingredients used for perfuming¹, as well as several other materials classified as odour “masking” agents, which is equivalent with regard to allergy. A mixture of seven fragrance chemicals and one natural extract, which have been identified as major fragrance allergens in the past (34), are used for diagnosing contact allergy to fragrance

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

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

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

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

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

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

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

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

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

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

4.3.2. Clinical epidemiology

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

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

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

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

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

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

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

Note: § Possibly included in (60).

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

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

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

Fragrance Mix II

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

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

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

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

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

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

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

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

***Myroxylon pereirae* (Balsam of Peru)**

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

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

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

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

Oil of turpentine

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

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

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

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

4.3.3. Population-based epidemiology

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

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

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

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

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

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

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

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

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

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

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

4.4.1. Clinical relevance

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

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

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

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

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

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

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

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

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

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

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

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

Medical history

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

NACDG 2009 study (65)

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

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

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

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

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

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

Frosch 2002 (a) study (67)

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

Frosch 2002 (b) study (97)

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

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

Frosch 2005 study (35)

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

Danish Contact Dermatitis Group 2005-2008 (69)

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

Cosmetic products

Fragrance formulae from cosmetic products

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

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

IVDK “own perfumes” study (106)

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

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

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

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

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

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

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

4.4.3. Elicitation in diagnostic patch tests without clinical history

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

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

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

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

4.5. Socio-economic impact of contact allergy

4.5.1. Health related quality of life

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

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

4.5.2. Occupational restrictions

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

4.5.3. Costs to health care/health economics

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

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

4.6. Allergen avoidance

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4.7. Conclusions

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

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

5. Activation of weak or non-sensitising substances into sensitisers - prehapten and prohapten

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

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

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

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

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

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

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

5.1. Prehapten

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

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

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

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

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

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

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

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

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

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

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

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

Notes: § Bicentric or multicentre studies.

(ox.) Oxidised.

* Stabilised: according to the manufacturer contained additional substances aimed at limiting oxidation.

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

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

Reactions to ox. D- limonene and/or ox. L- limonene ^a	Pos. to ox. D- limonene: 41	14	34	11	27	11	27	(134)*		
	Neg. to ox. D- limonene: 1771	113	6.4	91	5.1	62	3.5			
	Pos. to ox. L- limonene: 36	11	31	12	33	9	25			
	Neg. to ox. L- limonene: 1776	116	6.5	80	4.5	64	3.6			
Reactions to any of ox. linalool, myrcene, caryophyllene	Pos. to any of the tested ox. subst.: 31	12	39	6	31	12	39	(133)*		
	Neg. to any of the tested ox. subst: 1480	93	6	63	4	46	3			
		Pos. to FM I		Pos. to FM II		Pos. to MP		Pos. to coloph.		
		n	%	n	%	n	%	n	%	
Reactions to ox. linalool	Pos. at test conc. 4%: 30	8	26.7	5	16.7	10	33.3	5	16.7	(135)*
	Pos. at test conc. 6%: 55	12	21.8	8	14.5	11	20	8	14.5	
	Pos. at test conc. 11%: 72	14	19.4	9	12.5	14	19.4	9	12.5	
	Total pos. at any test conc: 75/1004	n.g.		n.g.		n.g		n.g.		
	Total neg. at any test conc: 929/1004	56	6.0	29	3.1	45	4.8	24	2.6	

Notes: * Bicentric or multicentre studies.

n.g. Not given.

(ox.) Oxidised.

5.2. Prohaptens

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

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

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

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

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

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

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

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

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

5.3. Conclusions

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

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

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

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

- Further experimental and clinical research in the area of abiotic and/or metabolic activation of fragrance substances is clearly needed to increase the safety for the consumer. Compounds suspected to act as prehaptenes and/or prohaptenes should be considered as allergens, unless it could be demonstrated that they do not become activated by one of the described pathways.

6. Retrieval of evidence and classification of fragrance substances

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

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

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

6.1. Retrieval of evidence

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

6.1.1. Search strategy for clinical data

Method of literature search:

1. Manual search of the issues of the journal “Contact Dermatitis” (for the 26 “annex substances”, which were re-evaluated in the present opinion, starting 1999) up to October 2010, identifying all studies with fragrance substances.
2. PubMed search of CAS numbers identified in the previous opinion, reviews and already identified clinical studies, respectively, and manual screening of identified publications (narrowed for the last 10 years for the 26 “annex substances”), if necessary narrowing the search results by adding “dermatitis” or “allergy”. For example, for citral: 5392-40-5 AND (dermatitis or allergy), translated into
 "5392-40-5"[EC/RN Number] AND
 (
 ("dermatitis"[MeSH Terms] OR "dermatitis"[All Fields])
 OR
 ("hypersensitivity"[MeSH Terms] OR "hypersensitivity"[All Fields] OR "allergy"[All Fields] OR "allergy and immunology"[MeSH Terms] OR ("allergy"[All Fields] AND "immunology"[All Fields]) OR "allergy and immunology"[All Fields])
)
3. Manual search of all RIFM reviews published in supplement issues of “Food and Chemical Toxicology²” in the past 20 years. In case of the least evidence on human sensitisation the substances were preliminarily selected and further research initiated.

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

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

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

6.1.2. Collection of experimental (LLNA) data

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

6.2. Grading of evidence

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

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

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

6.2.1. Quality of a clinical study

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

- (i) Case reports or small case series, focusing on patients with positive (test) reactions to the target substance, sometimes including a set of non-exposed, possibly non-diseased “control patients”; these should present a concise summary of all relevant aspects of the patient's history, diagnostic procedures and possibly further outcomes.
- (ii) Clinical series in which results of a group of patients patch tested with the target substance, often combined with other substances, are presented. In the latter type of report, usually only a minority of patients tested show a positive reaction to the test substance. This implies that the majority of patients can be used to illustrate the proportion of irritant, doubtful and negative reactions. The degree of detail on the patients' histories is usually limited in such studies, compared to case reports.

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

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

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

6.2.2. Quality of an experimental study

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

6.2.3. Quality of “other” evidence

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

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

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

6.3. Aggregating evidence for a final conclusion

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

6.3.1. Established contact allergen in humans

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

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

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

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

6.3.2. Established contact allergen in animals

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

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

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

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

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

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

To qualify as a *possible contact allergen*, if classification as “established ...” or as “likely ...” contact allergen is not applicable, *at least one* of the following criteria must be met:

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

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

6.4. Conclusions

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

7. Reported fragrance allergens from the clinical perspective

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

7.1. Tabular summary of evaluated individual fragrance chemicals

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

7.2. Tabular summary of evaluated natural extracts/essential oils

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

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

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

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

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

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

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

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

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

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

⁴ <http://ec.europa.eu/consumers/cosmetics/cosing/>

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

Notes: r.t. Rarely tested.

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

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

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

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

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

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

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

7.3. Conclusions

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

8. Animal data

8.1. Predictive tests and sensitising potency categories

The animal test methods used in harmonised classification of substances, according to their potential to cause skin sensitisation, are the guinea pig maximisation test (GPMT), the Buehler test⁶ and the local lymph node assay (LLNA)⁷. These methods are used in hazard identification and risk assessment for regulatory purposes under REACH⁸. For registration in REACH, the LLNA is the preferred method for measuring skin sensitisation potential in animals, and justification for the use of other methods needs to be provided. According to the directives on classification and labelling⁹, substances and preparations meeting positive criteria in these tests shall be classified as sensitising and assigned the symbol “Xi” and the risk phrase “R43: May cause sensitisation by skin contact”; or, according to the recent regulation on classification, labelling and packaging (CLP¹⁰) “H317: May cause an allergic skin reaction”.

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

Positive results from the OECD guideline animal tests mentioned above which are sufficient to classify a substance as a skin 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 $SI \geq 3$). For positive LLNAs, an EC3 value is calculated which gives the estimated concentration of a chemical necessary to give a 3-fold increase in proliferative activity compared to vehicle-treated controls.

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

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

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

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

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

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

¹⁰ Regulation No. 1272/2008.

8.1.1. LLNA data

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

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

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

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

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

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

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

Notes: * Material with low levels of oxidation according to (164)

n/a: Not applicable (mixture of compounds).

M: EC3 based on molar concentration

8.1.2. LLNA data on oxidised fragrance substances

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

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

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

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

Notes: Pure: Purified before testing as most commercially available fragrance substances are not pure.

Not ox.: Not purified but used as it was delivered as this is a complex mixture and not a specific substance.

Ox. x w: Oxidised by air exposure during x weeks.

* Acetone:olive oil.

8.2. Methodological considerations

EC3 mean values

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

Vehicle

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

Number of doses and animals

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

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

Units for concentrations

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

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

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

8.3. Summary of animal data by LLNA

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

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

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

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

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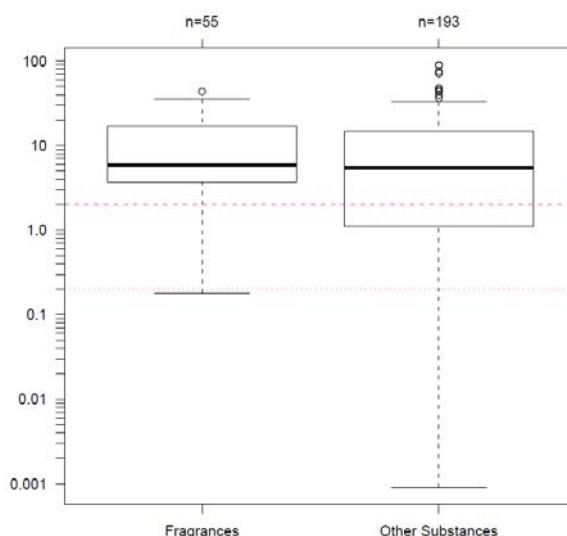


Figure 8-1: The distribution of fragrance chemicals and a variety of other chemicals (e.g. biocides, dyes, plastic materials), taken from the three references (164-166), are depicted as boxplots on a logarithmic scale. The bottom of the box denotes the 1st quartile (25% percentile), the thick line in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

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

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

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

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

8.4. Conclusions

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Table 9-1: Predicted sensitisers.

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

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

Note: § Classified as R43.

Table 9-2: Possible sensitisers.

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

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

Note: [§] Classified as R43.

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

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

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

Table 9-4: Not predictable.

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

9.1. General results

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

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

9.2. Conclusions

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

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

10. Exposure

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

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

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

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

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

10.1. Concentrations and quantities used

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

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

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

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

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

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

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

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

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

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

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

Opinion on fragrance allergens in cosmetic products

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

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

Opinion on fragrance allergens in cosmetic products

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

Notes: HICC Hydroxyisohexyl-3-cyclohexene carboxyaldehyde.

- Fragrance not detected in any product.

n.a. Not analysed.

* Benzyl alcohol could not be determined in 49% of the products due to interference.

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

Opinion on fragrance allergens in cosmetic products

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

INCI name of fragrance	PCP (n = 70)	MP (n = 59)	HP (n = 57)	WP (n = 44)	Cos (n = 39)	Deo (n = 17)	Dent (n = 14)	Total (n = 300)
Linalool	46	47	17	42	26	12	0	190 (63%)
Limonene	34	45	29	43	18	11	9	189 (63%)
Citronellol	23	24	21	37	25	15	0	145 (48%)
Geraniol	19	26	15	36	18	12	0	126 (42%)
BPMP	30	27	21	27	13	8	0	126 (42%)
Hexyl cinnamal	37	20	22	22	14	10	0	125 (42%)
Benzyl salicylate	23	23	10	31	15	12	0	114 (38%)
Alpha-isomethyl ionone	15	20	7	24	28	10	0	104 (35%)
Coumarin	12	27	8	23	12	8	0	90 (30%)
Lyr TM	17	24	3	24	15	5	0	88 (29%)
Eugenol	13	26	4	22	6	6	3	80 (27%)
Citral	2	28	6	29	7	2	0	74 (25%)
Benzyl benzoate	8	9	3	31	11	8	0	70 (23%)
Benzyl alcohol	9	8	1	30	9	3	1	61 (20%)
Hydroxycitronellal	5	6	1	30	6	4	0	52 (17%)
Isoeugenol	2	5	0	17	0	3	0	27 (9%)
Cinnamic alcohol	4	2	0	13	4	2	0	25 (8%)
Farnesol	1	3	0	17	2	0	0	23 (8%)
Amyl cinnamal	5	0	3	7	5	2	0	22 (7%)
Cinnamal	3	4	0	7	0	0	3	17 (6%)
Evermia prunastri/oak moss	0	3	0	5	5	0	0	13 (4%)
Benzyl cinnamate	2	0	0	8	0	0	0	10 (3%)
Evermia furfuracea/tree moss	1	5	0	3	0	0	0	9 (3%)
Anisyl alcohol	0	0	0	1	0	0	0	1 (0.3%)
Amyl cinnamic alcohol	0	0	0	0	0	0	0	0
Methyl heptine carbonate	0	0	0	0	0	0	0	0

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

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

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

Opinion on fragrance allergens in cosmetic products

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

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

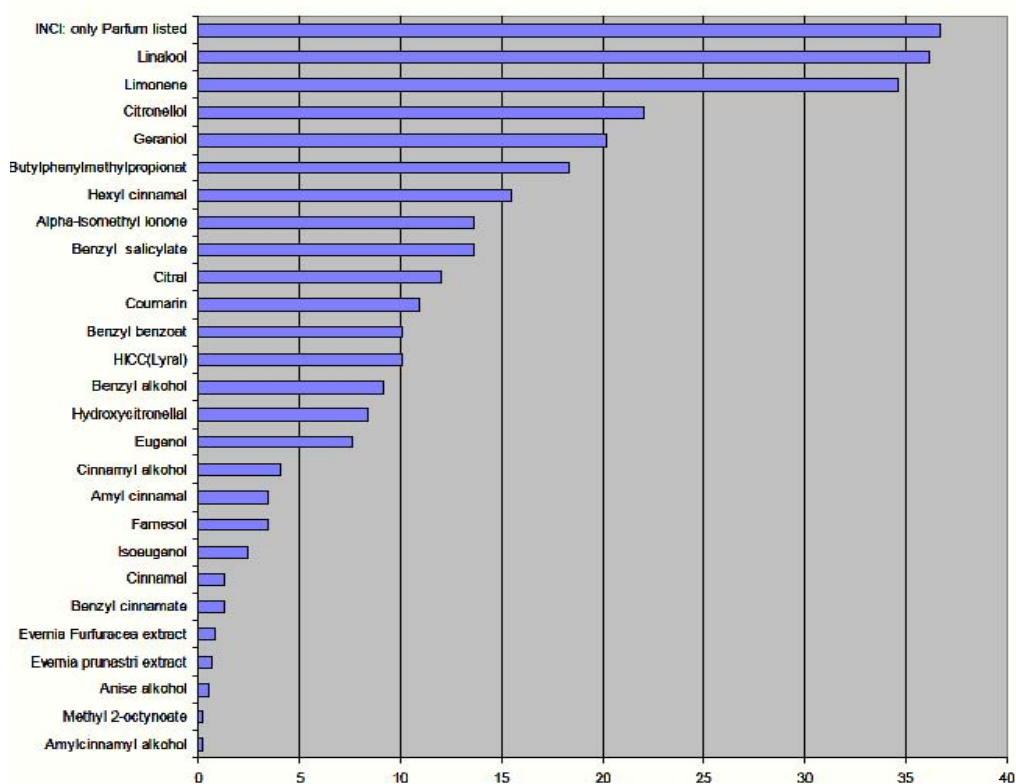


Figure 10-1: Frequency of occurrence in 3,000 consumer products of the 26 fragrance allergens that are required to be labelled in cosmetics and detergents (CVUA Karlsruhe, Germany, 2006/2007), according to (105).

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

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

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

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

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

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

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

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

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

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

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

Notes: n.d. Not detected.

* $P < 0.05$ (chi-square test).

SD: Standard deviation.

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

10.2. Global exposure (household and occupational exposures)

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

Topical pharmaceutical products

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

Childrens products and toys

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

Clothing

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

Cleaning agents and other household products

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

Candles

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

Food

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

Occupational exposure

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

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

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

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

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

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

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

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

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

10.3. Exposures related to particular anatomical sites

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

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

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

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

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

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

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

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

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

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

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

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

10.4. Conclusion

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

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

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

11. Dose-response relationships and thresholds

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

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

11.1. Induction

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

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

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

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

Notes: 1) Exposure per mg/cm²/day is based on 8.5 mg/cm²/day for deodorants, 2.2 for perfumes and 4.2 for hand creams as it is these exposure levels that are used by the IFRA.
 2) Cosmetic Directive Annex III: Hydroxycitronellal restricted to 1% in all products and isoeugenol to 0.02% in all products.

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

It was, among other things, concluded that:

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

and that:

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

11.2. Elicitation

11.2.1. General considerations

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

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

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

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

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

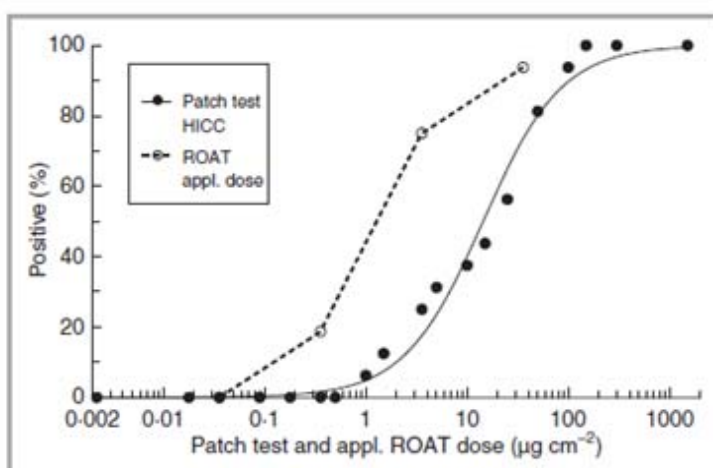


Figure 11-1: The fitted dose-response curve for patch test (solid line) is seen to be displaced to the right compared to the observed response from repeated open applications of the same allergen (HICC). It means that a smaller dose per application is needed to elicit a response than by one single occluded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

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

Volatility effects in skin sensitisation

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

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

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

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

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

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

11.2.2. Studies on specific fragrance ingredients

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

Overview of results

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

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

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

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

Note: HICC hydroxyisohexyl 3-cyclohexene carboxaldehyde.

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

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

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

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

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

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

Summary of results for specific fragrance ingredients

Chloroatranol (constituent of *Evernia prunastri*)

In ROAT a dose of 0.025 µg/cm² to 0.125 µg/cm² in ethanol elicited reactions in 92% to 100% of sensitised subjects.

In patch testing the ED10% was 0.0004 µg/cm².

Cinnamal

In ROAT a dose of 0.26 µg/cm² gave a response in 11% when applied as deodorant in the axilla and 82% responded to 2.63 µg/cm².

The ED50 in patch testing was 96 µg/cm².

HICC

In ROAT a dose of 0.0357 µg/cm² gave no response, while the dose that elicited a reaction in 10% of the sensitised test group (in ethanol) ranged from 0.064 µg/cm² to 1.2 µg/cm². The dose in a cream base was 4.9 µg/cm².

In ROAT a dose of 15.3 µg/cm² to 126.2 µg/cm² in ethanol elicited reactions in 61% to 89% of sensitised subjects.

The ED10 in patch testing ranged from 0.66-0.9 µg/cm².

Hydroxycitronellal

In ROAT a dose of 0.94 µg/cm² gave a response in 57% when applied in a deodorant in the axilla and 100% responded to 9.40 µg/cm².

The no-effect level in patch testing was below 0.036 µg/cm².

Isoeugenol

In ROAT a dose of 2.2 µg/cm² a response in 42% and 9.0 µg/cm² in 67%, when applied in ethanol on the arm. With a deodorant applied to the skin of the axillary, a dose of 0.167 µg/cm² caused a response in 23% and 77% reacted to 1.67 µg/cm².

The ED50 in patch testing was 32 µg/cm².

The no-effect in patch testing was below 0.15 µg/cm².

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

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

General thresholds

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

The reasonably uniform data generated on the above fragrance ingredients are in agreement with a recent “meta-analysis” of dose-response data of different allergens, incorporating some of the same studies as mentioned above, but also other allergens, such as preservatives and metals. The ED₁₀ at patch testing varied by a factor of 7 from the lowest to the highest value and the median was 0.82 µg/cm² if the three outliers formaldehyde (1997), nickel (1999) and methyldibromo glutaronitrile (2004) were left out and 0.84 µg/cm² if included (see Table 11-6 and Figure 11-2 below: (280)). An explanation of these results could be that thresholds in elicitation is less dependent on the antigenic properties of the individual substance (inherent potency) than thresholds of induction and more on the level of sensitivity of the individual, i.e. the level of T-cell clones able to recognise the antigen, which is not present in naïve not-sensitised, individuals. This seems plausible, based on both the recent clinical evidence (280) and guinea pig QSAR evidence (281). It provides the basis for a general approach in establishing safe thresholds for substances of concern.

The consequences of a limit of 0.8 µg/cm² for the product types most important for fragrance allergy are calculated below.

The calculation is based on:

- The generally safe exposure level, which is the median ED₁₀ value (the dose which will elicit allergic contact dermatitis in 10% of sensitised eczema patients) under patch test conditions: 0.8 µg/cm² (280).

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

Equation:

Safe concentration in product = (Generally safe exposure level ($0.8 \mu\text{g}/\text{cm}^2$)/daily exposure to product ($\mu\text{g}/\text{cm}^2/\text{day}$)) x 100 (for %).

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

	Estimated daily exposure level (g) (Table 3 SCCS NoG)	Mean exposed skin surface (cm^2) (Table 2 SCCS NoG)	Exposure /cm^2/day in grams	Exposure /cm^2/day in μg ($1\text{g} = 1 \times 10^6 \mu\text{g}$)	Concentration limit in product % in product: (GEL/daily exposure) x 100
Body lotion	7.82 g	15,670 cm^2	0.000499	499	0.16%
Face cream	1.54 g	565 cm^2	0.002725	2725	0.03%
Hand cream	2.16 g	860	0.002511	2511	0.03%
Deodorant aerosol spray ethanol based	1.43 g	200 cm^2	0.007150	7150	0.01%
Perfume spray	not given	?	0.00221 ¹⁾	2210	0.04%

Note: 1) $2.21 \text{ mg}/\text{cm}^2/\text{day}$ from Technical dossier Quantitative Risk Assessment.

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

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

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

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

Study	Number of patients	ED₁₀ ($\mu\text{g}/\text{cm}^2$)	95 % interval
MCI/MI	12	1.05	0.17–2.27
Formaldehyde	20	20.1	4.09–43.9
Nickel 1997	24	1.58	0.32–4.04
Nickel 1998	19	0.8	0.078–2.59

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

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

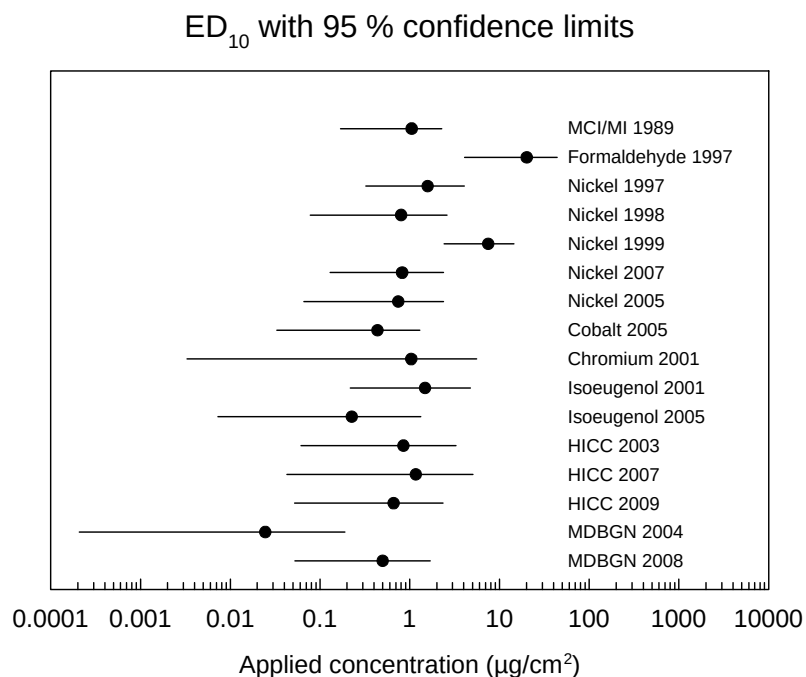


Figure 11-2: The threshold data with 95% confidence intervals from Table 11-6 presented graphically, after (280).

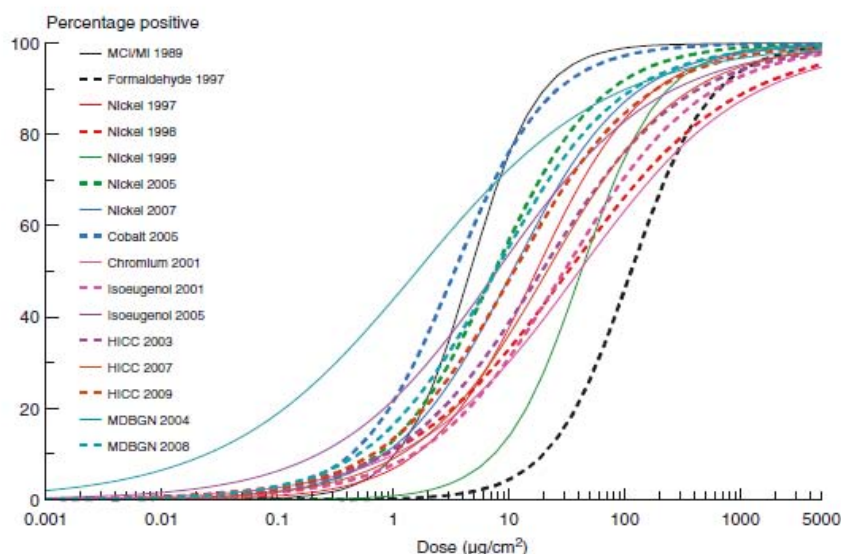


Figure 11-3: The fitted dose-response curves from the studies in Table 11-6, which are the basis for estimation of the ED10 value, after (280).

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

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

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

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

The approach taken by the SCCS is based on scientific evidence published in peer-reviewed journals (283)(284)(285)(286)(102, 224, 263)(264, 279)(287) in the past 20 years. The meta-analysis deriving the general threshold limit at $0.8 \mu\text{g}/\text{cm}^2$ limit has been published (280) in a peer-reviewed journal. The use of threshold limits based on elicitation data is a well established methodology which has been applied (with success) in EU to prevent further cases of induction and elicitation (primary and secondary prevention) in the case of nickel allergy, chromium in cement, chromium in shoes in

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

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

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

Thus in general the potency profile of the fragrance substances of concern is not very different from those included in the model to provide the suggested general safe threshold.

The approach is targeting the relevant end-point, namely, allergic contact dermatitis. The mere consideration of potency of the allergen, according to the LLNA (EC3), is insufficient in identifying the size of the problems of contact allergy/allergic contact dermatitis. Additional information is needed from clinical and epidemiological studies, exposure assessment and dose-elicitation studies. For instance, the elicitation thresholds of e.g. HICC (EC3: 17.1) and isoeugenol (EC3: 0.54) are very similar (0.85 µg/cm² and 0.89 µg/cm², respectively) despite very different potencies. Both are frequent causes of contact allergy.

It should be noted that the general threshold is only suggested to be used for substances of concern if no specific data of sufficient quality exist to set an individual safe threshold. In cases where specific data of sufficient quality are available, these data should be used to set an individual safe threshold.

The general threshold is indicative of a safe level for the majority of sensitised individuals, but does not preclude that the most sensitive subset of the population may react upon exposure to the allergen. These levels are based on patch tests and take no account of anatomical sites of exposure, frequency of exposure or vehicle effects. Therefore, any limitations in exposures are not substitutes for providing information to the consumer about the presence of a substance in a product as a certain fraction of sensitised individuals will still need to avoid specific exposures.

Based on experience, limitations in exposure based on elicitation thresholds will, apart from helping the sensitised consumer, also significantly reduce the risk of induction. This is the case for nickel allergy, where the restrictions in the EU nickel directive are based on elicitation threshold, leading to a significant reduction in new cases of sensitisation in young women (288) and in a reduction in morbidity, i.e. elicitation (289). Another example is restriction of chromium VI in cement (290).

It is not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist, and the model providing the general use concentration limit (0.01%) has been based on chemicals only.

The SCCP concluded in 2004 that Chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in consumer products because they are extremely potent allergens (239). The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents.

11.3. Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported individual fragrance chemical causing allergy since the 1999 opinion on fragrance allergy. In total, reports of about 1500 cases have been published in the scientific literature (see chapter 7.1 and Annex I to this opinion), while the second most

frequently reported individual chemical was cinnamal with around 350 published cases. Only a minority of the cases seen by clinicians is published and only a (small) proportion of those with allergic contact dermatitis seeks or has the possibility to seek medical attention.

Natural extracts such as *Myroxylon pereirae* and turpentine (oil) have been more frequently reported, but while HICC is a synthetic fragrance chemical, where the only source of exposure is fragrances, the natural extracts are used in many other contexts than fragrances/cosmetics.

Of patients tested by the Danish monitoring network of dermatologists 2.4% were found to be allergic to HICC in 2005-2008 (with no decreasing trend from 2003 to 2007 (291)) (for more studies see chapter 4.3.2); in 70% of the cases the reaction was of current relevance, i.e. causing disease (69). This is in agreement with the results of a recent German study with HICC, where 48 out of 51 patients (94.1%) with a positive patch test reaction to HICC also reacted in a repeated open application test, simulating normal use conditions of cosmetics containing HICC (105). In a Danish study 69% of 14 HICC allergic individuals developed allergic contact dermatitis from use of cosmetics containing HICC in realistic amounts (102).

On the basis of the high frequency of allergy to HICC, in 2003 the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) recommended 0.02% (200 ppm) as maximum amount of HICC in cosmetic products (292). This has not been implemented and no restrictions apply in the Cosmetic Directive.

The fragrance industry, via the International Fragrance Association (IFRA), has its own safety guidelines. Up until 2003 HICC was used without any restriction; in 2003 a limit of 1.5% HICC in any kind of product was introduced. In 2008 this was changed according to the new risk assessment model (QRA) applied by the fragrance industry to different levels in 11 different product types derived from the QRA (see 11.1). Limits from 0.11% in lip products to 1.5% in hair styling products were set. In 2009 a further lowering was made of the limits by industry with the following reasoning: "The industry firmly believes and continues to support thresholds based on induction rather than elicitation. However, given the exceptional situation in Europe, the fragrance industry elected to take further restrictive action on this material" (293). An overview of the IFRA restrictions is given in the table below.

Table 11-7: Restriction for HICC independent of the QRA according to (293).

IFRA QRA Category	Product type that drives the category	Consumer exposure level 2003–2008 (%)	IFRA Standard July 2008 (%)	IFRA Standard July 2009 (%)
Category 1	Lip products	1.5	0.11	0.02
Category 2	Deodorants/ antiperspirants	1.5	0.15	0.02
Category 3	Hydroalcohols for shaved skin	1.5	0.60	0.2
Category 4	Hydroalcohols for unshaved skin	1.5	1.5	0.2
Category 5	Hand cream	1.5	1.0	0.2
Category 6	Mouthwash	1.5	1.5	Not applicable*
Category 7	Intimate wipes	1.5	0.3	0.02

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Category 8	Hair styling aids	1.5	1.5	0.2
Category 9	Rinse-off hair conditioners	1.5	1.5%	0.2%
Category 10	Hard surface cleaners	1.5	1.5%	0.2%
Category 11	Incidental or non-skin contact	15	Not restricted	Not restricted

Note: HICC Hydroxyisohexyl 3-cyclohexene carboxaldehyde.

QRA Quantitative risk assessment.

* Not applicable because HICC is not approved for flavour use.

As an update since the presentation of the pre-consultation version of the opinion, surveillance data on HICC from two European countries have become available, covering the period 2002-2011 (IVDK/Germany (294)) and 2003-2011 (Danish contact dermatitis group (295)), respectively. The first analysis identified a slight decrease, which was considered “not overwhelming in absolute terms”, namely, from 2.3% in 2002 to 2.1% in 2011 (crude prevalences, Figure 11-4). Thus, despite statistical significance, the decrease is too slight to be interpreted as relevant improvement. In the Danish study, some fluctuation around a mean prevalence of about 2.5% was noted, but no trend (Figure 11-5). It is reported that 74% of the positive reactions were regarded as clinically relevant.

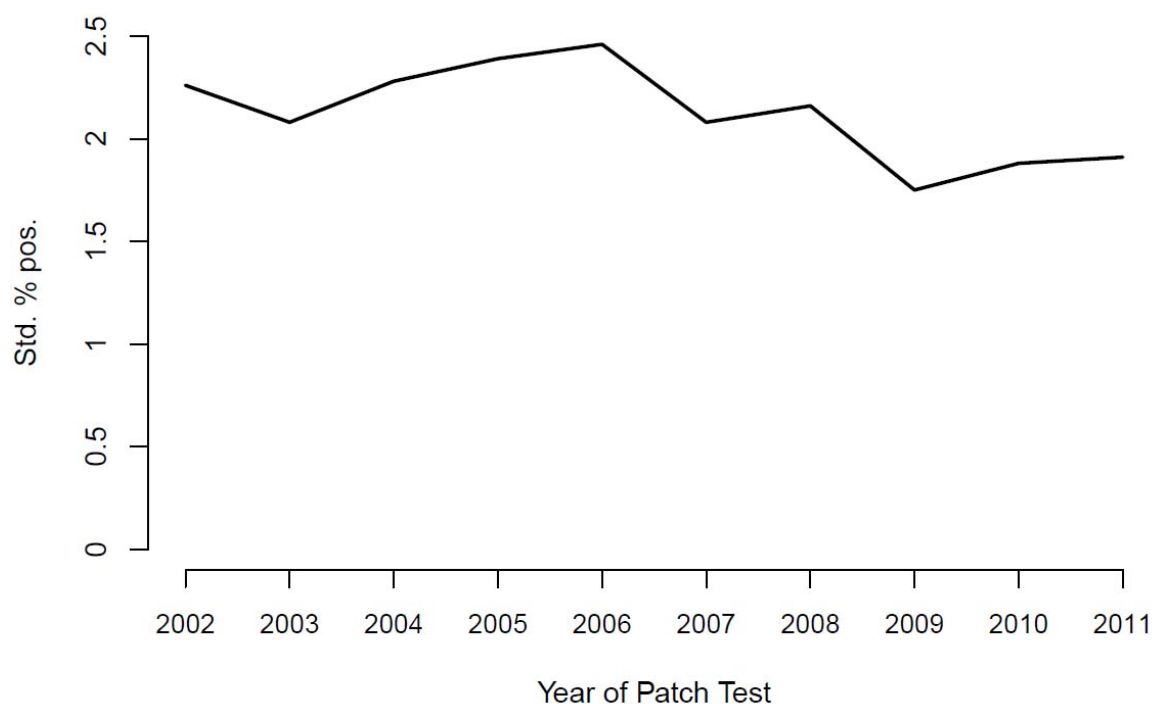


Figure 11-4: Time trend of hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitisation prevalence [standardised prevalence of positives (%)] during 2002-2011. The decrease over time is statistically significant, after **(294)**.

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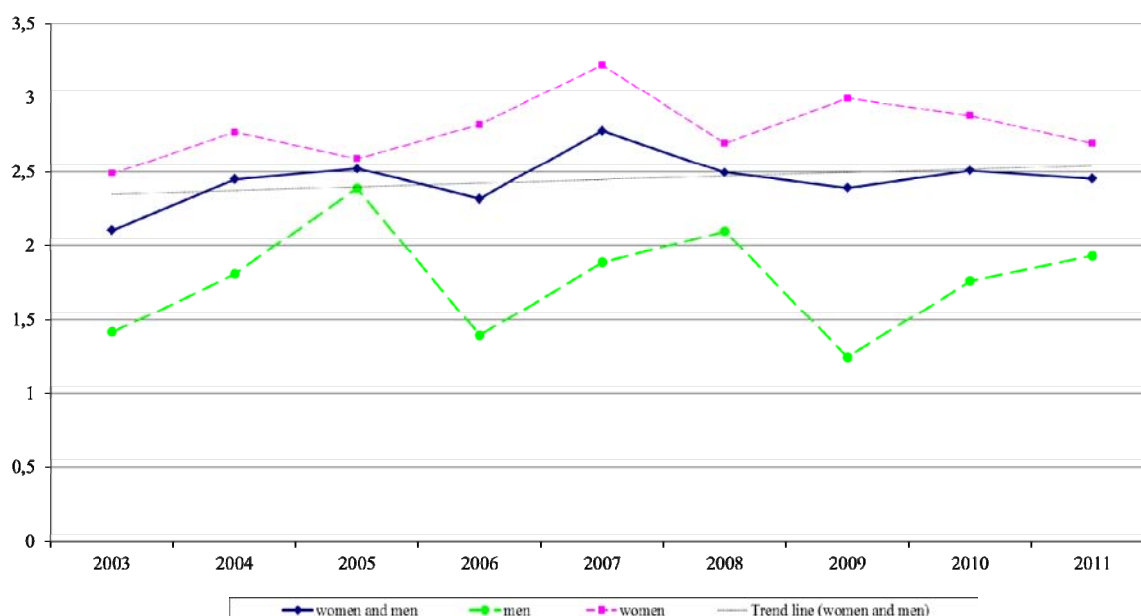


Figure 11-5: Prevalence of positive patch test reactions to hydroxyisohexyl 3-cyclohexene carboxaldehyde over time in 37 860 subjects tested by the Danish Contact Dermatitis Group (295).

11.4. Conclusion

- A dose-response relationship between exposure to contact allergens and induction of allergy (sensitisation) as well as elicitation is well established. This means that in principle, thresholds can be identified which are safe for the consumer.
- A model for dermal sensitisation quantitative risk assessment has been developed (QRA) and implemented by the fragrance industry. This model relies on thresholds, no effect or low-effect levels, established in healthy human volunteers and/or in animal experiments. The SCCP has previously reviewed this methodology and concluded that: "There is no confidence that the levels of skin sensitisers identified by the dermal sensitisation QRA are safe for the consumer."
- Elicitation data can provide thresholds indicative for the safe use of those substances which have already caused significant problems in the consumer. In this context, "safe use" means that the thresholds will protect the majority of consumers from allergic contact dermatitis, but does not preclude that the most sensitive subset of the population may react upon exposure to the allergen.
- Furthermore, based on experience from intervention studies, such thresholds will also be sufficiently low to protect (most of) the non-sensitised consumers from developing contact allergy.
- Elicitation levels have been studied specifically for the fragrance chemicals cinnamal, hydroxycitronellal and isoeugenol. These studies, however, are not adequate to derive safe thresholds for the individual substances directly from the data.
- In the absence of adequate substance specific data it is possible to use a general threshold. Based on a statistical analysis of the available data in the scientific literature, a threshold of $0.8 \mu\text{g}/\text{cm}^2$ was derived. This corresponds to 0.01% (100 ppm) limit in cosmetic products indicative for safe use.

- It is not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist and the model providing the general threshold (0.01%) has been based on individual chemicals only. However the maximum use concentration applies to the identified chemicals both if added as chemicals or as an identified constituent of a natural ingredient. This will also reduce the risk of sensitisation and elicitation from natural extracts.
- For substances for which there are no clinical data of concern, models such as the dermal sensitisation QRA approach may, after refinement and validation, be used to suggest a safe level of exposure prior to incorporation into products. However, aggregated exposures must be incorporated in the dermal sensitisation QRA model.
- HICC has for more than 10 years been recognized as an important allergen with more cases documented in the scientific literature than for any other fragrance chemical in this period. HICC has been shown to be a significant cause of disease as many of those with contact allergy to HICC had also reactions to cosmetics, which contained or were likely to contain HICC. Since 2003 attempts have been made by the fragrance industry to contain the outbreak of HICC allergy, but with no convincing success so far. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Therefore, HICC should not be used in consumer products in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized.
- The SCCP concluded in 2004 that chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in consumer products because they are extremely potent allergens. The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents, despite efforts to reduce the allergen content (296).

12. Data gaps and research needed

In the course of working on this opinion, the following points are highlighted as important data gaps, ordered by research area:

12.1. Clinical and epidemiological research

- Clinical data on more fragrance substances are needed to assess more fully the epidemiology of fragrance contact allergy and pin-point the culprit substances for induction and elicitation of contact allergy in man.
- Data from a broader range of EU countries on the clinical and epidemiological picture of fragrance contact allergy is needed, as difference in exposure and use habits are expected across Europe.
- A co-ordinated strategy for data collection should be developed.
- Very little is known about susceptible groups of the population, e.g. up to 10% of the European population carry mutations, which impair 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 prehapten, 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 prehapten and prohaptens to increase the possibility to diagnose allergy from these type of substances.
- There is a need for more experimental research to further establish the impact of the behaviour of fragrance substances when applied on the skin (including factors such as volatility, autoxidation, skin penetration, reactivity in skin and bioactivation).

12.2. Non-human studies

- Several studies in the industry submission (164) were of insufficient quality, not following the OECD guidelines.
- In some cases it was found that either very few concentrations points had been used in LLNAs, or concentrations were insufficient for achieving a 3-fold increase of the SI.

A sufficient number of doses (concentrations) should be applied in LLNAs (at least 5) so that interpolation (for deriving an EC3 value) can rely on more than two or three actual data points to be more reliable. SCCS therefore suggests a change in the OECD guideline 429. (It is important to remember that the production of unreliable data is a waste of animals.) Moreover, the maximum concentration should be high enough to achieve a > 3-fold increase in SI, as far as this is possible with the substance/vehicle combination chosen.

- Data on experimental results are often not published, but available only on file in the companies having performed the tests. Access to such results would be important for the scientific community, e.g. in the context of REACH, or independently, either to the public domain, or to a Public Trustee.
- The OECD guideline 429 recommends several vehicles. It is well known that a difference in the EC3 value can be obtained for the same substance depending on which vehicle is used in the LLNA. Thus, as an additional control, supplementary to the guideline based LLNA control, a clinically relevant solvent or the commercial formulation in which the test substance is marketed may be used.
- As long as no validated *in vitro* method exists, more research is needed. Until one or more method(s) have been decided to fulfil the requirements for substituting *in vivo* testing, the *in vivo* testing for prediction of skin sensitisation has to be used.
- Applying only mechanism-based QSAR (QMM) as a tool in non-animal based risk assessment for skin sensitisation is of limited value for fragrance substances. This is due to major information gaps in the present model when addressing substances that act via abiotic or metabolic activation, and the high incidence of such substances in fragrances. Therefore, further experimental and clinical research in the area of abiotic and/or metabolic activation of fragrance substances is needed to increase the safety for the consumer, i.e. experimental studies which include air oxidation and bioactivation.
- Further experimental investigations of the sensitisation potential of fragrance substances are needed to determine the impact of the volatility of the substance as well as the effect of the vehicle on skin penetration/absorption and reactivity.
- From a clinical perspective it is important for the individual who is sensitised to one fragrance substance to know if they must also avoid other fragrance substances that can cause allergic contact dermatitis due to cross-reactivity with the original sensitiser. Prediction of risks for cross-reactivity requires sound application of theoretical principles in combination with well-designed experimental studies. This is a field that has not been studied very much so far and needs to be focused on much more in the future.
- Quantitative structure activity relationship (QSAR) models should be further developed, combining, as appropriate, information from *in silico*, *in chemico* and *in vitro* methods as possible. Prediction of different activation pathways should be included.
- Effect estimates such as proportions of sensitised humans or animals, or mean stimulation indices, EC3 values and other derivations should ideally be accompanied by an interval estimate (confidence interval) to address precision (297).

13. Opinion

Contact allergy to fragrances is a common, significant and relevant problem in Europe. The studies since the SCCNFP opinion on fragrance allergy in consumers in 1999 (SCCNFP/0017/98) (SCCNFP 1999) have confirmed that the 26 fragrance allergens, identified by the SCCNFP, are still relevant fragrance allergens for consumers because of their exposure from cosmetic products. Additional exposure to many of these 26 fragrance allergens also occurs from the use of other consumer products, such as detergents, toys, etc. Some of these fragrance substances are also used as preservatives.

The overall trend of fragrance contact allergy appears to have been stable for the last 10 years, as some causes of fragrance allergy have decreased and others increased. From the few population-based studies, it can be estimated that the frequency of contact allergy to fragrance ingredients in the general population in Europe is 1-3%. This is based on the limited testing with eight common fragrance allergens (FM I) out of the approximately 2500 fragrance ingredients listed in CosIng and indicative of the substances that may be present in fragrance compounds. However, the real prevalence of contact allergy to fragrance substances may be higher if the testing were to be performed with the full spectrum of fragrance allergens, including oxidised substances, where relevant.

Among eczema patients in the European population, around 16% are sensitised to fragrance ingredients. The disease can be severe and generalised, with a significant impairment of quality of life and potential consequences for fitness for work.

Contact sensitisation, and its clinical manifestation, allergic contact dermatitis, can be prevented if the exposure to known contact allergens is reduced or abolished (primary prevention). Experiences so far, have indicated that not all substances that later turned out to be significant contact allergens after human exposure, were predicted by experimental studies, e.g. the preservative methyldibromo glutaronitrile and the fragrance chemical HICC. Thus, a significant exposure of the population may occur before a substance is established as an important contact allergen in man.

Elicitation of allergic contact dermatitis occurs when a consumer sensitised to a certain substance is re-exposed to the substance in question. Prevention at this stage, termed secondary prevention, can be achieved if use of the allergen in products is eliminated or reduced to a tolerable level (general prevention), or if the patients succeed in avoiding all sources of exposure (individual prevention). Ingredient listing of individual fragrance allergens has been shown to be an important tool to enable consumers with an identified allergy to reduce/avoid relevant exposures. Moreover, ingredient listing is also of great importance to ensure that an adequate diagnosis of fragrance contact allergy can be made without undue delay. If the information given on the presence of fragrance allergens is incomplete, diagnosis of fragrance contact allergy may be missed.

The SCCNFP, in its 1999 opinion, identified 26 fragrance allergens for which information should be provided to consumers concerning their presence in cosmetic products. This was implemented in the European Cosmetics legislation (298) as ingredient labelling of these 26 fragrance substances (Annex III, entries 67-92). However, safe use concentrations for these substances in cosmetic products have not yet been determined and much new evidence concerning fragrance allergy has been published since 1999. The present opinion updates the SCCNFP opinion with a systematic and critical review of the scientific literature up to October 2010. This review addresses the issue of contact allergy to fragrance substances, including natural extracts and updates the list of fragrance allergens relevant to consumers. Clinical, epidemiological and experimental studies were evaluated, as well as modelling studies performed, to establish lists of: (i) established fragrance allergens; (ii) likely fragrance allergens; and (iii) possible fragrance allergens. The review also includes fragrances, which on modification by oxidation or by enzyme mediated processes, can produce allergens. Available dose-response data have been

examined to answer whether safe thresholds can be established for the most frequent fragrance allergens.

13.1. Question 1

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

In order to answer this question, the SCCS has used clinical and epidemiological data to identify known fragrance allergens. These were categorised as *established contact allergens in humans* (see Table 13-1).

Where sufficient animal evidence was present, these substances were categorised as established contact allergens in animals (Table 13-2). For a number of other fragrance substances, combinations of limited clinical data together with SAR considerations have been applied to indicate likely fragrance allergens in man (Table 13-3). Finally, SAR has also been applied to substances that lack human data to identify fragrance chemicals that have the structural potential to be contact allergens. Substances with insufficient human data were also considered as possible fragrance allergens. For these further tests (experimental/clinical data) are required (Table 13-4).

Table 13-1: Established contact allergens in humans.

For categorisation of importance (+ to +++) see chapter 7.1. Allergens of special concern are substances where between 100 and 1,000 cases (+++) and more than 1,000 (+++++) have been published. These are set in bold. Fragrance substances identified as allergens in the 1999 opinion of SCCNFP (1) are marked with an asterisk.

“ox.” = oxidised; “non-ox.” = non-oxidised; “r.t.” = rarely tested (see chapter 7)

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
Individual chemicals		
ACETYLCEDRENE	32388-55-9	+
AMYL CINNAMAL *	122-40-7	++
AMYL CINNAMYL ALCOHOL *	101-85-9	++
AMYL SALICYLATE	2050-08-0	+
trans-ANETHOLE	4180-23-8	+ (r.t.)
ANISE ALCOHOL *	105-13-5	+
BENZALDEHYDE	100-52-7	+
BENZYL ALCOHOL *	100-51-6	++
BENZYL BENZOATE *	120-51-4	++
BENZYL CINNAMATE *	103-41-3	++
BENZYL SALICYLATE *	118-58-1	++
BUTYLPHENYL METHYLPROPIONAL *	80-54-6	++
CAMPHOR	76-22-2 / 464-49-3	+ (r.t.)
beta-CARYOPHYLLENE (ox.)	87-44-5	Non-ox.: +, ox.: +

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
CARVONE	99-49-0 / 6485-40-1 / 2244-16-8	+ (r.t.)
CINNAMAL*	104-55-2	+++
CINNAMYL ALCOHOL*	104-54-1	+++
CITRAL*	5392-40-5	+++
CITRONELLOL*	106-22-9 / 1117-61-9 / 7540-51-4	++
COUMARIN*	91-64-5	+++
(DAMASCENONE) ROSE KETONE-4	23696-85-7	+ (r.t.)
alpha-DAMASCONE (TMCHB)	43052-87-5 / 23726-94-5	++
cis-beta-DAMASCONE	23726-92-3	+
delta-DAMASCONE	57378-68-4	+
DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	151-05-3	+
EUGENOL*	97-53-0	+++
FARNESOL*	4602-84-0	++ - +++
GERANIOL*	106-24-1	+++
HEXADECANOLACTONE	109-29-5	+ (r.t.)
HEXAMETHYLINDANOPYRAN	1222-05-5	++
HEXYL CINNAMAL*	101-86-0	++
HYDROXYISOHEXYL CARBOXALDEHYDE (HICC)* 3-CYCLOHEXENE	31906-04-4 / 51414-25-6	++++
HYDROXYCITRONELLAL*	107-75-5	+++
ISOEUGENOL*	97-54-1	+++
alpha-ISOMETHYL IONONE*	127-51-5	++
(DL)-LIMONENE*	138-86-3	++ (non-ox.); +++ (ox.)
LINALOOL*	78-70-6	++ (non-ox.) +++ (ox.)
LINALYL ACETATE	115-95-7	+ (non-ox.) ++ (ox.)
MENTHOL	1490-04-6 / 89-78-1 / 2216-51-5	++
6-METHYL COUMARIN	92-48-8	++
METHYL 2-OCTYNOATE*	111-12-6	++
METHYL SALICYLATE	119-36-8	+
3-METHYL-5-(2,2,3-TRIMETHYL-3-	67801-20-1	++ (r.t.)

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

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

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

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

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

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

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

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

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

n.a.: not applicable (natural mixture)

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

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

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

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

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

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

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

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

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

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

*including their respective esters

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

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

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

Conclusions - Question 1

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

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

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

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

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

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

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

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

13.2. Question 2

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

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

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

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

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

If no adequate data are available, for substances posing a high risk to the consumer (like the 12 listed in Table 13-5), the use of a general threshold may be considered. A threshold of 0.8 µg/cm² has been derived based on a statistical analysis of the available data in the scientific literature, including two fragrance allergens. This corresponds to 0.01% (100 ppm) limit in cosmetic products indicative for safe use. This approximation may hold for weak to strong allergens. However, some strong and extreme sensitisers may require lower individual thresholds. As an example, chloroatranol, present in the natural product *Evernia prunastri* and in *Evernia furfuracea*, has been shown to have an elicitation threshold of 0.0004 µg/cm² under experimental conditions similar to those yielding above results. On the other hand, for very weak sensitisers, this generic threshold may be too conservative.

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

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

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

Conclusions - Question 2

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

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

The dose elicitation studies available indicate that a general level of exposure of up to 0.8 µg/cm² (0.01%) may be tolerated by most consumers with contact allergy to fragrance allergens. The SCCS considers that this level of exposure could be efficient in limiting elicitation unless there is substance specific data, either experimental or clinical, to the contrary.

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

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

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

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

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

13.3. Question 3

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

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

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

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

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

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

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

Based on the clinical data, oxidised limonene and oxidised linalool are allergens of high concern (Table 13-5) which pose a high risk of sensitisation to the consumer. For these substances the presence of the oxidised fraction represented by the peroxide content should not be higher than 10 ppm. Alternatively, the suggested general threshold dose/area of 0.8 µg/cm² (100 ppm in cosmetic products) could be applicable to the total oxidised fraction, i.e. not only peroxides but also secondary oxidation products such as aldehydes and epoxides.

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

Table 13-6: Known prehaptens and prohaptens.

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

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

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

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

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

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

Conclusions - Question 3

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

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

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

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

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

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

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

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

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

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

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

14. List of abbreviations

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

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

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Annex I - Catalogue of fragrance allergens**Contents**

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Single chemicals

Often, results with the single constituents of the FM I or, yet more rarely, FM II, are presented in one paper. As the main ordering of this annex is by allergen, core information on these studies is presented in a tabular format and referenced by a unique acronym in the single sections, to avoid redundancy. Regarding nomenclature, terms which are often not officially an INCI Name but Perfuming Name as listed by CosIng are used. "Current Regulation" refers to the EU Cosmetics Directive only.

Table 55: Background information on studies reporting results with (all) single constituents of the FM I (**amyl cinnamal, cinnamyl alcohol, cinnamal, eugenol, geraniol, hydroxycitronellal, isoeugenol, EVERNIA PRUNASTRI**)

Reference	Country	Study period, Patients	Comments by reviewers
Larsen 2002 c (1)	7 industrial countries worldwide	Prior to 2002 n=218 patients with known contact allergy to fragrance ingredients	Test concentrations identified as non-irritating in serial dilution testing in 20 healthy volunteers
Utrecht 1999 (2)	Utrecht, The Netherlands	1994-1998 n=757 patients with suspected ACD to cosmetics	All patients tested with FM I and single constituents
Sheffield 1999 (3)	UK	1994-1995 n=744, 40 of these positive to FM I and tested with single constituents	
IVDK 2007 (4)	Germany + one centre in Austria and Switzerland each	01/2003 – 12/2004, n=1658 to 21325, see text, consecutive patients	
Hungary 2002 (5)	Hungary, multicentre study,	1998-1999, n=3604 patients	recruitment not clear, presumably consecutive patients
Groningen 2009 (6)	Groningen, The Netherlands	04/2005-06/2007 n=320	patients selected according to history or site suspicious of contact allergy to fragrance ingredients
IVDK 2010 (7)	Germany, Switzerland and one centre in	2005-2008 n=36961 tested with FM I, n=4167 with FM II and	

	Austria	all SC	

Table 56: Results of PTing with single constituents of the FM I in patients positive to the FM I (as percent)

N(pos) to FM I, ref.	<i>Evernia prun.</i>	Isoeu g.	Hydroxy citron.	Cinna mal	Cinnamy I alcohol	Eugen ol	Gera-niol	Alpha-amyl cinnam al
N=160 (5)	13.1%	14.8 %	2.5%	8.1%	20.6%	8.8%	7.5%	5.0%
N= 991 (8)	18.4%	11.2 %	10.1%	6.1%	6.1%	6.6%	4.6%	2.4%
N=50 (2)	19.6%	14.3 %	8.9%	8.9%	7.1%	5.4%	2.7%	0%
n=40 Sheffield 1999 (3)	30%	20%	2.5%	12.5 %	10%	5%	0%	0%
N=226 Coimbra 2000 (9)	22.1%	19.9 %	6.6%	13.3 %	7.9%	14.6 %	8.4%	4.4%
N=655 IVDK 2010 (7)	29.8%	18.0 %	12.8%	11.6 %	9.6%	6.7%	4.7%	2.8%

Table 57: Background information on studies reporting results with (all) single constituents of the FM II (**citronellol, citral, coumarin, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), Farnesol, alpha-Hexyl-cinnamic aldehyde**)

Reference	Country	Study period, Patients	Comments by reviewers
IVDK 2007 (4)	Germany + one centre in Austria and Switzerland each	01/2003 – 12/2004, n=1658 to 21325, see text, consecutive patients	
EU 2005 (10)	6 European centres	10/2002 – 06/2003, n=1701	Applied in consecutive patients
Groningen 2009 (6)	Groningen, The Netherlands	04/2005-06/2007 n=320	patients selected according to history or site suspicious of contact allergy to fragrance ingredients

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

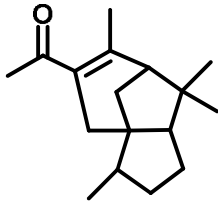
Table 58: Background information on studies reporting results with several fragrance compounds not, or only partly, corresponding to mixes (later created) or with essential oils

Reference	Country	Study period, Patients
deGroot 2000 (12)	The Netherlands (multicentre)	09/1998-04/1999 n=1825 consecutive patients
An 2005 (13)	South Korea (multicentre)	04/2002 – 06/2003 n=422 consecutive patients
Sugiura 2000 (14)	Nagoya, Japan	1990-1998 n=1483 patients with suspected cosmetic dermatitis
Frosch 1995 (15)	11 European depts.	Prior to 1995 n=1069 consecutive patients
Frosch 2002 a (16)	6 European depts.	10/1997-10/1998 n=1855 consecutive patients
Frosch 2002 b (17)	6 European depts.	Prior to 2002 n=1606 consecutive patients
Coimbra 2000 (9)	Portugal	07/1989-06/1999 n=226 with FM I SC n=67 also with other fragrances
Larsen 1977 (18)	US	1977 n=20 “perfume-sensitive patients”
Larsen 2001 (19)	worldwide multicentre	? (prior to 2001) n=178 patients with known contact allergy to fragrance ingredients
Belsito 2006 (20)	North American (5 US, 1 Canadian) depts.	2003 n=1603 patients
NACDG 2009 (21)	US and Canada	2005-2006 n= 4454 patients
Wöhrl 2001 (22)	“FAZ” clinic Vienna	1997-2000 n=747 of 2660 consecutive patients tested with special series
EECDRG 1995 (15)	European, multicentre	Different fragrances, tested in 2 concentrations, in sets of about 100 patients each in different centres
Goossens 1997 (23)	Leuven, Belgium	1978-1987 n=111 “Japanese perfume series” (highly selected patients)

Opinion on fragrance allergens in cosmetic products

Reference	Country	Study period, Patients
Malten 1984 (24)	Dutch multicentre	N=182 patients with suspected cosmetic dermatitis tested with 22 fragrance compounds
DeGroot 1985 (25)	Dutch	N=179 patients with suspected cosmetic dermatitis tested with 16 fragrance compounds
Rudzki 1976 (26)	Warsaw, Poland	N=200 consecutive patients
Rudzki 1986 (27)	Warsaw, Poland	N=86 patients of 299 (of 5315) patients with positive reaction to FM I tested with essential oils series
Santucci 1987 (28)	Rome, Italy	N=1500 consecutive patients; n=63 reacting positively to FM I re-tested with extended fragrance series
Nakayama 1974 (after (29))	Japan	N=183 patients with cosmetic dermatitis
IVDK 2010c (30)	Germany, Switzerland and one centre in Austria	15682 patients tested with at least one essential oil in different test series
Trattner/David (31)	Tel Aviv, Israel	N=641 consecutive patients

Catalogue of single chemicals evaluated

ACETYLCEDRENE	
CAS # 32388-55-9	
EC # 251-020-3	
1-[(3R,3aR,7R,8aS)-2,3,4,7,8,8a-Hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl]-ethanone	
Other names 1-(2,3,4,7,8,8a-Hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl)-, [3R-(3a,3aβ,7β,8aα)]-Ethanone; 1H-3a,7-Methanoazulene, Ethanone deriv.; Acetyl-α-cedrene; Lixetone; Vertofix	

Current regulation: /

Clinical

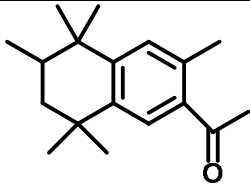
data:

In the Frosch 2002 a study, a total of 0.2% had positive PT reactions (16). In the Frosch 1995 dose-finding pilot study, 1 positive reaction to 1% and none to 5% "Vertofix ®" in pet., tested in 100 consecutive patients in Stockholm, were observed (15). In a case report, a 28-year-old patient with axillary dermatitis after using 2 different deodorants tested positive not only to HICC, but also to acetyl cedrene (tested 10.8% in diisopropylene glycol (20 healthy controls negative) (32). In this case report it is stated that "Acetyl cedrene (Vertofix Coeur) is a complex reaction mixture of which a principal constituent is methyl cedryl ketone".

Additional

information:

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

6-ACETYL-1,1,2,4,4,7-HEXAMETHYLTETRALINE	
CAS # 21145-77-7	
EC # 216-133-4 / 244-240-6	
1-(5,6,7,8-Tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)-ethanone	
AHMT (perfume), AHTN, Extralide, Fixolide, Musk tonalid, NSC 19550, Tentarome, Tetralide, Tonalid, Tonalide.	

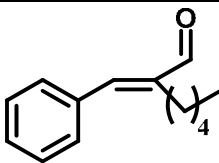
Current regulation: Annex III, part 1, entry 182

Clinical

data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% "Tonalide ®" in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

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

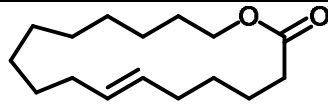
AMYL CINNAMAL	
CAS # 122-40-7	
EC # 204-541-5	
2-(Phenylmethylene)-heptanal	
Cinnamaldehyde, α-amyl- (4CI); Cinnamaldehyde, α-pentyl- (6CI,7CI,8CI); 2-(Phenylmethylene)heptanal; 2-Benzylideneheptanal; Amylcinnamaldehyde; Amylcinnamic acid aldehyde; Amylcinnamic aldehyde; Flomine; Jasminal; Jasminaldehyde; Jasmine aldehyde; NSC 6649; Pentylcinnamaldehyde; α-Amyl-β-phenylacrolein; α-Amylcinnamal; α-Amylcinnamaldehyde; α-Pentylcinnamaldehyde	

Current regulation: Annex III, part 1, entry 67

Clinical data:
In the “background information” section of the 1999 opinion (33), amyl cinnamal (synonymous: alpha amyl cinnamaldehyde) has been classified as frequently reported contact allergen because it has been identified as a cause of allergic reactions in persons with eczema from cosmetic products.

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=4, i.e., 0.2% (95% CI: 0.1 – 0.5%) positive reactions to this compound (1% pet.) in 2062 consecutively PTed patients (4). In the Groningen 2009 study, no positive reactions to this allergen, tested at 2% pet., were observed (6). The Larsen 2001 study yielded 2.3% positive reactions in 178 patients with known contact allergy to fragrance ingredients (test concentration: 5% pet.) (19). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=2 (0.3%) positive reactions to amyl cinnamal (22). The IVDK 2010 study, 0.26% (95% CI: 0 – 0.60%) of 1214 consecutively tested patients reacted to the compound, while 0.61% (95% CI: 0.36 – 0.86%) of 4375 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7).

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

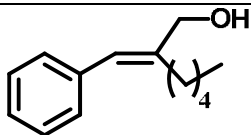
AMBRETTOLIDE	
CAS # 7779-50-2	
EC # 231-929-1	
Oxacycloheptadec-7-en-2-one	
1-Oxa-7-cycloheptadecen-2-one; 16-Hydroxy-6-hexadecenoic acid lactone; 16-Hydroxy-6-hexadecenoic acid ω-lactone	

Current regulation: /

Clinical data:
The Larsen 2001 study, using omega-6-hexadecenlactone (HDL, 5% pet.) as test concentration, diagnosed 3.4% positive reactions in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information:

Ambrettolide is 1 of 2 components of Ambrette seed oil (obtained from *Hibiscus abelmoschus* L., *Malvaceae*) responsible for the musk odour. In Surburg/Panten, the compound has the chemical name (Z)-7-hexadecen-16-olide (or Hexadec-7-en-16-olide according to CosIng), CAS 123-69-3 (34).

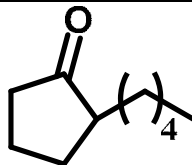
AMYL CINNAMYL ALCOHOL	
CAS # 101-85-9	
EC # 202-982-8	
2-(Phenylmethylene)-heptan-1-ol,	
2-Benzylidene- (6Cl,8Cl)1-heptanol; 2-Amyl-3-phenyl-2-propen-1-ol; 2-Benzylidene-1-heptanol; 2-Pentyl-3-phenyl-2-propen-1-ol; Buxinol; α-Amylcinnamic alcohol; α-Amylcinnamyl alcohol	

Current regulation: Annex II, Part 1, entry 74

Clinical data:
In the “background information” section of the 1999 opinion, amyl cinnamyl alcohol is mentioned to cross-react with amyl cinnamal. Moreover, this compound has been identified as a cause of allergic reactions in a notable number of persons with eczema from the use of cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.4% (95% CI: 0.1 – 0.7%) positive reactions in 1977 consecutively PTed patients (4). The IVDK 2010 study, 0.79% (95% CI: 0.54 – 1.04%; percentages standardised for age and sex) of 5650 patients PTed reacted to the compound (7). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6).

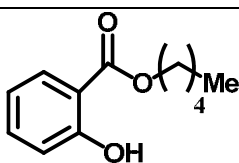
Additional information: A RIFM review is available (35) where selected clinical studies published until 1994 were considered.

AMYLCYCLOPENTANONE	
CAS # 4819-67-4	
EC # 225-392-2	
2-Pentylcyclopentanone	
2-Pentyl-1-cyclopentanone; 2-Pentylcyclopentanone; 2-Pentylcyclopenten-1-one; 2-n-Amylcyclopentanone; 2-n-Pentyl cyclopentanone; Delphone	

Current regulation: /

Clinical data:
In the Larsen 2001 study, none of 178 patients with contact allergy to fragrance ingredients reacted positively to this ingredient, PTed at 5% pet. (19).

Additional information: /

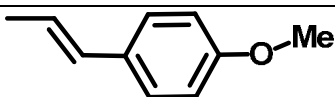
AMYL SALICYLATE	
CAS # 2050-08-0	
EC # 218-080-2	
Pentyl-2-hydroxybenzoate	
Amyl ester salicylic acid, (4CI); Pentyl ester salicylic acid, (6CI,8CI); 2-Hydroxybenzoic acid pentyl ester; Amyl salicylate; NSC 403668; NSC 44877; NSC 46125; Pentyl salicylate	

Current regulation: /

Clinical data:

In the Frosch 2002 a study, a total of n=3 (0.2%) had positive PT reactions (16). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% amyl salicylate and 1 positive reaction to 5% amyl salicylate were observed in 100 consecutive patients patch tested in Stockholm (15).

Additional information:
A RIFM review is available (36). It is a “top 100” substance (IFRA, pers. comm.2010)

trans-ANETHOLE	
CAS # 4180-23-8	
EC # 224-052-0 / 203-205-5	
1-Methoxy-4-(1E)-1-propen-1-yl-benzene	
(E)-p-Propenyl-anisole (8CI); (E)-1-Methoxy-4-(1-propenyl)-benzene; 1-Methoxy-4-(1E)-1-propenyl-benzene (9CI); (E)-1-(4-Methoxyphenyl)propene; (E)-1-p-Methoxyphenylpropene; (E)-Anethol; (E)-Anethole (REACH, EINECS); E-Anethole (INCI); 1-Methoxy-4-[(1E)-1-propenyl]benzene; (E)-1-Methoxy-4-(1-propenyl)-benzene (CosIng); NSC 209529; trans-1-(4-Methoxyphenyl)-1-propene; trans-1-(p-Methoxyphenyl)-1-propene; trans-1-(p-Methoxyphenyl)propene; trans-1-p-Anisylpropene; trans-4-(1-Propenyl)anisole; trans-Anethol; trans-Anethole; trans-p-Anethole; trans-p-Methoxy-β-methylstyrene	

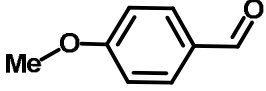
Current regulation: /

Clinical data:

A case of a 64 year old patient, who developed severe cheilitis and a loss of taste has been described (37). Both were reversible after the cessation of use of previous toothpastes. The patch test was strongly positive to anethole (isoform not given) 5% pet.; this was found an ingredient of the causative toothpaste. Two cases of occupational allergic contact dermatitis occurring in a traditional cake factory due to anise oil have been described, both testing (strongly) positive to anise oil (5% o.o.) and anethole (5% pet.) (38).

Additional information:
It is a “top 100” substance (IFRA, pers. comm.2010). trans-Anethole can be purified from star anise oil (34, 39), see 3.2., and is the main component of anise, star anise

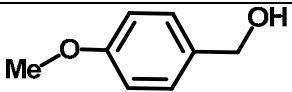
and fennel oils (38)

ANISALDEHYDE	
CAS # 123-11-5	
EC # 204-602-6	
4-Methoxy-benzaldehyde	
p-Methoxybenzaldehyde; p-Anisaldehyde; 4-Anisaldehyde; Aubepine; Crategine; NSC 5590; Obepin; p-Anisic aldehyde; Anisic aldehyde; p-Formylanisole.	

Current regulation: /

Clinical data: /

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

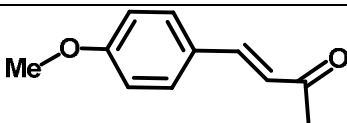
ANISYL ALCOHOL	
CAS # 105-13-5	
EC # 203-273-6	
4-Methoxy-benzenemethanol	
p-Methoxy-benzyl alcohol (8CI); (4-Methoxyphenyl)methyl alcohol; 4-(Hydroxymethyl)anisole; 4-(Methoxyphenyl)methanol; 4-Methoxy- α -hydroxytoluene; 4-Methoxybenzenemethanol; 4-Methoxybenzyl alcohol; Anise alcohol; Anisic alcohol; NSC 2151; [4-(Methoxy)phenyl]methanol; p-(Methoxyphenyl)methanol; p-Anisalcohol; p-Anisyl alcohol; p-Methoxybenzyl alcohol	

Current regulation: Annex III, part 1, n° 80

Clinical data:
In the “background information” section of the 1999 opinion, anisyl alcohol is classified as “less frequently reported allergen”; 2 studies were identified where 3 and 4 cases, respectively, with cosmetic dermatitis due to contact allergy to anisyl alcohol had been reported (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=1, i.e., 0.1% (95% CI: 0.00 – 0.3%) positive reactions in 2004 consecutively PTed patients, patch test concentration: 1% pet. (4). Similar results were obtained in the following period, with n=1 (and n=3 irritant and n=6 doubtful) reactions in 986 patients tested with 1% in pet. (30). In the Groningen 2009 study, no positive reactions to this allergen, tested at 5% pet., were observed in 320 patients (6). This test concentration has been regarded as relatively high by Hostynek and Maibach (40). The test concentration of Anisyl Alcohol has been further validated by Bruze et al. and 10% in pet was recommended as a non-irritant concentration for routine investigations (40a).

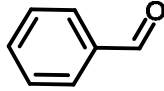
Additional information: /

ANISYLIDENE ACETONE	
CAS # 943-88-4	
EC # 213-404-9	
4-(4-Methoxyphenyl)-3-Buten-2-one	
1-(p-Methoxyphenyl)-1-buten-3-one; 4-(4-Methoxyphenyl)-3-buten-2-one; 4-(p-Methoxyphenyl)-3-buten-2-one; 4-Methoxybenzalacetone; 4-Methoxybenzylideneacetone; 4-Methoxystyryl methyl ketone; 4'-Methoxybenzylideneacetone; Anisalacetone; Methyl p-methoxystyryl ketone; NSC 31752; NSC 7946; p-Anisalacetone; p-Methoxybenzalacetone; p-Methoxybenzylideneacetone; p-Methoxystyryl methyl ketone	

Current regulation: Annex III, part 1, n° 443

Clinical data:
In the Malten 1984 study, 1.1% of 182 patients displayed a positive PT reaction to anisylidene acetone 2% pet. (24)

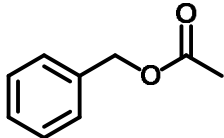
Additional information: /

BENZALDEHYDE	
CAS # 100-52-7	
EC # 202-860-4	
Benzaldehyde	
Artificial Almond Oil; Benzaldehyde FFC; Benzenecarbonal; Benzenecarboxaldehyde; Benzoic acid aldehyde; Benzoic aldehyde; NSC 7917; Phenylformaldehyde; Phenylmethanal	

Current regulation: /

Clinical data:
 In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions to benzaldehyde 5% pet. (22). The IVDK 2010 study, 6 weak positive reactions were observed, i.e., 0.16% (95% CI: 0.03 – 0.29%; percentages standardised for age and sex) of 2820 patients PTed reacted to the compound (7). A review is available in the Int. J. Toxicol. (41). In the case of a 19 year old pastry maker, Seite-Bellezza et al. report on immediate reactions to MP, cinnamal and benzaldehyde (tested at 5% pet.) subsiding after a few hours, in line with the patient's history (42).

Additional information: /

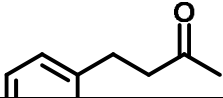
BENZYL ACETATE	
CAS #140-11-4	
EC # 205-399-7 / 202-940-9	
Benzyl acetate	
Benzyl ester acetic acid; Benzyl alcohol, acetate (6CI); (Acetoxymethyl)benzene; Benzyl ethanoate; NSC 4550; Phenylmethyl acetate; Methyl Phenylacetate; α-Acetoxytoluene ; Methyl alpha-Toluate	

Current regulation: /

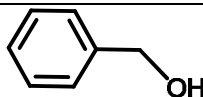
Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% benzyl acetate in pet., tested in 100 consecutive patients in Odense, DK, were observed (15). Benzyl acetate is a component of several natural mixtures, for example a major constituent of Narcissus abs., and a minor constituent of Jasmine abs. (17).

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

BENZYL ACETONE	
CAS # 2550-26-7	

EC # 219-847-4	
4-Phenyl-2-butanone	
4-Phenylbutan-2-one (REACH, EINECS); Benzylacetone; Methyl 2-phenylethyl ketone; Methyl phenethyl ketone; NSC 44829; NSC 813M; Phenethyl methyl ketone; 1-Phenyl-3-butanone; 2-Phenylethyl methyl ketone	
Current regulation: /	
Clinical data: /	
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (43).	

BENZYL ALCOHOL	
CAS # 100-51-6	
EC # 202-859-9	
Phenylmethanol	
Benzyl alcohol; (Hydroxymethyl)benzene; Benzenecarbinol; Benzylic alcohol; NSC 8044; Phenylcarbinol; Benzenemethanol; Phenylmethyl alcohol; Sunmorl BK 20; TB 13G; α-Hydroxytoluene; α-Toluenol	
Current regulation: Annex III, part 1, n° 45; Annex VI, part1, n ° 34	

Clinical data:
In the “background information” section of the 1999 opinion, benzyl alcohol is classified as allergen frequently causing allergic reactions. It has been found to cause allergic reactions in 1.2 to 15% of patients with eczema from cosmetic products (33). A CIR expert panel review is available in the Int. J. Toxicol. (44).

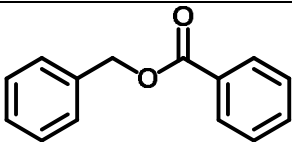
Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.7%) positive reactions in 2166 consecutively PTed patients (4). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen (6).

Both in terms of case reports (45-47) and clinical epidemiology data (0.22 % [95% CI: 0.16 – 0.28%] positive tested with benzyl alcohol in the context of a “topical drugs” series, n=26448 (7)) the relevance of this alternative exposure is highlighted. In a study from Alicante, Spain, 86 selected patients were tested with benzyl alcohol, yielding 2 positive reactions (48).

After application of saline soaks preserved with benzyl alcohol onto his stasis dermatitis, a 53 year old patient developed a rash, which was, according to test results obtained by J. D. Guin and J. Goodman, at least partly due to an immediate hypersensitivity to benzyl alcohol, as verified by an intense urticarial reaction at the test site lasting several days (49). According to 2 cases reported by A. A. Fisher, PT-proven, relevant delayed type hypersensitivity is not associated with immediate reactions in scratch or intradermal tests (50). D. W. Shaw describes a patient with allergic contact dermatitis caused by benzyl alcohol in a hearing aid impression material and in topical medications (51). Another contribution points to covert exposures to benzyl alcohol even in products labelled “fragrance free” (52) probably because benzyl alcohol is used as preservative, or an essential oil containing benzyl alcohol is used as cosmetic ingredient.

Additional information:

Benzyl alcohol is a component of several natural mixtures, including Myroxylon pereirae resin, which have been used for extraction, but is nowadays synthesised (53). It is permitted in certain foodstuffs (liquors: < 100 mg/l, sweets and cakes: < 250 mg/kg) under the coding “E 1519” (http://www.zusatzstoffe-online.de/zusatzstoffe/317.e1519_benzylalkohol.html, last accessed 2009-11-27). In addition to being a fragrance compound (which may be used, even in relatively high concentration, to scent topical medications (54)), benzyl alcohol is used as antioxidant in topical therapeutics or cosmetics. The German “Rote Liste” (<http://www.rote-liste.de>, last accessed 2009-11-11), for instance, lists 205 specialties containing benzyl alcohol. Benzyl alcohol may be used up to 1.0% as a preservative in cosmetic products according to the Cosmetic Directive 76/768/EEC

BENZYL BENZOATE	
CAS # 120-51-4	
EC # 204-402-9	
Benzyl benzoate	
Benzyl ester benzoic acid; Ascabin; Ascabiol; Benylate; Benzyl benzenecarboxylate; Benzyl benzoate; Benzyl phenylformate; Benzylets; Colebenz; NSC 8081; Nicca Sunsolt LM 7EX; Novoscabin; Pelemol B66; Peruscabin; Phenylmethyl benzoate; Scabagen; Scabanca; Scabcare BB; Scabide; Scabiozon; Scobenol; Vanzoate; Venzonate	

Current regulation: Annex III, part 1, n° 85

Clinical data:

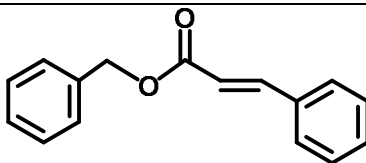
In the “background information” section of the 1999 opinion, benzyl benzoate is classified as “less frequently reported allergen”; in several studies, only single cases had been reported in each, except for patients sensitive to MP (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=1, i.e., 0.1% (95% CI: 0.00 – 0.3%) positive reactions in 2003 consecutively PTed patients, test concentration 1% pet. (4). In the subsequent period (2005-2008), n=1062 patients were tested in the IVDK 2010 study, with no positive reactions (7). In the Groningen 2009 study, no positive reactions to this allergen, tested at 5% pet., were observed in 320 patients (6). Thus, the pooled proportion of positive patch test reactions is 1 / 3385 (0.03%, exact upper 1-sided 95% CI: 0.14%)

Additional information:

Benzyl benzoate naturally occurs in MP resin and ylang-ylang oil. Nowadays it is synthesised and used for a variety of purposes (53). These include use as a scabicide (one brand specialty on the German market, using a concentration of 10% for children and 25% for adults), possibly with some differences among European countries. In France, a combination of benzyl benzoate 10% and sulfiram 2% is reported to be used most often (55). Hausen et al. review the older literature and mention a study identifying 1 sensitised patient in 73 patients treated for scabies (details not given) (53). According to the mandatory factsheet (see PDF “benzylbenzoate_infosheet_DE.pdf”) dermatitis after anti-scabies treatment is “rare”, in a range between 1:1000 and 1:10000.

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

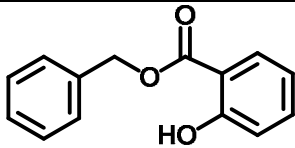
<i>BENZYL CINNAMATE</i>	
CAS # 103-41-3	
EC # 203-109-3	
Benzyl 3-phenylprop-2-enoate	
Benzyl ester cinnamic acid; 3-phenyl-phenylmethyl ester 2-propenoic acid; 3-Phenyl-2-propenoic acid benzyl ester; Benzyl 3-phenylpropenoate; Benzyl γ-phenylacrylate; Cinnamein; NSC 11780; NSC 44403	

Current regulation: Annex III, part 1, n° 81

Clinical data:
In the “background information” section of the 1999 opinion, benzyl cinnamate (synonymous: benzyl 3-phenyl-2-propenoate, cinnamein) is classified as “less frequently reported allergen”; one study of patients with contact allergy to cosmetic products was identified and further a study where benzyl cinnamate associated with contact sensitisation to MP (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.6%) positive reactions in 2042 consecutively PTed patients, test concentration 5% pet. (4). The IVDK 2010 study, n=4 weak positive were observed, amounting to 0.12% (95% CI: 0 – 0.25%; percentages standardised for age and sex) of 2872 patients PTed reacted to the compound (7). In the Groningen 2009 study, no positive reactions to this allergen, using the same test concentration, were observed in 320 patients (6). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions (22).

Additional information: A RIFM review is available (56).

BENZYL SALICYLATE	
CAS # 118-58-1	
EC # 204-262-9	
Benzyl 2-hydroxybenzoate	
Salicylic acid, Benzyl ester; Benzoic acid, 2-Hydroxy-, phenylmethyl ester; Benzyl o-hydroxybenzoate; NSC 6647	

Current regulation: Annex III, part 1, n° 75

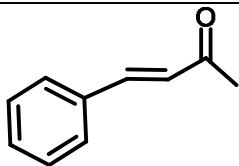
Clinical data:

In the “background information” section of the 1999 opinion (33), benzyl salicylate is classified among the frequent allergens, with 0.2 to 10% of patients with eczema from cosmetic products testing positively. In one study, benzyl salicylate accounted for 75% of reactions to commercial products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=2, i.e. 0.1% (95% CI: 0.01 – 0.4%) positive reactions in 2041 consecutively PTed patients (test concentration 1% pet.) (4). The IVDK 2010 study, 2 of 3775 patients PTed reacted weakly positive to the compound (7). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen, tested at 2% pet. (6). In the deGroot 2000 study, 10 of 1825 consecutive patients tested positive to benzyl salicylate (2% pet.), of these, 3 were not detected by the FM I (12). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions (22). Trattner/David found 2 positive cases in 641 consecutive eczema patients (31). In a study from Alicante, Spain, 86 selected patients were tested with benzyl salicylate, yielding 2 positive reactions (48).

Additional information:

It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010). A RIFM review is available, including internal results on, e.g. HRIPT, and a review of LLNA results, where benzyl salicylate is classified as “weak” allergen (57).

BENZYLIDENEACETONE	
CAS # 122-57-6	
EC # 204-555-1	
4-Phenyl-3-buten-2-one	
4-Phenylbut-3-en-2-one; 2-Butenone, 4-Phenyl- (2CI); Ketone, Methyl styryl (7CI); 1-Phenyl-1-buten-3-one; 2-Phenylethenyl methyl ketone; 2-Phenylvinyl methyl ketone; 4-Phenyl-3-buten-2-one; 4-Phenyl-3-butene-2-one; 4-Phenylbutenone; Acetocinnamone; Benzalacetone; Benzylideneacetone; Methyl 2-phenylvinyl ketone; Methyl phenylvinyl ketone; Methyl styryl ketone; Methyl β-styryl ketone; NSC 5605; Styryl methyl ketone	

Current regulation: Annex II, n° 356

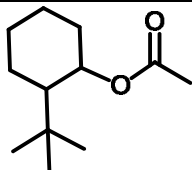
Clinical

data:

In the Malten 1984 study, none of 182 patients displayed a positive PT reaction to

benzylidene acetone 0.5% pet. (24).

Additional information: /

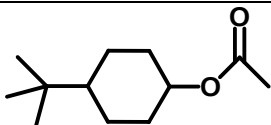
2-TERT-BUTYLCYCLOHEXYL ACETATE	
CAS # 88-41-5	
EC # 201-828-7	
2-(1,1-dimethylethyl)cyclohexyl acetate	
Cyclohexanol, 2-(1,1-dimethylethyl)-, acetate ; Cyclohexanol, 2-Tert-butyl-, acetate; 2-Tert-Butylcyclohexanol acetate; Verdox; o-Tert-Butylcyclohexyl acetate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Verdox ®” in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15)

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (58).

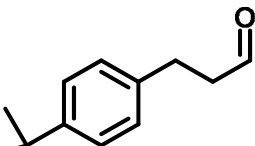
4-TERT-BUTYLCYCLOHEXYL ACETATE	
CAS # 32210-23-4	
EC # 250-954-9	
4-(1,1-Dimethylethyl)cyclohexyl acetate	
Boisinal A 464D; Cyclohexanol, 4-tert-Butyl-, acetate; Cyclohexanol, 4-(1,1-Dimethylethyl)-, acetate; 4-(1,1-Dimethylethyl)cyclohexyl acetate; 4-tert-Butylcyclohexanol acetate; Dorisyl; Madeflor; NSC 163103; Oryclone, Oryclone special, Oryclon extra; p-t-BCHA; p-tert-Butylcyclohexyl acetate; para-tert-Butylcyclohexyl acetate; PTBCHA; Velvetone; Verbeniax; Vertenex; Vertinate; Vertopol; Ylanate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Vertenex ®” in pet., tested in 107 consecutive patients in High Wycombe, were observed (15).

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (59).

p-tert -Butyldihydrocinnamaldehyde	
CAS # 18127-01-0	
EC # 242-016-2	

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

Current regulation: III/155

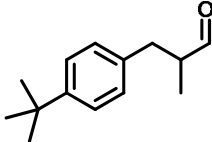
Clinical data: /

Additional

information:

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

http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=39132

BUTYLPHENYL METHYLPROPIONAL (Lilial®)	
CAS # 80-54-6	
EC # 201-289-8	
3-(4-tert-Butylphenyl)-2-methylpropanal	
p-t-Butyl-alpha-methylhydrocinnamic aldehyde; 2-(4-tert-Butylbenzyl)propionaldehyde (REACH, EINECS); 4-(1,1-Dimethylethyl)-α-methyl-benzenepropanal; Hydrocinnamaldehyde, p-tert-Butyl-α-methyl-; (±)-2-Methyl-3-(4-tert-butylphenyl)propanal; 2-Methyl-3-(4-tert-butylphenyl)propanal; 2-[(4-tert-Butylphenyl)methyl]propanal; 3-(4-tert-Butylphenyl)-2-methylpropanal; 3-(p-tert-Butylphenyl)-2-methylpropionaldehyde; 3-(p-tert-Butylphenyl)isobutylaldehyde; 4-(1,1-Dimethylethyl)-α-methylbenzenepropanal; 4-tert-Butyl-α-methylhydrocinnamic aldehyde; Lilestralis; Lilial; Lysmeral; NSC 22275; lilestral; p-tert-Butyl-α-methylhydrocinnamaldehyde; p-tert-Butyl-α-methylhydrocinnamic aldehyde; pt-Bucinal; α-Methyl-p-tert-butylhydrocinnamaldehyde; β-Lilial	

Current regulation: Annex III, part 1, n° 83

Clinical data:

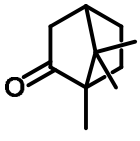
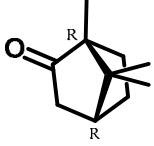
In the “background information” section of the 1999 opinion, lilial is classified as “less frequently reported allergen”; with 2 cases of contact allergy reported in 1 study of 176 eczema patients and 1 case with contact allergy to Lilial from a deodorant; a number of other reported positive cases were considered to possibly have been false positive (33).

Since the last SCCNFP-opinion of 1999, the Frosch 2002a study yielded 0.2% positive reactions to Lilial® (10% pet.) among the 1855 consecutive patients tested (16). The IVDK 2007 study yielded 0.4% (95% CI: 0.2 – 0.8%) positive reactions in 2004 patients consecutively tested (4). The IVDK 2010 study, 0.62% (95% CI: 0.04 – 1.21%; percentages standardised for age and sex) of 1947 patients PTed reacted to the compound (7). In the Groningen 2009 study, n=2, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at only 1% pet. (6). In the deGroot 2000 study, 9 of 1825 consecutively tested patients had a positive reaction to lilial® (5%

pet.) (12). Lilial® has been identified as constituent of perfumes used by a patient, causing ACD (60).

Additional information:

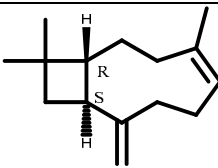
It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

CAMPHOR	 76-22-2
CAS # 76-22-2 / 464-49-3	
EC # 207-355-2 / 200-945-0	
1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one (76-22-2) (1R,4R)-1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one (464-49-3)	
76-22-2: DL-Bornan-2-one (REACH, EINECS); 2-Bornanone; Bornan-2-one, INCI name according to CAS; CAMPHOR/DL-bornan-2-one; Camphor; (±)-Camphor; DL-Camphor; 1,7,7-Trimethylnorcamphor; 2-Camphanone; Alphanon; Borneo camphor; Root bark oil; Spirit of camphor 464-49-3: (1R)-1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one; (1R,4R)-(+)-Camphor; (+)-2-Bornanone; (+)-Camphor; (1R)-(+)-Camphor; (1R)-Camphor; (1R,4R)-(+)-Camphor; (R)-(+)-Camphor; (R)-Camphor; Camphor; D-Camphor; D-(+)-Camphor; Alcanfor; Japanese camphor.	 464-49-3

Current regulation: /

Clinical data:
From the UK, a case of allergic contact dermatitis after application of Earex ® ear drops due to rectified camphor oil (tested 10% pet.) was reported (61). Application of a liquid rubefacient of Asian origin caused allergic contact dermatitis in a 58-year-old patient, according to the positive PT result with 10% camphor ("alcaonfor") in pet. due to this ingredient (62). In the US, a case of contact dermatitis due to "Vics VapoRub" has been reported (63).

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

beta-CARYOPHYLLENE	
CAS # 87-44-5	
EC # 201-746-1	
(1R,4E,9S)-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene	
(E)-(1R,9S)-(-)-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene; [1R-(1R*,4E,9S*)]-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene; (-)-(E)-Caryophyllene; (-)-Caryophyllene; (-)-E-Caryophyllene; (-)-trans-Caryophyllene; (-)-β-Caryophyllene; (E)-Caryophyllene; Caryophyllene; Caryophyllene B; NSC 11906; l-Caryophyllene; trans-Caryophyllene; β-Caryophyllen; β-Caryophyllene; (-)-β-Caryophyllene	

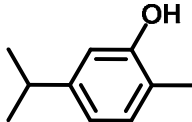
Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.6% positive reactions to caryophyllene (5% pet.) in 1606 consecutive were observed (17).

Additional information:

beta-Caryophyllene autoxidizes at air exposure. As the primary oxidation products, the hydroperoxides, are very unstable and immediately form epoxides with low sensitizing capacity, the increase in allergenic activity caused by autoxidation is comparably low (64). A multicenter study identified 0.5% positive reactions to oxidized *beta*-caryophyllene (3.0% pet.) in 1511 consecutive patients (65). Of these, 2 patients (0.1%) reacted to the major oxidation product (caryophyllene oxide) (3.9% pet.).

CARVACROL	
CAS # 499-75-2	
EC # 207-889-6	
2-Methyl-5-(1-methylethyl)-phenol	
2-Hydroxy-1-methyl-4-(1-methylethyl)benzene; 2-Hydroxy-p-cymene; 2-Methyl-5-(1-methylethyl)phenol; 2-Methyl-5-isopropylphenol; 3-Isopropyl-6-methylphenol; 5-Isopropyl-2-methylphenol; 5-Isopropyl-o-cresol; 6-Methyl-3-isopropylphenol; Antioxine; Dentol; Isopropyl o-cresol; Isothymol; NSC 6188; p-Cymen-2-ol	

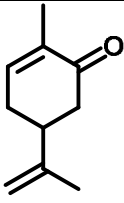
Current regulation: /

Clinical data:

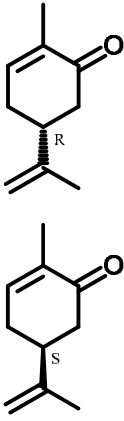
The DeGroot 1985 study identified 2 (1.1%) positive reactions among 179 patients using a 5% PT preparation of this compound – these reactions may have been at least partly due to an “excited back syndrome” and are thus of limited evidence (25). Meynadier et al. ¹¹ patch tested 28 patients with contact allergy to fragrance ingredients using 2% carvacrol in pet. Positive reactions were observed in 3 of 28 patients (after (66)).

Additional information:

Carvacrol is derived from p-cymene by sulfonation followed by alkali fusion. Carvacrol can also be derived from savory, thyme, marjoram, oregano, lovage root, and Spanish origanum oil (66). Carvacrol is a flavor ingredient that can be found in alcoholic beverages, baked goods, chewing gum, condiment relish, frozen dairy, gelatin pudding, non-alcoholic beverages, and soft candy at concentrations from 0.1 to 28.54 ppm (RIFM 2001, according to (66)).

CARVONE	
CAS # 99-49-0 / 6485-40-1 / 2244-16-8	
EC # 202-759-5 / 229-352-5 / 218-827-2	
2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (99-49-0)	

¹¹ Meynadier, J. M., J. Meynadier, J. L. Peyron, and L. Peyron. 1986. Clinical forms of skin manifestations in allergy to perfume. *Ann. Dermatol. Venerol.* 113:31–39.

(5R)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (6485-40-1) (5S)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (2244-16-8)	
99-49-0: p-Mentha-6,8-dien-2-one; (±)-Carvone; 2-Methyl-5-isopropenyl-2-cyclohexenone; 5-Isopropyl-2-methyl-2-cyclohexen-1-one; Carvone; DL-Carvone; Karvon; Limonen-6-one; NSC 6275; p-Mentha-1(6),8-dien-2-one 6485-40-1: R)-(-)-p-Mentha-6,8-dien-2-on); (-)-(5R)-Carvone; (-)-(R)-Carvone; (-)-Carvone; (-)-p-Mentha-6,8-dien-2-one; (4R)-(-)-Carvone; (R)-(-)-Carvone; (R)-Carvone; L-(-)-Carvone; L-Carvone; l-1-Methyl-4-isopropenyl-6-cyclohexen-2-one; l-Carvone 2244-16-8: (S)-(+)-p-Mentha-6,8-dien-2-one; (+)-Carvone; (S)-(+)-Carvone; (S)-(+)-p-Mentha-6,8-dien-2-one; (S)-Carvone; (+)-Carvone; D-(+)-Carvone; D-Carvone; Talent; d-1-Methyl-4-isopropenyl-6-cyclohexen-2-one; (S)-2-Methyl-5-(1-methylvinyl)cyclohex-2-en-1-one; d-Carvone	

Current regulation: /

Clinical data:

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

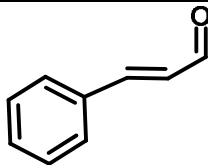
“Carvone has occasionally been reported as an allergen, usually in flavourings. Isomers of carvone have been either a mint or a rye flavour and aroma. We report a woman with positive patch-test reactions to carvone (newly added to the North American Contact Dermatitis Group standard series) and dermatitis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

Additional information:

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

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

¹² <http://www.ncbi.nlm.nih.gov/pubmed/20233552>

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

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

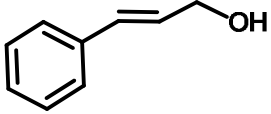
Clinical data:
In the “background information” section of the previous opinion (33), cinnamal, one of the 8 constituents of the FM I, is classified as frequent allergen, causing allergic reactions in a notable persons with eczema from cosmetic products in several studies (33).

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

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

Additional information:

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

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

Opinion on fragrance allergens in cosmetic products

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

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

Clinical data:

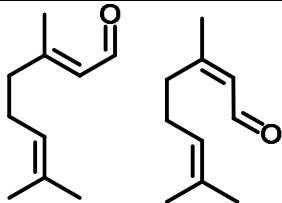
In the “background information” section of the previous opinion (33), cinnamyl alcohol, one of the 8 constituents of the FM I, is classified as frequent allergen, causing allergic reactions in a notable persons with eczema from cosmetic products (33).

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

Additional information:

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

A RIFM review is available (81)

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

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

Clinical data:

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

cosmetic products (33).

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

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

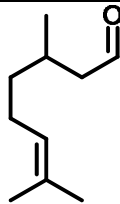
Additional information:

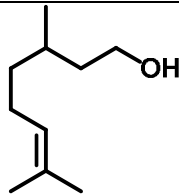
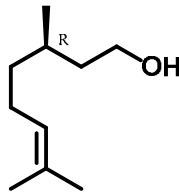
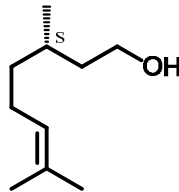
Citral is the mixture of two isomers: cis-citral (neral) and trans-citral (geranial).

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

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

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

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

CITRONELLOL			
CAS # 106-22-9 / 1117-61-9 / 7540-51-4			
EC # 247-737-6 / 214-250-5 / 231-415-7			
3,7-Dimethyl-6-octen-1-ol (106-22-9); (3R)-3,7-Dimethyl-6-octen-1-ol (1117-61-9); (3S)-3,7-Dimethyl-6-octen-1-ol (7540-51-4)			106-22-9
106-22-9: (±)-3,7-Dimethyl-6-octen-1-ol; (±)-Citronellol; (±)-β-Citronellol; 2,3-Dihydrogeraniol; 2,6-Dimethyl-2-octen-8-ol; Cephrol; Citronellol; Citronello 950; DL-Citronellol; Dihydrogeraniol; NSC 8779; Rodinol; dl-Citronellol; β-Citronellol			
1117-61-9: (R)-3,7-Dimethyl-6-octen-1-ol; (R)-(+)-3,7-Dimethyl-6-octen-1-ol; (+)-(R)-Citronellol; (+)-Citronellol; (+)-β-Citronellol; (3R)-(+)-β-Citronellol; (R)-(+)-Citronellol; (R)-(+)-β-Citronellol; (R)-Citronellol; (R)-β-Citronellol; D-Citronellol; d-Citronellol	1117-61-9	7540-51-4	
7540-51-4: (-)-3,7-Dimethyl-6-octen-1-ol; (-)-(S)-Citronellol; (-)-Citronellol; (-)-β-Citronellol; (S)-(-)-Citronellol; (S)-(-)-β-Citronellol; (S)-3,7-Dimethyl-6-octen-1-ol; (S)-Citronellol; (S)-β-Citronellol; L-Citronellol; l-Citronellol			

Current regulation: Annex III, part 1, n° 86

Clinical data:

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

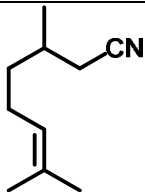
Since the last SCCNFP-opinion of 1999, in the Larsen 2002 c study, „DL citronello“ (5% in pet.) elicited positive PT reactions in 8.7% of the patients (1). In 1855 consecutive patients of the Frosch 2002 a study, 0.4% positive reactions were noted (16). In the EU 2005 study, 4 of 1701 patients (0.2%,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

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

Additional information:

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

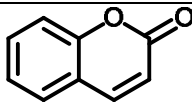
RIFM reviews have been published regarding L-citronellol (86), D-citronellol (87) and DL-citronellol (88). Another review is available by Hostynek and Maibach (89). It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

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

Current regulation: /

Clinical data: /

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

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

Current regulation: Annex III, part1, n° 77

Clinical data:

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

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

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

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

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

Additional information: ...

169

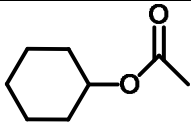
methylpropionaldehyde; methylpropionaldehyde(REACH, EINECS); methylhydrocinnamic aldehyde; Cyclamal; Cyclamen aldehyde; Cyclosal; Cyclosal perfume; Cymal; p-Isopropyl- α - methylhydrocinnamaldehyde; methylethyl)benzenepropanal; isopropylhydrocinnamaldehyde	3-p-Cumenyl-2- 4-Isopropyl- α - α -Methyl-4-(1-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

Current regulation: ...

Clinical
/

data:

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

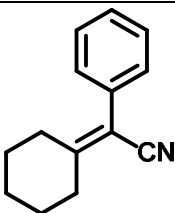
CYCLOHEXYL ACETATE	
CAS # 622-45-7	
EC # 210-736-6	
Cyclohexyletanoat	
Acetic acid cyclohexanyl ester; Acetoxycyclohexane; Cyclohexyl acetate; NSC 8772	

Current regulation: /

Clinical data:

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

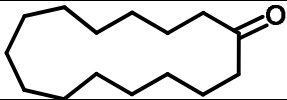
Additional information: A RIFM review is available (92).

<i>alpha</i>-CYCLOHEXYLIDENE BENZENEACETONITRILE	
CAS # 10461-98-0	
EC # 423-740-1	
α-Cyclohexylidenebenzeneacetonitrile	
alpha-Cyclohexylidene-benzeneacetonitrile (REACH); Δ 1 α -Phenyl- α -Cyclohexaneacetonitrile; 2-Cyclohexylidene-2-phenylacetonitrile; NSC 408284; Peonile (REACH)	

Current regulation: /

Clinical data: /

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

CYCLOPENTADECANONE	
CAS # 502-72-7	

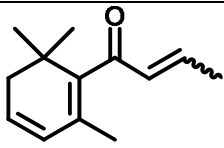
Opinion on fragrance allergens in cosmetic products

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

Current regulation: /

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

Additional information: ...

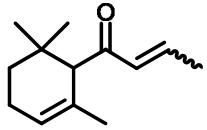
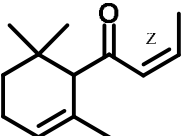
DAMASCENONE	
ROSE KETONE-4 (Not officially an INCI Name but Perfuming Name; Damascenone as such is not listed in CosIng)	
CAS # 23696-85-7	
EC # 245-833-2	
1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one	
1-(2,6,6-Trimethyl-1,3-cyclohexadienyl)-2-buten-1-one; 1-Crotonoyl-2,6,6-trimethyl-1,3-cyclohexadiene; 2,6,6-Trimethyl-1-(2-butenoyl)-1,3-cyclohexadiene; 2,6,6-Trimethyl-1-crotonyl-1,3-cyclohexadiene; Rose ketone # 4	

Current regulation: Annex III, part1, n° 160 (max. conc. 0.02%)

Clinical data: /

Additional information:

RIFM reviews are available (93, 94), quoting 1 negative, and 2 positive (2 of 37, 1 of 50 volunteers) HRIPTs with damascenone based on 2 LLNA, the EC3 values were calculated as 1.24% and 1.22%, respectively (94).

<i>alpha-DAMASCONE (TMCHB)</i>	 43052-87-5
CAS # 43052-87-5 / 23726-94-5	
EC # x / 245-845-8	
1-(2,6,6-Trimethylcyclohex-2-en-1-yl)but-2-enone (43052-87-5); (2Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one (23726-94-5)	
43052-87-5: 2,6,6-Trimethyl-1-crotonyl-2-cyclohexene; α -Damascone	 23726-94-5
23726-94-5: (Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one; (Z)- α -Damascone; cis- α -Damascone	

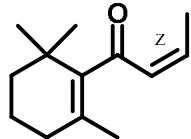
Current regulation: Annex III, part1, n° 157 (max. conc. 0.02%)

Clinical data:
In the Frosch 2002 b study, n=8 (0.5%) mostly strong positive PT reactions to consecutive patients were noted using a mixture of alpha and beta damascene, 0.1% pet. each (17). In human sensitisation experiments, after epicutaneous induction with 30% 1-(2,6,6-trimethylcyclohex-2-en-1-yl)but-2-enone (TMCHB, CAS # 43052-87-5) with adjuvant, to enhance response to this weak sensitiser, 8 of 30 patients were elicited by a challenge with 3% TMCHB 2 weeks later (95).

Additional information:

The former CAS # refers to alpha-Damascone or 1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-Buten-1-one. The latter CAS # refers to the identified ingredient cis-alpha-Damascone or (Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one, the content of which is restricted (SCCS-opinion 0392/00).

A RIFM review is available on alpha-damascone (96), quoting a number of partly positive HRIPT and other human studies, as well as different animal experiments. In 1 LLNA reported, an EC3 value of 3.3% was found. Another RIFM review is available for cis-alpha-damascone (97), supplying, however, no data on sensitisation.

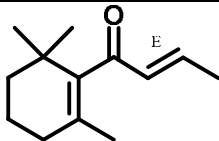
<i>cis-beta-DAMASCONE</i>	
CAS # 23726-92-3	
EC # 245-843-7	
(2Z)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one	
(Z)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (Z)- β -Damascone	

Current regulation: Annex III, part 1, n° 162 (max. conc. 0.02%)

Clinical data:
Regarding results of the Frosch 2002 b study, see under alpha-damascone.

Additional information:

A RIFM review is available (98), citing several negative and one positive HRIPTs, and a number of – mostly positive – animal experiments.

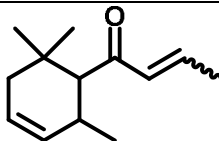
<i>trans-beta-DAMASCONE</i>	
CAS # 23726-91-2	
EC # 245-842-1	
(2E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one	
(E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (E)- β -Damascone; Damascone beta; trans-2,6,6-Trimethyl-1-crotonylcyclohex-1-ene; trans- β -Damascone; β -Damascone	

Current regulation: Annex III, part 1, n° 158 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (99), citing 2 negative HRIPT and 1 negative maximisation test, and a number of positive animal experiments (the EC3 value, based on 1 LLNA, was found to be 2.4%).

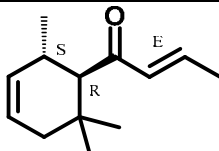
<i>delta-DAMASCONE</i>	
CAS # 57378-68-4	
EC # 260-709-8	
1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one	
δ-Damascone	

Current regulation: Annex III, part 1, n° 161 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (100), citing several positive HRIPT and 1 negative HRIPT. Cross sensitisation to alpha- and beta-damascone was demonstrated in 3 sensitised subjects. 2 LLNA studies are reported on, yielding EC3 values of 5.19% and 9.6%, resp.

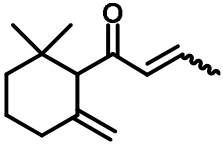
<i>trans-trans-delta-DAMASCONE</i>	
CAS # 71048-82-3	
EC # 275-156-8	
(2E)-rel-1-[(1R,2S)-2,6,6-Trimethyl-3-cyclohexen-1-yl]- 2-buten-1-one	
[1 α (E),2 β]-1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one; trans- δ -Damascone; δ -Damascone; trans, trans- δ -Damascone	

Current regulation: Annex III, part 1, n° 165 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (101), citing 1 positive HRIPT (2/15 with 1%).

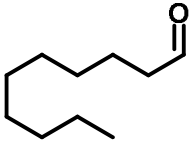
<i>gamma-DAMASCONE</i>	
CAS # 35087-49-1	
EC # 481-910-9	
1-(2,2-Dimethyl-6-methylenecyclohexyl)-2-buten-1-one	
γ-Damascone	

Current regulation: /

Clinical data: /

Additional information:

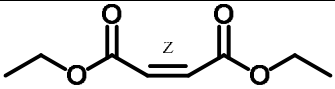
A RIFM review is available (102), citing 1 positive Buehler test and 1 LLNA study yielding an EC3 value of 4.6%

<i>DECANAL</i>	
CAS # 112-31-2	
EC # 203-957-4	
n-Decanal	
Capraldehyde; Capric aldehyde; Caprinaldehyde; Caprinic aldehyde; Decaldehyde; Decanaldehyde; Decyl aldehyde; Decylic aldehyde; NSC 6087; n-Decaldehyde; n-Decyl aldehyde	

Current regulation: /

Clinical data: /

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

<i>DIETHYL MALEATE</i>	
CAS # 141-05-9	
EC # 205-451-9	
(2Z)-Diethyl but-2-enedioate	
2-Butenedioic acid (2Z)-, diethyl ester; 2-Butenedioic acid (Z)-, diethyl ester; Maleic acid, diethyl ester; (2Z)-2-Butenedioic acid diethyl ester; Diethyl (Z)-2-butenedioate; Ethyl maleate; Staflex DEM	

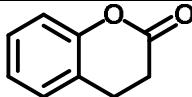
Current regulation: Annex II, n° 426

Clinical

data:

In the Malten 1984 study, 3.2% of 182 patients displayed a positive PT reaction to diethyl maleate 0.1% pet. (24). In this study, it has been noted that “in the max. test and clinically this is a strong sensitiser having caused patch test sensitisation (42%)”

Additional information: /

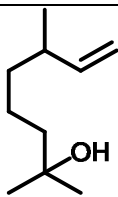
DIHYDROCOUMARIN	
CAS # 119-84-6	
EC # 204-354-9	
3,4-Dihydro-2H-1-benzopyran-2-one	
Hydrocoumarin; Hydrocinnamic acid, o-hydroxy-, δ -lactone; 2-Chromanone; 3,4-Dihydro-1H-benzopyran-2-one; 3,4-Dihydrocoumarin; Dihydrocoumarin; Melilotin; Melilotin (coumarin); Melilotol	

Current regulation: Annex II, n° 427

Clinical data:

In the Malten 1984 study, 3.7% of 182 patients displayed a positive PT reaction to dihydrocoumarine 5% pet. (24).

Additional information: /

DIHYDROMYRCENOL	
CAS # 18479-58-8	
EC # 242-362-4	
(±)-2,6-Dimethyloct-7-en-2-ol	
1,1,5-Trimethyl-6-heptenol; 2,6-Dimethyl-7-octen-2-ol; 3,7-Dimethyl-1-octen-7-ol; 2,6-Dimethyl-7-octen-2-ol (INCI)	

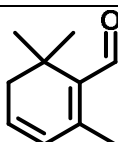
Current regulation: /

Clinical data: /

Additional information:

A RIFM review is available (103), listing 2 negative HRIPTs and 1 negative human maximisation test.

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

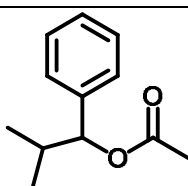
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	
CAS # 116-26-7	
EC # 204-133-7	
2,6,6-Trimethyl-1,3-cyclohexadiene-1-carboxaldehyde	
2,2,6-Trimethyl-4,6-cyclohexadien-1-aldehyde; 2,6,6-Trimethyl-1,3-cyclohexadiene-1-aldehyde; Safranal	

Current regulation: /

Clinical data: /

Additional information:

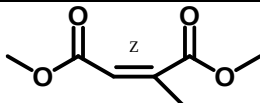
A RIFM review quotes one positive HRIPT (5 of 53) and one negative HRIPT (0 of 54) (93).

<i>DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)</i>	
CAS # 151-05-3	
EC # 205-781-3	
2-Methyl-1-phenylpropyl acetate	
Benzeneethanol, α,α-dimethyl-, acetate; Phenethyl alcohol, α,α-dimethyl-, acetate; 1,1-Dimethyl-2-phenylethyl acetate; 2-Methyl-1-phenyl-2-propyl acetate; 2-Methyl-1-phenylpropan-2-yl acetate; Benzyl dimethylcarbinol acetate; Benzyl dimethylcarbinyl acetate; Dimethylbenzylcarbinol acetate; Dimethylbenzylcarbonyl acetate; NSC 46123; α,α-Dimethylphenethyl acetate	

Current regulation: /

Clinical data:
In the Frosch 2002 a study, 0.2% positive PT reactions to consecutive patients were noted (16). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and one to 5% DMBCA in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

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

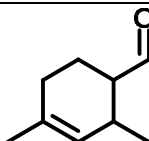
<i>DIMETHYL CITRACONATE</i>	
CAS # 617-54-9	
EC #	
(2Z)-Diethyl-2-metyl-but-2-enedioate	
(2Z)-2-methyl-2-Butenedioic acid, dimethyl ester; 2-Butenedioic acid, 2-methyl-, dimethyl ester, (Z)-; Citraconic acid, dimethyl ester; Dimethyl methylmaleate; Methylmaleic acid, dimethyl ester	

Current regulation: Annex II, n° 431

Clinical data:

In the Malten 1984 study, 3.7% of 182 patients displayed a positive PT reaction to dimethylcitraconate 12% pet. (24). In this paper, a human maximisation test positive in “4/44” is quoted.

Additional information: ...

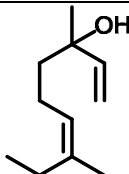
2,4-DIMETHYL-3-CYCLOHEXEN-1-CARBOXALDEHYDE	
CAS # 68039-49-6	
EC # 268-264-1	
2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde	
(Z)-Vertocitral C; 2,4-Dimethyl-3-cyclohexene-1-carboxaldehyde; 2,4-Dimethyl-3-cyclohexenecarboxaldehyde; 2,4-Dimethyl-3-cyclohexenylcarbaldehyde; Cyclal C; Ligustral; Tricyclal; Triplal; Tripral; Zestover	

Current regulation: /

Clinical data: /

Additional information:

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

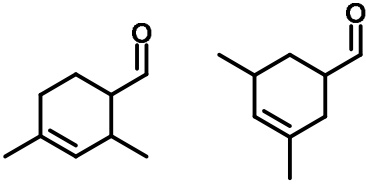
3,7-DIMETHYL-1,6-NONADIEN-3-OL	
CAS # 10339-55-6	
EC # 233-732-6	
(7Z)-3,7-Dimethyl-1,6-nonadien-3-ol	
Ethyl linalool; Methyllinalool	

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (104), citing 1 negative human maximisation test (n=25).

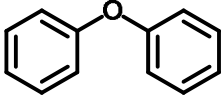
DIMETHYLTETRAHYDRO BENZALDEHYDE	
CAS # 68737-61-1	
EC # 272-113-5	
2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde 3,5-Dimethyl-cyclohex-3-ene-1-carboxaldehyde	
Hivertal; Vertocitral	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 2.3% positive PT reactions were observed with the isomer mixture, tested 5% pet., in 178 patients with known contact allergy to fragrance ingredients (19).

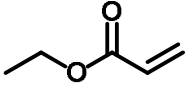
Additional information: /

DIPHENYL ETHER	
CAS # 101-84-8	
EC # 202-981-2	
Phenyl ether	
1,1'-oxybis-Benzene; Barrel Therm 330; Benzene, phenoxy-; Biphenyl oxide; Chemcryn JK-EB; Diphenyl ether; Diphenyl oxide; NSC 19311; Oxybisbenzene; Phenoxybenzene; Phenyl oxide	

Current regulation: /

Clinical data: /

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

ETHYL ACRYLATE	
CAS # 140-88-5	
EC # 205-438-8	
Ethyl 2-propenoate	
Acrylic acid ethyl ester (6CI,8CI); 2-Propenoic acid ethyl ester; Ethyl 2-propenoate; Ethyl acrylate; Ethyl acrylic ester; Ethyl propenoate; NSC 8263	

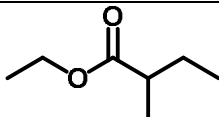
Current regulation: Annex II, n° 435

Clinical data:

In the Malten 1984 study, n=1 (0.5%) of 182 patients displayed a positive PT reaction to ethyl acrylate 1% pet. (24). In the NACDG 2009 multicentre study, 0.9% of

consecutive patients (n=4428) had a positive PT reaction (21).

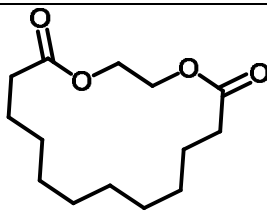
Additional information: /

ETHYL 2-METHYLBUTYRATE	
CAS # 7452-79-1	
EC # 231-225-4	
Ethyl 2-methylbutyrate	
Butyric acid, 2-methyl-, ethyl ester (6CI,7CI,8CI); (±)-Ethyl 2-methylbutanoate; 2-Methylbutanoic acid ethyl ester; 2-Methylbutyric acid ethyl ester; Ethyl 2-methylbutanoate; Ethyl 2-methylbutyrate; Ethyl α-methylbutyrate; NSC 1103	

Current regulation: /

Clinical data: /

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

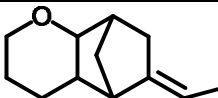
ETHYLENE DODECANEDIOATE	
CAS # 54982-83-1	
EC # 259-423-6	
1,4-Dioxacyclohexadecane-5,16-dione	
Cyclic ethylene dodecanedioate; Ethylene dodecanedioate; Musk 144; Musk C-14	

Current regulation: /

Clinical data:

In the Larsen 2002 c study on 218 patients with known contact allergy to fragrance ingredients, this compound caused 0.9% positive PT reactions at 5% pet. (1).

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

6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	
CAS # 93939-86-7	
EC # 300-376-9	
6-Ethylideneoctahydro-5,8-methano-2H-1-benzopyran	

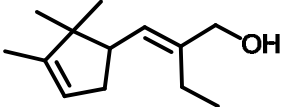
Current regulation: /

Clinical

data:

In the Larsen 2001 study, no positive PT reactions were observed with this compound, tested 5% pet., in 178 patients with known contact allergy to fragrance ingredients (19).

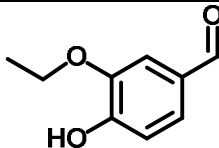
Additional information: /

2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL	
CAS # 28219-61-6	
EC # 248-908-8	
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol	
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol; 2-Ethyl-4-(2',2',3-trimethylcyclopent-3'-enyl)but-2-enol; Bacdanol; Bangalol; Dartanol; Finanol; Levosandol; Radjanol; Sanjinol	

Current regulation: /

Clinical data: /

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

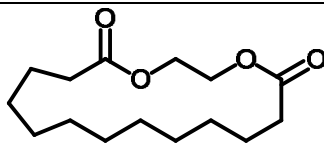
ETHYL VANILLIN	
CAS # 121-32-4	
EC # 204-464-7	
3-Ethoxy-4-hydroxybenzaldehyde	
2-Ethoxy-4-formylphenol; 3-Ethoxy-4-hydroxybenzaldehyde; 3-Ethylvanillin; 4-Hydroxy-3-ethoxybenzaldehyde; Arovanillon; Bourbonal; Ethavan; Ethovan; Ethylprotal; Ethylvanillin; NSC 1803; NSC 67240; Protocatechuic aldehyde ethyl ether; Quantrovanil; Rhodiarome; Vanillal; Vanirom	

Current regulation: /

Clinical data:

The case of a 28-year-old metal grinder with allergic contact dermatitis to a “cutting oil reodorant” has been reported, who tested positively not only to the cutting fluid, the reodorant, but also to several ingredients of the latter product, including “Vanillal S 10026”, 5% pet. (105).

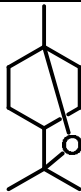
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

ETHYLENE BRASSYLATE	
CAS # 105-95-3	
EC # 203-347-8	
1,4-Dioxacycloheptadecane-5,17-dione	
Tridecanedioic acid, cyclic ethylene ester; Ethylene glycol, cyclic tridecanedioate; Astratone; Cyclic ethylene glycol tridecanedioate; Cyclic ethylene tridecanedioate; Emeressence 1150; Ethylene brassylate; Musk T; NSC 46155	

Current regulation: /

Clinical data: /

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

EUCALYPTOL	
CAS # 470-82-6	
EC # 207-431-5	
1,3,3-Trimethyl-2-Oxabicyclo[2.2.2]octane	
1,8-Epoxy-p-menthane; 1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane; 1,8-Cineol; 1,8-Cineole; 1,8-Epoxy-p-menthane; 2-Oxa-1,3,3-trimethylbicyclo[2.2.2]octane; Cajeputol; Cineol; Cineole; Eucalyptol; Eucalyptole; Eucalytol; Eucapur; Eukalyptol; NSC	

Opinion on fragrance allergens in cosmetic products

6171; Terpan; p-Cineole	

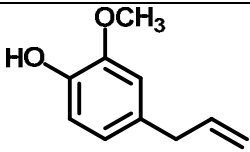
Current regulation: /

Clinical data: /

Additional information:

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

See also ***EUCALYPTUS SPP. LEAF OIL***; eucalyptol is the major ingredient there (up to 85%), but found in significant quantities also in a number of other essential oils (see 3.2).

EUGENOL	
CAS # 97-53-0	
EC # 202-589-1	
2-Methoxy-4-(2-propen-1-yl)-phenol	
Other names: 4-Allyl-2-methoxy-phenol; 1-Allyl-4-hydroxy-3-methoxybenzene; 2-Hydroxy-5-allylanisole; 2-Methoxy-1-hydroxy-4-allylbenzene; 2-Methoxy-4-(2-propenyl)phenol; 2-Methoxy-4-(2'-propenyl)phenol; 2-Methoxy-4-[2-allyl]phenol; 2-Methoxy-4-allylphenol; 3-(3-Methoxy-4-hydroxyphenyl)propene; 3-(4-Hydroxy-3-methoxyphenyl)-1-propene; 4-Allyl-1-hydroxy-2-methoxybenzene; 4-Allyl-2-methoxyphenol; 4-Allylguaiacol; 4-Hydroxy-3-methoxyallylbenzene; Allylguaiacol; Bioxeda; Caryophylllic acid; Dentogum; Eugenig acid; Eugenol; NSC 209525; NSC 8895; p-Allylguaiacol; p-Eugenol	

Current regulation: Annex III, part 1, n° 71

Clinical data:

In the “background information” section of the previous opinion (33), eugenol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 1.2% in consecutive PT patients and accounting for 4 to 16% of reactions to the FM I. Allergic reactions had been observed in 0.7 – 20% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.5% (95% CI: 0.3 – 1.0%) positive reactions in 2065 consecutively PTed patients (4). In the Groningen 2009 study, 1.3% (95% CI: 0.3 – 3.2%) had positive reactions to eugenol, tested at 2% pet., i.e., twice the commonly used concentration (6). F. Giusti et al. examined 1754 consecutive patients tested with eugenol 1% pet. in addition to the baseline series, 09/1998 - 01/2000. 21 patients (1.2%) reacted positively to eugenol (106). In the An 2005 study, 8 of 422 consecutive patients, i.e., 1.9%, had positive reaction (13) (test concentration 2%). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 2.5% positive reactions (22). The IVDK 2010 study, 0.44% (95% CI: 0.04 – 0.84%) of 1214 consecutively tested patients reacted to the compound, while 1.57% (95% CI: 1.19 – 1.95%) of 4801 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to eugenol (48).

Moreover, eugenol is capable of inducing immediate type reactions of the airways, as illustrated by the well-documented case of a 30 year old hairdresser who developed severe occupational bronchial asthma due to eugenol (107). A case of urticaria after dental treatment with eugenol-containing material was reported from India (108); however, occasional cases are also reported from Europe (109). Occupational exposure to eugenol / zinc oxide type dental restorative material, which is apparently less frequently used nowadays, may lead to occupational sensitisation to eugenol, as illustrated by a case report (110).

Additional information:

Eugenol is the main component (80-95%) of clove oil, but also found in citronella oil, pimento leaf oil and cinnamon bark oil (see section 3.2).

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

FARNESOL

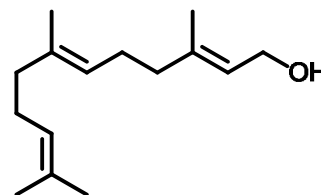
CAS # 4602-84-0

EC # 225-004-1

3,7,11-Trimethyl-2,6,10-Dodecatrien-1-ol

Farnesol; 3,7,11-Trimethyl-2,6,10-dodecen-1-ol; FCI 119a; Farnesyl alcohol; NSC 60597; Nikkosome

Current regulation: Annex III, part 1, n° 82



Clinical data:

In the “background information” section of the 1999 opinion, farnesol is classified as “less frequently reported allergen”; in 1 study of patients with cosmetic dermatitis 2 cases with contact allergy to farnesol had been reported; in other studies, positive reactions were seen in patients with positive PT reactions to MPR (33).

Since the last SCCNFP-opinion of 1999, farnesol is used not only for its scent, but also for its (slight) antimicrobial activity, useful, for instance, in deodorants. Thus, axillary dermatitis is a relatively typical presentation (111). In a multicentre study based on 1997/98 PT data, 0.5% positive reactions in consecutive patients were noted (Frosch 2002 a (16)). Farnesol is included in the FM II. In the original publication on single constituents of the FM II, 6 of 1701 consecutive patients reacted positively to farnesol 5%, ie., 0.35% (95% CI: 0.13 – 0.77%) (10). In a study on consecutive patients tested in 2003, 38 of 4238 patients had positive reactions to farnesol 5% pet. (0.9%, 95% CI: 0.6 – 1.2%) (4)(IVDK 2007). (A paper on farnesol previously published by the IVDK (112) presents results included in this later analysis.) In a series from Nagoya, Japan, 1.1% positive reactions in 1483 patients with suspected cosmetic dermatitis were observed (tested at 5% pet.) (14). In the Groningen 2009 study, 0.9% (95% CI: 0.2 – 2.7%) had positive reactions (6).

Additional information:

“Farnesol is an acyclic primary sesquiterpene alcohol found in essential oils such as lemongrass, citronella, tuberose blossom, sandalwood and orange blossom” (23). A RIFM review is available (113).

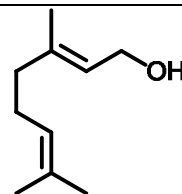
GERANIOL

CAS # 106-24-1

EC # 203-377-1

(2E)-3,7-Dimethyl-2,6-octadien-1-ol

(E)-3,7-Dimethyl-2,6-octadien-1-ol; (E)-Geraniol; (E)-Nerol; 3,7-Dimethyl-trans-2,6-octadien-1-ol; Geraniol; Geranyl alcohol; Lemonol; MosquitoSafe; NSC 9279; trans-3,7-Dimethyl-2,6-octadien-1-ol; trans-Geraniol; β-Geraniol



Current regulation: Annex III, part 1, n° 78

Clinical data:

In the “background information” section of the previous opinion (33), geraniol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 0.4% in consecutive PT patients and accounting for 3 to 7% of reactions to the FM I. Allergic reactions had been observed in 1.2 – 30% of patients with

eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.5% (95% CI: 0.2 – 0.9%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at 2%, i.e. twice the usual concentration (6). In a series from Nagoya, Japan, 0.3% positive reactions in 1483 patients with suspected cosmetic dermatitis were observed (tested at the unusually high concentration of 5% pet.) (14). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=7 (0.9%) positive reactions (22). The IVDK 2010 study, 0.39% (95% CI: 0.10 – 0.69%) of 1214 consecutively tested patients reacted to the compound, while 0.87% (95% CI: 0.63 – 1.10%) of 5695 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=17 reacted positively to geraniol (48).

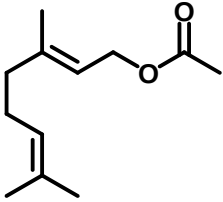
The fact that geraniol also occurs in food flavourings, and can elicit signs and symptoms of manifest contact sensitisation, is illustrated by the case of a 19 year old Japanese woman with cheilitis due to geraniol, improving after avoidance of respective foodstuff (114). A 20 year old Japanese woman with urticaria at the site of application of cosmetics with generalisation (contact urticaria syndrome grade 2), which A. Yamamoto et al. diagnosed as immediate type hypersensitivity to geraniol (without CA) (115).

Additional information:

Geraniol is a component of Palmarosa oil (CYMBOPOGON MARTINI see below), geranium oil (about 40%), citronella oil (30-40%), rose oil, lavender oil, and jasmine oil. It is sensitive to heat which induces autooxidation and isomeric with linalool (53).

Geraniol forms oxidation product with increased sensitizing capacity both via spontaneous autooxidation at air exposure and via metabolic oxidation. Geraniol and neral together with hydroperoxide have been identified as oxidation products when geraniol autooxidizes (84). Geraniol and neral were also identified as metabolites of geraniol (85). This explains the simultaneous reactions to geraniol and citral seen by (4).

A review is available by Hostynek and Maibach (116) and by RIFM (117). It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

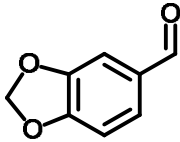
GERANYL ACETATE	
CAS # 105-87-3	
EC # 203-341-5	
(2E)-1-Acetate-3,7-dimethyl-2,6-octadien-1-ol	
(E)-Acetat-3,7-dimethyl-2,6-Octadien-1-ol; Geraniol acetate; (E)-3,7-Dimethyl-2,6-octadien-1-ol acetate; (E)-3,7-Dimethyl-2,6-octadienyl acetate; Acetic acid (2E)-3,7-dimethyl-2,6-octadienyl ester; Acetic acid geraniol ester; Bay pine (oyster) oil; Geranyl acetate; Geranyl ethanoate; NSC 2584; trans-1-Acetoxy-3,7-dimethyl-2,6-octadiene; trans-3,7-Dimethyl-2,6-octadien-1-yl acetate; trans-Geranyl acetate; β-Geranyl acetate	

Current regulation: /

Clinical data: /

Additional information:

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

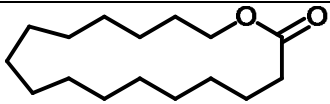
HELIOTROPINE	
CAS # 120-57-0	
EC # 204-409-7	
1,3-Benzodioxole-5-carboxaldehyde	
Piperonal; 2H-Benzo[3,4-d]-1,3-dioxolan-5-ylformaldehyde; 3,4-(Methylenedioxy)benzaldehyde; 3,4-Dihydroxybenzaldehyde methylene ketal; 3,4-Dimethylenedioxybenzaldehyde; 5-Formyl-1,3-benzodioxolane; 5-Formyl-1,3-benzodioxole; 5-Formylbenzodioxole; Benzo[1,3]dioxole-5-carbaldehyde; Benzo[d][1,3]dioxole-5-carboxaldehyde; Heliotropin; Heliotropine; NSC 26826; Piperonaldehyde; Piperonylaldehyde; Protocatechuic aldehyde methylene ether	

Current regulation: /

Clinical data:

In the Frosch 2002 b study, n=2 (0.2%) positive reactions to “piperonal” (1% pet.) and n=6 (0.4%) to “piperonal” (5% pet.), respectively, in 1606 consecutive were observed (17). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% heliotropine in pet., tested in 106 consecutive patients in Barcelona, were observed (15).

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

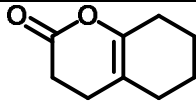
HEXADECANOLACTONE	
CAS # 109-29-5	
EC # 203-662-0	
Oxacycloheptadecan-2-one	
o-Lactone-16-hydroxy-hexadecanoic acid; 1,16-Hexadecanolide; 16-Hexadecanolactone; Cyclohexadecanolide; Dihydroambrettolide; Hexadecanoic acid, 16-Hydroxy-, o-lactone; Hexadecanolactone; Hexadecanolide; Juniperic acid lactone; NSC 33546	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 1 of 178 patients with previously diagnosed contact allergy to fragrance ingredients had a positive PT reaction to this compound, tested 5% pet. (19). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reactions to 5% “hexadecanolide” (13).

Additional information: /

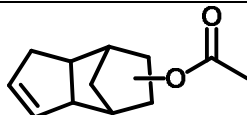
HEXAHYDROCOUMARIN	
CAS # 700-82-3	
EC # 211-851-4	
3,4,5,6,7,8-Hexahydro-2H-1-benzopyran-2-one	
3,4,5,6,7,8-Hexahydro-coumarin; δ -Lactone-2-hydroxy-1-cyclohexene-1-propanoic acid; 3,4,5,6,7,8-Hexahydrocoumarin; Hexahydrocoumarin; Δ -1,6-2-Oxabicyclo(4.4.0)decen-3-one	

Current regulation: Annex II, n° 1135

Clinical data: /

Additional information:

A RIFM review is available (93), p. S115 ff, citing a number of positive human sensitisation experiments.

3α,4,5,6,7,7α-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	
CAS # 54830-99-8	
EC # 259-367-2	
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-indenol Acetate	
Acetoxidyhydrodicyclopentadiene; Cyclacet; Dicyclopentenyl acetate; Dicylat; Tricyclo[5.2.1.0 ^{2,6}]dec-3-enyl acetate; Tricyclodecenyl acetate	

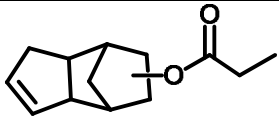
Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 1 to 5% "Cyclacet ®" in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

Additional information:

Produced by IFF under the brand name "Cyclacet" (<http://www.iff.com/Ingredients.nsf/0/1C9F2CB39EB1EF6480256993002FBC14>, last accessed 2010-07-08).

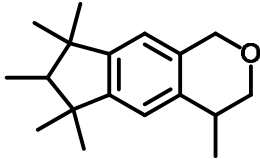
HEXAHYDRO-METHANOINDENYL PROPIONATE	
CAS # 68912-13-0	
EC # 272-805-7	
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-indenol propanoate	

3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-indenyl propionate (Mixture of Isomers); Dicyclopentadiene propionate; tricyclodecenyl propionate; Tricyclo[5.2.1.0 ^{2,6}]dec-3-enyl propionate; Verdyl propionate	

Current regulation: /

Clinical data: /

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

HEXAMETHYLINDANOPYRAN	
CAS # 1222-05-5	
EC # 214-946-9	
1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[γ]-2-benzopyran	
1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[γ]-2-benzopyrane; 1,3,4,6,7,8-Hexahydro-4,6,6,8,8,8-hexamethylcyclopenta-2-benzopyran; Abbalide; Galaxolide; Galaxolide 50; Galaxolide 50BB; Galaxolide 50IPM; Galaxolide White; HHCB; Pearlide	

Current regulation: /

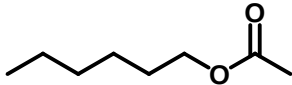
Clinical data:

In the Frosch 2002 a study, n=3 (0.2%) had positive reactions to the compound, tested 10% in isopropyl myristate (with 1 patient reacting positively to the diluent) (16). The Larsen 2001 study, testing with HHCB 7% pet., found 3.4% positive reactions in 178 patients with known contact allergy to fragrance ingredients (19). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had a positive reaction to “Galaxolide 50”, tested at 5% (13) (test concentration 2% pet.). The DeGroot 1985 study identified 3 (1.7%) positive reactions among 179 patients using a 25% PT preparation of HHCB (25). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% “Galaxolide 50 ®” in pet., tested in 100 consecutive patients in Stockholm, were observed (15).

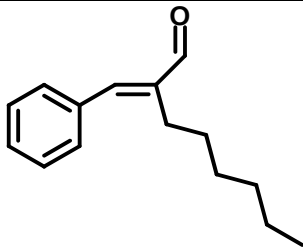
Additional information:

[0403/00 - Opinion concerning Hexahydro-hexamethyl-cyclopenta\(γ\)-2-benzopyran \(HHCB\)](#)

[0610/02 - Opinion on Hexahydro-hexamethyl-Cyclopenta \(γ\)-2-Benzopyran \(HHCB\)](#) (no restrictions) It is a “top 100” substance (IFRA, pers. comm.2010).

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

acid;; NSC 7323; n-Hexyl acetate; n-Hexyl ethanoate	
Current regulation: /	
Clinical data: /	
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).	

HEXYL CINNAMAL	
CAS # 101-86-0	
EC # 202-983-3	
α-Hexyl-cinnamaldehyde	
2-(Phenylmethylene)octanal; 2-Hexyl-3-phenyl-2-propenal; 2-Hexylcinnamaldehyde; Hexyl cinnamic aldehyde; NSC 406799; NSC 46150; α-Hexylcinnamaldehyde; α-Hexylcinnamic aldehyde; α-Hexylcinnamyl aldehyde; α-n-Hexyl-β-phenylacrolein; α-n-Hexylcinnamaldehyde	
Current regulation: Annex III, part 1, n° 87	

Clinical data:

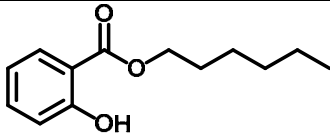
In the “background information” section of the 1999 opinion, hexyl cinnamal (synonymous: alpha-hexyl cinnamal, AHCA) is classified as “less frequently reported allergen”; 2 studies with 1 case and 1 study with 7 cases of contact allergy to this compound in patients with eczema from cosmetic products were found (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 0.3% positive PT reactions to consecutive patients were noted (16). In the subsequent EU 2005 study, 2 of 1701 patients had positive reactions to AHCA, and n=16 doubtful or irritant to AHCA at 10% in pet. (10). The IVDK 2007 study yielded n=3, i.e. 0.2% (95% CI: 0.03 – 0.4%) positive reactions in 2019 consecutively PTed patients, using 10% pet. as test concentration (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, using a lower test concentration of 5% pet. (6).

Additional information:

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

Hexyl cinnamal is regarded as “a recommended positive control for skin sensitization testing”, e.g., in the context of the LLNA (118).

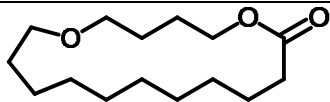
HEXYL SALICYLATE	
CAS # 6259-76-3	
EC # 228-408-6	
Hexyl-2-hydroxybenzoate	
Salicylic acid, hexyl ester; 1-Hexyl salicylate; Hexyl salicylate; n-Hexyl salicylate	
Current regulation: /	

Clinical data:

None of the 218 patients with known contact allergy to fragrance ingredients reacted positively to this compound (tested at 5% in pet.) in the Larsen 2002 c study (1).

Additional information:

In a RIFM review, 2 human sensitisation experiments are mentioned which yielded no evidence of sensitising potential (HRIPT, n=103, maximisation test, n=22) (119). It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

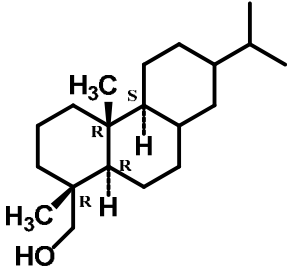
HIBISCOLIDE	
CAS # 6707-60-4	
EC # 229-755-6	
1,6-Dioxacycloheptadecan-7-one	
Undecanoic acid, 11-(4-hydroxybutoxy)-, o-lactone; 12-Oxa-1,16-hexadecanolide; Cervolide; Musk 781; NSC 34741; 12-Oxahexadecan-16-olide	

Current regulation: /

Clinical data:

None of the 178 patients with known contact allergy to fragrance ingredients reacted positively to "12-oxahexadecanolide" (tested at 5% in pet.) in the Larsen 2001 study (19).

Additional information: /

HYDROABIETYL ALCOHOL, when used as a fragrance ingredient	
CAS # 13393-93-6	
EC # 236-476-3	
(1R,4aR,4βS,10aR)-Tetradecahydro-1,4a-dimethyl-7-(1-methylethyl)-1-Phenanthrenemethanol	
Tetradecahydro-1,4a-dimethyl-7-(1-methylethyl)-phenanthrenemethanol; Tetrahydroabietyl alcohol	1-

Current regulation: AnnexII, n° 440

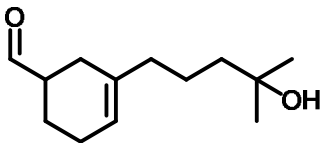
Clinical data:

In the deGroot 2000 study, 17 of 1825 consecutively tested patients had positive reactions to hydroabietyl alcohol (10% pet.) (12).

Additional information:

Commercial hydroabietyl alcohol consists of di- and tetrahydroabietyl alcohol together with non-modified colophony (120)

HYDROXYISOHEXYL	3-CYCLOHEXENE	

CARBOXALDEHYDE (HICC) regioisomers	 51414-25-6
CAS # 31906-04-4 / 51414-25-6	
EC # 250-863-4 / 257-187-9	
4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (31906-04-4) 3-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (51414-25-6)	

31906-04-4: 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexenecarboxaldehyde; 4-(4-Methyl-4-hydroxyamyl)cyclohex-3-ene carboxaldehyde; Lyrall

Current regulation: Annex III, part 1, n° 79

Clinical data:

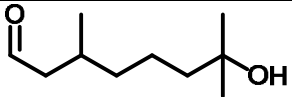
In the “background information” section of the previous opinion (33) HICC is classified as frequent allergen, causing allergic reactions in about 2.8% in consecutive PT patients, two thirds of these being relevant (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 2.7% of the 1855 consecutive patients reacted positively to HICC (5% pet.) (16). In the EU 2005 study, 28 of 1701 patients (1.7%, 95% CI: 1.1 – 2.4%) reacted positively to 5% HICC in pet. (10). In 21325 patients PTed consecutively in the IVDK 2007 study, 2.4% (95% CI: 2.2 – 2.6%) positive reactions were noted to 5% HICC in pet. (4). Similar to other studies, HICC was the most common single fragrance allergen among 320 patients tested in the Groningen 2009 study, with 3.1% (95% CI: 1.5 – 5.7%) positive reactions despite testing with a lower concentration of 2% pet. (6). In the An 2005 study, 7 of 422 consecutive patients, i.e., 1.7%, had positive reaction (13). The Belsito 2006 study (20) yielded a relatively low prevalence of 0.4% (7 of 1603; exact 95% CI (recalculated): 0.17 – 0.90%) positive reactions with 5% HICC in pet. and even less with lower test concentrations; possible reasons for the much lower prevalence were discussed. The IVDK 2010 study, 2.36% (95% CI: 2.19 - 2.53%) of 37270 consecutively tested patients reacted to HICC (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=8 reacted positively to HICC (48).

Further clinical data with a focus on quantitative dose-response (see also section 4.3), is discussed in (121).

Among the early case reports, S.A. Hendriks reported the case of a 20 year old patient developing axillary dermatitis after 5 months use of a deodorant containing HICC (122).

Additional information: /

HYDROXYCITRONELLAL	
CAS # 107-75-5	
EC # 203-518-7	
7-Hydroxy-3,7-dimethyl-octanal	

(±)-Hydroxycitronellal; 3,7-Dimethyl-7-hydroxyoctanal; 7-Hydroxy-3,7-dimethyloctanal; 7-Hydroxycitronellal; Citronellal hydrate; Citronellal, hydroxy-; Cyclalial; Cyclosia; Cyclosia base; Fixol; Hydroxycitronellal; Laurine; Lilyl aldehyde; Muguet synthetic; Muguetine principle; NSC

Opinion on fragrance allergens in cosmetic products

406740; Phixia

Current regulation: Annex III, part 1, n° 72

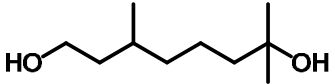
Clinical data:

In the “background information” section of the previous opinion (33), hydroxycitronellal, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 0.75% in consecutive PT patients and accounting for 6 to 16% of reactions to the FM I. Allergic reactions had been observed in 10 – 45% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 1.3% (95% CI: 0.9 – 1.9%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 2.2% (95% CI: 0.9 – 4.5%) had positive reactions to this compound, tested at 2% pet., i.e., twice the commonly used concentration (6). The Sugiura 2000 study observed 1% positive PT reactions (test concentration 5% pet.) in 1483 patients tested for suspected cosmetic dermatitis (14). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.5% positive reactions (22). The IVDK 2010 study, 1.17% (95% CI: 0.48 – 1.85%) of 1214 consecutively tested patients reacted to the compound, while 2.95% (95% CI: 2.43 – 3.47%) of 4359 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were tested with hydroxycitronellal, yielding 6 positive reactions (48).

Additional information:

Hydroxycitronellal is a synthetic fragrance, which only recently has been found in a few essential oils, e.g., of a *Narcissus* species and in essential oils of pepper (53)

HYDROXYCITRONELLOL	
CAS # 107-74-4	
EC # 203-517-1	
3,7-Dimethyl-7-octanediol	
2,6-Dimethyl-2,8-octanediol; 3,7-Dimethyl-1,7-octanediol; 3,7-Dimethyloctan-1,7-diol; Citronellol, hydroxy-; Hydroxyciol; Hydroxycitronellol; NSC 406140; NSC 67886	

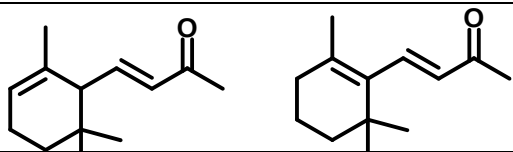
Current regulation: /

Clinical data:

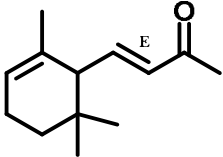
This compound elicited 6.0% positive PT reactions in 218 fragrance sensitive individuals (Larsen 2002 c, (1)).

Additional information:

A RIFM review is available, reporting results of a human induction study (maximisation test) in 25 volunteers, yielding no evidence of sensitisation (123).

IONONE isomeric mixture	
CAS # 8013-90-9	
EC # 232-396-8	

Ionone	
Irisone, mixture of alpha- and beta ionone	
Current regulation: /	
Clinical data: / (see single isomers)	
Additional information:	
It is a “top 100” substance, further specified with “mixed isomers” (IFRA, pers. comm.2010).	
INCI: “MIXED IONONES”, with CAS # 14901-07-6 / 6901-97-9 / 8013-90-9 (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=35383 , last accessed 2010-07-13).	
A RIFM review is available on “ionone” (124), quoting negative human and experimental results.	

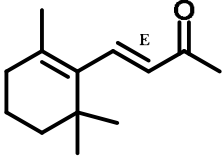
<i>alpha-IONONE</i>	
CAS # 127-41-3	
EC # 204-841-6	
(3E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-Buten-2-one	
(E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-Buten-2-one; (5E)-Ionone; (E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; (E)-α-Ionone; (±)-trans-α-Ionone; (±)-α-Ionone; 4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one;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

Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% alpha-ionone in pet., tested in 205 consecutive patients, were observed (15).

Additional information: A RIFM review is available (125).

<i>beta-IONONE</i>	
CAS # 79-77-6	
EC # 201-224-3	
(3E)-4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	
(E)-4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one; (E)-β-Ionone; Ionone beta; trans-β-Ionone; β-Ionone	

Current regulation: /

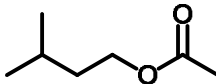
Clinical data:

In the Frosch 1995 dose finding pilot study, no positive reaction to 1% and 5% beta-

ionone in pet., tested in 205 consecutive patients, were observed (15).

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (126).

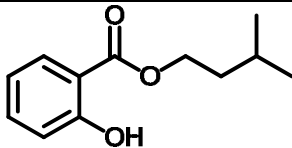
ISOAMYL ACETATE	
CAS # 123-92-2	
EC # 204-662-3	
3-Methylbutyl acetate	
1-Butanol, 3-methyl-, acetate; Acetic acid, isoamyl ester; Isopentyl alcohol, acetate; 3-Methyl-1-butanol acetate; 3-Methyl-1-butyl acetate; 3-Methylbutyl acetate; 3-Methylbutyl ethanoate; Acetic acid 3-methyl-1-butyl ester; Acetic acid 3-methylbutyl ester; Acetic acid isopentyl ester; Banana oil; Isoamyl acetate; Isoamyl alcohol acetate; Isoamyl ethanoate; Isopentyl acetate; Isopentyl ethanoate; NSC 9260; Pear oil; i-Amyl acetate; iso-Amyl acetate; iso-Pentyl acetate	

Current regulation: /

Clinical data: /

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

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

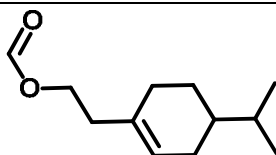
ISOAMYL SALICYLATE	
CAS # 87-20-7	
EC # 201-730-4	
3-Methylbutyl-2hydroxybenzoate	
Isopentyl 2-Hydroxybenzoate; Isopentyl salicylate; Salicylic acid, isopentyl ester (6CI,8CI); Isopentyl alcohol, salicylate; 3-Methylbutyl salicylate; Isoamyl o-hydroxybenzoate; Isoamyl salicylate; Isopentyl salicylate; NSC 7952	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified 1 (0.6%) positive reactions among 179 patients using a 50% PT preparation of this compound – this reaction may have been due to an “excited back syndrome” and is thus a limited evidence (25). In the Frosch 1995 dose finding pilot study, no positive reaction to 1% and 5% isoamyl salicylate in pet., tested in 95 consecutive patients, were observed (15).

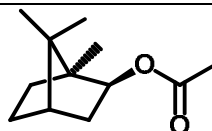
Additional information: A RIFM review is available (127).

ISOBERGAMATE	
CAS # 68683-20-5	
EC # 272-066-0	
4-(Isopropyl)cyclohexadiene-1-ethyl formate	
Structure is incompletely defined 4-(1-Methylethyl)-1,?-cyclohexadiene-1-ethyl formate 4-(Isopropyl)cyclohexadiene-1-ethyl methanoate; menthadienyl formate; Menthadiene-7-methyl formate	

Current regulation: Annex III, part 1, n° 170

Clinical data: /

Additional information: A RIFM review is available (128).

ISOBORNYL ACETATE	
CAS # 125-12-2	
EC # 204-727-6	
(1R,2R,4R)-1,7,7-trimethyl-Bicyclo[2.2.1]hept-2-yl acetate	
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-Trimethyl-, acetate, (1R,2R,4R)-rel- ; Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, exo-; Isoborneol, acetate; (±)-Isobornyl acetate; Isobornyl acetate; NSC 62486; Pichtosin; Pichtosine; exo-Bornyl acetate	

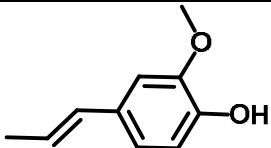
Opinion on fragrance allergens in cosmetic products

Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% isobornyl acetate in pet., tested in 107 consecutive patients in High Wycombe, were observed (15).

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

ISOEUGENOL	
CAS # 97-54-1	
EC # 202-590-7	
2-Methoxy-4-(1-propen-1-yl)-phenol	
Phenol, 2-methoxy-4-(1-propenyl)- ; Phenol, 2-methoxy-4-propenyl-; 1-(3-Methoxy-4-hydroxyphenyl)-1-propene; 2-Methoxy-4-(1-propenyl)phenol; 2-Methoxy-4-propenylphenol; 3-Methoxy-4-hydroxy-1-propenylbenzene; 4-Hydroxy-3-methoxy-1-propenylbenzene; 4-Hydroxy-3-methoxy-β-methylstyrene; 4-Propenylguaiacol; Isoeugenol; NSC 6769	

Current regulation: Annex III, part 1, n° 73

Clinical data:

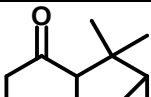
In the “background information” section of the previous opinion (33), isoeugenol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 1.9% in consecutive PT patients and accounting for 6 to 22% of reactions to the FM I. Allergic reactions had been observed in 2 – 25% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 1.3% (95% CI: 0.8 – 1.8%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 1.3% (95% CI: 0.3 – 3.2%) had positive reactions to isoeugenol, tested at 2% pet., i.e., twice the commonly used concentration (6). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 5.4% positive reactions (22). At St Johns Institute of Dermatology in London 3636 subjects were patch tested with isoeugenol 2001-2005, 97 of whom were positive. Year-on-year incidence showed an increasing trend, with an overall incidence of 2.67% (129). The IVDK 2010 study, 1.62% (95% CI: 0.87 – 2.38%) of 1214 consecutively tested patients reacted to the compound, while 3.41% (95% CI: 2.90 – 3.92%) of 5747 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=11 reacted positively to isoeugenol (48).

Additional information:

Isoeugenol occurs in a cis- (CAS 5912-86-7) and a trans-isomers (CAS 5932-68-3), the latter dominating in trade products (82-88%) (53).

Isoeugenyl methyl ether caused 7.3% positive reactions in the Larsen 2002 c study (1). A number of derivatives of isoeugenol, such as isoeugenyl acetate, transisoeugenol, isoeugenyl benzoate, isoeugenyl phenylacetate, isoeugenyl methyl ether and benzyl isoeugenyl have been examined in 2261 consecutive patients; a varying proportion of positive patch test reactions and a varying proportion of concomitant reactions with isoeugenol have been observed (130). In an earlier study, 5 of 7 patients positive to isoeugenol also displayed positive reactions to isoeugenol acetate (1.2% eth.) (131) (see also section 5 and 6).

ISOLONGIFOLENEKETONE	
CAS # 33407-62-4	

EC # 245-890-3	
1,3,4,6,7,8a-Hexahydro-1,1,5,5-tetramethyl-2H-2,4a-methanonaphthalen-8(5H)-one	
Hexahydro-1,1,5,5-tetramethyl-2H-2,4a-methanonaphthalen-8(5H)-one	

Current regulation: /

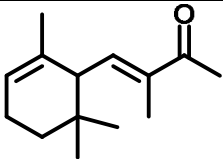
Clinical data:

The Larsen 2001 study identified 1 in 178 patients with known contact allergy to fragrance ingredients who reacted positively in the PT (5% pet.) (19).

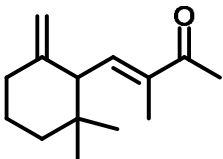
Additional information:

Not listed in CosIng under this CAS #. Other CAS # reported in RIFM review ¹³:

- 29461-14-1 CosIng: INCI name "ISOLONGIFOLENE KETONE EXO";
- 23787-90-8 CosIng: INCI name "ISOLONGIFOLANONE";
- 29461-13-0: CosIng: INCI name "HEXAHYDRO-TETRAMETHYLMETHANONAPHTHALEN-8-ONE".

<i>alpha-ISOMETHYL IONONE</i>	
CAS 127-51-5	
EC 204-846-3	
3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	
4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-methyl-3-buten-2-one; Cetone Alpha; Isomethyl-α-ionone; NSC 66432; α-Cetone	

Current regulation: Annex III, part 1, n° 90

<i>gamma-Methylionone</i>	
CAS 7388-22-9	
EC /	

According to CosIng, "alpha-ISOMETHYL IONONE" (CAS # 127-51-5) and "gamma-Methylionone" (CAS # 7388-22-99) are synonyms, with one CAS number, and one preferred chemical name. The substance(s) are accordingly treated in the 1999 opinion (33) as one. As this treatment is also found in the literature, both substances are reviewed together.

¹³ Opdyke, D. L. J.; Letizia, C. **Monographs on fragrance raw materials. Isolongifolanone.** Food and Chemical Toxicology (1983), 21(6), 859

Clinical data:

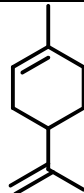
In the “background information” section of the 1999 opinion, “gamma-methylionone” is classified as “less frequently reported allergen”; 1 study with 2 cases and 2 studies with 1 case were found among patients with eczema from cosmetic products (33).

The IVDK 2007 study yielded $n=1$, i.e. 0.1% (95% CI: 0.00 – 0.2%) positive reactions in 2004 consecutively PTed patients (4). In the subsequent period (2005-2008), $n=986$ patients were tested in the IVDK 2010 study, with no positive reactions (7). In the Groningen 2009 study, $n=2$, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at only 1% pet. (6). In a Korean study with 422 consecutive patients, 2.1% reacted positively to “alpha isomethyl ionone (gamma-methylionone), CAS # 127-51-5”, tested 5% pet. (13)

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010) under the label of “alpha-ISOMETHYL IONONE (CAS # 127-51-5)”.

A RIFM review is available, listing 4 human sensitisation experiments employing different study protocols – all yielding negative results (132). Another review is available by Hostynek and Maibach (133), both referring to “alpha-ISOMETHYL IONONE (CAS # 127-51-5)”.

(DL)-LIMONENE	
CAS # 138-86-3	
EC # 231-732-0	
1-Methyl-4-(1-methylethenyl)-cyclohexene	
p-Mentha-1,8-diene; (±)-Dipentene; (±)-Limonene; (±)-α-Limonene; 1,8-p-Menthadiene; 1-Methyl-4-(1-methylethenyl)cyclohexene; 1-Methyl-4-isopropenyl-1-cyclohexene; 1-Methyl-4-isopropenylcyclohexene; 1-Methyl-p-isopropenyl-1-cyclohexene; 4-Isopropenyl-1-methyl-1-cyclohexene; 4-Isopropenyl-1-methylcyclohexene; Cajeputen; Cajeputene; Cinen; Cinene; DL-Limonene; Dipenten; Dipentene; Eulimen; Flavor orange; Goldflush II; Kautschin; Limonen; Limonene; NSC 21446; NSC 844; Nesol; Orange X; Orange flavor; PC 560; Roti-Histol; SF 001; dl-Limonene; α-Limonene	

Current regulation: Annex III, part1, n° 88, 167, 168

Clinical data:

In the “background information” section of the 1999 opinion, d-limonene (CAS 5989-27-5) is classified as “less frequently reported allergen in relation to cosmetic exposure”; with contact allergy to oxidised limonene not infrequently reported in the literature (33).

Since 1999, several studies have been performed using limonene where the oxidation state is not given, but intended to be low. In one study, 0.6% positive reactions to limonene (3% pet.) were observed in 1606 consecutive patients (17). The IVDK 2007 study yielded n=3, i.e. 0.1% (95% CI: 0.03 – 0.4%) positive reactions in 2396 patients consecutively PTed with limonene (2% pet.) (4). The IVDK 2010 study, 0.28% (95% CI: 0 – 0.57%; percentages standardised for age and sex) of 1241 patients PTed with dipentene reacted to the compound (7). In the Groningen 2009 study, no positive reactions to this allergen, tested at 2% pet., were observed in 320 patients (6).

Regarding selected case reports, a case of a 40 year old citrus fruit picker with work related hand dermatitis and bronchial asthma has been described, who tested extreme positive to DL-limonene (2% pet.), and, less extremely, to citronellol and to the biocide dichlorophene (134). Moreover, limonene is used as a solvent in technical applications and cleaning and can lead to allergic contact dermatitis (e.g., a histopathology technicians (135, 136) or a painter and decorator (137)). In “water-free” hand cleansers it is reported to be used in concentrations around 10 – 20% (137). Wax polishes may contain dipentene and have caused one reported case of occupational ACD in a car mechanic (138). Another case of occupational ACD from dipentene in honing oil has been reported (139). In a case series from Sweden, 2 of 105 car mechanics patch tested for occupational contact dermatitis had positive reactions to oxidised d-limonene (5% pet.) (140).

Additional information:

Limonene is a monocyclic monoterpene existing in two enantiomers: (R)-(+)-limonene (CAS 5989-27-5) and (S)-(-)-limonene (CAS 5989-54-8). Racemic limonene is known as dipentene.

The allergenicity of limonene is closely related to oxidation (71, 72, 141, 142). It has been demonstrated that both enantiomers, R-(+)- and S-(-)-limonene spontaneously autoxidize, and that the primary oxidation products formed, the hydroperoxides, are strong and clinically relevant contact allergens. Among 2411 consecutive patients in a multi-centre European study, 63 (2.6 % [95%CI: 2.0-3.3]) reacted to oxidised (R)-(+)-and/or (S)-(-)-limonene (3.0% pet.) (72). In other multi-studies also, a considerable proportion of patients showed

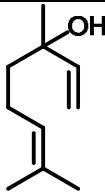
positive patch test reactions to oxidised R-(+)- limonene, e.g.,

- between 0.3% and 5.1% of subgroups of 2800 patients in Stockholm and Leuven, depending on test concentration, oxidation state and department(141),
- between 0.3% and 6.5% in 4 different departments in altogether 2273 patients (72, 143).

The primary oxidation products are the major allergens forming specific antigens (Bråred-Christensson J, Matura M, Bäcktorp C, Börje A, Nilsson JLG, Karlberg A-T. Hydroperoxides form specific antigens in contact allergy. Contact Dermatitis 2006; 55: 230-237.).

Current IFRA standards emphasise “a peroxide value of less than 20 millimoles peroxides per litre, determined according to the FMA method” (<http://www.ifraorg.org/Home/Code,+Standards+Compliance/IFRA+Standards/page.aspx/56>, last accessed 2009-11-11). For a more general discussion see section 5.

There is no scientific rationale for the difference in peroxide value allowed for limonene (20 millimoles peroxides per litre) compared to linalool (10 millimoles peroxides per litre). Specific values for hydroperoxides, which are allergens, would be desirable.

LINALOOL	
CAS # 78-70-6 (isomeric mixture)	
EC # 201-134-4; 245-083-6	
See: http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=27933	
3,7-Dimethyl-1,6-octadien-3-ol	
(±)-Linalool; 2,6-Dimethyl-2,7-octadien-6-ol; 2-Methyl-1-prenyl-3-buten-2-ol; 3,7-Dimethyl-1,6-octadiene-3-ol; 3,7-Dimethyl-3-hydroxy-1,6-octadiene; L 260-2; Linalol; Linalool; Linalyl alcohol; Linanol; NSC 3789; dl-Linalool; β-Linalool	

Current regulation: Annex III, part 1, n° 84

Clinical data:
In the “background information” section of the 1999 opinion, linalool in non-oxidized form is classified as “less frequently reported allergen”; with 4 cases of contact allergy reported in 2 studies on patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, studies have been performed on contact allergy to linalool, oxidation state not given, but intended to be low. In the Larsen 2002 c study, none of the 218 patients with known contact allergy to fragrance ingredients had a positive reaction to linalool 5% pet., as prepared specially for this study (1). The IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.6%) positive reactions in 2401 patients consecutively tested with stabilised linalool (10% pet.) (4). The IVDK 2010 study, 1 patient had a weak, and another a ++ reaction among the n=985 patients tested with 10% linalool (stabilised) in pet. (7). In the Groningen 2009 study, n=2, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6). The deGroot 2000 study with 1825 consecutively tested patients yielded 3 positive reactions to linalool (12). The DeGroot 1985 study found no positive reactions among 179 patients using a 30 % PT preparation of linalool (25).

Additional information:

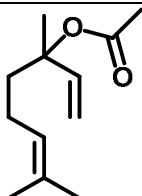
The allergenicity of linalool is closely related to oxidation and the primary oxidation products, the hydroperoxides, are the main allergens (144). In a clinical study 2002-

2003 in 6 European centres including 1511 consecutive patients, 1.3% showed a positive reaction to oxidized linalool (2.0% pet.) and 1.1% to the hydroperoxide fraction (65). A recent dose-response study in Sweden including 3400 patients in two test centres showed a positive reaction in 5.3% of the 1725 patients tested with oxidized linalool 6% pet. (145).

A review by RIFM is available both regarding linalool (146) and linalool “and related esters” (147). Another review is available by Hostynek and Maibach (148).

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

Additional CAS numbers exist for the single isomers: CAS # 126-90-9 (S-isomer), CAS # 126-91-0 (R-isomer); however, in the studies reviewed the isomeric mixture has been used throughout.

LINALYL ACETATE	
CAS # 115-95-7	
EC # 204-116-4	
3,7-Dimethyl-1,6-octadien-3-yl acetat	
1,6-Octadien-3-ol, 3,7-dimethyl-, acetate; Linalool acetate K; (±)-Linaloyl acetate; (±)-Linalyl acetate; 1,5-Dimethyl-1-vinyl-4-hexenyl acetate; 3,7-Dimethyl-1,6-octadien-3-yl acetate; 3-Acetoxy-3,7-dimethyl-1,6-octadiene; Acetic acid linalool ester; Bergamiol; Bergamol; Bergamot mint oil; Linalyl acetate; NSC 2138; dl-Linalool acetate	

Current regulation: /

Clinical data:
In 100 patients tested in Odense, DK, in the early 90s, no positive reactions were observed with 1 and 5% linalyl acetate in pet. (15). In the Frosch 2002 a study, testing with linalyl acetate (10% pet.), 0.2% positive PT reactions to consecutive patients were noted (16). Similarly, the RIFM review mentioned quotes a number of studies where no allergic reactions to this compound had been observed, with the exception of one positive reaction in a Dutch study in 1988(149).

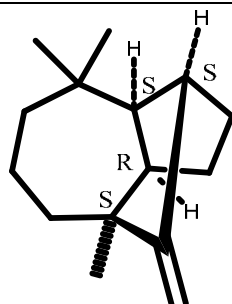
Additional information:

This is the main component of lavender oil (30%), also part of bergamot oil, neroli oil, peppermint oil, lemon oil and jasmine oil (53).

Linalyl acetate autoxidizes spontaneously at air exposure and the major allergens, the hydroperoxides, are the primary oxidation products (150). The pattern of autoxidation is similar to that for linalool and as the acetate can be metabolically hydrolysed to the corresponding alcohol cross reactions to allergens from oxidized linalool should be possible. This was indicated in a study of lavender oil and oxidised linalyl acetate which elicited positive PT reactions in some patients with known contact allergy to oxidised linalool (n=3) (151).

A RIFM review is available reporting 7 human sensitisation experiments yielding few or no cases of sensitisation (152).

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

Longifolene	
CAS # 475-20-7	
EC # 207-491-2	
(1S,3aR,4S,8aS)-Decahydro-4,8,8-trimethyl-9-methylene-1,4-methanoazulene	
1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, (1S,3aR,4S,8aS)-(+)-; 1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1α,3aβ,4α,8aβ)]-; (+)-Longifolene; Junipen; Junipene; Kuromatsuen; Kuromatsuene; Longifolen; NSC 150808; d-Longifolene; α-Longifolene	

Current regulation: /

Clinical data: /

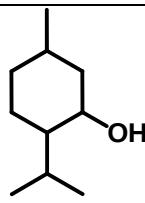
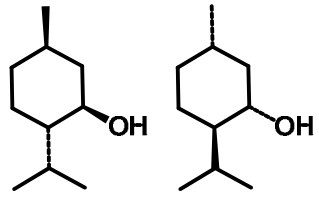
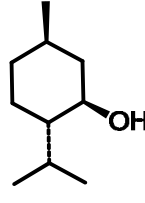
Additional information:

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

http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=77412

This substance is listed in the Register of Flavouring Substances pursuant to Article 3(1) of Regulation EC No. 2232/96 (28 Oct 1996) that lays down a procedure for flavouring substances used or intended for use in or on foodstuffs. Adopted February 23, 1999.

A RIFM review is available citing one negative human maximisation test (n=25) with 10% pet. (153).

MENTHOL	 1490-04-6
CAS # 1490-04-6 / 89-78-1 / 2216-51-5	
EC # 216-074-4 / 239-388-3 / 218-690-9	
5-Methyl-2-(1-methylethyl)-cyclohexanol (1490-04-6) (1R,2S,5R)-rel-5-Methyl-2-(1-methylethyl)-cyclohexanol (89-78-1) (1R,2S,5R)-5-Methyl-2-(1-methylethyl)-cyclohexanol (2216-51-5)	
Other names: 1490-04-6: Menthol; 1-Methyl-4-isopropyl-3-cyclohexanol; 2-Isopropyl-5-methylcyclohexan-1-ol; 2-Isopropyl-5-methylcyclohexanol; 3-Hydroxy-p-menthane; 5-Methyl-2-(1-methylethyl)cyclohexanol; 5-Methyl-2-isopropylcyclohexanol; Menthyl alcohol; p-Menthan-3-ol 89-78-1: (1a,2β,5a)-5-Methyl-2-(1-methylethyl)-cyclohexanol; cis-1,3,trans-1,4-Menthol; dl-Menthol; (1R,2S,5R)-rel-5-Methyl-2-(1-methylethyl)cyclohexanol; (±)-Menthol; DL-Menthol; Fisherman's Friend Lozenges; Hexahydrothymol; Menthacamphor; Menthol; Menthomenthol; NSC 2603; Peppermint camphor; Racementhol; Therapeutic Mineral Ice; Thymomenthol; rac-Menthol	 89-78-1  2216-51-5

2216-51-5: (1R,2S,5R)-5-Methyl-2-(1-methylethyl)-cyclohexanol; [1R-(1 α ,2 β ,5 α)]-5-Methyl-2-(1-methylethyl)-cyclohexanol; (1R,3R,4S)-(-)-Menthol; (-)-Menthol; (-)-Menthyl alcohol; (-)-trans-p-Methan-cis-3-ol; (1R)-(-)-Menthol; (1R,2S,5R)-(-)-Menthol; (1R,2S,5R)-2-Isopropyl-5-methylcyclohexan-1-ol; (1R,2S,5R)-2-Isopropyl-5-methylcyclohexanol; (R)-(-)-Menthol; 1R-Menthol; L-Menthol; L-Mentholum; Levomenthol; NSC 62788; l-(-)-Menthol; l-Menthol

Current regulation: /

Clinical data:

Among 512 patients referred from a dental department for diagnostic work-up of various intraoral symptoms and complaints within 4 years, 10 patients had positive (+ to +++) PT reactions to menthol 5% pet. at D4, mostly reporting dramatic improvement after cessation of use of peppermint-containing oral products (154). In 63 patients positive to the FM I, 1 had a positive PT reaction to menthol, 5% pet., in the Santucci 1987 study (28). The IVDK 2010 study, 1 of 1147 patients tested with 1% menthol in pet. had a weak positive reaction to menthol (7).

A case of contact allergy to “peppermint and menthol” in a transdermal therapeutic system with flurbiprofen for lumbar pain has been described (155). Moreover, a case of rhinitis caused by different menthol-containing products, diagnostically proven by repeatedly positive urticarial reactions after application of 2% menthol in pet. or 5% peppermint oil in pet., has been reported (156). “A case of asthma due to menthol is reported in a 40-year-old woman with no history of asthma or any other allergy. During the last two years, the patient had presented dyspnoea, wheezing and nasal symptoms when exposed to mentholated products such as toothpaste and candies. The aetiology was suggested by the history of exposure and diagnosis was established by skin tests and bronchial challenge with menthol. The patient achieved control of symptoms by avoiding menthol and its derivatives.” (157).

Additional information:

Menthol is an ingredient of several essential oils, like peppermint oil, and has been identified as causative allergen in case reports listed above.

Four stereoisomeric forms are known. Natural menthol occurs as L-form (CAS 2216-51-5), trade products are DL-menthol (CAS 1490-04-6). D-form: CAS 89-78-1, racemic: CAS 15356-70-4. Sensitive to light, air and heat (53).

L-menthol and menthol (isomer not specified) are “top 100” substances (IFRA, pers. comm.2010). RIFM reviews are available regarding “menthol” (158), D-menthol (159), L-menthol (160), DL-menthol (161) and menthol, racemic (162). A CIR expert panel review is available (163).

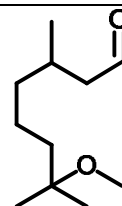
METHOXYCITRONELLAL

CAS # 3613-30-7

EC # 222-784-5

7-Methoxy-3,7-dimethyl-octanal

7-Methoxy-3,7-dimethyloctanal; 7-Methoxy-6,7-dihydrocitronellal; 7-Methoxycitronellal; Methoxycitronellal; Methoxydihydrocitronellal

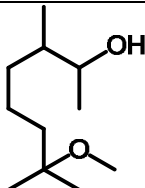


Current regulation: /

Clinical data:

Nakayama et al. found 1974 (after (29)) 12 “strong positive” and 10 “weak positive” reactions to methoxycitronellal (unknown test concentration), with cross-reactions to hydroxycitronellal (proportion not given), in 183 patients.

Additional information: /

METHOXYTRIMETHYLHEPTANOL	
CAS # 41890-92-0	
EC # 255-574-7	
7-Methoxy-3,7-dimethyl-2-octanol	
3,7-Dimethyl-7-methoxy-2-octanol; Dihydromethoxyelgenol; Elesant; Osyrol	

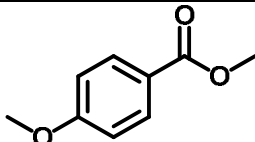
Current regulation: /

Clinical data:

In the Larsen 2002 c study, 0.9% of the patients with known contact allergy to fragrance ingredients had a positive PT reaction to this ingredient not reported as allergen previously (1).

Additional information:

A RIFM review is available (128) citing 1 negative maximisation test (n=27).

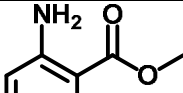
METHYL <i>p</i>-ANISATE	
CAS # 121-98-2	
EC # 204-513-2	
Methyl-4-methoxybenzoate	
p-Anisic acid, methyl ester; 4-(Methoxycarbonyl)anisole; 4-Methoxybenzoic acid methyl ester; Methyl p-anisate; Methyl p-methoxybenzoate; NSC 7324; p-Methoxybenzoic acid methyl ester	

Current regulation: /

Clinical data:

In the Malten 1984 study, n=1 (0.5%) of 182 patients displayed a positive PT reaction to methyl anisate 4% pet. (24).

Additional information: /

METHYL ANTHRANILATE	
CAS # 134-20-3	

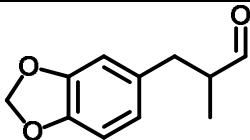
EC # 205-132-4	
Methyl 2-aminobenzoate	
Anthranilic acid, methyl ester; 2-(Methoxycarbonyl)aniline; 2-Aminobenzoic acid methyl ester; 2-Carbomethoxyaniline; Bird Shield; Grain 96-1; Methyl 2-aminobenzoate; Methyl 6-aminobenzoate; Methyl anthranilate; Methyl o-aminobenzoate; NSC 3109; ReJex-iT; ReJex-iT AP 50; ReJex-iT TP 40; Sunarome UVA; [2-(Methoxycarbonyl)phenyl]amine; o-(Methoxycarbonyl)aniline; o-Aminobenzoic acid methyl ester; o-Carbomethoxyaniline	

Current regulation: /

Clinical data:

In 91 Israeli patients with a positive or doubtful reaction to FM I or MP methyl anthranilate was tested (conc. not given), with a negative result (164).

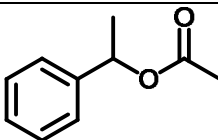
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

METHYLENEDIOXYPHENYL METHYLPROPANAL	
CAS # 1205-17-0	
EC # 214-881-6	
3-(1,3-Benzodioxol-5-yl)-2-methylpropanal	
Hydrocinnamaldehyde, α-methyl-3,4-(methylenedioxy)-; 2-Methyl-3-(3,4-methylenedioxyphenyl)propanal; 2-Methyl-3-(3,4-methylenedioxyphenyl)propionaldehyde; 3-(3,4-Methylenedioxyphenyl)-2-methylpropanal; Heliobouquet; Heliofresh; Heliogan; Helional; Helipropanal; NSC 22282; Tropional; α-Methyl-1,3-benzodioxole-5-propanal; α-Methyl-3,4-(methylenedioxy)hydrocinnamaldehyde	

Current regulation: /

Clinical data: /

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

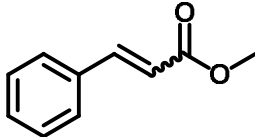
METHYLBENZYL ACETATE	
CAS # 93-92-5	
EC # 202-288-5	
1-Phenylethyl acetate	
Benzenemethanol, α-methyl-, acetate ; Benzyl alcohol, α-methyl-, acetate ; (±)-Styrallyl acetate; (±)-α-Methylbenzyl acetate; (±)-α-Phenethyl acetate; 1-Acetoxy-1-phenylethane; 1-Phenylethyl acetate; Gardeniol II; Gardenol; Methyl phenyl carbinyl acetate; Methylphenylcarbinol acetate; NSC 2397; Styrallyl acetate;	

Styrylallyl acetate; dl-1-Phenylethyl acetate; sec-Phenethyl acetate; sec-Phenylethyl acetate; α-Methylbenzenemethanol acetate; α-Methylbenzyl acetate; α-Methylbenzyl alcohol, acetate; α-Phenethyl acetate; α-Phenylethyl acetate

Current regulation: /

Clinical data: /

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

METHYL CINNAMATE	
CAS # 103-26-4	
EC # 203-093-8	
Methyl 3-phenylprop-2-enoate	
3-Phenyl-2-propenoic acid methyl ester; Cinnamic acid, methyl ester; 3-Phenyl-2-propenoic acid methyl ester; 3-Phenylacrylic acid methyl ester; Methyl 3-phenyl-2-propenoate; Methyl 3-phenylacrylate; Methyl 3-phenylpropenoate; Methyl cinnamate; Methyl cinnamylate; NSC 9411; SemaSORB 9815	

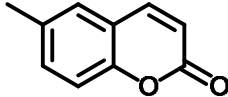
Current regulation: /

Clinical data:

Patch tests with some components of Peru balsam were carried out at 8 worldwide centers in 142 patients who had previously reacted to 25% MP. Reactions to methyl cinnamate (dose and vehicle not reported) were observed in 6 of 142 patients (no further details reported) (165).

Additional information:

A RIFM review is available (166), reviewing, e.g., a number of animal studies with conflicting results. See also under Myroxylon pereirae.

6-METHYL COUMARIN	
CAS # 92-48-8	
EC # 202-158-8	
6-Methylchromen-2-one	
Coumarin, 6-methyl-; 6-MC; 6-Methyl-2H-1-benzopyran-2-one; 6-Methyl-2H-chromen-2-one; 6-Methylbenzopyrone; 6-Methylcoumarin; 6-Methylcoumarinic anhydride; NSC 5870; Toncarine	

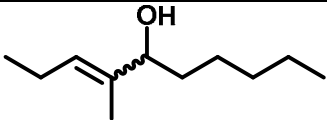
Current regulation: Annex III, part 1, n° 46

Clinical data:

Two of 24 white volunteers developed a photoallergic reaction after single epicutaneous exposure with 5% methyl coumarin in ethanol and UV-A radiation (16 J/cm²). After a photomaximisation test, 6 of 10 subjects developed photocontact allergic reactions

(167). Cardoso et al. report on 2 photoallergic patch test reactions to this substance, which were apparently clinically relevant, in 83 Portuguese patients tested (168). Similar results (2 of 76 patients with positive photopatchtest) were reported from New York (169).

Additional information: /

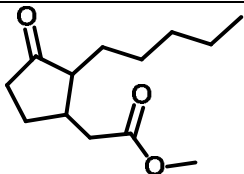
METHYL DECENOL	
CAS # 81782-77-6	
EC # 279-815-0	
4-Methyl-3-decen-5-ol	

Current regulation: /

Clinical data: /

Additional information:

A RIFM review is available (170), reporting 1 negative HRIPT (n=50). It is a “top 100” substance (IFRA, pers. comm.2010).

METHYL DIHYDROJASMONATE	
CAS # 24851-98-7	
EC # 246-495-9	
Methyl 2-(3-oxo-2-pentyl cyclopentyl) acetate	

Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester; Kharismal; MDJ; Methyl (3-oxo-2-pentylcyclopentyl)acetate; Methyl 3-oxo-2-pentylcyclopentane ethanoate; Hedione

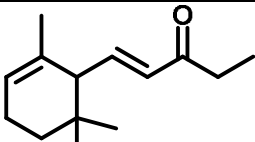
Current regulation: /

Clinical data:

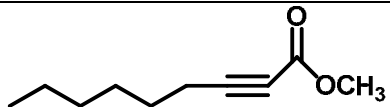
In the Frosch 2002 b study, 3 of 1606 consecutive patients (0.2%) showed positive reactions to hedione (5% pet.) (17). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% hedione in pet., tested in 100 consecutive patients in Belfast, were observed (15).

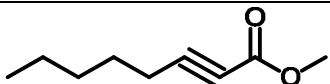
Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). An older RIFM review exists (128) citing 1 negative human maximisation test (n=25).

METHYL IONONE (mixture of isomers)	
CAS # 1335-46-2	
EC # 215-635-0	

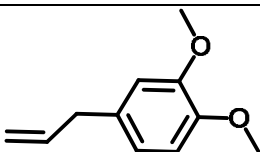
1-(2,6,6-Trimethyl-1-cyclohex-2-enyl)pent-1-en-3-one	
6-Methylionone	
Current regulation: /	
Clinical data:	
See METHYLIONANTHEME for one clinical case report. Regarding methyl ionone gamma, the Frosch 1995 dose-finding pilot study found no positive reaction to 1% and 5% of this substance in pet., tested in 100 consecutive patients in Belfast (15).	
Additional information:	
It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (171).	

METHYL OCTINE CARBONATE	
CAS # 111-80-8	
EC #	
Methyl 2-octynoate	
Methyl 2-Nonynoate, MOC	
Current regulation: Annex III, part 1, n°173	
Clinical data:	
English and Rycroft reported a case of a 19-year-old laboratory technician working in the fragrance industry, who developed hand dermatitis after contact with methyl heptine and methyl octane carbonates; patch testing was strongly positive to both compounds at 1% in MEK (172).	
Additional information: /	

METHYL 2-OCTYNOATE	
CAS # 111-12-6	
EC # 203-836-6	
Methyl oct-2-ynoate	
M2O; Methyl heptin carbonate; Folione; Methyl hept-1-yne-1-carboxylate; Methyl pentylacetylenecarboxylate; NSC 72098; Vert de violette artificiel	
Current regulation: Annex III, part 1, n° 89	

had positive reactions to this allergen, tested at only 2% pet. (6). In a previous case report of a fragrance laboratory assistant with work-related ACD both methyl heptin and methyl octin carbonate had been found sensitisers – probably due to their very similar chemical structure (172). In a recent bi-centric study with 350 eczema patients who were consecutively tested with 1% and 2% M2O in pet.; 0.8% positive reactions were observed. However, in 3 additional cases active sensitisation, with first reactions appearing 2 to 4 weeks after the patch test, and prompt reactions in the 2 cases repeat-patch tested, was observed (174).

Additional information: /

METHYL EUGENOL	
CAS # 93-15-2	
EC # 202-223-0	
1,2-Dimethoxy-4-(prop-2-enyl)benzene	
4-Allylveratrole; Eugenyl methyl ether extra; 1,2-Dimethoxy-4-allylbenzene; 1,3,4-Eugenol methyl ether; 1-(3,4-Dimethoxyphenyl)-2-propene; 1-Allyl-3,4-dimethoxybenzene; 3,4-Dimethoxy-1-(2-propenyl)benzene; 3,4-Dimethoxyallylbenzene; 3-(3,4-Dimethoxyphenyl)propene; 4-Allyl-1,2-dimethoxybenzene; Benzene, 4-allyl-1,2-dimethoxy-; Chavibetol methyl ether; Ent 21040; Eugenol methyl ether; Eugenyl methyl ether; Methyl eugenol ether; Methyl eugenyl ether; Methylchavibetol; NSC 209528; NSC 8900; O-Methyleugenol; Veratrole methyl ether; Veratrole, 4-allyl-	

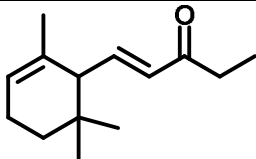
Current regulation: Annex II, 451

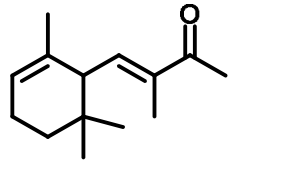
Clinical data:

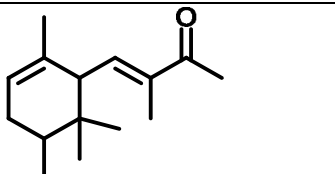
In a previous study by Larsen et al (2002 c), 1.8% of patients with contact allergy to fragrance ingredients reacted positively to this compound (1).

Additional information:

Quote from the SCCS-opinion [0373/00](#): “Methyleugenol should not be intentionally added as a cosmetic ingredient. However, when fragrance compounds containing methyleugenol naturally present in essential oils are used as components in cosmetic products, the highest concentration of methyleugenol in the finished products must not exceed 0.01 % in fine fragrance, 0.004 % in eau de toilette, 0.002 % in a fragrance cream, 0.0002 % in other leave-on products and in oral hygiene products, and 0.001% in rinse-off products.” (The reason is genotoxicity and carcinogenicity).

METHYLIONANTHEME	
CAS # 55599-63-8	
EC #	
(1E)-2-Methyl-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-1-penten-3-one mixt. with (3E)-3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	

8-Methyl- α -ionone-10-methyl- α -ionone mixt.; Iralia Mixture	
Current regulation: ...	
Clinical	data:
One case of ACD has been reported, caused by an E.d.C. (175).	
Additional information:	
Patented by GIVAUDAN SA 1933, is composed of isomeric n-methylionones and iso-methylionones. Methylionone has CAS # 1335-94-0 (not in CosIng) and 1335-46-2 (METHYL α -IONONE ISOMERS); other names: Methyl- α -cyclocitrilydenacetone; Iralia; Isoaldeine (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.detail&id=41456 , last accessed 2010-07-14).	

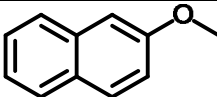
5-METHYL-<i>alpha</i>-IONONE	
CAS # 79-69-6	
EC # 201-219-6	
4-(2,5,6,6-Tetramethyl-2-cyclohexen-1-yl)-3-buten-2-one	
Methyl- α -Ionone; 6-Methyl- α -ionone; α -Irone	
Current regulation: /	

Clinical data:

In the Frosch 2002 b study, 5 of 1606 consecutive patients (0.3%) showed positive reactions to α -irone (10% pet.) (17).

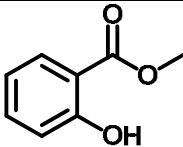
Additional information:

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

METHYL beta-NAPHTHYL ETHER	
CAS # 93-04-9	
EC # 202-213-6	
2-Methoxynaphthalene	
beta-Naphthyl methyl ether; methyl 2-naphthyl ether; Nerolin (old); NSC 4171; Yara yara; β-Methoxynaphthalene; β-Naphthol methyl ether; β-Naphthyl methyl ether; 2-Methoxynaphthalene; Methyl β-naphthyl ether; 2-Naphthol methyl ether; 2-Naphthyl methyl ether; 6-Methoxy-2-naphthalene	

Clinical data: /

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

METHYL SALICYLATE	
CAS # 119-36-8	
EC # 204-317-7	
Methyl 2-hydroxybenzoate	
Other names: Salicylic acid, methyl ester; 2-(Methoxycarbonyl)phenol; 2-Carbomethoxyphenol; 2-Hydroxybenzoic acid methyl ester; Analgit; Anthrapole ND; Ben Gay; Exagien; Flucarmit; Methyl ester of 2-hydroxy benzoic acid; Methyl o-hydroxybenzoate; Methyl salicylate; NSC 8204; Wintergreen oil; o-Hydroxybenzoic acid methyl ester; “Oil of wintergreen”	

Current regulation: /

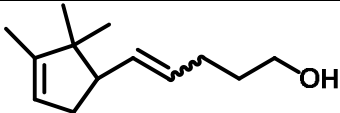
Clinical data:

The deGroot 2000 study yielded 7 positive reactions to methyl salicylate (2% pet.) in 1825 consecutive patients (12).

A case of ACD following the application of a compress bandage containing methyl salicylate has been reported, using 2% “o.o.” as PT concentration; the dose per area of methyl salicylate in the occlusive bandage was not reported (177). A similar case was reported in 1977, positive to 2% methyl salicylate in olive oil, with elicitation of pruritus and erythema after oral ingestion of acetyl salicylic acid (178).

Additional information:

A RIFM review is available (179) providing an overview on 3 human sensitisation experiments (e.g., the HRIPT) which were all negative, and clinical data. In a number of older PT studies, positive test results were seen in 6 of 4600, 3 of 183, 3 of 241, 17 of 585, 1 of 70, all employing a test concentration of 2%, usually in pet., according to above review. Methyl salicylate may occur in topical analgesic (OTC) medications, in Germany, for instance, in “Camphopin® Salbe” („Rote Liste 2010“).

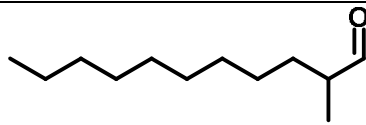
3-METHYL-5-(2,2,3-TRIMETHYL-3-CYCLOPENTENYL)PENT-4-EN-2-OL	
CAS # 67801-20-1	
EC # 267-140-4	
3-Methyl-5-(2,2,3-trimethyl-1-cyclopent-3-enyl)pent-4-en-2-ol	
3-Methyl-5-(2,2,3-trimethyl-3-cyclopenten-1-yl)-4-penten-2-ol; 3-Methyl-5-(2,2,3-trimethylcyclopent-3-enyl)pent-4-en-2-ol; Ebanol	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 1 of 178 patients with known contact allergy to fragrance ingredients exhibited a positive PT reaction to "MTCP", tested 5% pet. (19). In the An 2005 study, 12 of 422 consecutive patients, i.e., 2.8%, had positive reactions to "ebanol", tested at 5% (13).

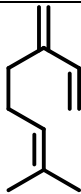
Additional information: /

METHYLUNDECANAL	
CAS # 110-41-8	
EC # 203-765-0	
2-Methylundecanal	
Aldehyde c-12 mna; undecenal, 2-methyl-; 2-Methyl-1-undecanal; Aldehyde M.N.A.; Methyl n-nonyl acetaldehyde; Methylnonylacetaldehyde; NSC 46127	

Current regulation: /

Clinical data: /

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

MYRCENE	
CAS # 123-35-3	
EC # 204-622-5	
7-Methyl-3-methylideneocta-1,6-diene	
2-Methyl-6-methylene-2,7-octadiene; 7-Methyl-3-methylene-1,6-octadiene; NSC 406264; β-Geraniolene; β-Myrcene	

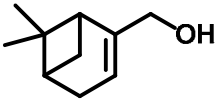
Current regulation: /

Clinical data:

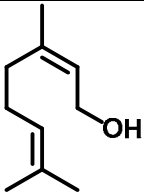
In a clinical study in 6 European centres, including 1511 consecutive patients, 1 patient had a positive reaction to oxidized myrcene (65).

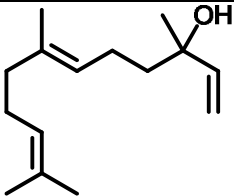
Additional information:

Myrcene autoxidizes spontaneously and rapidly at air exposure. In experimental studies on beta-myrcene an EC3 value of 4.3% was seen for a sample air-exposed 10 weeks (Sköld M. Contact allergy to autoxidized fragrance terpenes (180).

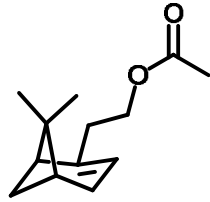
MYRTENOL	
CAS # 515-00-4	
EC # 208-193-5	

(7,7-Dimethyl-4-bicyclo[3.1.1]hept-3-enyl)methanol
(-)-Pin-2-ene-10-ol; 2-Pinen-10-ol; (6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methanol; (±)-Myrtenol; 6,6-Dimethyl-2-(hydroxymethyl)bicyclo[3.1.1]hept-2-ene; NSC 408846; α-Pinene-10-ol
Current regulation: /
Clinical data: /
Additional information:
A RIFM review exists (181), citing 2 of 3 HRIPT studies with 1 case of sensitisation to myrtenol each.

NEROL	
CAS # 106-25-2	
EC # 203-378-7	
(2Z)-3,7-Dimethylocta-2,6-dien-1-ol	
2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-; (Z)-3,7-Dimethyl-2,6-octadien-1-ol; (Z)-Geraniol; (Z)-Nerol; 2-cis-3,7-Dimethyl-2,6-octadien-1-ol; 3,7-Dimethyl-cis-2,6-octadien-1-ol; Nerol 900; Neryl alcohol; cis-3,7-Dimethyl-2,6-octadien-1-ol; cis-Geraniol; β-Nerol; cis-geraniol – i.e., isomeric to geraniol	
Current regulation: /	
Clinical data:	
In the Larsen 2002 c study, 6.0% of the fragrance sensitive patients reacted positively to 5% in pet. (1).	
Additional information:	
A RIFM review is available (182) citing (negative) human sensitisation experiments, an older study from Japan and the Larsen 2002 c study (see above).	
Regarding autoxidation studies – see geraniol.	

Nerolidol (isomer not specified)	
CAS # 7212-44-4	
EC # 230-597-5	
3,7,11-Trimethyl-1,6,10-odecatrien-3-ol	
Nerolidol; (±)-Nerolidol; FCI 119b; Nerodilol	
Current regulation: /	
Clinical data: /	
Additional information:	

RIFM review is available (183) citing the occurrence of “3 positive reactions in 2273 patients”. Another RIFM review is available on cis-nerolidol (184), mentioning that no data on this compound are available.

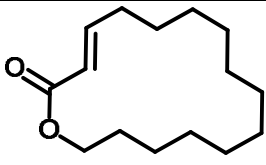
NOPYL ACETATE	
CAS # 128-51-8	
EC # 204-891-9	
2-(7,7-Dimethyl-4-bicyclo[3.1.1]hept-3-enyl)ethyl acetate	
2-Norpinene-2-ethanol, 6,6-Dimethyl-, acetate; Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-, acetate; 2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl acetate; 7,7-Dimethylbicyclo[3.1.1]hept-2-ene-2-ethanol acetate; Citroviol; NSC 1286; NSC 404963; Nopol acetate; Nopyl acetate	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified 2 (1.1%) positive reactions among 179 patients using a 25% PT preparation of this compound – reactions may have at least partly been due to an “excited back syndrome” and thus a limited evidence (25).

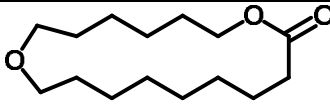
Additional information: /

OXACYCLOHEXADECENONE	
CAS # 34902-57-3	
EC # 609-040-9	
(3E)-Oxacyclohexadec-3-en-2-one	
Globalide; Oxacyclohexadecen-2-one	

Current regulation: /

Clinical data: /

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

OXALIDE	
CAS # 1725-01-5	
EC # 217-033-3	
1,8-Dioxacycloheptadecan-9-one	
Nonanoic acid, 9-[(6-hydroxyhexyl)oxy]-, o-lactone; 10-Oxa-16-hexadecanolide; Oxalide; Oxalide T	

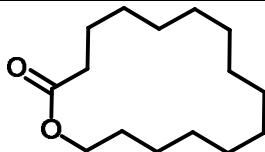
Current regulation: /

Clinical data:

In the Larsen 2001 study, none of 178 patients with known contact allergy to fragrance ingredients exhibited a positive PT reaction to "10-oxahexadecanolide", tested 5% pet. (19).

Additional information:

A RIFM review is available (128), citing a negative maximisation test (n=29).

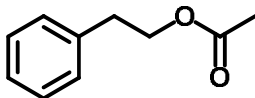
PENTADECALACTONE	
CAS # 106-02-5	
EC # 203-354-6	
1-Oxacyclohexadecan-2-one	
Pentadecanoic acid, 15-hydroxy-, ξ -lactone; 1,15-Pentadecanolide; 15-Hydroxypentadecanoic acid lactone; 15-Pentadecanolide; 15-Pentadodecanolactone; 2-Pentadecalone; CPE 215; Cyclopentadecanolide; Exaltolide; Macrolide Supra; Muskalactone; NSC 36763; Pentadecalactone; Pentadecanolactone; Pentadecanolide; Pentalide; Thibetolide; cpd Supra; ω -Pentadecalactone; angelica lactone; hexaltolide	

Current regulation: /

Clinical data: /

Additional information:

It is a "top 100" substance (IFRA, pers. comm.2010). The substance has been used for clinical olfactory testing in the 60ies under the name of exaltolide.

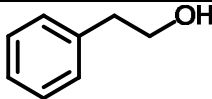
PHENETHYL ACETATE	
CAS # 103-45-7	
EC # 203-113-5	
2-Phenylethyl acetate	
Acetic acid, phenethyl ester ; Phenethyl alcohol, acetate; 2-Phenethyl acetate; 2-Phenylethyl acetate; Benzylcarbiny acetate; NSC 71927; Phenethyl acetate; Phenylethyl ethanoate; β-Phenethyl acetate; β-Phenylethanol acetate; β-Phenylethyl acetate	

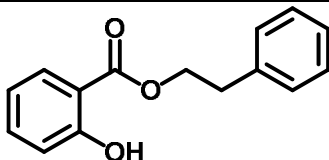
Current regulation: /

Clinical data: /

Additional information:

It is a "top 100" substance (IFRA, pers. comm.2010). Exposure via plants (*Tanacetum parthenium*) is possible (185).

PHENETHYL ALCOHOL	
CAS # 60-12-8	
EC # 200-456-2	
2-Phenylethanol	
Phenethyl alcohol; (2-Hydroxyethyl)benzene; 2-Phenethanol; 2-Phenethyl alcohol; 2-Phenyl-1-ethanol; 2-Phenylethyl alcohol; Benzyl carbinol; Ethanol, 2-phenyl-; NSC 406252; PEA; Phenethanol; Phenethylol; Phenylethanol; Phenylethyl alcohol; β -(Hydroxyethyl)benzene; β -PEA; β -Phenethanol; β -Phenethyl alcohol; β -Phenethylol; β -Phenylethanol; β -Phenylethyl alcohol	
Current regulation: /	
Clinical data:	
The DeGroot 1985 study identified 1 (0.6%) positive reactions among 179 patients using a 25% PT preparation of phenylethyl alcohol (25). In the Frosch 1995 dose-finding pilot study, no positive reaction to this compound, tested 1% pet. in 100 consecutive patients in Odense, DK, was observed (15).	
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).	

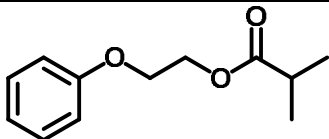
PHENETHYL SALICYLATE	
CAS # 87-22-9	
EC # 201-732-5	
2-Phenylethyl 2-hydroxybenzoate	
Salicylic acid, phenethyl ester; 2-Phenylethyl salicylate; Benzylcarbinyl salicylate; NSC 72035; Phenethyl salicylate	

Current regulation: /

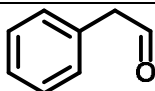
Clinical data: /

Additional information:

A RIFM review exists (186), quoting a negative human maximisation test and a number of animal experiments, including cross-sensitisation experiments with benzyl salicylate. One LLNA study is reported yielding an EC3 value of 2.1%.

PHENOXYETHYL ISOBUTYRATE	
CAS # 103-60-6	
EC # 203-127-1	
2-Phenoxyethyl 2-methylpropanoate	
Isobutyric acid, 2-phenoxyethyl ester; Ethanol, 2-phenoxy-, isobutyrate; 2-Phenoxyethyl isobutyrate; NSC 227210; NSC	

406209; Phenoxyethyl isobutyrate; β -Phenoxyethyl isobutyrate	
Current regulation: /	
Clinical data: /	
Additional information:	
It is a "top 100" substance (IFRA, pers. comm.2010).	

PHENYLACETALDEHYDE	
CAS # 122-78-1	
EC # 204-574-5	
2-Phenylacetaldehyde	
Benzylcarboxaldehyde; Hyacinthin; NSC 406309; Phenacetaldehyde; Phenylacetaldehyde; Phenylacetic aldehyde; Phenylethanal; α-Phenylacetaldehyde; α-Tolualdehyde; α-Toluic aldehyde	

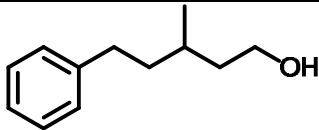
Current regulation: /

Clinical data:

In the Malten 1984 study, 1.1% of 182 patients displayed a positive PT reaction to phenylacetaldehyde 2% pet. (24). In a case report, Sanchez-Politta et al. describe a 26-year-old worker in a perfume factory, who suffered from a spill of pure phenylacetaldehyde and became sensitised, as proven by positive patch tests with 0.5%, 1% and 2% (10 healthy controls negative) (187).

Additional information:

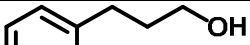
SCCS opinion: [1153/08 - Opinion on "Dermal Sensitization Quantitative Risk Assessment" \(QRA: Citral, farnesol and phenylacetaldehyde\)](#)

PHENYLISOHEXANOL	
CAS # 55066-48-3	
EC # 259-461-3	
3-Methyl-5-phenylpentan-1-ol	
3-Methyl-5-phenyl-1-pentanol; 3-Methyl-5-phenylpentanol; 5-Phenyl-3-methylpentanol; Mefrosol; Phenoxanol	

Current regulation: /

Clinical data: /

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

PHENYLPROPANOL	

Opinion on fragrance allergens in cosmetic products

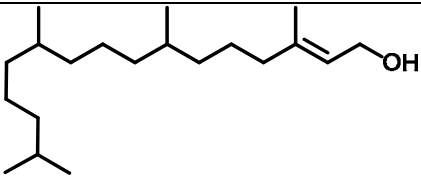
CAS # 122-97-4	
EC # 204-587-6	
3-Phenylpropan-1-ol	
(3-Hydroxypropyl)benzene; 1-Hydroxy-3-phenylpropane; 3-Benzenepropanol; 3-Hydroxy-1-phenylpropane; 3-Phenyl-1-propanol; 3-Phenyl-n-propanol; 3-Phenylpropanol; 3-Phenylpropyl alcohol; Dihydrocinnamyl alcohol; Hydrocinnamic alcohol; Hydrocinnamyl alcohol; NSC 16942; γ-Phenylpropanol; γ-Phenylpropyl alcohol; Phenethyl Carbinol	

Current regulation: /

Clinical data:

The Larsen 2002 c study yielded 0.9% positive reactions in 218 patients with contact allergy to fragrance ingredients (1).

Additional information: ...

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

Current regulation: /

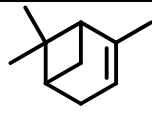
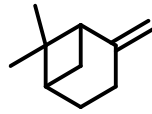
Clinical

data:

/

Additional information:

Phytol is a main constituent of Jasmin abs. with 7.4% reported content (17). In a human maximization study involving 25 subjects, there was one case of contact sensitization to 10% phytol (6900 µg/cm²), applied in petrolatum, as reported in a RIFM review (188).

alpha-PINENE and beta-PINENE	
CAS # 80-56-8 (alpha-Pinene); CAS # 127-91-3 (beta-Pinene)	 80-56-8
EC # 201-291-9 (alpha-Pinene; according to CAS service: 219-445-9); EC # 204-872-5 (beta-Pinene; according to CAS service: 245-424-9)	 127-91-3
2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene (80-56-8)	
6,6-Dimethyl- 2-methylenebicyclo[3.1.1]heptane (127-91-3)	

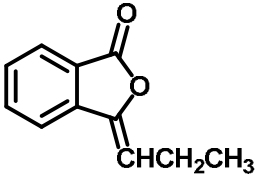
80-56-8: 2-Pinene; (±)-2-Pinene; (±)-α-Pinene; Acintene A; NSC 7727; PC 500; PC 500 (terpene); Sylvapine A; α-Pinene 127-91-3: 2(10)-Pinene ; (±)-2(10)-Pinene; (±)-6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane; (±)-β-Pinene; 6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane; NSC 21447; NSC 406265; NSC 59190;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

Clinical data:

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

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

Additional information: /

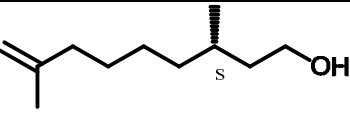
PROPYLIDENE PHTHALIDE	
CAS # 17369-59-4	
EC # 241-402-8	
3-Propylidene-2-benzofuran-1-one	
3-Propylidene-1(3H)-isobenzofuranone; Propylidenephthalide; Celeriax; Propylidenephthalide	3-

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

Clinical data:

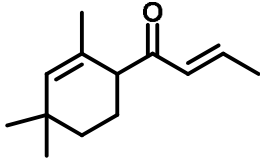
In the Malten 1984 study, 2.6% of 182 patients displayed a positive PT reaction to ethyl acrylate 1% pet. (24). In this paper, "3/25" positive results in human maximisation tests are listed.

Additional information: /

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

Opinion on fragrance allergens in cosmetic products

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

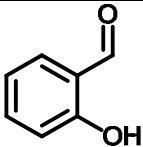
<i>trans</i>-ROSE KETONE-5	
CAS # 39872-57-6	
EC # 254-663-8	
(2E)-1-(2,4,4-Trimethylcyclohex-2-en-1-yl)but-2-en-1-one	
alpha-Isodamascone; trans-2,4,4-Trimethyl-1-crotonyl-2-cyclohexene; (E)-1-(2,4,4-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one	

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

Clinical data: /

Additional information:

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

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

Current regulation: /

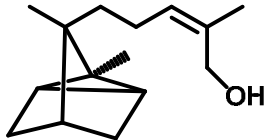
Clinical

data:

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

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

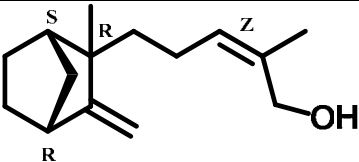
Additional information: Along with other derivatives of salicylic acid, salicylaldehyde is found in the bark of several trees, such as willow or aspen, and can cause allergic contact dermatitis by this exposure (196).

<i>alpha-SANTALOL</i>	
CAS # 115-71-9	
EC # 204-102-8	
(R Z)- 5-(2,3-dimethyltricyclo[2.2.1.0^{2,6}]hept-3-yl)-2-methylPent-2-en-1-ol	
2-Penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.0 ^{2,6}]hept-3-yl)-2-methyl-, [R(Z)]-; 2-Penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.0 ^{2,6}]hept-3-yl)-2-methyl-, stereoisomer; α-Santalol; Tricyclo[2.2.1.0 ^{2,6}]heptane, 2-penten-1-ol deriv.; (+)-(Z)-α-Santalol; (+)-α-Santalol; (Z)-α-Santalol; Sandal; Santalol a; cis-α-Santalol; d-α-Santalol	

Current regulation: /

Clinical data: / (see beta-santalol)

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

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

Current regulation: /

Clinical

data:

A RIFM review is available for alpha-santalol (197) and on “santalol” (CAS # 11031-45-1 (198)). The former review cites a Japanese study: “Between April 1979 and August 1990, a total of 3123 male and female patients were patch tested to 2% santalol (.alpha. or .beta. not specified) in petrolatum. Reactions were observed in 47/3123 (1.5%) of the patients. The incidence of positive reactions from 1979 to 1990 was 1.5%. The rate of reactions observed was higher during the earlier period of the patch testing than the later stage (Utsumi et al., 1992)¹⁵.” In another Japanese study cited by the RIFM review “... patch tests were conducted with 0.05–0.5% santalol (specified as santalol 1) in a base cream or in 99% ethanol. Patches consisted of a piece of 1 cm² lint with a 2 cm² cellophane disc placed on the lint and then covered with a 4 cm² plaster. Patches were applied to the back, the forearm, and the inside of the upper arm for 24–48 h. Reactions were observed in 15 patients and questionable reactions were observed in 10 patients out of the total 427 participating. A second sample of santalol (specified as santalol 2) was tested on 214 patients. Reactions were observed in three patients and questionable reactions were observed in six patients (Takenaka et al., 1986)¹⁶.” Moreover, “The Mid-Japan Contact Dermatitis Research group (MJDCRG) conducted a 6-year (1976–1981) patch test study on facial dermatoses patients with various fragrance materials. During the year 1979, a total of 327 patients were tested with a mixture of .alpha. and .beta. santalol at concentrations of 10%, 2%, and 1% in white petrolatum. Reactions were observed in 1.5%, 0.6% and 0.6% of the 327 patients tested at concentrations 10%, 2%, and 1%, respectively (MJDCRG, 1984)¹⁷.”

The Goossens 1997 study found 5 of 111 patients positive to “santalol 10% pet.” (isoform not specified) – all sensitised to other fragrance allergens as well (23). In the Larsen 2001 study, patch testing with “2-methyl-5-(2,3-dimethyltricyclo[2.2.1.0(2,6)]hept-3-yl-2-pentenol(.alpha.-form) and 2-methyl-5-(2-methyl-3-methylenebicyclo[2.2.1]hept-3-yl-2-penten-1-ol(beta-form) 5% pet.” (no CAS numbers given) yielded a total of 2 positive reactions among the 178 patients with known contact allergy to fragrance ingredients (19).

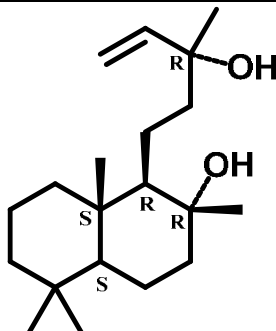
Additional information: “There is no one CAS number for the mixture. The alpha form has a CAS No. 115-71-9 and the beta form is 37172-32-0 (this # is trans-.beta.-santalol). There was no reported use of these materials in the last two IFRA Surveys (8 years total)” (A.M. Api, pers. comm., 2010).

Following a precautionary principle, both isoforms – often not differentiated in reports – are considered as one and considered as established contact allergen in humans

¹⁵ Utsumi, M., Sugai, T., Shoji, A., Watanabe, K., Asoh, S., Hashimoto, Y., 1992. Incidence of positive reactions to sandalwood oil and its related fragrance materials in patch tests and a case of contact allergy to natural and synthetic sandalwood oil in a museum worker. *Skin Research* 34, 209–213

¹⁶ Takenaka, T., Hasegawa, E., Takenaka, U., Saito, F., Odaka, T., 1986. Fundamental studies of safe compound perfumes for cosmetics Part 1. The primary irritation of compound materials to the skin. *Unknown Source*, 313–329.

¹⁷ Mid-Japan Contact Dermatitis Research Group, 1984. Determination of suitable concentrations for patch testing of various fragrance materials. A summary of group study conducted over a 6-year period. *Journal of Dermatology*, 11(1), 31–35.

SCLAREOL	
CAS # 515-03-7	
EC # 208-194-0	
(1R,2R,8aS)-1-[(3R)-3-Hydroxy-3-methylpent-4-enyl]-2,5,5,8a-tetramethyl-3,4,4a,6,7,8-hexahydro-1H-naphthalen-2-ol	
(αR,1R,2R,4aS,8aS)-α-Ethenyldecahydro-2-hydroxy-α,2,5,5,8a-pentamethyl-1-naphthalenepropanol; [1R-[1α(R*),2β,4αβ,8α]] - α-ethenyldecahydro-2-hydroxy-α,2,5,5,8a-pentamethyl-1 Naphthalenepropanol; (13R)-Labd-14-ene-8,13-diol; Sclareol; (-)-Sclareol; [1R-[1.alpha.(R*),2.beta.,4α.beta.,8α.alpha.]]-2-hydroxy-α,2,5,5,8a-pentamethyl-α-vinyldecahydronaphthalene-1-propan-1-ol	

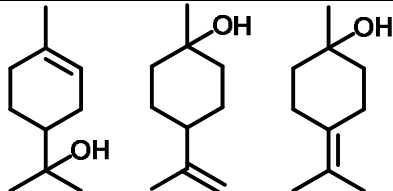
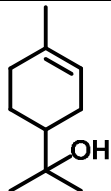
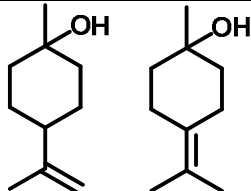
Current regulation: /

Clinical data: /

Additional information:

An older RIFM review exists (128), reporting several human maximisation tests with different samples of sclareol, yielding partly positive, partly negative results. A more recent RIFM review is available (199), citing no clinical data, but several maximisation studies, one of which was positive in a few volunteers, which was apparently due to an impurity.

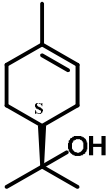
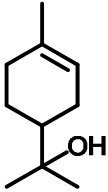
[0986/06 - Opinion on Sclareol \(sensitisation only\)](http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_056.pdf)
(http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_056.pdf)

TERPINEOL	
CAS # 8000-41-7	
EC # 232-268-1	
Mixtures of isomers	
Terpineol 318, mixture of terpineol isomers alfa, beta, gamma	 alfa gamma  beta

Current regulation: /

Clinical data:
A RIFM review is available (200), citing negative human induction studies and one clinical study "Takenaka 1986", finding 4 of 312 patients with 0.05% to 0.5% terpineol in a cream base and in ethanol, resp., and 2 negative clinical studies of limited size. In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% terpineol in pet., tested in 100 consecutive patients in Belfast, were observed (15).

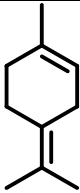
Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010).

<i>alpha-TERPINEOL</i>	 10482-56-1
CAS # 10482-56-1 / 98-55-5	
EC # 233-986-8 / 202-680-6	
2-[(1S)-4-Methyl-1-cyclohex-3-enyl]propan-2-ol (10482-56-1)	
2-(4-Methyl- 1-cyclohex-3-enyl)propan-2-ol (98-55-5)	
10482-56-1: (S)-(-)-p-Menth-1-en-8-ol; (-)-α-Terpineol; (S)-(-)-Terpineol; (S)-(-)-α-Terpineol; (S)-α-Terpineol; l-α-Terpineol 98-55-5: p-Menth-1-en-8-ol; (±)-α-Terpineol; 1,1-Dimethyl-1-(4-methylcyclohex-3-enyl)methanol; 1-p-Menthen-8-ol; 2-(4-Methyl-3-cyclohexenyl)-2-propanol; 4-(2-Hydroxy-2-propyl)-1-methylcyclohexene; 8-Hydroxy-p-menth-1-ene; NSC 21449; NSC 403665; PC 593; Pine Oil 593; Terpineol 350; dl-α-Terpineol; α,α,4-Trimethyl-3-cyclohexene-1-methanol; α-Terpineol	 98-55-5
Current regulation: /	

Clinical data:

A RIFM review is available (201) specifically on (-)-alpha-terpineol stating that “no data is available” regarding skin sensitisation. Another RIFM review is available on alpha-terpineol (202). In the Frosch 2002 b study, 1 of 1606 consecutive patients showed a positive reaction, but 11 patients doubtful reactions to alpha-terpineol (5% pet.) (17). The DeGroot 1985 study identified no positive reactions among 179 patients using a 15% PT preparation of terpineol (mixed isomers) (25). In 63 patients positive to the FM I, 2 had a positive PT reaction to alpha terpineol, 5% pet., in the Santucci 1987 study (28). A clinical series from Portugal, addressing contact allergy to oil of turpentine diagnosed in 30 patients, used a series with pure terpenes. A total of 3 of 30 patients reacted positively to alpha-terpineol (189)

Additional information: see also terpineol (mixture of isomers). Comments on turpentine under pinene.

<i>Terpinolene</i>	
CAS # 586-62-9	
EC # 209-578-0	
1-Methyl-4-propan-2-ylidenecyclohexene	
p-Mentha-1,4(8)-diene; 1-Methyl-4-(1-methylethylidene)-cyclohexene; 4-Isopropylidene-1-methylcyclohexene; Isoterpinene; Nofmer TP; Terpinolen; Terpinolene; α-Terpinolene; δ-Terpinene	

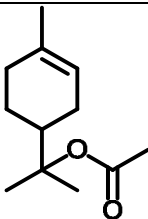
Current regulation: Annex III, part 1, n° 133 (Peroxide value less than 10 mmoles/L in substance)

Clinical data:

A 49-year-old machine cleaner developed occupational contact dermatitis due to the cleaner, which gave a positive patch test result at 1:10 000 in water. Of the ingredients identified by chromatography, only .delta.-3-carene and terpinolene, tested 5% pet.,

gave a positive result (negative in 10 controls) (203). Eleven patients sensitised to tea tree oil showed positive reactions to alpha-terpinene, terpinolene and ascaridol (204).

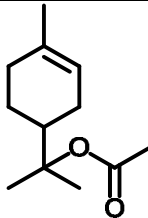
Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010)

TERPINEOL ACETATE (Isomer mixture)	
CAS # 8007-35-0	
EC # 232-357-5	
4-Methyl-1-propan-2-yl-1-cyclohex-2-enyl acetate	
Terpinyl acetate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% terpinyl acetate in pet., tested in 106 consecutive patients in Barcelona, were observed (15)

Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010)

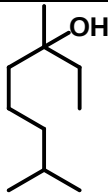
alpha-TERPINYL ACETATE	
CAS # 80-26-2	
EC # 201-265-7	
2-(4-Methyl-1-cyclohex-3-enyl)propan-2-yl acetate	
3-Cyclohexene-1-methanol, α,α,4-trimethyl-, acetate; p-Menth-1-en-8-ol, acetate; (±)-α-Terpineol acetate; (±)-α-Terpinyl acetate; 2-(4-Methyl-3-cyclohexen-1-yl)-2-propyl acetate; Terpinyl acetate; α-Terpineol acetate; p-Menth-1-en-8-yl acetate; 1-Methyl-1-(4-methylcyclohex-3-enyl)ethyl ethanoate; (±)-.alpha.,.alpha.,4-trimethylcyclohex-3-ene-1-methyl acetate	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified no positive reactions among 179 patients using a 10% PT preparation of “terpinyl acetate” (25).

Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010)

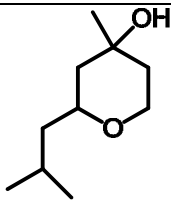
Tetrahydrolinalool	
CAS # 78-69-3	
EC # 201-133-9	
3,7-Dimethyloctan-3-ol	
2,6-Dimethyl-6-octanol; 3,7-Dimethyloctan-3-ol; Linalool tetrahydride; NSC 128151; Tetrahydrolinalool	

Current regulation: /

Clinical data:
/

Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010). A RIFM

review is available (205) quoting 1 negative human maximisation test.

TETRAHYDRO-METHYL-METHYLPROPYL)-PYRAN-4-OL	
CAS # 63500-71-0	
EC # 405-040-6	
4- Methyl-2-(2-methylpropyl)tetrahydro-2H-4-pyranol	
2-(2-Methylpropyl)-4-hydroxy-4-methyltetrahydropyran; 2-Isobutyl-4-hydroxy-4-methyltetrahydropyran; 2-Isobutyl-4-methyltetrahydropyran-4-ol; 4-Hydroxy-4-methyl-2-(2-methylpropyl)tetrahydropyran; Florosa; Rozanol	

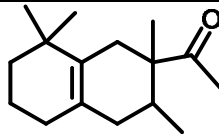
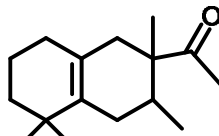
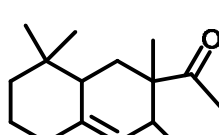
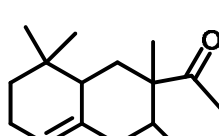
Current regulation: /

Clinical

data:

/

Additional information: It is a “top 100” substance (IFRA, pers. comm.2010).

TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES		54464-57-2
CAS # 54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9		54464-59-4
EC # 259-174-3 / 259-175-9 / 268-978-3 / 268-979-9		68155-66-8
1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone (54464-57-2)		68155-67-9
1-(1,2,3,4,5,6,7,8-Octahydro-2,3,5,5-tetramethyl-2-naphthalenyl)-ethanone (54464-59-4)		
1-(1,2,3,5,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone (68155-66-8)		
1-(1,2,3,4,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone (68155-67-9)		
54464-57-2: 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)ethanone; 1',2',3',4',5',6',7',8'-Octahydro-2',3',8',8'-tetramethyl-2'-acetoneaphthone; 7-Acetyl-1,2,3,4,5,6,7,8-octahydro-1,1,6,7-tetramethylnaphthalene; Amberonne; Ambralux; Iso Ambois Super; Iso-E Super; Isocyclemon E; OTNE; Orbitone		

Current regulation: /

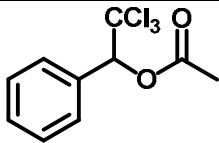
Clinical

data:

In the Frosch 2002 a study, 0.2% of 1855 consecutive patients reacted to the compound (brand name mentioned: „Iso E. Super“, 5% pet.) (16). In the Frosch 1995 dose-finding pilot study, 1 positive reaction both to 1% and 5% “Iso E Super ®” in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15). The Larsen 2001 study yielded 1.7% positive reactions (5% pet.) in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information: According to CosIng: "Mixture of isomers: 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one; 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,5,5-tetramethyl-2-naphthyl)ethan-1-one; 1-(1,2,3,5,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one (68155-67-9); 1-(1,2,3,4,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one (68155-66-8) ", (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40504>, last accessed 2009-11-11).

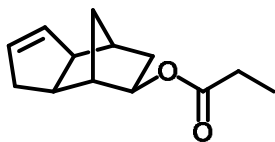
It is a "top 100" substance (IFRA, pers. comm. 2010)

TRICHLOROMETHYL PHENYL CARBINYL ACETATE	
CAS # 90-17-5	
EC # 201-972-0	
2,2,2-Trichloro-1-phenylethyl acetate	
Benzenemethanol, α-(trichloromethyl)-, acetate; Benzyl alcohol, α-(trichloromethyl)-, acetate (Trichloromethyl)phenylcarbinyl acetate; (±)-α-(Trichloromethyl)benzyl acetate; 2-Acetoxy-1,1,1-trichloro-2-phenylethane; Crystal rose; NSC 165582; Rosacetol; Rosephenone; Rosetone; Rosone; α-(Trichloromethyl)benzyl acetate	

Current regulation: /

Clinical data: /

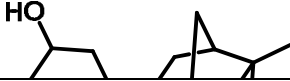
Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010)

TRICYCLODECENYL PROPIONATE	
CAS # 17511-60-3	
EC # 241-514-7	
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-inden-6-yl propionate	
4,7-Methano-1H-inden-6-ol, 3α,4,5,6,7,7α-Hexahydro-, propanoate; 4,7-Methanoinden-6-ol, 3α,4,5,6,7,7α-Hexahydro-, propionate; Cyclaprop; Florocyclene; Greenyl propionate; Tricyclo(5.2.1.02,6)dec-3-en-8-yl propionate.	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010).

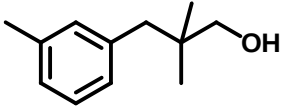
3-(5,5,6-TRIMETHYLBICYCLO[2.2.1]HEPT-2-YL)-CYCLOHEXAN-1-OL	

CAS # 3407-42-9	
EC # 222-294-1	
3-(5,5,6-Trimethyl-6-bicyclo[2.2.1]heptanyl)cyclohexan-1-ol	
3-(5,5,6-Trimethyl-2-norbornyl)-cyclohexanol; 3-(5,5,6-Trimethylbicyclo[2.2.1]hept-2-yl)cyclohexan-1-ol; 3-Hydroxy-1-(5-isocamphyl)cyclohexane; Sandela	

Current regulation: /

Clinical data: /

Additional information: part of “synthetic sandalwood oil”.

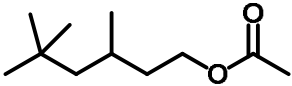
TRIMETHYL-BENZENEPROPANOL (Majantol)	
CAS # 103694-68-4	
EC # 403-140-4	
2,2-Dimethyl-3-(3-methylphenyl)propan-1-ol	
2,2-Dimethyl-3-(3-tolyl)propan-1-ol; 3-(2,2-Dimethyl-3-hydroxypropyl)toluene	

Current regulation: /

Clinical data:

In the Larsen 2002 c study, majantol (conc. not given, elsewhere reported as 5% pet.) caused positive PT reactions in 3.2% of patients with known contact allergy to fragrance ingredients. In a later study by the IVDK, 0.5% (95% CI: 0.3 – 0.7%) consecutive patients displayed a positive reaction to majantol 5% pet. (206). In the IVDK 2010 study, majantol was tested both in n=2189 consecutive patients, yielding 0.36 % (95% CI: 0.12—0.60%) positive reactions, and in the context in a special series, applied in an aimed fashion to n=4972 patients, yielding 0.76% (95% CI: 0.49—1.03%) (standardised) positive reactions (7). In a recent study from Copenhagen, DK, 6 of 722 patients tested with this compound were found positive, 2 of these to material used earlier provided by Symrise, 4 to material by Allmiral/Hermal/Trolab used later instead. There was no significant difference between these proportions obtained with batches of majantol from different production processes (207).

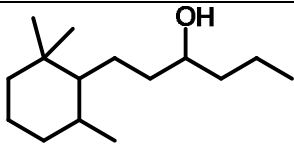
Additional information: /

TRIMETHYLHEXYL ACETATE	
CAS # 58430-94-7	
EC # 261-245-9	
3,5,5-Trimethylhexyl acetate	
1-Hexanol, 3,5,5-trimethyl-, acetate; Vanoris; neononyl acetate	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010)

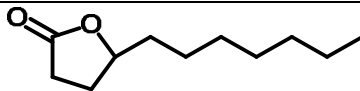
TRIMETHYL-PROPYLCYCLOHEXANEPROPANOL (TMCH)	
CAS # 70788-30-6	
EC # 274-892-7	
1-(2,2,6-Trimethylcyclohexyl)hexan-3-ol	
Other names: 2,2,6-Trimethyl-alpha-propylcyclohexanepropanol (REACH, EINECS); .alpha.-Propyl-2,2,6-trimethylcyclohexanepropanol; 6-(2,2,6-Trimethylcyclohexyl)-4-hexanol; Finotimber; Timberol	

Current regulation: /

Clinical data:

In the Larsen 2001 study, none of 178 patients with contact allergy to fragrance ingredients reacted positively to this ingredient, PTed at 5% pet. (19).

Additional information: /

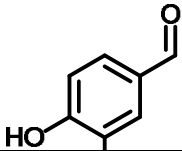
<i>gamma</i>-UNDECALACTONE	
CAS # 104-67-6	
EC # 203-225-4	
5-Heptyltetrahydrofuran-2-one	
Undecanoic acid, 4-hydroxy-, γ -lactone; (RS)- γ -Undecalactone; ($\pm$)- γ -Undecalactone; 4-Hydroxyundecanoic acid lactone; 4-Undecanolide; 5-Heptyldihydro-2(3H)-furanone; NSC 406421; NSC 46118; NSC 76413; Neutralizing agent 350120-1; Peach lactone; Peche Pure; Persicol; γ -(n-Heptyl)- γ -butyrolactone; γ -Heptyl- γ -butyrolactone; γ -Heptylbutyrolactone; γ -Undecalactone; γ -Undecanolactone; γ -Undecanolide; γ -n-Heptylbutyrolactone	

Current regulation: /

Clinical data: /

Additional information:

It is a "top 100" substance (IFRA, pers. comm. 2010)

VANILLIN	
CAS # 121-33-5	
EC # 204-465-2	

4-Hydroxy-3-methoxybenzaldehyde	
2-Methoxy-4-formylphenol; 3-Methoxy-4-hydroxybenzaldehyde; 4-Formyl-2-methoxyphenol; 4-Hydroxy-5-methoxybenzaldehyde; 4-Hydroxy-m-anisaldehyde; H 0264; Lioxin; NSC 15351; NSC 403658; NSC 48383; Rhovanil; Vanillaldehyde; Vanillic aldehyde; Vanillum; m-Methoxy-p-hydroxybenzaldehyde; p-Hydroxy-m-methoxybenzaldehyde; p-Vanillin	

Current regulation: /

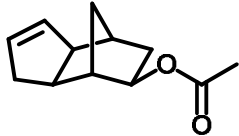
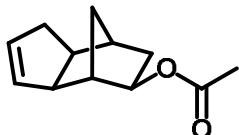
Clinical data:

In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to vanillin was observed (3). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reaction to vanillin 10 % pet. (22). The IVDK 2010 study, n=10, i.e., 0.19% (95% CI: 0.07 – 0.32%; percentages standardised for age and sex) of 4377 patients PTed reacted to the compound, tested 10% pet. (7). In n=102 patients with a positive reaction to MPR, 19 compounds of this natural mixture were tested, among these, vanillin, to which none reacted positively (208). In 21 patients with contact allergy to propolis, 2 also reacted to vanillin (10% pet.) (209).

A 13-year-old girl with recurrent (peri-)cheilitis after application of a vanilla lip salve tested strongly positive to this salve (as is), "Vanilla 10% pet." (unclear, whether natural extract or vanillin) and MPR (210). Trattner/David identified 1 / 641 consecutive patients with positive reaction to vanillin (31).

Additional information:

Naturally occurring in the fruit of *Vanilla planifolia* after a fermentation process, in styrax, clove oil, potatoes, wood, including Myroxylon pereirae resin, and other material (53). Nowadays, vanillin is synthesised from eugenol, guajakol and lignin residues from paper production, however, not fully achieving the subtle scent and taste of the natural material (53). It is a "top 100" substance and classified as R43 (IFRA, pers. comm. 2010).

VERDYL ACETATE	
CAS # 2500-83-6/ 5413-60-5	
EC # 219-700-4 / 226-501-6	
3a,4,5,6,7,7a-Hexahydro-4,7-methanoinden-6-yl acetat (2500-83-6)	2500-83-6
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-inden-5-yl acetat (5413-60-5)	
2500-83-6: 4,7-Methano-1H-inden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methanoinden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; NSC 142428; NSC 94573	
5413-60-5: 4,7-Methano-1H-inden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methanoinden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methano-3a,4,5,6,7,7a-hexahydroinden-6-yl acetate; 8-Acetoxytricyclo[5.2.1.0 ^{2,6}]dec-3-ene; Greenyl acetate;	5413-60-5

Opinion on fragrance allergens in cosmetic products

Herbaflorat; Jasmacyclene; NSC 6598	

Current regulation: /

Clinical data: /

Additional information:

In CosIng, both above CAS numbers are listed under “verdyl acetate” (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41289>, last accessed 2010-07-19).

In the CAS, there are 2 separate entries; moreover, there are 2 separate RIFM reviews:

- # 2500-83-6: Other names: Tricyclo[5.2.1.0^{2,6}]dec-4-en-8-yl acetate (REACH, EINECS, INCI Name according to CAS); 3a,4,5,6,7,7a-Hexahydro-4,7-methanoinden-6-yl Acetate; Tricyclodecen-4-yl 8-Acetate. It is a “top 100” substance (IFRA, pers. comm. 2010). A RIFM review is available, stating that “no data is available” regarding the skin sensitising properties of the substance (211).
- # 5413-60-5: Other names: 3a,4,5,6,7,7a-hexahydro-4,7-methanoinden-6-yl acetate (REACH, EINECS, INCI Name according to CAS), 4,7-Methano-3a,4,5,6,7,7a-hexahydroinden-6-yl acetate; 4,7-Methanoinden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 8-Acetoxytricyclo[5.2.1.0^{2,6}]dec-3-ene; Tricyclodecenyl acetate; Greenyl acetate; Herbaflorat; Jasmacyclene; NSC 6598; Verdyl acetate. It is a “top 100” substance (IFRA, pers. comm. 2010). A RIFM review is available (212), citing 2 negative human maximisation tests and 1 negative HRIPT.

Natural extracts / essential oils

Natural raw materials in terms of extracts are used in the fragrance and flavour industry for various reasons. Most importantly, several naturally occurring mixtures have a very complex composition and sensory nature which cannot (fully) be achieved by synthetic material. Moreover, several compounds cannot be synthesised at a competitive price, and the demand for perfumes based on natural materials is considerable (34).

The three main methods used to concentrate plant fragrance substances as essential oils comprise steam distillation, mechanical processes from the epicarp of Citrus fruits ("pressing") and dry distillation. An Essential oil is „obtained by steam distillation with addition of water in the still (hydrodistillation) or without addition of water in the still (directly by steam“)(213). Essential oil of fruit juice is „obtained by from a fruit juice during its concentration or during UHT (flash pasteurization) treatment“ (213). Cold pressed essential oil is „obtained by mechanical processes from the epicarp of the fruit of a Citrus, at ambient temperature“(213). Citrus peel oils, apart from distilled Citrus oils, are produced with various methods (214). The oil consists of a high volume of volatile terpenes, mostly monoterpenes but also contains small amounts of non-volatile compounds such as dyes, waxes and furocoumarines.

The method of solvent extraction is generally applied in the separation of heat-labile materials or if an essential oil can only be obtained in very low yield, e.g. from blossoms. It is also used if the non-volatile components are desired for their fixative properties, e.g. in the preparation of resinoids from exudates. The most important extracts are termed: (i) concrete: an extract „obtained from a fresh plant natural raw material by extraction with a solvent“¹⁸, containing not only volatile, but also a large proportion of non-volatile substances such as waxes; and (ii) absolute: „product, obtained by extraction with ethanol from a concrete, a floral pomade, a resinoid or a supercritical fluid extract. The ethanolic solution is generally cooled down and filtered in order to eliminate the «waxes»; the ethanol is then eliminated by distillation“¹⁹. Resinoids, used for their fixative properties, are „obtained from a dry plant natural raw material by extraction with a solvent“²⁰. The products are usually highly viscous and thus might sometimes be diluted, e.g. with phthalates or benzyl benzoate. Oleoresins are extracts „of spice or aromatic herb“ by „treating a natural raw material with a solvent, then, after filtration if necessary, the solvent is eliminated“²¹.

Regarding clinical data in terms of contact allergy to fragrance ingredients, the main focus of case report or clinical studies regarding essential oils and natural extracts, respectively, is on general dermatological patients with complaints related to use of cosmetics etc. However, series of cases with occupational exposure to essential oils with occupational allergic contact dermatitis have also been reported (e.g., masseurs,

¹⁸ ISO/DIS 9235

¹⁹ ISO/DIS 9235

²⁰ ISO/DIS 9235

²¹ ISO/DIS 9235

physiotherapists (215, 216), aromatherapists (217-221), beauticians doing massages (222); for further details, e.g., PT results with various essential oils, see original case reports. "Current Regulation" refers to the EU Cosmetics Directive only.

Catalogue of natural extracts / essential oils evaluated

ACORUS CALAMUS ROOT OIL

CAS 84775-39-3; EC 283-869-0

Calamus Oil; "Sweet Flag Oil"

(*Acorus calamus*, ext. = INCI name)

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to "calamus" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to "calamus" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Acorus calamus* L. (sweet flag calamus). *Acorus Calamus* Root Oil is an essential oil obtained from the rhizomes of the calamus, *Acorus calamus* L., Araceae. It contains beta-asarone (up to 96%, depending on ploidy, and with this, origin (34)), calamene (about 4%), calamol (about 3%) alpha-asarone (about 1%), camphene (about 1%) and some beta-pinene and asaronaldehyde (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41330>, last accessed 2010-01-29. Use is restricted due to potential toxicity of beta-asarone (34).

CANANGA ODORATA and Ylang-ylang oil

Ylang-ylang and cananga oils are essential oils that are obtained from two subspecies of the cananga tree (34). In the INCI nomenclature, both are not differentiated.

CANANGA ODORATA FLOWER EXTRACT

CAS 83863-30-3; EC 281-092-1
(ylang-ylang, ext.) INCI name:
CANANGA ODORATA EXTRACT

CANANGA ODORATA FLOWER OIL

CAS 8006-81-3, 68606-83-7; EC / (oils,
ylang-ylang) INCI name: CANANGA
ODORATA OIL

Current regulation: ...

Clinical data:

Ylang-ylang oil

ISO 4720:2009 nomenclature: *Cananga odorata* (Lam.) Hook. f. et Thomson *forma*

genuina)

In the Larsen 2002 c study, “synthetic ylang-ylang oil” caused 6.4% positive reactions in 218 patients with known contact allergy to fragrance ingredients (1). In a Japanese study, M. Sugawara et al. noted a significant decline of the proportion of patients reacting positively to “ylang-ylang oil 5% pet.” from 1971 to 1989, the overall number in patients with cosmetic dermatitis amounting to 176 of 1438 (12.2%, 95% CI: 10.6 – 14.0%) (223). In the Frosch 2002 b study, two fractions of Ylang-Ylang oil (I and II) were separately tested, each at 10% pet. Fraction I yielded 2.6%, fraction II 2.5% positive test reactions (no data on concomitant reactivity given) (17). The deGroot 2000 study, with 1825 consecutively tested patients, found 18 positive PT reactions to “ylang-ylang oil”, tested at 4% in pet. (12). The Sugiura 2000 study with 1483 patients with suspected cosmetic dermatitis observed 0.8% positive PT reactions with ylang-ylang oil (5% pet.) (14). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with ylang-ylang oil (2% pet.) 13.4% positive reactions (9). The Belsito 2006 study (20) yielded 0.6% positive reactions to ylang-ylang oil. The subsequent NACDG 2009 study identified 1.5% positive reactions in 4434 patients PTed with 2% “ylang-ylang oil” (21). The IVDK 2010c study found 2.5% positive reactions in 3175 consecutively tested patients, and 3.9% in 2155 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to ylang-ylang oil and 3 to “cananga oil” (48).

Cananga oil

ISO 4720:2009 nomenclature: *Cananga odorata* (Lam.) Hook. f. et Thomson *forma macrophylla*. For Oil of cananga (*Cananga odorata* (Lam.) Hook. f. et Thomson, *forma macrophylla*) an ISO standard exists: ISO 3523:2002. Cananga oil is produced by steam distillation of the flowers of *Cananga odorata* (DC.) Hook f. et Thomson subsp. *macrophylla* (*Annonaceae*). The composition resembles that of “ylang-ylang III”, but with a higher content of caryophyllene (30-40%). Cananga oil originates almost exclusively in Java; annual production about 50 t. The oil is used mainly in perfuming soaps where it is more stable than ylang-ylang oils due to its lower ester content (34).

Sugiura et al. (2000) found 1.1% positive reactions to “cananga oil”, tested 5% pet. (14). Cananga oil (2% pet.) mentioned in the same Portuguese study already cited (9) yielded 10.4% positive reactions. In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to cananga odorata oil tested at 2% concentration (13).

Studies with both oils

The Goossens 1997 study found 3 of 111 patients positive to “ylang-ylang oil 5% pet.”, and 4 to “cananga oil 15% pet.” – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found 1 positive reaction in 200 patients to “cananga” and 4 to “ylang-ylang” essential oil, both tested 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=10 (11.6%) positive reactions to “cananga” and n=8 (9.3%) to “ylang-ylang” essential oils, each tested at 2% pet. (27). Nakayama et al. found 1974 (after (29)) 11 “strong positive” and 15 “weak positive” reactions to “Cananga oil” and 9 and 16, resp., to “Ylang-ylang oil” (unknown test concentration) in 183 patients.

A number of case reports highlight the possibility of occupational contact and sensitisation, e.g. (222, 224).

Additional information:

Ylang-ylang oil

The composition of this essential oil is defined by a standard: ISO 3063:2004. Ylang-ylang oils are obtained by steam distillation of freshly picked blossoms of *Cananga odorata* (DC.) Hook f. et Thomson subsp. *genuina* (Annonaceae). The oil is produced mainly in Madagascar and the Comoro islands. Four fractions are collected at progressively longer distillation times and are known as “extra”, “I”, “II” and “III”. The composition of the various oil fractions depends on the duration of distillation. The first fraction has the highest content of strongly odiferous constituents such as p-cresyl methyl ether (5-16%), methyl benzoate (4-9%), (-)-linalool (7-24%), benzyl acetate (5.5-17.5%), and geranyl acetate (2.5-14%). The other fractions contain increasing amounts of sesquiterpene hydrocarbons such as caryophyllene, germacrene-D, and (E,E)-alpha-farnesene (> 70% in “ylang-ylang III”). Components such as p-cresol, eugenol and isoeugenol are important for odour, although they are present only in low concentration (34). According to (30) the maximum observed concentration in ylang-ylang I and II are (in %): germacrene-D (28); (E,E)-alpha-farnesene (21); caryophyllene (17); linalool (I: 19.0; II: 9.5); benzyl benzoate (8.0); farnesol (4.0); benzyl salicylate (4.0); (E,E)-farnesyl acetate (3.5); geraniol (2.5); isoeugenol (0.8); benzyl alcohol (0.5); eugenol (0.5); p-cresyl methyl ether (I: 5.0; II 3.5); methyl benzoate (I: 5.5; II: 3.5); benzyl acetate (I: 10.0; II: 5.0); geranyl acetate (I: 15.0; II: 12.0).

CEDRUS ATLANTICA BARK OIL

CAS 92201-55-3; EC 295-985-9
(*Cedrus atlantica*, ext. = INCI) /
8000-27-9; EC / (Oils,
cedarwood) INCI name: CEDRUS
ATLANTICA OIL

Cedarwood oil

Current regulation: /

Clinical data:

In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=5 (0.7%) positive reactions to cedarwood oil 10% pet. (22). (The exact origin of “cedarwood oil” in this study is not clear.) The IVDK 2010 c study identified 0.8% positive reactions in 6223 patients tested in the context of a special series with a cedarwood oil tagged with CAS # 8000-27-9 (30).

Additional information:

Cedrus Atlantica Bark Oil is the volatile oil obtained from the bark of *Cedrus atlantica*, Pinaceae

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55309>, last accessed 2010-01-05). The main odiferous component is alpha-atlantone [32207-08-2] (39)

Nomenclature also used: *Cedrus atlantica* wood oil (*Cedrus atlantica* (Endl.) G.Manetti ex Carrière)²²

See also *Juniperus virginiana*.

²² ISO 4720:2009 nomenclature

CEDRUS DEODARA WOOD OILCAS 91771-47-0; EC 294-939-5 (*Cedrus deodara*, ext.)*Cedarwood oil*

Current regulation: /

Clinical data:

The Rudzki 1976 study found 3 positive reactions in 200 patients to “cedarwood” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to “Himalayan cedarwood” essential oil 2% pet. (27). (The labelling in the latter report points to *Cedrus deodara* as source of “cedarwood oil” in these 2 Polish studies.)

Additional information:

Cedrus Deodara Wood Oil, Himalayan cedarwood oil (*Cedrus deodara* (Roxb. ex D.Don) G. Don)²³, is the volatile oil obtained by steam distillation of the stumps of the Deodar Cedar, *Cedrus deodara*, *Pinaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55311>, last accessed 2010-01-29).

Several other conifer species are called cedars, and the corresponding oils vary considerably in composition. These include Cedar leaf oil (*Thuja* oil) produced by steam distillation of fresh leaves and branch ends of *Thuja occidentalis* L. (*Cupressaceae*) from North America, containing a minimum of 60% thujone [8007-20-3] [90131-58-1] (34). Texas cedarwood oil is produced by steam distillation of chopped wood of *Juniperus mexicana* Schiede (*Cupressaceae*), containing alpha-cedrene (15-25%), thujopsene (25-35%), cedrol 20% minimum [8000-27-9] [91722-61-1] (34). Chinese cedarwood oil is similar to Texas cedarwood oil, obtained by steam distillation of *Cupressus funebris* Endl., *Cupressaceae* (*Chamaecyparis funebirs* (Endl.) France), which is a weeping cypress [8000-27-9] [85085-29-6] (34).

CINNAMOMUM CASSIA LEAF OIL

94961-46-6 [invalid] / 8007-80-5; EC / (Oils, cassia) INCI name: CINNAMONUM CASSIA OIL

*Cassia Oil; Cassia leaf Oil; Cinnamon Oil Chinense***CINNAMOMUM ZEYLANICUM BARK OIL**CAS 84649-98-9; EC 284-635-0 (*Cinnamomum zeylanicum*, ext. = INCI)*Cimmamon Bark Oil Ceylon; Cinnamon Oil Ceylon*

Current regulation: /

Clinical data:

The Rudzki 1976 study found 2 positive reactions in 200 patients to “cassia” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=24

²³ ISO 4720:2009 nomenclature

(27.9%) positive reactions to "cassia" essential oil 2% pet. (27).

A 32 year old Spanish physiotherapist developed vesicular hand dermatitis after using a "balsam from ash extract" cream. PTing revealed positive reactions to this cream, the FM I, eugenol, and 2 components of the cream: "cinnamon oil" (0.5% pet.) and clove oil (1% pet.) (225).

Additional information:

ISO 4720:2009 nomenclature: *Cinnamomum tsumu* Helms, syn. *Cinnamomum cassia* auct. and *Cinnamomum zeylanicum* Blume syn. *Cinnamomum verum* J. Presl, respectively. Cassia oil (Chinese cinnamon oil) is obtained by steam distillation of the leaves, twigs, and bark of *Cinnamomum aromaticum* Nees (*C. cassia* Blume, *Lauraceae*). In contrast to cinnamon bark oil (see below), cassia oil contains a considerable amount of 2-methoxycinnamal (3-15%), in addition to its main constituent, cinnamal (70-88%). Cassia oil is predominantly used in flavouring soft drinks, with an annual production of a few hundred tons (34). For Oil of cassia, Chinese type (*Cinnamomum aromaticum* Nees, syn. *Cinnamomum cassia* Nees ex Blume) an ISO standard exists: ISO 3216:1997

Cinnamomum Zeylanicum Bark Oil is the volatile oil expressed from the bark of the Ceylon Cinnamon, *Cinnamomum zeylanicum*, *Lauraceae*. It contains mainly cinnamaldehyde (34), e.g. 50-60%, and lesser quantities of eugenol (4-8%), phellandrene

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75370>, last accessed 2009-11-16). For Oil of cinnamon leaf, Sri Lanka type (*Cinnamomum zeylanicum* Blume) an ISO standard exists: ISO 3524:2003

Cinnamomum Cassia Leaf Oil is the volatile oil obtained by steam distillation from the leaves and twigs of the Chinese Cinnamon, *Cinnamomum cassia* (L.), *Lauraceae*. It contains 80% eugenol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75368>, last accessed 2009-11-16). The cinnamon leaf oil produced by steam distillation of the leaves of *Cinnamomum zeylanicum* Blume (*C. verum* J.S. Presl) similarly has a content of 70-83% eugenol (34).

Considering the content of well-known allergenic compounds, the essential oil is considered an Established contact allergen in humans,

CITRUS AURANTIUM AMARA FLOWER OIL

CAS 8016-38-4, 68916-04-1; EC /
(Oils, neroli) /

Neroli oil

CITRUS AURANTIUM AMARA PEEL OIL
EXPRESSED

72968-50-4; EC 277-143-2 (Orange,
sour, ext.)

"Bitter Orange Oil"

INCI names: CITRUS AURANTIUM
AMARA ...

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "neroli oil" (2% pet.) 6.6% positive reactions (9). The Rudzki 1976 study found 3 positive reactions in 200 patients to "bitter orange" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%)

positive reactions to “bitter orange” essential oil 2% pet. (27). The IVDK 2010 c study identified 0.7% positive reactions in 6220 patients tested in the context of a special series (30)

Additional information:

ISO 4720:2009 nomenclature: *Citrus aurantium* L., syn. *Citrus amara* Link, syn. *Citrus bigaradia* Loisel, syn. *Citrus vulgaris* Risso. For Oil of neroli (*Citrus aurantium* L. spp. *aurantium*, syn. *Citrus aurantium* L. spp. *amara* var. *pumilia*) an ISO standard exists: ISO 3517:2002. Citrus Aurantium Peel Oil Expressed is an essential oil expressed from the fresh epicarps of the Sour Orange, *Citrus aurantium*, Rutaceae. It contains D-limonene (about 90%), citral, decanaldehyde, methyl anthranilate, linalool, terpineol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41394>, last accessed 2010-01-29). The aldehyde content is lower and the ester content (e.g., linalyl and geranyl acetate) is higher than in sweet orange oil (34). It is predominantly used for flavouring alcoholic beverages. According to (30) the maximum observed concentration in neroli oil are (in %): linalool (44); limonene (18); β -pinene (17); linalyl acetate (15); *trans*- β -ocimene (8); geranyl acetate (5); *trans*-nerolidol (5); (*E,E*)-farnesol (4); myrcene (4); farnesol (4,0); geraniol (3,5); citral (0,3) (30).

CITRUS AURANTIUM AMARA LEAF OIL

72968-50-4; EC 277-143-2 (Orange, sour, ext.)

Petitgrain oil Paraguay / ... bigarade

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to “Petitgrain bigarade” and “Petitgrain Paraguay” essential oil each, both tested at 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to “Petitgrain bigarade” and n=4 (4.6%) to “Petitgrain Paraguay” essential oil each, both tested at 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Citrus sinensis* L. Pers. X *Citrus aurantium* L. ssp. *amara* var. *pumilia*. Petitgrain oils in general are steam distilled from the leaves of citrus trees. Citrus Aurantium Leaf Oil is an essential oil obtained from the leaves of the Sour Orange, *Citrus aurantium*, Rutaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41392>, last accessed 2010-02-10). Petitgrain oil Paraguay is obtained from an acclimatised variety of the bitter orange tree. Main constituents are linalool (15-30%) and linalyl acetate (40-60%). A number of trace constituents contribute essentially to the odour (34). Petitgrain oil bigarade is derived from the same species of tree grown in France, Italy, Spain and North Africa (34). For Oil of bitter orange petitgrain, cultivated (*Citrus aurantium* L.) an ISO standard exists: ISO 8901:2003.

Considering the content of well-known allergenic compounds, the essential oil is regarded as an established contact allergen in humans

CITRUS BERGAMIA PEEL OIL EXPRESSED

CAS 89957-91-5, 8007-75-8; EC

	289-612-9 (<i>Bergamot, ext.</i>)
<i>Bergamot Oil, Bergamot Orange Oil</i>	INCI: CITRUS AURANTIUM BERGAMIA EXTRACT

Current regulation: /

Clinical data:

The Rudzki 1976 study found 3 positive reactions in 200 patients to “Bergamot” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found no positive reaction to “Bergamot” essential oil 2% pet. (27). In 63 patients positive to the FM I, 2 had a positive PT reaction to bergamot oil, 2% pet., in the Santucci 1987 study (28). A case report from Zacher and Ippen describes 2 patients with allergic contact dermatitis due to bergamot oil (191), one a worker in a perfume factory, the other sensitised by non-occupational use of cosmetics.

Additional information:

ISO 4720:2009 nomenclature: *Citrus bergamia* (Risso et Poit.), syn. *Citrus aurantium* L. subsp. *bergamia* (Wight et Arnott) Engler. Citrus Bergamia Peel Oil Expressed is an essential oil expressed from the epicarps of the Bergamot, *Citrus bergamia* risso, Rutaceae. It contains 35-45% L-linalyl acetate, about 6% linalool, D-limonene, DL-limonene and bergaptene (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=se_arch.details&id=41398, last accessed 2009-11-27). According to Surburg/Panten: linalyl acetate 22-36%, linalool 3-15%, geranial 0.25-0.5%, citral 1%, with a relatively low terpene content of 25-50% (34, 39). Bergaptene content by HPLC is 0.18-0.38% (34). Annual production from Italy, Brazil, Spain and Ivory Coast is 100 to 150 t. For Oil of bergamot [*Citrus aurantium* L. subsp. *bergamia* (Wight et Arnott) Engler], Italian type an ISO standard exists: ISO 3520:1998.

CITRUS LIMONUM PEEL OIL EXPRESSED	CAS 84929-31-7, 8008-56-8; EC 284-515-8 (<i>Lemon, ext.</i>)
<i>Lemon oil</i>	INCI names: CITRUS MEDICA LIMONUM ...

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with “lemon oil” (2% pet.) 4.5% positive reactions (9). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=2 (0.3%) positive reactions to “lemon oil” 2% pet. (22).

The Rudzki 1976 study found 1 positive reaction in 200 patients to “lemon” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to “lemon” essential oil 2% pet. (27). The IVDK 2010 c study identified 0.3% positive reactions in 6467 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Citrus limon* (L.) Burm. f. According to (30) the maximum observed concentration in lemon oil are (in %): limonene (80); β -pinene (16.5); γ -terpinene (12); citral (3.0); geranial (2.0); neral (1.2); β -bisabolene (0.9); geranyl

acetate (0.7); neryl acetate (0.6); linalool (0,3); geraniol (0,2) (30). An ISO standard exists for Oil of lemon [Citrus limon (L.) Burm. f.], obtained by expression: ISO 855:2003. The composition of lemon oil depends on the variety of lemon and the country of origin, see table from (34).

Table 3. Specifications for qualities of lemon oils of different origins

Parameter	Type	Mediterranean		Equatorial	
	American Origin				
	Coast	Desert	Italy	Spain	Ivory coast, Brazil
d_{20}^{20}	0.851–0.857	0.849–0.854	0.850–0.858	0.849–0.858	0.845–0.854
n_D^{20}	1.4370–1.4760	1.4370–1.4760	1.4370–1.4760	1.4370–1.4760	1.4370–1.4790
α_D^{20}	+57° to +65°6'	+67° to +78°	+57° to +66°	+57° to +66°	+57° to +70°
Composition by GC [area %]					
β -Pinene	9–14	10–13	10–16.5	10–16.5	7–16
Limonene	63–70	70–80	60–68	60–70	59–75
γ -Terpinene	8.3–9.5	6.5–8	8–12	8–12.8–12	6–12
Neral	0.6–0.9	0.3–0.6	0.6–1.2	0.4–1	0.2–1.2
Geraniol	1.0–2	0.5–0.9	0.8–2	0.6–2	0.5–2
Evaporation residue [weight %]					
	1.75–3.9		1.5–3.9	1.5–3.9	1.5–4
Carbonyl value					
	8–14	6.25–12	11–17	11–17	6–17
CD value	min. 0.2	min. 0.2	0.45–0.9	0.4–0.9	0.2–0.96

CITRUS PARADISI PEEL OIL

Grapefruit oil, expressed

CAS 8016-20-4 ; EC /

INCI: CITRUS GRANDIS OIL

Current regulation: II/358 R1

Clinical data: /

Additional information:

Citrus Paradisi Peel Oil is the volatile oil expressed from the peel of the Grapefruit, Citrus paradisi, Rutaceae
http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=55434

It is a “top 200” substance and classified as R43 (IFRA, pers. comm.2010)

CITRUS SINENSIS (syn.: AURANTIUM DULCIS) CAS 97766-30-8, 8008-57-9, EC
 PEEL OIL EXPRESSED 307-891-8 (Orange, sweet,

(Sweet) Orange oil

Valencia, ext. = INCI) / 8028-48-6; EC 232-433-8 (Orange, sweet, ext.)

INCI names: CITRUS AURANTIUM DULCIS ...

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "orange oil" (2% pet.) 4.5% positive reactions (9). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reactions to orange oil 2% pet. (22). The Rudzki 1976 study found 1 positive reaction in 200 patients to "sweet orange" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "sweet orange" essential oil 2% pet. (27). In the Frosch 1995 dose-finding pilot study, neither positive nor irritant reaction to 1% and 5% "orange oil Bras." in pet., tested in 205 consecutive patients in Dortmund and Göttingen, were observed (15). The IVDK 2010 c study identified 0.2% positive reactions in 6246 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Citrus sinensis* (L.) Osbeck. For Oil of sweet orange (*Citrus sinensis* (L.) Osbeck), CAS 8008-57-9, obtained by mechanical treatment, an ISO norm exists: ISO 3140:2005. The oils have a high terpene hydrocarbon content (> 90%, mainly (+)-limonene. Important for aroma are aldehydes, mainly decanal and citral, and aliphatic and terpenoid esters. The sesquiterpene aldehydes alpha-sinensal [17909-77-2] and beta-sinensal [6066-88-8] contribute particularly to the special sweet aroma (34). According to (30) the maximum observed concentration in sweet orange oil are (in %): *limonene* (95.0); *linalool* (0.7); n-decanal (0.7); *citral* (0.3); alpha-sinensal (0.05); beta-sinensal (0.06) (30). Worldwide production is more than 30000 tons / year. Main uses comprise the flavouring of beverages and confectioneries and perfuming E.d.C, soaps and household products.

For the latter uses relevant here, both "Orange peel oil, sweet (*Citrus sinensis* (L.) Osbeck) (8008-57-9)", "Orange peel, sweet, extract (*Citrus sinensis* L. Osbeck) (8028-48-6)" and "Orange, sweet, Valencia, ext. (97766-30-8)" are among the top 100 used fragrance materials and classified as R43 (IFRA, pers. comm. 2010).

ORANGE OIL TERPENES (CAS # 68647-72-3) are a "top 100 mixture of substances and classified as R43 (IFRA, pers. comm.2010). Other names: ORANGE, SWEET, TERPENES (REACH); Terpenes and Terpenoids, sweet orange-oil (REACH). The CAS entry refers to a group of substances "Terpenes and Terpenoids, sweet orange-oil" (REACH).

CITRUS TANGERINA ...

Oil of tangerine

CAS 223748-44-5; EC /

[no info in CAS database]

Current regulation: /

Clinical data:

In a 17 year old girl, the perfume used for 3 months caused ACD due to the ingredient "oil of tangerine", with a strong positive PT reaction (to 2% or 10% in pet.; 50 controls

negative) (226).

Additional information:

Citrus Tangerina Peel Oil is the volatile oil expressed from the peel of the ripe fruit the Tangerine, *Citrus Tangerina*, *Rutaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55441>, last accessed 2010-01-29); (*Citrus tangerina* Tanaka).

CORIANDRUM SATIVUM HERB OIL

CAS 84775-50-8; EC 283-880-0
(Coriander, ext.)

Coriander oil

INCI: CORIANDRUM SATIVUM
EXTRACT

Current regulation: /

Clinical data:

The Rudzki 1976 study found 2 positive reactions in 200 patients to “coriander” essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to “coriander” essential oil 2% pet. (27).

Additional information:

Coriander Sativum Herb Oil is an essential oil obtained from the herbs of the Coriander, *Coriandrum sativum* L., *Umbelliferae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39388>, last accessed 2010-01-29). The main component of coriander oil is linalool (by GC: 65-78%) and mono- and polyunsaturated fatty aldehydes contributing to the particular aroma. In contrast to the seed oil, coriander leaf oil contains these aldehydes as main constituents, e.g. 2-deccanal and 2-dodecanal (34). For Oil of coriander fruits (*Coriandrum sativum* L.) an ISO standard exists: ISO 3516:1997.

CYMBOPOGON OILS

Cymbopogon oils are produced from several aromatic grasses that belong to the genus *Cymbopogon* Speng. (*Poaceae*). The oils are obtained by steam distillation of the aerial parts of the plants (34).

The composition of the essential oil derived from *Cymbopogon flexuosus* (Nees ex Steudel) J.F. Watson is defined by a standard: ISO 4718:2004, as is the oil derived from *Cymbopogon citratus*: 3217:1974.

CYMBOPOGON CITRATUS LEAF OIL

Cymbopogon citratus (DC.) Stapf.²⁴

CAS 89998-14-1; EC 289-752-0
(*Cymbopogon citratus*, ext. =
INCI)

²⁴ ISO 4720:2009 nomenclature

Lemon Grass Oil; Indian Verbena Oil; Indian Melissa Oil

CYMBOPOGON SCHOENANTHUS OIL

Cymbopogon flexuosus (Nees ex Steudel) J.F. Watson²⁵

CAS 8007-02-1; EC 289-754-1 (oils, lemongrass) / 89998-16-3; EC 289-752-0 (Cymbopogon Schoenanthus, ext. = INCI)

Lemon Grass Oil

Current regulation: /

Clinical data:

The Frosch 2002 b study on 1606 consecutive patients reported 1.6% positive reactions to "lemongrass oil (East India), CAS 8007-02-1", PTed at 2% pet. (17). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 3 positive reactions to lemongrass oil were observed (3). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=6 (0.8%) positive reactions to lemongrass oil 2% pet. (22). The IVDK 2010 c study identified 0.6% positive reactions in 2435 consecutively tested patients and 2.3% positive reactions in 8445 patients tested in the context of a special series (30).

Additional information:

Cymbopogon Citratus Leaf Oil is an essential oil obtained from the leaves of the Lemon Grass, *Cymbopogon citratus* (DC., ex Nees), *Poaceae*. It contains citral (75-85%), methylheptenone, citronellal, geraniol, limonene (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39457>, last accessed 2009-11-12). According to Surburg/Panten, by GC: neral (31-40%), geranial (40-50%) (34).

Indian lemongrass oil is obtained by the so-called Indian variety of lemongrass, *Cymbopogon flexuosus* (Nees ex Steud.) Stapf. Content by GC: 25-35% neral, 35-47% geranial (34).

Cymbopogon Schoenanthus Oil is the volatile oil obtained by the steam distillation of fresh Lemon Grass, *Cymbopogon schoenanthus* (L.), *Poaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75419>, last accessed 2009-11-12). According to (30) the maximum observed concentration in lemongrass oil are (in %): citral (85.0); geraniol (7.0); limonene (4.0); geranyl acetate (2.2); caryophyllene (1.6); trans-isocitral (1.4); 6-methyl 5-hepten-2-one (1.3); caryophyllene oxide (1.2); 4-nonanone (1); citronellol (0.8); eugenol (0.3); linalool (0.2) (also according to (227))

In a LLNA study by RIFM, the lemongrass oil as used was reported to contain 68.8% citral, 6.7% limonene, 6.1% geraniol, 2.2% geranyl acetate, 1.6% caryophyllene, 1.4% trans-isocitral, 1.3% 6-methyl 5-hepten-2-one, 1.2% caryophyllene oxide and 1% 4-nonanone, according to analyses of the supplier. The EC3 value was calculated to be 6.5% (227).

CYMBOPOGON MARTINI HERB EXTRACT

CAS 84649-81-0; EC 283-461-2 (Cymbopogon Martini, ext)

²⁵ ISO 4720:2009 nomenclature

INCI: CYMBOPOGON MARTINI OIL*Palmarosa oil*

Current regulation: /

Clinical data: /

Additional information:

ISO 4720:2009 nomenclature: *Cymbopogon martini* (Roxb.) Will. Watson var. *motia* and var. *sofia*. Cymbopogon Martini Herb Extract is an extract obtained from the herbs of the plant, *Cymbopogon martini*, Gramineae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39460>, last accessed 2009-11-24), namely, by steam distillation of wild or cultivated *Cymbopogon martini* (Roxb.) J.F. Wats., collected when in blossom (34). The main constituent is geraniol (72-94%) (34).

In a LLNA study by RIFM, the palmarosa oil as used was reported to contain 79.4% geraniol, 9.4% geranyl acetate and 1.9% caryophyllene, according to analyses of the supplier. The EC3 value was calculated to be 9.6% (227).

CYMBOPOGON NARDUS HERB OIL

CAS 89998-15-2; EC 289-753-6 (*Cymbopogon nardus*, ext. = INCI)

Citronella Oil (Sri Lanka)

CYMBOPOGON WINTERIANUS HERB OIL

CAS 91771-61-8; EC 294-954-7 (*Cymbopogon Winterianus*, ext. = INCI)

Citronella Oil (Java)

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 5 positive reactions in 200 patients to "citronella" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=1 (1.1%) positive reactions to "citronella" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Cymbopogon nardus* (L.) W. Watson var. *lenabatu* Stapf. and *Cymbopogon winterianus* Jowitt, respectively. Cymbopogon Nardus Herb Oil is an essential oil obtained from the herbs of the plant, *Cymbopogon* (syn: *Andropogon*) *nardus* (L.), Gramineae. The Ceylon citronella oil contains geraniol (about 60%), citronellal (about 15%), camphene, limonene, linalool, borneol. According to Surburg/Panten, the Sri Lankan oil contains citronellal (3-6%), borneol (4-7%), citronellol (3-8.5%), geraniol 15-23%) and methyl isoeugenol (7.11%) (34).

The Java citronella oil contains 25-50% citronellal, 25-45% geraniol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.h.details&id=39469>, last accessed 2009-11-24). Cymbopogon Winterianus Herb Oil as a synonym for Java citronella oil is obtained from the herbs of the plant, *Cymbopogon winterianus*, Gramineae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=sea>

[rch.details&id=39472](#), last accessed 2009-11-24). This oil, produced in Taiwan and Java, contains citronellal (31-40%), geraniol (20-25%), citronellol (8.5-14%), geranyl acetate (2.5-5.5%), citronellyl acetate (2-4%) and many minor components. Annual worldwide production is currently at around 1000 t (34). For Oil of citronella, Sri Lankan type (*Cymbopogon nardus* (L.) W. Watson var. *lenabatu* Stapf.) an ISO standard exists: ISO 3849:2003, for Oil of citronella, Java type the ISO 3848:2001.

In a LLNA study by RIFM, the citronella oil as used was reported to contain 36.6% citronellal, 20.6% geraniol, 4.1% limonene, 3.7% geranyl acetate, 3.0% citronellyl acetate, 2.6% elemol, 2.2% beta-bourbonene, 1.9% delta-cadiene, 1.6% isopugenol I, 1.4% germacrene D and eugenol and linalol at < 1%, according to analyses of the supplier. The EC3 value was calculated as > 50 % (227).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

EUCALYPTUS SPP. LEAF OIL

CAS 92502-70-0; EC 296-357-7
(*Eucalyptus*, ext. = INCI)

Eucalyptus Oil

CAS 8000-48-4; EC / (Oils,
eucalyptus) INCI: *EUCALYPTUS*
GLOBULUS OIL

Current regulation: /

Clinical data:

In a study with 218 fragrance sensitive patients, 1.8% reacted positively to 10% eucalyptus oil (pet.) (1). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to "eucalyptus oil" was observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=4 (0.6%) positive reactions to eucalyptus oil 2% pet. (22). The Rudzki 1976 study found 3 positive reactions in 200 patients to "eucalyptus" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=1 (1.1%) positive reactions to "Eucalyptus" essential oil 2% pet. (27). The IVDK 2010 c study identified 0.2% positive reactions in 6680 patients tested in the context of a special series (30).

In a professional athlete, the use of an "analgesic and anti-inflammatory cream" over 2 years lead to ACD, which was attributed to eucalyptol (eucalyptus oil, 1% pet., 25 controls negative), the sole ingredient of the cream eliciting a positive PT reaction (228)

Additional information:

ISO 4720:2009 nomenclature: *Eucalyptus globulus* Labill. Eucalyptus oils are produced from plants belonging to the genus *Eucalyptus* (*Myrtaceae*), which includes about 500 species in Australia, the country of origin, alone. At present, few of the oils, which are used to characterise species, are commercially important (34). Some species are rich in 1,8-cineole (80-85% content). Other species contain less cineole, but 10-22% alpha-pinene. *E. citriodora* predominantly contains citronellal (min. 75% by GC), with some citronellol and isopulegol (5-10% each) (34). *E. dives* contains (-)-piperitone and 15-25% alpha-phellandrene (34). According to (30) the maximum observed concentration in eucalyptus oil are (in %): 1,8-cineole (58; 70-80 after rectification); α -pinene (22); limonene (8); para-cymene (5); trans-pinocarveol (5); aromadendrene (10); globulol (2.5) [the latter 2 components only traces after rectification] (30).

For Crude or rectified oils of *Eucalyptus globulus* (*Eucalyptus globulus* Labill.) an ISO standard exists: ISO 770:2002.

It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

EUGENIA CARYOPHYLLUS LEAF / FLOWER OIL

CAS 8000-34-8; EC / (Oils, clove)

Clove oil

INCI: EUGENIA CARYOPHYLLUS OIL

Current regulation: /

Clinical data:

In the Larsen 2002 c study, 19.3% of patients with known contact allergy to fragrance ingredients reacted positively to "clove bud oil" (10 % pet.) (1). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "clove oil" were observed (3). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with clove oil (2% pet.) 13.4% positive reactions (9). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.6% positive reactions 2% pet. (22). The Rudzki 1976 study found 2 positive reactions in 200 patients to "clove" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=12 (13.3%) positive reactions to "clove" essential oil 2% pet. (27). The IVDK 2010 c study identified 1.5% positive reactions 6893 patients tested in the context of a special series (30).

A 32 year old Spanish physiotherapists developed vesicular hand dermatitis after using a "balsam from ash extract" cream. PTing revealed positive reactions to this cream, the FM I, eugenol, and 2 components of the cream: cinnamon oil (0.5% pet.) and clove oil (1% pet.) (225).

Additional information:

ISO 4720:2009 nomenclature: *Syzygium aromaticum* (L.) Merr. & L. M. Perry syn. *Eugenia caryophyllus* (Spreng.) Bullock & S. G. Harrison. Standards regarding the composition of clove oil are available: ISO 3141:1997, ISO 3142:1997, ISO 3143:1997. Clove oils are produced from the clove tree *Syzygium aromaticum* (L.) Merr. et L.M. Perry [*Eugenia caryophyllus* (Spreng.) Bullock ex S.G. Harrison]. The content of clove bud, clove leaf and clove stem oil has, with little variation, been determined by GC as 75-92% eugenol, 2-17% caryophyllene and 0.2-15% eugenyl acetate – the latter compound found in particularly high concentration in bud oil (34). According to another source, the following maximum content (%) has been observed regarding the constituents listed: eugenol (92,0);

caryophyllene (17); eugenyl acetate (15); isoeugenol (0.5) (30).

In a LLNA study by RIFM, the clove leaf oil as used was reported to contain 85.3% eugenol, 9.9% caryophyllene and 2.2% alpha humulene, according to analyses of the supplier. The EC3 value was calculated to be 7.1% (227).

EVERNIA FURFURACEA LICHEN EXTRACT

CAS 90028-67-4; EC 289-860-8
(*Evernia furfuracea*, ext. = INCI)

Tree moss extract

Current regulation: /

Clinical data:

The Larsen 1977 study in 20 “perfume-sensitive patients” yielded n=6 positive reactions with “treemoss abs. in benzyl benzoate, 5% petrolatum” (18). In the IVDK 2007 study, 2.7% (95% CI: 2.0 – 3.6%) of 1658 consecutive patients had a positive reaction to “tree moss absolute” (4). In the Groningen 2009 study, 2.5% (95% CI: 1.1 – 4.9%) had positive reactions to the allergen, tested at 2%, i.e., twice the commonly used concentration, and not in pet., but in diethylphthalate (6). The IVDK 2010 study, 6.02% (95% CI: 4.90 – 7.14%; percentages standardised for age and sex) of 1947 patients PTed reacted to the compound (7).

Additional information:

Syn.: *Pseudoevernia furfuracea* (L.) Zopf (53). The lichen grows on the bark of pine and fir trees. The extraction process with carbohydrate solvents yields a “concrete” (2-5% yield) which, in a next step eliminating waxy compounds, is extracted with warm alcohol and subsequent cooling, yielding an “absolute” (40-60% yield) (53).

EVERNIA PRUNASTRI

CAS 90028-68-5; EC 289-861-3
(*Evernia prunastri*, ext. = INCI)

Oak moss abs.

Current regulation: Annex III, part 1, n° 91

Clinical data:
In the “background information” section of the 1999 opinion, oak moss extract is classified as “most frequently reported allergen”; in consecutive PT patients, about 2.8% positive reactions had been reported (33). The German MAK commission has labelled oak moss extract as ‘sensitising to the skin’ (229).

Since the last SCCNFP-opinion of 1999, a “polymer based method” was developed to reduce the natural content of these two compounds from around 1 - several percent to < 75 ppm for atranol and < 25 ppm for chloratranol. However, PTing 14 subjects with previous positive PT reactions to the “oak moss” allergen preparation with the modified *Evernia prunastri* material still elicited positive reactions in 8/14 subjects; thus, the reduction in allergen content was deemed unsafe for the consumer (230). In a study of 885 consecutive eczema patients tested in Gentofte, Denmark, 3.2% had a positive or follicular patch test response to oak moss absolute. Two types of oak moss absolute were tested, one contaminated by resin acids and one without any detectable resin acids. There was no difference in reactivity between the two types of oak moss absolute

(231). The IVDK 2007 study yielded 2.2% (95% CI: 1.6 – 3.0%) positive reactions in 2063 consecutively tested patients (4). In the Groningen 2009 study, 1.9% (95% CI: 0.7 – 4.0%) had positive reactions to oak moss, tested at 2% pet., i.e., twice the commonly used concentration (6). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reaction (13) (test concentration 2% pet.). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 5.0% positive reactions (22). The IVDK 2010 study, 1.81% (95% CI: 1.07 – 2.56%) of 1213 consecutively tested patients reacted to the compound, while 5.59% (95% CI: 4.90 – 6.27%) of 4482 of patients tested in a more aimed manner, partly as breakdown testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were tested with *E. prunastri* extract, yielding 2 positive reactions (48).

L. Kanerva et al. report on a 41 year old female hairdresser in whom oak moss abs. contained in a perming solution (concentration in the product unknown) was unequivocally identified as allergen causing (i) occupational hand dermatitis and (ii) scalp dermatitis after application to the own hair (232). Another case of occupational hand dermatitis in a grinding engineer was, at least partly, attributable to contact sensitisation to "oak moss resin" contained in a soluble oil (233).

Additional information:

Source: *Evernia prunastri* (Oak moss) (*Evernia prunastri* var. *prunastri* L. Ach). Oak moss is extracted as described above. Chloratranol and atranol are the degradation products of chloratranorin and atranorin, resp., which are recognised as the main sensitisers in *Evernia prunastri* extracts.

ILLICIIUM VERUM FRUIT OIL

CAS 84650-59-9, 8007-70-3; EC 283-518-1

"Anise Oil", Star anise oil

(Star anise, *Illicium verum*, ext. = INCI)

Current regulation: /

Clinical data:

In a study involving 100 consecutive patients, Rudzki and Grzywa found (i) a relatively high frequency of active sensitisation to star anise oil (n=5) tested with 0.5, 1 and 2% concentration (most likely in yellow petrolatum, as the other allergens in this series). Later patch testing with constituents of this essential oil (1%) in 3 patients yielded positive results to anethole in 3 cases, and to alpha-pinene and safrole in the 1 case tested to these substances. 34% of the consecutive patients reacted positively to star anise oil at 1%, which was considered as (marginally) non-irritating PT concentration (234).

Additional information:

ISO 4720:2009 nomenclature: *Illicium verum* Hook. f. *Illicium Verum* Fruit Oil is an essential oil distilled from the fruits of the Star Anise, *Illicium verum*, Illiciaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40297>, last accessed 2010-01-29). The main component is trans-anethole (86-93%), which can be purified from star anise oil. Main uses are alcoholic beverages, food flavouring and oral care products (34, 39). For Oil of star anise, Chinese type (*Illicium verum* Hook. f.) an ISO standard exists: ISO 11016:1999.

JASMINUM GRANDIFLORUM FLOWER EXTRACT	CAS 84776-64-7; EC 283-993-5 (Jasmine, <i>Jasminum grandiflorum</i> , ext. = INCI)
<i>Jasmine abs.</i>	
JASMINUM OFFICINALE FLOWER OIL	CAS 90045-94-6; EC 289-960-1 (Jasmine, <i>Jasminum officinale</i> , ext. = INCI)
JASMINUM OFFICINALE OIL	CAS 8022-96-6; EC / (Oils, jasmine) INCI: JASMINUM OFFICINALE OIL

Current regulation: /

Clinical data:

In the Frosch 2002 b study, a total of 1.2% of 1606 consecutive patients had a positive PT to "jasmine absolute", tested 5% in pet. (17). The deGroot 2000 study yielded 13 positive reactions to "jasmine, synthetic" in 1825 consecutively tested patients (12). In the early Larsen 1977 study, 18 of 20 "perfume sensitive patients" reacted to "Jasmin synthetic" 10% pet. (18), while 7 reacted to "Jasmin absolute" (10% pet.) – all of these also positive to the synthetic fragrance. The Sugiura 2000 study set in Nagoya, Japan, yielded 1% positive PT reactions in 1483 patients PTed for suspected cosmetic dermatitis, using 5% pet. as test concentration (14). The Larsen 2001 study in 178 patients with known contact allergy to fragrance ingredients found 16.9% positive reactions to jasmine absolute (10% pet.) (19). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had a positive reaction to Jasmine officinale oil (Jasmine absolute, Egyptian), tested at 2% (13). In the NACDG 2009 study, 1.1% of 4447 patients tested with "Jasmine absolute 2% pet." were found PT-positive (21). The Belsito 2006 study (20) yielded 0.4% positive reactions to "jasmine absolute". The Goossens 1997 study found 5 of 111 patients positive to "jasmine absolute" (10% pet.) – all sensitised to other fragrance allergens (23). In 63 patients positive to the FM I, 13 had positive PT reactions to "jasmine absolute", 2% pet., and 12 to "jasmine synthetic", 2% pet. in the Santucci 1987 study – the amount of concomitant reactivity was not examined (28). Nakayama et al. found 1974 (after (29)) 19 "strong positive" and 25 "weak positive" reactions to "jasmin oil" (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 1.5% positive reactions in 3668 consecutively tested patients and 1.2% positive reactions in 982 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were tested with jasmine absolute, yielding 3 positive reactions, and with "Jasmine synthetic", also resulting in 3 positive reactions (48).

Additional information:

Jasminum Grandiflorum Flower Extract is an extract obtained from the flowers of the Spanish Jasmine, *Jasminum grandiflorum* L., Oleaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39752>, last accessed 2009-11-12).

Jasminum Officinale Flower Oil is an essential oil obtained by molecular distillation of the flowers from the Jasmine, *Jasminum officinale* L., Oleaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39754>, last accessed 2009-11-25).

Jasminum Officinale Oil is the volatile oil obtained from the flowers of the Jasmine, *Jasminum officinale* L., Oleaceae

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=34776>, last accessed 2010-01-05); this latter extract is used by Almirall/Hermal/Trolab for the preparation of a PT allergen.

Jasmine absolute is obtained by solvent extraction, via concrete, from the flowers of *J. grandiflorum* (L.) Aiton from China and India. The main volatile compound is benzyl acetate, however, minor compounds such as indole [120-72-9], cis-jasmone [488-10-8] and methyl jasmonate [1211-29-6] contribute to the typical jasmine fragrance (34). Reported compounds include the following (maximum observed concentration given in parentheses): benzyl acetate (28); benzyl benzoate (24.0); phytol acetate (9); isophytol (8.5); phytol (7.4); linalool (7.0); eugenol (4.0); squalene (4); indole (3.5); benzyl alcohol (2.5); cis-jasmone (2.5); methyl linolenate (2.0); methyl palmitate (1.4); p-cresol (1.0); cis-3-hexenyl benzoate (1.0); benzyl salicylate (0.4); jasmin lactone (0.9); methyl jasmonate (0.7); isoeugenol (0.4) ((30), also according to (17))

JUNIPERUS VIRGINIANA OIL

CAS 8000-27-9; EC / (Oils, cedarwood) [this also refers to *Cedrus atlantica* ...] / 85085-41-2; EC 285-370-3 (Juniper, *Juniperus virginiana*, ext. = INCI)

JUNIPERUS VIRGINIANA WOOD OIL

CAS 85085-41-2; EC 285-370-3

Cedar Wood Oil (Virginian)

Current regulation: /

Clinical data:

In the Frosch 2002 b study, a total of 0.6% of 1606 consecutive patients had a positive PT to "cedarwood oil (Moroccan and Chinese 1:1)", tested 10% in pet. (17). After application of Penaten-baby™ oil as immersion oil for dermatoscopy a patient developed multiple patches of eczema at the application sites. Investigation revealed that the oil was kept in a bottle previously used for *Juniperus virginiana* oil, to which contact sensitisation was verified by patch testing (235).

Additional information:

ISO 4720:2009 nomenclature: *Juniperus virginiana* L.. *Juniperus Virginiana* Oil is the volatile oil obtained from the fruits and leaves of the Red Cedar, *Juniperus virginiana* L., Cupressaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=78070>, last accessed 2010-01-05)

Juniperus Virginiana Wood Oil is an essential oil obtained from the wood and twigs of the Red Cedar, *Juniperus virginiana* L., Cupressaceae. It contains chiefly (alpha and beta) cedrene and cedral (cedar camphor), cuparene, thujopsene, widdrol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39767>, last accessed 2009-11-12)(235). According to Surburg/Panten by GC: alpha-cedrene 22-35%, thujopsene 10-25%, cedrol 16-25% (34).

See also *Cedrus atlantica*. According to (30) the maximum observed concentration in cedar wood oil are (in %): α-cedrene (32); thujopsene (25); cedrol (25); β-cedrene (6); widdrol (5) and cuparene (traces) (30).

For Oil of cedarwood, Virginian (*Juniperus virginiana* L.) an ISO standard is available: ISO 4724:2004. For Oil of cedarwood, Texas (*Juniperus mexicana* Schiede) an ISO standard exists: ISO 4725:2004.

LAURUS NOBILIS OIL

CAS 8002-41-3; EC / (Oils, laurel)
 INCI: LAURUS NOBILIS OIL /
 8007-48-5; EC / (Oils, sweet
 bay)/ 84603-73-6; EC 283-272-5
 (Laurus nobilis, ext.) INCI:
 LAURUS NOBILIS EXTRACT

Laurel oil

Current regulation: Annex II, n° 359 (seed oil)

Clinical data:

In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=4 (0.6%) positive reactions to "laurel oil" 2% pet. (22).

After sensitisation by a one-time occlusive application a 36 year old Turkish patient developed widespread allergic contact dermatitis 3 days after massage with olive oil containing Laurus nobilis oil; sensitisation was proven by a strong positive reaction to the commercial test preparation and the massage oil previously used (236). Topical application of laurel oil for knee arthropathy led to an erythema exudativum multiforme-like rash on the legs of a 63 year old patient; interestingly, laurel oil yielded a "target like" strongly positive PT reaction in this case (237). In an earlier Turkish case with a similar history, the EEM-like appearance was lacking; however, a very intense, edematous reaction was noted (238). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "laurel oil" were observed (3). The IVDK 2010 c study identified 1.0% positive reactions in 6297 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Laurus nobilis* L. Laurel leaf oil is obtained by steam distillation of leaves from *Laurus nobilis* L. (Lauraceae), an evergreen cultivated primarily in the Mediterranean countries. The main components are 1,8-cineole (30-70%), linalool (about 10%) and eugenol (34). According to (30) the maximum observed concentration in laurel oil are (in %): 1,8-cineole (70); β -caryophyllene (11); linalool (11); limonene (5.0); eugenol (2.0); geraniol (0.3) (30).

LAVANDULA HYBRIDA HERB OIL

CAS 91722-69-9; EC 294-470-6
 (Lavender, *Lavandula hybrida*,
 ext. = INCI)

Lavandin Oil

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "lavandin" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to "lavandin" essential oil 2% pet. (27). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% lavandin oil in pet., tested in 205 consecutive patients in Dortmund and Göttingen, and just 1 irritant reaction to

the higher concentration, were observed (15).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula angustifolia* Mill. x *Lavandula latifolia* Medik. *Lavandula* *hybrida*, *Labiatae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39789>, last accessed 2010-01-29. Nomenclature according to Surburg/Panten: *Lavandula* x *intermedia* Lois, which is a hybrid of lavender and spike (see below) (34). The oils from the most important variants, abrial and grosso, contain linalool (24-38%), linalyl acetate (20-38%) as well as 1,8-cineole (4-11%), and camphor (6-11%) (34). A third variant is called super because of its high concentration of linalyl acetate (35-47%), more closely resembling lavender oil (34). For Oil of lavandin Grosso (*Lavandula angustifolia* Mill. x *Lavandula latifolia* Medik.), French type an ISO standard exists: ISO 8902:2009, for Oil of lavandin Abrial (*Lavandula angustifolia* Miller x *Lavandula latifolia* Medikus), French type a different ISO standard: ISO 3054:2001.

It is a "top 100" substance (IFRA, pers. comm.2010)

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

LAVANDULA OFFICINALIS FLOWER OIL

CAS 84776-65-8, 8000-28-0; EC 283-994-0 (*Lavender*, *Lavandula angustifolia angustifolia*, ext. = *INCI*)

Lavender oil

Current regulation: /

Clinical data:

In a large series from Nagoya, Japan, 1483 patients were tested with lavender oil 20% in pet., with overall 3.7% positive reactions from 1990 to 1998. However, within this period, a sharp increase was noted in 1997 and 1998, which as attributed to changed exposure by M. Sugiura et al. (14). On the individual level, relevance of positive reactions remained unclear in about half of the cases. The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "lavender absolute" (2% pet.) 6.6% positive reactions (9). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to "Lavandula augustifolia oil" (Lavender absolute) 2% (13). The Goossens 1997 study found 4 of 111 patients positive to "lavender oil 20% pet." – all of them sensitised to other fragrance allergens (23). The Rudzki 1976 study found no positive reaction in 200 patients to "lavender" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "lavender" essential oil 2% pet. (27). Nakayama et al. found 1974 (after (29)) 6 "strong positive" reactions to "Lavender oil" (unknown test concentration) in 183 patients. In a study from Alicante, Spain, 86 selected patients were tested with "lavender absolute", yielding 2 positive reactions (48).

R. Goiriz et al. report on a case of photo contact allergy (10 controls negative) in a 45 year old woman developing after application of a ketoprofen-containing topical gel ("Fastum") (239). A physiotherapist developed acute, recurrent dermatitis after use of "Difflam® gel", scented with lavender oil. Both the gel and lavender oil (2% pet.) tested positive; avoidance resulted in clearing (240). In a study on 218 patients with known

contact allergy to fragrance ingredients, Larsen (2002 c) found positive reactions to 10% lavender oil (pet.) in 2.8% of these (1). A case of vulvovaginitis with spread and affecting the dominant hand applying various tea tree and lavender oil creams was reported by S. Varma; the PT with 10% lavender oil abs. in pet. (50 controls negative) was positive (241). In two cases, facial "pillow dermatitis" due to lavender oil, applied to the pillows, developed, confirmed by positive PT to lavender abs. (2% pet.) (242).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula angustifolia* Mill. *Lavandula officinalis* Flower Oil is an essential oil obtained from the fresh flowering tops of the Lavender, *Lavandula officinalis* (syn: *L. vera*), *Labiatae*. It contains 30-40% esters calculated as linalyl acetate, linalool, pinene, limonene, geraniol, some eucalyptol (cineol) (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40370>, last accessed 2009-11-09). According to Surburg/Panten, lavender oil is obtained by steam distillation of freshly cut flowering tops of *Lavandula angustifolia* Mill. (Lamiaceae). Main constituents according to GC are linalyl acetate (25-45%), cis-ocimene (4-10%), trans-ocimene (1.5-6%), 1,8-cineole ($\leq 1\%$) camphor ($\leq 0.5\%$), linalool (25-38%), 1-terpinen-4-ol (2-6%) and lavandulyl acetate [25905-14-0] ($\geq 2\%$) (34).

In addition to distillation, both *Lavandula officinalis* and Lavandin are also solvent extracted, yielding concretes and, after ethanol extraction, absolutes, which are said to have a longer-lasting odour (34).

For Oil of lavender (*Lavandula angustifolia* Mill.) an ISO standard exists: ISO 3515:2002.

LAVANDULA SPICA HERB OIL

CAS 97722-12-8; EC 307-762-6
(Lavender, *Lavandula spica*, ext.
= INCI

"Spike Oil"

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "spike" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=8 (9.3%) positive reactions to "spike" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula latifolia* Medik. *Lavandula Spica* Herb Oil is an essential oil distilled from the flowering herbs of the Spikenard, *Lavandula spica* (syn: *Lavandula latifolia*), *Labiatae*. It contains eucalyptol (35%), camphor, linalool, borneol, terpineol, D-camphene and sesquiterpenes (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40372>, last accessed 2010-01-29). According to Surburg/Panten, Spanish spike lavender oil is steam distilled from the flowering tops of *Lavandula latifolia* Medik.. The main components are linalool (34-50%), 1,8-cineole (16-39%) and camphor (8-16%) (34). For Oil of spike lavender (*Lavandula latifolia* (L.f.) Medikus), Spanish type an ISO standard exists: ISO 4719:1999

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

LITSEA CUBEBA FRUIT EXTRACT

CAS 90063-59-5, 68855-99-2; EC 290-018-7 (*Litsea cubeba*, ext.)
 INCI: LITSEA CUBEBA OIL

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 3 positive reaction in 200 patients to “Litsea cubeba” essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to this essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Litsea cubeba* (Lour) Pers. Litsea Cubeba Fruit Extract is an extract obtained from the fruits of the plant, *Litsea cubeba*, Lauraceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40036>, last accessed 2009-11-24. The content by GC is: neral (25-33%), geranial (38-45%) – i.e. about ¾ citral, for which the extract had previously served as a raw material (34); direct use for perfuming is limited to household products (39). For Oil of Litsea cubeba (*Litsea cubeba* Pers.) an ISO standard exists: ISO 3214:2000.

In a LLNA study by RIFM, the “Litsea cubeba oil” as used was reported to contain 85.7% citral, 2.9% limonene, 1.7% linalool, 1.4% citronellal and < 1% caryophyllene and methyl heptanone, according to analyses of the supplier. The EC3 value was calculated as 8.4 % (227).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

MENTHA ARVENSIS LEAF OIL

CAS 68917-18-0 ; EC /

Cornmint oil

INCI: MENTHA ARVENSIS OIL

Current regulation: /

Clinical data: /

Additional information:

Mentha Arvensis Leaf Oil is the oil derived from the leaves of the Horse Mint, *Mentha arvensis* L., Labiatae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=57860)

It is a “top 200” substance and classified as R43 (IFRA, pers. comm.2010)

MENTHA PIPERITA OIL

CAS 8006-90-4; EC / (Oils, peppermint) INCI: MENTHA

*PIPERITA OIL / 84082-70-2; EC
282-015-4 (Peppermint, ext.) INCI
names: MENTHA PIPERITA ...*

Peppermint oil

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.6% of 1606 consecutive patients reacted positively to "peppermint oil (American)", tested 2% in pet. (17). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "peppermint oil" were observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reactions to peppermint oil 2% pet. (22). Among 512 patients referred from a dental department for diagnostic work-up of various intraoral symptoms and complaints within 4 years, 6 patients had positive (+ to +++) PT reactions to "peppermint oil" 1% pet. at D4, mostly combined with positive reactions to menthol (see above) and reporting dramatic improvement after cessation of use of peppermint-containing oral products (154). The Rudzki 1976 study found 1 positive reaction in 200 patients to "Peppermint" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=6 (6.9%) positive reactions to "peppermint" essential oil 2% pet. (27). In 63 patients positive to the FM I, 3 had positive PT reactions to peppermint oil, 2% pet., in the Santucci 1987 study (28). The IVDK 2010 c study identified 0.6% positive reactions in 6546 patients tested in the context of a special series (30).

An unusual case of "baboon-like" allergic contact dermatitis of the vulva after drinking excessive amounts of a herbal tea containing, among other ingredients, peppermint. While the PT reaction to peppermint oil was only weak to doubtful, dramatic improvement after cessation and prompt relapse after repeat ingestion proved the diagnosis (243). Recurrent foot and lower leg dermatitis after the application of a "foot spray" (containing peppermint oil) was diagnosed as allergic contact dermatitis due to this ingredient in a 59 year old golf player (244). In another case, ACD after application of a transdermal system for the treatment of lumbar pain was attributed to CA to peppermint oil (2% pet.) and its main ingredient menthol (1% pet.) (155). In a patient with toothpaste-induced cheilitis, not only *M. piperita*, but also *M. arvensis*, but not *M. spicata* or *cardica* extracts (all tested 1% pet.), as well as natural and synthetic menthol caused positive PT reactions (245).

Additional information:

ISO 4720:2009 nomenclature: *Mentha x piperita* L. A standard by ISO exists for Oil of peppermint (*Mentha x piperita* L.): ISO 856:2006. A review by the Cosmetic Ingredient Review Expert Panel, Washington, DC on the "Final report on the safety assessment of *Mentha Piperita* (Peppermint) Oil, *Mentha Piperita* (Peppermint) Leaf Extract, *Mentha Piperita* (Peppermint) Leaf, and *Mentha Piperita* (Peppermint) Leaf Water" is available (163), stating that "Peppermint Oil is used at a concentration of < or = 3% in rinse-off formulations and < or = 0.2% in leave-on formulations. Peppermint Oil is composed primarily of menthol and menthone. Other possible constituents include pulegone, menthofuran, and limone. According to Surburg/Panten: (-)-menthol (34-46%), (-)-menthone (15-27%), (-)-menthyl acetate (2.5-7%) and menthofuran [17957-94-7] (0.5-6%) (34). According to (30) the maximum observed concentration in peppermint oil are (in %): (-)-menthol (49); (-)-menthone (28); (-)-menthyl acetate (8); mentofuran (8); isomenthone (8); neo menthol (6); pulegone (3.5); limonene (3.0); linalool (0.4) (30). Most of the safety test data concern Peppermint Oil. The oil is considered to present the "worst case scenario" because of its many constituents, so data on the oil were considered relevant to the entire group of ingredients. ... Repeated

intradermal dosing with Peppermint Oil produced moderate and severe reactions in rabbits” concluding that “with the limitation that the concentration of pulegone in these ingredients should not exceed 1%, it was concluded that Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Extract, Mentha Piperita (Peppermint) Leaves, Mentha Piperita (Peppermint) Water are safe as used in cosmetic formulations”.

MENTHA SPICATA HERB OIL

CAS 84696-51-5, 8008-79-5; EC 283-656-2 (Spearment, ext.)

Spearment oil

INCI: MENTHA VIRIDIS EXTRACT

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.8% of 1606 consecutive patients reacted positively to “spearment oil (American)”, tested 2% in pet. (17). The CAS # quoted (8008-79-5) refers, according to CosIng, to MENTHA VIRIDIS LEAF OIL, the volatile oil obtained from the dried tops and leaves of the Garden Mint, *Mentha viridis* L., Labiatae. The Larsen 2001 study diagnosed 5.0% positive reactions in 178 patients with known contact allergy to fragrance ingredients, using this oil at 5% pet. test concentration (19). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reactions to “Mentha viridis oil” 5% (13). PT results with toothpaste ingredients were positive in 7 patients, of whom 4 had strong positive reactions to spearment (246).

Additional information:

ISO 4720:2009 nomenclature: *Mentha spicata* L. *Mentha Spicata* Oil is an essential oil obtained from the herbs of the Spearment, *Mentha spicata* L., Labiatae (syn: *Mentha viridis* L., Labiatae). It contains carvone (more than 50%), limonene, pinene (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40394>, last accessed 2009-11-11). According to Surburg/Panten, the content is limonene (9-16.5%), (-)-carvone (60-70%), menthone (0-0.2%) and viridiflorol (0-0.5%) (34). Exposure by toothpastes, and subsequent contact allergic reaction of the lips or the oral mucosa, have been reported (e.g., (247, 248)). L-Carvone is a component of the oil from *Mentha spicata* (spearment) (53) and had been tested with positive results in “toothpaste cases”, even at a concentration as low as 0.067% (68).

For Oil of spearment -- Part 1: Native type (*Mentha spicata* L.) an ISO standard exists: ISO 3033-1:2005, for Oil of spearment -- Part 2: Chinese type (80 % and 60 %) (*Mentha viridis* L. var. *crispa* Benth.), redistilled oil: ISO 3033-2:2005, for Oil of spearment -- Part 3: Indian type (*Mentha spicata* L.), redistilled oil: ISO 3033-3:2005 and for Oil of spearment -- Part 4: Scotch variety (*Mentha x gracilis* Sole): ISO 3033-4:2005.

MYROXYLON PEREIRAE RESIN

CAS 8007-00-9; EC 232-352-8 (Balsams, Peru)

Balsam of Peru

INCI: MYROXYLON PEREIRAE / Balsams, Peru

Current regulation: Annex III, part1, n° 154

Clinical data:

This natural mixture has been employed as screening agent in Baseline series worldwide for many decades. Hence, a wealth of data is available; table 3.2 – 1 summarises results of the past 10 years.

Additional information:

ISO 4720:2009 nomenclature: *Myroxylon pereirae* (Royle) Klotzsch, syn. *Myroxylon balsamum* var. *pereirae* (Royle) Harms. *Myroxylon pereirae* resin (MPR, Balsamum peruvianum) is harvested from the balsam of Peru tree, *Myroxylon balsamicum* (L.) HARMS var. *pereirae* (ROYLE) HARMS, synonymous *Myroxylon pereirae* (ROYLE) KLOTZSCH (249) after thermal stress, almost exclusively in El Salvador. Main constituents of the pleasantly, vanilla-like smelling dark brown liquid are benzyl esters of cinnamic and benzoic acid (35 – 75%), up to 30% cinnamic acid, up to about 10% benzoic acid, approximately 5% alpha- and beta-nerolidol, benzyl alcohol and mostly less than 1% cinnamyl alcohol, benzyl ferulate and -isoferulate, cinnamic acid amyl ester, coniferyl alcohol, coniferyl benzoate, eugenol, isoeugenol, farnesol, vanillin, and several trace constituents (250-253). The composition of MPR varies with the origin and other factors; moreover, MPR is sometimes blended with other natural mixtures such as turpentine, styrax or colophonium (249).

MPR can be used to improve taste or smell in gargling solutions, cosmetic products such as soaps, shampoo or lipsticks, as well as sweets, tobacco and beverages (249, 254). According to EU legislation and IFRA guidelines MPR should not be used in products intended for skin contact; however, extracts and distillates of MPR may be used in a concentration of < 0,4% (IFRA-Guidelines, www.ifraorg.org (255)). E. Temesvári et al. report on the interesting case of severe ACD with subsequent hypopigmentation after a “temporary henna tattoo”, which was, unexpectedly, not due to p-phenylene diamine, but to the oil used to disperse the pigment, which presumably contained allergens also included in the FM I and MPR, both of which were extreme positive on a later PT (256).

In addition to delayed type hypersensitivity reactions, MPR (and some of his constituents such as benzoic acid (257)) are capable of eliciting (non-immunological) urticarial immediate reactions (258-260). In one case, the immediate reaction to MPR (and to FM I) at the test site spread systemically in terms of a generalised urticaria, while no delayed type reactions were observed to the PT (261). Generally, there is apparently no association of immediate reactions to MPR (and cinnamal or cinnamyl alcohol) and contact sensitisation to these compounds (262). In animal experiments the sensitising potency of MPR was clearly established (250), with coniferyl benzoate identified as single compound with the most marked potency (252). However, due to the limited chemical stability of this compound it is unclear whether other, more stable compounds are, in fact, more important allergens, such as cinnamic acid and (iso-) ferulic acid esters or oxidised constituents of the resin fraction (263).

Table 3.2.2 – 1: Results with contact allergy to fragrance ingredients screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: **Myroxylon pereirae resin** (Balsam of Peru) 1). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the reviewers.

Country	Population	Years	No. tested	Crude % positive (95% CI) §
Tel Aviv, Israel (264) #	Consecutive patients	1999-2000	943	6.6 % (5.1 – 8.4) §
South Korea (13)	Consecutive patients	04/2002 – 06/2003	422	7.3% (5.1 – 10.3%) §

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Tel Aviv, Israel (265)	Consecutive patients	1998-2004	2156	3.6 (2.9 – 4.5) § %
Manipal, India (266)	Dermatitis patients	1989-1998	1780	n=17
Tehran, Iran (267)	Consecutive patients	2002-2004	250	2.4 (0.9 – 5.2) § %
Sevilla, Spain (268)	Consecutive patients	2002-2004	863	5.8 (4.3 – 7.6) § %
Ankara, Turkey (269)	Consecutive patients	1992-2004	1038	2.1 (1.3 – 3.2) § %
Vienna, Austria (22)	Consecutive patients of one clinic	1997-2000	2660	5.4% (4.6 – 6.3%) §
Czech Republic (270)	Consecutive patients	1997-2001	12058	7.3% (6.8 – 7.8) §
Copenhagen, Denmark (271)	Consecutive patients	1985-2007	16173	3.9 (3.6 – 4.2) § %
Sweden (272)	Consecutive patients	2000	3790	6.5%
9 European countries (273) §	Consecutive patients	2002-2003	9672	6.1 %
Germany, 3 Swiss + 1 Austrian Dept. (7)	Consecutive patients	2005-2008	36919	8.0% (7.7 – 8.3%)
10 depts. From 7 EU countries (274) *	Consecutive patients	1996-2000	26210	6.0 %
USA (Canada) (20)	Probably consecutive patients	2003	1603	6.6%
NACDG 2009 (21)	Consecutive patients	2005-2006	4449	11.9%

§ Calculated by reviewers, where possible (if actual numbers were given)

Probably included in (265)

\$ > 5-fold difference between departments

* About 4-fold difference between departments

NARCISSUS SPP. EXTRACT / OIL

CAS: diverse

Narcissus abs.

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 1.3% positive reactions to “narcissus absolute” (2% pet.) were observed in 1606 consecutive (17). The extract used by the PT allergen provider Almirall/Hermal/Trolab has the CAS number 90064-25-8. The IVDK 2010 c study identified 0.5% positive reactions in 2445 consecutively tested patients and 0.6% positive reactions in 809 patients tested in the context of a special series (30).

Additional information:

Commonly used: *Narcissus poeticus* L. According to (30) the maximum observed concentration in Narcissus abs. are (in %): α-terpineol (23.7); trans-Isoeugenol methyl ether (20); benzyl benzoate (20); coumarin (5.7); benzyl alcohol (4.0); Δ³-carene (3.4); cinnamyl alcohol (2.5); phenylethyl alcohol (2.2); ethyl palmitate (2.2); phenylpropyl acetate (1.7); 1,8-cineole (1.5); caryophyllene (1.0); benzyl acetate (0.7); isoeugenol (0.5); farnesol (0.3) (also according to (17)) (30).

OCIMUM BASILICUM HERB OIL

CAS 84775-71-3; EC 283-900-8 (*Ocimum basilicum*, ext. = INCI)

Basil Oil (sweet)

Current regulation: /

Clinical data:

/

Additional information:

ISO 4720:2009 nomenclature: *Ocimum basilicum* L. For Oil of basil, methyl chavicol type (*Ocimum basilicum* L.) an ISO standard exists: ISO 11043:1998. *Ocimum Basilicum* Herb Oil is an essential oil obtained from the herbs of the Sweet Basil, *Ocimum basilicum* L., *Labiatae*. (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40474>, last accessed 2009-11-24). The chemical composition varies greatly with the origin (34):

- Basil oil of the methylchavicol type (Réunion type) is extracted from flowering tops or whole plants from Réunion, Comores, Madagascar, but also other countries such as Egypt. Mainly used for seasoning food. Content by GC: methylchavicol 75-87%, linalool 0.5-3%
- Basil oil, linalool type is produced mainly in the Mediterranean area. Content by GC: Linalool 45-62%, methylchavicol trace to 30%, eugenol 2-15%
- Indian Basil oil is produced exclusively in India. Content by GC: methylchavicol trace to 70%, linalool 25%.

In a LLNA study by RIFM, the basil oil as used was reported to contain 51% linalool, 10.4% eugenol, 7.7% cineol, 3.7% bergamotene, 2.7% germacrene D, 2.7% cadinol and 1.3% cadinene, according to analyses of the supplier. The EC3 value was calculated to be < 2.5% (227).

PELARGONIUM GRAVEOLENS FLOWER OIL

CAS 90082-51-2; EC 290-140-0 (*Pelargonium graveolens*, ext. = INCI) / 8000-46-2; EC / (Oils, geranium) INCI: GERANIUM

Geranium Oil Bourbon

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "geranium oil Bourbon" (2% pet.) 7.4% positive reactions (9). In the Larsen 2001 study, 8.4% positive reactions were observed in 178 patients with known contact allergy to fragrance ingredients ("geranium oil Bourbon", 10% pet.) (19). The Goossens 1997 study found 3 of 111 patients positive to "geranium oil 20% pet." – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found 3 positive reactions in 200 patients to "geranium" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to "geranium" essential oil 2% pet. (27). Nakayama et al. found 1974 (after (29)) 3 "strong positive" reactions to "Geranium oil" (unknown test concentration) in 183 patients, Trattner/David 1 / 641 consecutive patients positive to "Geranium oil" (31). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=8 reacted positively to geranium oil bourbon (48).

Additional information:

ISO 4720:2009 nomenclature: *Pelargonium x ssp.* For Oil of geranium (*Pelargonium X ssp.*) an ISO standard exists: ISO 4731:2006 *Pelargonium Graveolens* Flower Oil is the volatile oil obtained from the flowers of the Bourbon Geranium, *Pelargonium graveolens* L. Hér. Ex Aiton, *P. roseum* Willdenow (and other nondefined hybrids that have developed in different regions of the world) Geraniaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=57527>, last accessed 2009-11-16)(34). The Bourbon type (Réunion, Madagascar) is more valuable than the North African and Chinese products, and differs in characteristic components: (-)-6,9-guaiadiene [36577-33-0] 5-9% in the Bourbon type, and 10-epi-gamma-eudesmol [15051-81-7] 3-6% in the African type, in addition to the main components (-)-citronellol, isomenthone, formates and tiglates. Chinese oil is similar to Bourbon oil, however, it contains more citronellol (32-43%) and lower amounts of linalool (2-4.5%) and geraniol (5-12%) (34).

In a LLNA study by RIFM, the geranium oil as used was reported to contain 41.1% citronellol, 9.8% 2,6-guiadine, 6.2% isomethone, 4.9% geraniol, 2.2% cis-rose oxide, 2.1% linalool, 1.5% geranyl formate, 1.3% phenyl ethyl tiglate, 1.0% trans-rose oxide, and geranyl tiglate and alpha-pinene at < 1%, according to analyses of the supplier. The EC3 value was calculated to be > 50% (227).

PELARGONIUM ROSEUM LEAF OIL

CAS 90082-55-6; EC 290-144-2
(*Pelargonium roseum*, ext. =
INCI)

Geranium Oil; Rose Geranium Oil

Current regulation: /

Clinical data:

In the Sugiura 2000 study, among 1483 patients with suspected cosmetic dermatitis, 2.1% positive PT reactions to "geranium oil" (tested 20% in pet.) were observed (14).

Additional information:

Pelargonium Roseum Leaf Oil is an essential oil obtained from the leaves of the plant, Pelargonium roseum, Geraniaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40565>, last accessed 2009-11-16).

PIMENTA RACEMOSA LEAF/FRUIT OIL

CAS 85085-61-6; EC 285-385-5

Bay oil (34)

Current regulation: /

Clinical data:
/

Additional information:

ISO 4720:2009 nomenclature: *Pimenta racemosa* (Mill.) J.W. Moore. For Oil of bay [*Pimenta racemosa* (Mill.) J.W. Moore] an ISO standard exists: ISO 3045:2004 Pimenta Racemosa Leaf/Fruit Oil is an essential oil obtained from the fruits of the plant, Pimenta racemosa, Myrtaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41014>, last accessed 2010-02-10).

Steam distillation of the leaves of Pimenta racemosa (Mill.) J.W. Moore (Myrtaceae) yields bay oil, which consists of myrcene (20-30%), eugenol (42-56%) and chavicol (8-13%) (34).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

Pinus mugo leaf and twig oil and extract

CAS 90082-72-7, 8000-26-8; EC 290-163-6

Dwarf pine needle oil
(German: Latschenkiefernöl)

Current regulation: Annex III, part 1, 109

Clinical data:

In the Frosch 2002 b study, 0.7% positive reactions to dwarf pine needle oil (2% pet.) were observed in 1606 consecutive (17). The Rudzki 1976 study found 4 positive reactions in 200 patients to "Pine needle" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "pine needle" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Pinus mugo* Turra syn. *Pinus montana* Mill.) Pinus Mugo Twig Oil is an essential obtained from the twigs of the Pine, Pinus mugo, Pinaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.h.details&id=41476&back=1>, last accessed 2010-03-09). Pinus Mugo Twig Leaf Extract is an extract obtained from the twigs leaves of the Pine, Pinus mugo, Pinaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.h.details&id=41476&back=1>, last accessed 2010-03-09).

[h.details&id=41473&back=1](#), last accessed 2010-03-09).

Dwarf pine needle oil is obtained from *Pinus mugo* Turra subsp. *mugo* and subsp. *pumilio* (Haenke) Franco (34). For Oil of dwarf pine (*Pinus mugo* Turra) an ISO standard exists: ISO 21093:2003. American pine oils contain almost no 3-carene or camphene (34).

PINUS PUMILA TWIG LEAF EXTRACT / OIL

CAS 97676-05-6; EC 307-681-6
(Pine, *Pinus pumila*, ext. = INCI)

Dwarf pine needle oil

Current regulation: Annex III, part 1, 114

Clinical data: /

Additional information:

Pinus Pumila Twig Leaf Extract obtained from the twigs leaves of the Pine, *Pinus pumila*, Pinaceae

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41483&back=1>, last accessed 2009-11-12), *Pinus Pumila* Twig Leaf Oil is the essential oil obtained from the twigs leaves of the Pine, *Pinus pumila*, Pinaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41484&back=1>, last accessed 2009-11-12). Main constituents are alpha-pinene (60-70%) and beta-pinene (20-25%). (34) Occurrence from Siberia to Japan, classified as Endangered Species

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

POGOSTEMON CABLIN OIL

CAS 8014-09-3; EC / (Oils, patchouli) / 84238-39-1; EC 282-493-4 (Patchouli, ext.)

Patchouli oil

INCI: POGOSTEMON CABLIN / Patchouli, ext.

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.8% positive reactions to patchouli oil (10% pet.) in 1606 consecutive were observed (17). Nakayama et al. found 1974 (after (29)) 3 “strong positive” and 8 “weak positive” reactions to “Patchouli oil” (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 0.6% positive reactions in 2446 consecutively tested patients and 1.4% positive reactions in 828 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Pogostemon cablin* (Blanco) Benth. syn. *Mentha cablin* Blanco. An ISO standard is available for Oil of patchouli (*Pogostemon cablin* (Blanco) Benth.): ISO 3757:2002. *Pogostemon Cablin* Leaf Oil is an essential oil obtained from the fermented leaves of the Patchouli, *Pogostemon cablin* (syn: *Pogostemon patchouli*),

Labiatae (Lamiaceae (34)). It contains patchouli alcohol, beta-patchoulene, azulene, eugenol, sesquiterpenes (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40927>, last accessed 2009-11-12). Although the sesquiterpene alcohol (-)-patchoulol [5986-55-0] is the main component of patchouli oil (27-35%), the compound largely contributing to the characteristic odour is norpatchoulol [41429-52-1] (0.35-1%). Other constituents include (+)-alpha-bulnesene [6391-11-0] (13-21%), (-)-alpha-guajene [3691-12-1] (11-16%), (-)-β-patchoulene [514-51-2] (1.8-3.5%) and (-)-seychellene [20085-93-2] (1-3%) (34). According to (30) the maximum observed concentration in patchouli oil are (in %): (-)-patchoulol (35); (+)-alpha-lulnesene (21); (-)-alpha-guajene (16); β-pinene (6); (-)-β-patchoulene (3.5); (-)-seychellene (3); pogostol (2.5); α-pinene (2.5); norpatchoulol (1) (30).

It is a "top 100" substance (IFRA, pers. comm.2010).

ROSE FLOWER OIL (ROSA SPP.)

CAS 8007-01-0; EC / (Oils, rose)

ROSA ALBA FLOWER EXTRACT

CAS 93334-48-6; EC 297-122-1
(Rose, *Rosa alba*, ext. = INCI)

ROSA CANINA FLOWER OIL

CAS 84696-47-9; EC 283-652-0
(Rose, *Rosa canina*, ext.) INCI:
ROSA CANINA

ROSA CENTIFOLIA FLOWER OIL

CAS 84604-12-6, EC 283-289-8
(Rose, *Rosa centifolia*, ext.) INCI:
ROSA CENTIFOLIA / Rose, *Rosa centifolia*, ext.

ROSA DAMASCENA FLOWER OIL

CAS 90106-38-0; EC 290-260-3
(Rose, *Rosa Damascena*, ext. = INCI)

ROSA GALLICA FLOWER OIL

CAS 84604-13-7; EC 283-290-3
(Rose, *Rosa Gallica*, ext.) INCI:
ROSA GALLICA

ROSA MOSCHATA OIL

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ROSA RUGOSA FLOWER OIL

CAS 92347-25-6; EC 296-213-3
(Rose, *Rosa rugosa*, ext.)

Current regulation: /

Clinical data:

In the Sugiura 2000 study, 1483 patients with suspected cosmetic dermatitis were PTed with "rose oil Bulgaria" (2% pet.), yielding 0.4% positive reactions (14); Trattner/David found 2 / 641 consecutive patients positive to "Rose oil (Bulgarian)" (31). The Bulgarian rose oil usually corresponds to *Rosa Damascena* Flower Oil (http://en.wikipedia.org/wiki/Rose_oil, last accessed 2009-11-16). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "rose Bulgarian oil" (2% pet.) 4.5% positive reactions (9). One case of contact allergy to "Bulgarian rose oil (2 % pet.)" – and geraniol – in a 48-year-old female with ACD after application of "Eau de Rochas" E.d.C. was diagnosed, among 326 patients with suspected contact allergy to fragrance ingredients had tested negative (275). However, other rose oils are also used (and capable of eliciting ACD) as illustrated by the case of a 27 year old woman who developed ACD after using "Rose Absolute Eau ® eau de

parfum", a "non-scented" body lotion and a number of other topicals. PTing revealed a number of (previously) relevant reaction, including "Rose centifolia" (5% alc.) and "Rose oil Bulgarian" (2% pet.) essential oil preparations (276). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to "Rose oil Bulgarian", tested at 2% concentration (13). Nakayama et al. found 1974 (after (29)) 4 "strong positive" reactions to "Rose oil Bulgarian" (unknown test concentration) in 183 patients. In a study from Alicante, Spain, 86 selected patients were tested with rose oil absolute, yielding 6 positive reactions (48).

Additional information:

ISO 4720:2009 nomenclature: *Rosa x damascena* Mill. and *Rosa sertata* X *Rosa rugosa*. Rose Flower Oil is the volatile oil obtained from the flowers of *Rosa* spp. , rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59362>, last accessed 2009-11-16). "Rose oil, meaning either rose otto (attar of rose, attar of roses) or rose absolute, is the essential oil extracted from the petals of various types of rose. Rose ottos are extracted through steam distillation, while rose absolutes are obtained through solvent extraction or supercritical carbon dioxide extraction, with the absolute being used more commonly in perfumery" (http://en.wikipedia.org/wiki/Rose_oil, last accessed 2009-11-17). There are several more specifically named flower extracts used for masking or perfuming:

- Rosa Alba Flower Extract is an extract obtained from the flowers of the Rose, *Rosa alba* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40969>, last accessed 2009-11-16).
- Rosa Canina Flower Oil is the volatile oil obtained from the flowers of the Hip Rose, *Rosa canina* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59263>, last accessed 2009-11-16).
- Rosa Centifolia Flower Oil is the volatile oil obtained from the flowers of the Cabbage Rose, *Rosa centifolia* (L.), Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79757>, last accessed 2009-11-16).
- Rosa Damascena Flower Oil is the volatile oil obtained from the flowers of the Damask Rose, *Rosa damascena*, Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79760>, last accessed 2009-11-16).
- Rosa Gallica Flower Oil is the volatile oil obtained from the flowers of the French Rose, *Rosa gallica* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59346>, last accessed 2009-11-16).
- Rosa Moschata Oil is the oil obtained from the Musk Rose, *Rosa moschata*, Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79761>, last accessed 2009-11-16).
- Rosa Rugosa Flower Oil is the volatile oil obtained from the flowers of the Rose, *Rosa rubiginosa* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=83588>, last accessed 2009-11-16).

Apparently, the *Rosa Damascena* and the *Rosa centifolia* are the species most commonly used for extraction of essential rose oils, the former mostly grown in Bulgaria, Turkey, Russia, India and China, the latter more commonly in Morocco, France and Egypt (276). Main constituents by GC are: citronellol (20-49%), geraniol (6-23%), nerol (3-12%) and phenylethyl alcohol (up to 3.5%) (34).

For Oil of rose (*Rosa x damascena* Miller) an ISO standard exists: ISO 9842:2003.

ROSMARINUS OFFICINALIS FLOWER OILCAS 84604-14-8; EC 283-291-9
(Rosemary, ext.)

"Rosemary Oil"

INCI: ROSMARINUM OFFICINALIS
/ Rosemary, ext.

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to "rosemary" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "rosemary" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Rosmarinus officinalis* L. Rosmarinus Officinalis Flower Oil is an essential oil obtained from the leaves and fresh flowering tops of the Rosemary, *Rosmarinus officinalis* L., Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40978>, last accessed 2010-01-29). Major constituents are: 1,8-cineole (17-55%), alpha-pinene (9-26%), camphor (5-22%) and verbenone [18309-32-5] as traces in North African oils, but between 0.7 and 2.5% in Spanish oils (34). For Oil of rosemary (*Rosmarinus officinalis* L.) an ISO standard exists: ISO 1342:2000.

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

SALVIA spp. HERB OIL

Sage oil

SALVIA OFFICINALIS LAVANDULIFOLIA HERB OILCAS 97952-71-1; EC 308-365-0
(Sage, *Salvia officinalis* *lavandulifolia*, ext. = INCI)**SALVIA LAVANDULIFOLIA HERB OIL**CAS 90106-49-3; EC 290-272-9
(Sage, *Salvia lavandulifolia*, ext. = INCI)**SALVIA SCLAREA FLOWER OIL**CAS 84775-83-7; EC 283-911-8
(Sage, *Salvia sclarea*, ext.) INCI: SALVIA SCLAREA / Sage, *Salvia sclarea*, ext.**SALVIA HISPANICA HERB OIL**CAS 93384-40-8; EC 297-250-8
(Sage, *Salvia hispanica*, ext. = INCI)

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "Clary sage", 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to "clary sage" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Salvia officinalis* L. *Salvia Officinalis Lavandulifolia* Herb Oil is an essential oil obtained from the herbs of the Sage, *Salvia officinalis* L. spp. *lavandulifolia*, Lamiaceae, Syn. Dalmatian sage (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41084>, last accessed 2010-01-29).

Salvia Lavandulifolia Herb Oil is an essential oil obtained from the herbs of the Sage, *Salvia lavandulifolia*, Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40987>, last accessed 2010-01-29).

Salvia Sclarea Flower Oil is an essential oil obtained from the flowers and foliage of the Clary Sage, *Salvia sclarea* L., Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41086>, last accessed 2010-01-29).

Salvia Hispanica Herb Oil is an essential oil obtained from the herbs of the Spanish Sage, *Salvia hispanica* L., Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40985>, last accessed 2010-01-29).

Clary sage oil is obtained by steam distillation of flowering tops and foliage of cultivated *Salvia sclarea* L. (Lamiaceae). Main constituents are linalyl acetate (56-78%) and linalool (6.5-24%) (34). Dalmatian sage oil is steam distilled from partially dried leaves of *S. officinalis* L. (Lamiaceae). The content by GC is: alpha-thujone (18-43%), beta-thujone (3-8.5%), 1,8-cineole (5.5-13%), camphor (3-8.5%) as main constituents (34). Spanish sage oil does not contain thujone, but mainly camphor (15-36%) and 1,8-cineole (11-30%), and is used mainly in pharmaceutical preparations and technical perfumery (34). For Oil of sage, Spanish (*Salvia lavandulifolia* Vahl) an ISO standard exists: ISO 3526:2005, for Oil of Dalmatian sage (*Salvia officinalis* L.): ISO 9909:1997.

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

SANTALUM ALBUM WOOD OIL

CAS 84787-70-2; EC 284-111-1
(Sandalwood, ext.) INCI:
SANTALUM ALBUM / Sandalwood,
ext.

Sandalwood oil ([East] India)

SANTALUM ALBUM OIL

CAS 8006-87-9; EC / (Oils,
sandalwood)

Sandalwood oil ([East] India)

Current regulation: /

Clinical data:

In the Sugiura 2000 study, 1483 patients with suspected cosmetic dermatitis were PTed with "sandalwood oil" (2% pet.), yielding 0.8% positive reactions (14). In the Frosch 2002 b study, "sandalwood oil (East India)" is mentioned with a CAS # 8015-65-4, which, however, is attributed to AMYRIS BALSAMIFERA BARK OIL, see above. Assuming that this CAS # is erroneous, study results are considered to be valid for *S. album* wood oil, tested at 2% and 10% concentration, yielding 0.4% and 0.9% positive reactions,

respectively (17). Out of 6 of 15 patients with a positive reaction to the higher concentration no clinical relevance was found, compared to 2 of 7 patients positive to the lower concentration (17). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "sandalwood oil" (2% pet.) 6.6% positive reactions (9). In the An 2005 study, 10 of 422 consecutive patients, i.e., 2.4%, had positive reactions to "Santalum album oil" 2% (13). The Goossens 1997 study found 4 of 111 patients positive to "sandalwood oil 10% pet." – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found no positive reaction in 200 patients to "sandalwood", 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to "sandalwood" essential oil 2% pet. (27). In 63 patients positive to the FM I, 1 had a positive PT reaction to sandalwood oil, 2% pet., in the Santucci 1987 study (28). Nakayama et al. found 1974 (after (29)) 6 "strong positive" and 8 "weak positive" reactions to "Sandalwood oil" (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 1.3% positive reactions in 3671 consecutively tested patients and 1.8% positive reactions in 1002 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were tested with sandalwood oil, yielding 2 positive reactions (48).

Additional information:

ISO 4720:2009 nomenclature: *Santalum album* L. *Santalum Album* Oil is the volatile oil obtained from the heartwood of the Sandalwood, *Santalum album* L., Santalaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=80209>, last accessed 2009-11-26).

Santalum Album Wood Oil is an essential oil obtained from the wood of the Sandalwood, *Santalum album* L., Santalaceae. It contains 75% santalol isomers (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41092>, last accessed 2009-11-12), typically up to 55% .alpha.-santalol and up to 24% .beta.-santalol (30). East Indian sandalwood oil consists almost exclusively of closely related sesquiterpenoids; by far the main constituents are the alcohols alpha-santalol [115-71-9] (41-55%) and cis-beta-santalol [77-42-9] (16-24%), the latter being mainly responsible for the specific odour (34, 39).

An ISO standard regarding the composition of "*Santalum album* oil" is available: ISO 3518:2002. "Sandalwoods" are labelled as *Amyris balsamifera*, *Eremophila mitchelli*, *Fusanus acuminatus* (= *Santalum acuminatum*), *Santalum album*, *S. austrocaledonicum*, *S. latifolium*, *S. spicatum* and *S. yasi*. The majority of currently available trade oils, reportedly from *S. album*, contained approximately 50-70% santalols (Z-alpha and Z-beta), as analysed with gas chromatography-mass spectrometry (GC-MS) (277). A review on the toxicological properties of "*Santalum album* oil" is available (278).

AMYRIS BALSAMIFERA BARK OIL (*Sandalwood oil (Caribbean)*), CAS 8015-65-4; EC / (Oils, amyris) / 90320-49-3; EC 90320-49-3 (*Amyris balsamifera*, ext. = INCI name) is used as a cheap substitute for East Indian Sandalwood in perfumes and cosmetics. Originally cultivated primarily in Haiti where it was known as 'candle wood' and used as a torch by locals due to the tree's high oil content (<http://www.amphora-retail.com/sandalwood-amyris-essential-10ml-p-107.html>, last accessed 2009-11-12). The major components are sesquiterpenoids such as valerianol, elemol, β -eudesmol and epi-gamma-eudesmol (39). For Oil of amyris (*Amyris balsamifera* L.) an ISO standard exists: ISO 3525:2008. *Amyris Balsamifera* Bark Oil is the volatile oil distilled from the bark of the tree, *Amyris balsamifera*, Rutaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=74455>, last accessed 2009-11-12).

SANTALUM SPICATA WOOD OILCAS 8024-35-9; EC 296-618-5
(Sandalwood oil, Western
Australia)*Sandalwood oil (Australia)*

Current regulation: /

Clinical data:

In clinical studies, mostly *S. album* wood oil had been used (see above); in a number of studies this is not clear.

Additional information:

ISO 4720:2009 nomenclature: *Santalum spicatum* (R.Br.) A. DC, syn. *Eucarya spicata* (R.Br.) Sprag & Summ. For Oil of Australian sandalwood (*Santalum spicatum* (R.Br.) A.DC.) an ISO standard exists: ISO 22769:2009. Santalum Spicata Wood Oil is an essential oil obtained from the wood of the Australian Sandalwood, *Santalum spicata*, Santalaceae. It contains 75% santalols and 10% farnesol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41093>, last accessed 2009-11-12). This oil also contains santalols as main constituents but differs somewhat in the remaining composition. Today, it makes up a considerable part of the sandalwood oil market (34).

Considering the content of well-known allergenic compounds (santalols), this essential oil is regarded as established contact allergen in humans.

TAGETES PATULA FLOWER OILCAS 91722-29-1; EC 294-431-3
(*Tagetes patula*, ext. = INCI)*"Marigold Oil; Tagetes Oil"*

Current regulation: /

Clinical data:

In an aromatherapist, an essential oil solvent-extracted from *Tagetes patula*, patch tested at 1.5% in grapeseed oil (vehicle negative, 7 controls negative to essential oils) resulted in a +++ reaction, in accordance with a work-related bilateral hand dermatitis (217).

Additional information:

Tagetes Patula Flower Oil is an essential oil obtained by hydrodistillation of the flowers of the *Tagetes*, *Tagetes patula* L., *Compositae*. It contains mainly D-limonene, ocimene, 2,6-dimethyloct-7-en-4-one (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41506>, last accessed 2010-01-28). According to Surburg/Panten, tagetes oil is steam distilled from the flowering plants of *Tagetes minuta* L. (*T. glandulifera* Schrank., *Asteraceae*). Main components comprise cis-ocimene, dihydrotagetone, tagetone, and cis- and trans-ocimenone (34, 39).

THYMUS spp. HERB OIL

THYMUS VULGARIS HERB OIL

CAS 84929-51-1, 8007-46-3; EC 284-535-7 (Thyme, Thymus vulgaris, ext.)

"Thyme oil"

INCI: THYMUS VULGARIS / Thyme, Thymus vulgaris, ext.

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to "thyme" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to "thyme" essential oil 2% pet. (27). In 63 patients positive to the FM I, none had a positive PT reaction to thymol, 1% pet., in the Santucci 1987 study (28).

Additional information:

ISO 4720:2009 nomenclature: *Thymus vulgaris* L. *Thymus vulgaris* Herb Oil is an essential oil obtained from the herbs of the Thyme, *Thymus vulgaris* L., Lamiaceae. It contains 20-40% thymol and carvacrol, cymene, pinene, linalool, bornyl acetate (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41133>, last accessed 2010-01-29).

Other species are used for extraction, e.g., *Thymus Mastichina* (CAS 84837-14-9), *Thymus Serpillum* (CAS 84776-98-7), *Thymus Zygis* (CAS 85085-75-2), according to CosIng. The main constituent is thymol (37-56%) (34). For Oil of thyme containing thymol, Spanish type [*Thymus zygis* (Loefl.) L.] an ISO standard exists: ISO 14715:2010, for Oil of Spanish wild marjoram (*Thymus mastichina* L.): ISO 4728:2003.

TURPENTINE (oil)

CAS 8006-64-2 / 9005-90-7 / 8052-14-0; EC 232-350-7 / 232-688-5 / -

Current regulation: III/124 ; III/125 ; III/126

Clinical data:

Oil of turpentine has been patch tested in a number of baseline series, i.e., in consecutive patients, although not included in the European Baseline series.

In a series of 24 patients with occupational contact dermatitis from the pottery industry, Lear at al. found 14 to be sensitised to "Indonesian oil of turpentine" and 8 to alpha-pinene (190)

Table 3.2.2 – 2: Overview of results with **Oil of turpentine** in patients patch tested for suspected allergic contact dermatitis. If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS.

Country	Population	Years	No. tested	Crude % positive (95% CI) §

Opinion on fragrance allergens in cosmetic products

Lisbon, Portugal (189); virtually no .delta.-3-carene	Consecutive patients	1979-1983	4316	2.3 % (1.9 – 2.8) §
Birmingham, UK (190)	Potters with occup. dermatitis hand	6 months; prior to 1996	24	14 / 24 pos. to “Indonesian turpentine”
Austria/Germany (IVDK) (279)	Consecutive patients	1992-1995	27658	0.47 % (0.39 – 0.55) §
Austria/Germany (IVDK) (280)	Consecutive patients	1996-2002	59478	Annual prevalences 1.6 to 4.4 %
Augsburg/Germany (281)	Population sample	1998	1141	1.2% (on population level!)
Europe (ESSCA) (273)	Consecutive patients	2002/03	3767	1.6 %
Austria/Germany/Switzerland (IVDK) (7)	Consecutive patients	2005-2008	37163	1.8 %

Additional information:

ISO 4720:2009 nomenclature: *Pinus pinaster* Aiton and *Pinus massoniana* Lamb. Turpentine, oil: Any of the volatile predominately terpenic fractions or distillates resulting from the solvent extraction of, gum collection from, or pulping of softwoods. Turpentine is a mixture of terpene hydrocarbons obtained from various species of *Pinus* http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=41521

The composition of oil of turpentine varies with its origin, in particular, the content of .delta.-3-carene, one of its main allergenic compounds (189, 279). Similarly, the peroxide degree may vary. The main constituents are .alpha.-pinene (50-72%), .beta.-pinene (6-15%), carenes (< 0.1-17%), camphene (up to 1%), dipentene (0.5-5%), along with a number of other substances (279).

It is a “top 200” substance and classified as R43 (IFRA, pers. comm.2010)

Verbena absolute (*Lippia citriodora* Kunth.)

CAS 8024-12-2, 84961-67-1; EC /)

Current regulation: Annex III, part 1, n° 206

Clinical data: /

Additional information:

ISO 4720:2009 nomenclature: *Aloysia citriodora* Palau syn. *Lippia citriodora* Kunth syn. *Aloysia triphylla* (L' Hér.) Kuntze. An older RIFM review is available citing several positive human maximisation studies both with “Verbena absolute” and “Verbena oil” (128).

VETIVERIA ZIZANOIDES ROOT OIL

CAS 8016-96-4; EC / (Oils, vetiver) / 84238-29-9; EC 282-490-8 (Vetiveria zizanioides, ext. = INCI)

"Vetiver oil; khas khas oil"

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "vetiver" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=9 (10.4%) positive reactions to "vetiver" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Vetiveria zizanioides* (L.) Nash. *Vetiveria Zizanioides* Root Oil is an essential oil distilled from the dried roots of the grass *Vetiveria zizanioides* (L.) Nash *Poaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41293>, last accessed 2010-01-29). Vetiver oil has a high sesquiterpene content. The ketones alpha-vetivone [15764-04-2] (6-12%) and beta-vetivone [18444-79-6] (4-10%), which usually form more than 10% of the oil, as well as khusimol [16223-63-5] (24-36%) and isovelencenol [22387-74-2] (12-24%) are the main constituents (in Bourbon oil, i.e., from Réunion) (34). For Oil of vetiver (*Vetiveria zizanioides* (L.) Nash) an ISO standard exists: ISO 4716:2002.

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Annex II - Animal Data

Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Substance		Vehicle (AOO=acetone:olive oil; DEP=diethyl phthalate; DMF=dimethyl formamide; DMSO=dimethyl sulphoxide; EtOH=ethanol; MEK=methyl ethyl ketone)	Conc. in vehicle (%, generally w/v)	No. animals per dose group	EC3 value *				Comment (deviation from OECD 429 etc)	Reference
INCI name (other name)	CAS no.				%	µg/cm ²	M	lowest for the substance (%)		
<i>Allyl phenoxyacetate</i>	7493-74-5	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	4	3.1	775	0.16	3.1		RIFM, 2007a
Amyl cinnamal	122-40-7	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	7.6	1900	0.38	7.6		RIFM, 2006a
Amyl cinnamal	122-40-7	4:1 AOO	-	4	10.6	2650	0.52		Elahi gives ref to Basketter et al 1999, but no data on the substance is found. It is not known if Elahi, Aptula and Roberts quote the same experiment	Elahi et al., 2004

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Amyl cinnamal	122-40-7	-	-	-	11	2750	0.54		Aptula gives ref to Kimber et al 2003, but no LLNA data on the substance is found. It is not known if Elahi, Aptula and Roberts quote the same experiment; original reference is not given.	Aptula et al., 2007
Amyl cinnamal	122-40-7	-	-	-	11	2750	0.54		Original ref not given.	Roberts et al., 2007
Amylcinnamyl alcohol	101-85-9	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	> 25	>6250	>1.22	> 25	Should have been tested at higher concentrations	RIFM, 2004a
Anise alcohol	105-13-5	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	5.9	1475	0.43	5.9		RIFM, 2005a
Benzaldehyde	100-52-7	-	-	-	-	-	-		No data in the ref	Roberts et al., 2007
Benzaldehyde	100-52-7	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2003
Benzyl alcohol	100-51-6	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	> 50	>12500	>4.62	> 50	Should have been tested at higher concentrations	RIFM, 2005b

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Benzyl benzoate	120-51-4	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	> 50	>12500	>2.36	> 50	Should have been tested at higher concentrations	RIFM, 2005c
Benzyl cinnamate	103-41-3	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	18.4	4600	0.77	18.4		RIFM, 2005d
Benzyl salicylate	118-58-1	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	2.9	725	0.13	2.9		RIFM, 2005e
<i>p</i> -tert-Butyl-dihydrocinnamaldehyde	18127-01-0	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	4.3	1075	0.23	4.3		RIFM, 2007b
Butylphenyl methylpropional (BMHCA)	80-54-6	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	2.9	725	0.14	2.9		RIFM, 2001a
Butylphenyl methylpropional (BMHCA)	80-54-6	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	4.1	1025	0.20			RIFM, 2001b
Butylphenyl methylpropional (BMHCA)	80-54-6	1:3 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	13.9	3475	0.68			RIFM, 2001c
Butylphenyl methylpropional (BMHCA)	80-54-6	1:3 DEP:EtOH	0.3, 1.0, 3.0, 10.0, 30.0	4	8.8	2200	0.43			RIFM, 2001d
Butylphenyl methylpropional (BMHCA)	80-54-6	4:1 AOO	1.0, 2.5, 5.0, 10.0, 25.0	4	16.8	4200	0.82			RIFM, 2001e

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Butylphenyl methylpropional (BMHCA)	80-54-6	4:1 AOO	1, 2.5, 10, 25, 50	4	18.7	4675	0.92		Basketter et al., 2001
Camellia sinensis leaf Tea Leaf Absolute	84650-60-2	DMF	0.5, 1.0, 2.5, 5.0, 10.0	4	> 5.0	>1250	N/a	> 5.0	Should have been tested at higher concentrations RIFM, 2005m
Cananga odorata leaf / flower oil Ylang Ylang Extra	8006-81-3	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	4	6.8	1700	N/a	6.8	RIFM, 2007f
Carvone	6485-40-1	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	10.7	2675	0.71		RIFM, 2007c
Carvone	6485-40-1	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	5.7	1425	0.38	5.7	RIFM, 2007d
Carvone	6485-40-1	4:1 AOO	6.0, 12, 20	4	13	3250	0.86		Nilsson et al., 2005
Cinnamal	104-55-2	3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.2	50	0.015	0.2	RIFM, 2003a
Cinnamal	104-55-2	0.1% α -tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.2	50	0.015		RIFM, 2003b
Cinnamal	104-55-2	2.0% α -tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.6	150	0.045		RIFM, 2003c
Cinnamal	104-55-2	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1	0.1, 0.3, 1.0, 3.0, 10.0	4	0.7	175	0.053		RIFM, 2003d

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

EtOH:DEP

Cinnamal	104-55-2	0.1% Trolox C in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.7	175	0.053		RIFM, 2003e
Cinnamal	104-55-2	2.0% α -tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.8	200	0.060		RIFM, 2003f
Cinnamal	104-55-2	3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.9	225	0.068		RIFM, 2003g
Cinnamal	104-55-2	0.1% α -tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	1.1	275	0.083		RIFM, 2003h
Cinnamal	104-55-2	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	1.3	325	0.098		RIFM, 2003i
Cinnamal	104-55-2	0.1% Trolox C in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	1.4	350	0.11		RIFM, 2003j
Cinnamal	104-55-2	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Cinnamal	104-55-2	4:1 AOO	0.5, 1, 2.5, 5, 10	4	3.1	775	0.23		Basketter et al., 2001
Cinnamal	104-55-2	4:1 AOO	-	4	1.3	325	0.10		Elahi et al., 2004
Cinnamal	104-55-2	4:1 AOO	1, 2.5	-	1.4	348	0.11	Too few concentrations tested; few details given in ref	Smith and Hotchkiss, 2001
Cinnamal	104-55-2	4:1 AOO	1.0, 2.5, 5.0, 10.0, 25.0	4	1.7	425	0.13		Wright et al., 1995

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Cinnamal	104-55-2	MEK	1.0, 2.5, 5.0, 10.0, 25.0	4	1.1	275	0.083		Wright et al., 1996
Cinnamal	104-55-2	DMF	0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0	4	0.5	125	0.038		Wright et al., 1997
Cinnamal	104-55-2	propylene glycol	1.0, 2.5, 5.0, 10.0, 25.0	4	1.4	350	0.11		Wright et al., 1998
Cinnamal	104-55-2	DMSO	0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0	4	0.9	225	0.068		Wright et al., 1999
Cinnamal	104-55-2	90:10 EtOH:water	1.0, 2.5, 5.0, 10.0, 25.0	4	1.6	400	0.12		Wright et al., 2000
Cinnamal	104-55-2	50:50 EtOH:water	1.0, 2.5, 5.0, 10.0, 25.0	4	1.2	300	0.091		Wright et al., 2001
Cinnamyl alcohol	104-54-1	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Cinnamyl nitrile	1885-38-7	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	> 10	>2500	>0.77	> 10	Report: systemic toxicity at 25% and 50%. Should have been tested at higher concentrations RIFM, 2005f
Citral	5392-40-5	1:3 EtOH:DEP	0.4, 2.0, 4.0, 8.0, 20.0	4	1.2	300	0.079	1.2	RIFM, 2004b
Citral	5392-40-5	0.1% α -tocopherol in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	1.5	375	0.099		RIFM, 2003k

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Citral	5392-40-5	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	2.1	525	0.14		RIFM, 2003l
Citral	5392-40-5	0.1% Trolox C in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	3.7	925	0.24		RIFM, 2003m
Citral	5392-40-5	3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	4.6	1150	0.30		RIFM, 2003n
Citral	5392-40-5	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	4.6	1150	0.30		RIFM, 2003o
Citral	5392-40-5	3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	5.3	1325	0.35		RIFM, 2003p
Citral	5392-40-5	0.1% Trolox C in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	5.8	1400	0.38		RIFM, 2003q
Citral	5392-40-5	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	6.3	1575	0.41		RIFM, 2003r
Citral	5392-40-5	0.1% α -tocopherol in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	6.8	1700	0.44		RIFM, 2003s
Citral	5392-40-5	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Citronellol	106-22-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	43.5	10875	2.78	43.5	RIFM, 2004c

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Coumarin	91-64-5	DMF	10, 25, 50	4	>50	>12500	>3.42	>50	Should have been tested at higher concentrations	Vocanson et al., 2006
<i>Dibenzyl ether</i>	103-50-4	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	6.3	1575	0.32	6.3		RIFM, 2007e
Eugenol	97-53-0	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	5.3	1325	0.32	5.3		RIFM, 2001f
Eugenol	97-53-0	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	10.5	2625	0.64			RIFM, 2001g
Eugenol	97-53-0	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	10.7	2675	0.65			RIFM, 2001h
Eugenol	97-53-0	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	15.1	3775	0.92			RIFM, 2001i
Eugenol	97-53-0	4:1 AOO	2.5, 5.0, 10.0, 25.0, 50.0	-	11.9	2975	0.72			Basketter et al., 1999
Eugenol	97-53-0	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2003
Evernia furfuracea extract <i>Treemoss absolute</i>	90028-67-4	1:3 EtOH:DEP	5.0, 10.0, 20	4	> 20	>5000	N/a	> 20	Should have been tested at higher concentrations	RIFM, 2004k
Evernia furfuracea extract <i>Treemoss absolute</i>	90028-67-4	1:3 EtOH:DEP	10.0, 25.0	4	> 25	>6250	N/a		Too few concentrations tested	RIFM, 2004d

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Evernia prunastri extract <i>Oakmoss</i>	90028-68-5	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	3.88	970	N/a	3.88		RIFM, 2004j
Farnesol	4602-84-0	4:1 AOO	5.0, 10.0, 25.0	4	5.5	1375	0.25		Should also have been tested at lower concentrations	RIFM, 2004d
Farnesol	4602-84-0	4:1 AOO	5.0, 10.0, 25.0	4	4.1	1025	0.18	4.1	Should also have been tested at lower concentrations	RIFM, 2004d
Geraniol	106-24-1	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	5.6	1400	0.36	5.6		RIFM, 2001j
Geraniol	106-24-1	3:1 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	11.4	2850	0.74			RIFM, 2003t
Geraniol	106-24-1	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	11.8	2950	0.76			RIFM, 2001k
Geraniol	106-24-1	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	20.4	5100	1.32			RIFM, 2001l
Geraniol	106-24-1	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	25.8	6450	1.67			RIFM, 2001m
Geraniol	106-24-1	-	-	-	26	6500	1.69			Roberts et al., 2007
<i>trans-2-Hexenal</i>	6728-26-3	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5, 10	4	2.6	650	0.26	2.6		RIFM, 2005g
<i>trans-2-Hexenal</i>	6728-26-3	-	-	-	5.5	1375	0.56			Roberts et al., 2007

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Hexyl cinnamal	101-86-0	generally 4:1 AOO		-O162	5.3-14.7	1325-3675	0.25-0.68	5.3		"numerous accounts in the literature"
2-Hexylidene cyclopentanone	17373-89-6	1:3 EtOH:DEP	0.1, 0.5, 1.0, 2.5, 5.0	5	2.4	600	0.14	2.4		RIFM, 2008a
Hexyl salicylate	6259-76-3	1:3 EtOH:DEP	0.05, 0.25, 0.5, 1.0, 2.5	4	0.18	45	0.008	0.18		RIFM, 2006b
Hydroxycitronellal	107-75-5	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	19.3	4825	1.12	19.3		RIFM, 2001n
Hydroxycitronellal	107-75-5	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	19.7	4925	1.14			RIFM, 2001o
Hydroxycitronellal	107-75-5	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	22.2	5550	1.29			RIFM, 2001p
Hydroxycitronellal	107-75-5	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	26.4	6600	1.53			RIFM, 2001q
Hydroxycitronellal	107-75-5	AOO	25, 50, 100	-	-	-	-		EC3 value not given	Ashby et al., 1995
Hydroxycitronellal	107-75-5	4:1 AOO	2.5, 5, 10, 25, 50	4	33.0	8250	1.92			Basketter et al., 2001
Hydroxycitronellal	107-75-5	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2002
Hydroxycitronellal	107-75-5	-	-	-	25.25	6313	1.47			Estrada et al., 2003

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Hydroxycitronellal	107-75-5	4:1 AOO	10, 25	-	23	5750	1.34		Too few concentrations tested; few details given in ref	Smith and Hotchkiss, 2001
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	31906-04-4	4:1 AOO	1.0, 2.5, 5, 10, 25, 50	4	17.1	4275	0.81	17.1		RIFM, 2001r
<i>p</i> -Isobutyl- α -methyl hydrocinnamaldehyde	6658-48-6	70% EtOH	10.0, 25.0, 50.0, 100.0	4	9.5	2375	0.46	9.5	Should also have been tested at lower concentrations	RIFM, 2001w
<i>Isocyclocitral</i>	1335-66-6	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	4	7.3	1825	0.48	7.3		RIFM, 2006c
<i>Isocyclogeraniol</i>	68527-77-5	1:3 EtOH:DEP	5.0, 10.0, 25.0, 50.0	4	> 25	>6250	>1.62	> 25	Should have been tested at higher concentrations	RIFM, 2005h
Isoeugenol	97-54-1	4:1 AOO	0.5, 5.0	6	0.54	145	0.033	0.54	Too few concentrations tested	RIFM, 2001s
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.6	150	0.037			RIFM, 2002a
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.76	191	0.046			RIFM, 2002b
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.79	199	0.048			RIFM, 2002c

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Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.19	296	0.072	RIFM, 2001t
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.28	320	0.078	RIFM, 2004e
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	6	1.54	385	0.094	RIFM, 2001u
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.95	488	0.119	RIFM, 2001v
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0		3.3	825	0.20	Basketter et al., 1999
Isoeugenol	97-54-1	-	-	-	-	-	-	No data in the ref (poster abstract) Basketter et al., 2002
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.3	325	0.079	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	3.3	825	0.20	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.8	450	0.11	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	3.1	775	0.19	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.6	400	0.097	Loveless et al., 1996
Isoeugenol	97-54-1	AOO	0.5, 1.0, 2.5, 5.0, 10.0	4	1.0	250	0.061	Wright et al., 2001
Isoeugenol	97-54-1	MEK	0.5, 1.0, 2.5, 5.0, 10.0	4	1.0	250	0.061	Wright et al., 2001
Isoeugenol	97-54-1	DMF	0.5, 1.0, 2.5, 5.0, 10.0	4	1.4	350	0.085	Wright et al., 2001

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Isoeugenol	97-54-1	propylene glycol	0.5, 1.0, 2.5, 5.0, 10.0	4	2.5	625	0.15			Wright et al., 2001
Isoeugenol	97-54-1	DMSO	0.5, 1.0, 2.5, 5.0, 10.0	4	0.9	225	0.055			Wright et al., 2001
Isoeugenol	97-54-1	90:10 EtOH:water	0.5, 1.0, 2.5, 5.0, 10.0	4	1.8	450	0.11			Wright et al., 2001
Isoeugenol	97-54-1	50:50 EtOH:water	0.5, 1.0, 2.5, 5.0, 10.0	4	4.9	1225	0.30			Wright et al., 2001
<i>Jasmine absolute (Grandiflorum)</i>	8022-96-6	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	5.9	1475	N/a	5.9		RIFM, 2006d
Jasminum Sambac Flower CERA / Extract / Water	91770-14-8	1:3 EtOH:DEP	10.0, 25.0, 50.0, 75.0, 100.0	4	35.4	9100	N/a	35.4		RIFM, 2006e
<i>d-Limonene**</i>	5989-27-5	EtOH	10.0, 20.0, 50.0, 75.0, 100.0	4	< 10	< 250	<0.73	< 10	Should also have been tested at lower concentrations	RIFM, 2004l
<i>d-Limonene**</i>	5989-27-5	3:1 EtOH:DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	22.0	5500	1.61			RIFM, 2004m
<i>d-Limonene**</i>	5989-27-5	1:3 EtOH:DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	38.0	9500	2.79			RIFM, 2004n
<i>d-Limonene**</i>	5989-27-5	DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	63.0	15.75	4.62			RIFM, 2004o
<i>d-Limonene**</i>	5989-27-5	4:1 AOO	25, 50, 100	4	68.5	17125	5.03			Warbrick et al., 2001

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Linalool**	78-70-6	-	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Menthadiene-7-methyl formate	68683- 20-5	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	> 10	> 2500	>0.51	> 10	Should have been tested at higher concentrations	RIFM, 2008c
4-Methoxy- α -methyl benzenpropanal	5462- 06-6	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	23.6	5900	1.32	23.63		RIFM, 2004f
α -Methyl cinnamic aldehyde	101-39- 3	-	-	-	4.5	1125	0.31	4.5		Roberts et al., 2007
Methylenedioxyphenyl methylpropanal	1205- 17-0	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	16.4	4100	0.85	16.4		RIFM, 2005i
6-Methyl-3,5-heptadien-2- one	1604- 28-0	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	> 5	> 1250	>0.40	> 5	Should have been tested at higher concentrations	RIFM, 2008d
α -iso-Methylionone	127-51- 5	1:3 EtOH:DEP	10.0, 25.0, 50.0, 75.0, 100.0	4	21.8	5450	1.06	21.8		RIFM, 2005j
Methyl octine carbonate	111-80- 8	-	-	-	2.5	635	0.15	2.5		Roberts et al., 2007

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Methyl 2-octynoate	111-12-6	1:3 EtOH:DEP	0.5, 1.0, 2.0, 5.0, 10.0	4	< 0.5	< 125	<0.032	< 0.5	Should also have been tested at lower concentrations	RIFM, 2005k
2-Methoxy-4-methylphenol	93-51-6	-	-	-	5.8	1450	0.42	5.8		Roberts et al., 2007
1-Octen-3-yl acetate	2442-10-6	1:3 EtOH:DEP	7.5, 15.0, 30.0	5	> 30	> 7500	>1.76	> 30	Should have been tested at higher concentrations	RIFM, 2004g
Perillaldehyde <i>p</i> -Mentha-1,8-dien-7-al	2111-75-3	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	9.3	2325	0.62			RIFM, 2008b
Perillaldehyde <i>p</i> -Mentha-1,8-dien-7-al	2111-75-3	-	-	-	8.1	2025	0.54	8.1		Roberts et al., 2007
Balsam oil, Peru (<i>Myroxylon pereirae</i> Klotzsch)	8007-00-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	3.95	987	N/a	3.95		RIFM, 2004h
Peru balsam absolute	8007-00-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	2.5	625	N/a	2.5		RIFM, 2004i
Peru balsam absolute	8007-00-9	1:3 EtOH:DEP	0.5, 1.0, 2.5	4	>2.5	>625	N/a			RIFM, 2004i
Phenylacetaldehyde	122-78-1	4:1 AOO	2.5, 5, 10, 25, 50	4	3.0	750	0.25	3.0		Basketter et al., 2001

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<i>Phenylacetaldehyde</i>	122-78-1	-	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2003
<i>3-Propylidenephthalide</i>	17369-59-4	4:1 AOO	5, 10, 20	4 or 5	3.7	925	0.21	3.7	Should also have been tested at lower concentrations	Gerberick et al., 2004
Tetramethyl acetyloctahydronaphthalenes (OTNE)	54464-57-2	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	25.14	6285	1.07	25.14		RIFM, 2005l
Trimethylbenzenepropanol <i>Majantol</i>	103694-68-4	4:1 AOO	3.0, 10.0, 30.0	4	~30	~7500	~1.68	30	Should have been tested at higher concentrations	RIFM, 2002d
Vanillin	121-33-5	4:1 AOO	2.5, 5, 10, 25, 50	4	>50.0	>1250	>3.3	>50.0		Basketter et al., 2001

* source of EC3 value value: % given in the RIFM report or references; µg/cm2 given in the RIFM report and RIFM poster; M calculated by SCCS working group

**material with low levels of oxidation according to RIFM, 2009

- = no data given; A216

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Annex III - Tabular summary of dose-elicitation studies in sensitised patients**Contents**

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Chloroatranol

Chloroatranol (allergen in oak moss absolute: <i>Evernia prunastri</i>) (1)	
Design	blinded, randomised with regard to doses and controlled
Test subjects	13 patients previously identified as sensitized to chloroatranol and oak moss absolute
Controls	10 healthy controls
Substance	Purity: >99%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	200 ppm to 0.0063 ppm (10 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction
ROAT	volar aspect of forearms
area	3 x 3 cm ²
applications/day	two
dose	chloroatranol in ethanol: Step 1: 5 ppm Step 2: 25 ppm
dose/application/cm ²	step 1: 0.025 µg step2: 0.125 µg
control substance	ethanol
definition of positive	erythema in at least 25% and at least one papule
period	two weeks for each step
Results	
PT ED10% (95% CI)	0.013 (0.002-0.03) ppm =0.0004 µg/cm ²
PT ED50% (95% CI)	0.15 (0.077-0.295) ppm =0.0045 µg/cm ²
PT no effect level (observed)	/
ROAT	Cumulative responses
Step 1 (5 ppm)	12/13 (92%)
Step 2 (25 ppm)	13/13 (100%)
Controls	Negative
Other information	None relevant

In a subsequent study chloroatranol and atranol, both ingredients in *Evernia prunastri*, were tested in equimolar concentrations in serial dilution in 10 eczema patients with known sensitization to chloroatranol and oak moss. A positive response was defined as any degree of reaction. Ethanol was included as the control and gave no response. No use tests were done and no control subjects included.

Results: All patients reacted to the highest concentrations of the two substances. For both substances there was a significant dose-dependence and the estimated difference in elicitation potency of chloroatranol relative to atranol was 217%. The dose-response curve is seen in figure 1 below (2).

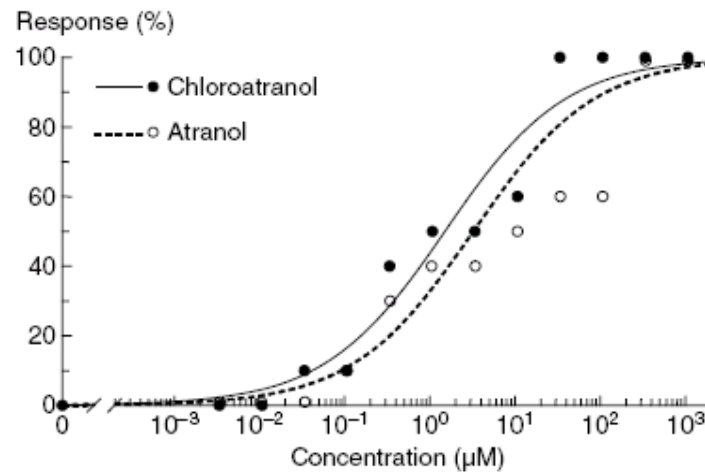


Fig. 1. Observed response rates and fitted parallel logistic dose-response curves for atranol and chloroatranol in equimolar concentrations at patch testing. The response was dichotomized and any reaction other than zero was classified as positive.

Cinnamal

Cinnamal (3)	
Design	blinded, randomised and controlled
Test subjects	18 patients with a positive patch test to cinnamal and additional 4 with a doubtful response
Controls	20 healthy controls
Substance	Purity: >98%
Patch test	20 mg solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.01% (7 steps)
-control/vehicle	petrolatum
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of responses
ROAT	outer aspect of upper arm
area	5 x 5 cm ²
applications/day	two with atomizer pump
dose	Step 1: 0.02% Step 2: 0.1% Step 3: 0.8%
dose/application/cm ²	Not given
control substance	ethanol
definition of positive	The response was classified as positive no matter the degree of reaction.
period	two weeks for each step; total maximum 6 weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	0.24% = 96 µg/cm ² (calculated from the data in the paper)
PT no effect level(observed)	0.01 % in pet. = 0.4 µg/cm ²
ROAT	Cumulative responses
Step 1 (0.02%)	0/18
Step 2 (0.1%)	8/18 (44 %)
Step 3 (0.8%)	13/18 (72 %)
Controls	No eczema reactions were seen
Other information	2 patients and 2 controls developed immediate reactions to the cinnamal solution

Cinnamal (4)	
Design	blinded, randomised doses and controlled
Test subjects	17 patients with a positive patch test to cinnamal (8 patients in part 1 and 9 in part two)
Controls	20 controls (non-sensitised dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2 % to 0.00006 % (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting a + reaction
ROAT	Axilla
area	10 x 10 cm ² (estimated)
applications/day	two with roll on deodorant (89-700 mg per application of solution) average cases: 263 mg/application controls: only range given
dose	Part one: Step 1: 0.032% Step 2: 0.1% Step: 0.32% Part two: Step 1: 0.01% Step 2: 0.032% Step 3: 0.1%
dose/application/cm ²	Part two estimated: step one: 0.26 µg; step two: 0.84 µg; 2.63 µg
control substance	Deodorant matrix
definition of positive	eczematous reaction covering at least 25% of test area
period	Part one: one week with each concentration: maximum three weeks Part two: two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level(observed)	0.002%
ROAT	Cumulative responses
Step 1 (0.01)	2/9 (22%)
Step 2 (0.032)	6/9 (67%)
Step 3 (0.1)	8/9 (88%)
Controls	No reactions were seen
Other information	Only reactions seen to the cinnamal-containing deodorants at ROAT, difference to matrix axilla ($p<0.001$) and all control

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	persons negative ($p < 0.001$)

Hydroxycitronellal

Hydroxycitronellal (5)	
Design	blinded, randomised doses and controlled
Test subjects	7 patients with a positive patch test to hydroxycitronellal
Controls	7 controls (non-sensitised dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	4% to 0.00006% (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting + reaction
ROAT	Axilla
area	10 x 10 cm ² (estimated)
applications/day	two with roll on deodorant (172-591 per application of solution) average cases: 294 mg/application controls: only range given
dose	Step 1: 0.032% Step 2: 0.1% Step: 0.32%
dose/application/cm ²	Estimated: step 1: 0.94 µg; step 2: 2.94 µg; step 3: 9.40 µg
control substance	Deodorant matrix
definition of positive	eczematous reaction covering at least 25% of test area
period	two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level(observed)	<0.00012 %
ROAT	Cumulative responses
Step 1 (0.032)	4/7 (57%)
Step 2 (0.1)	5/7 (71%)
Step 3 (0.32)	7/7 (100%)
Controls	No reactions were seen
Other information	Reactions were only seen to the hydroxycitronellal-containing deodorant at ROAT, difference to matrix treated axilla ($p<0.001$) and all control persons negative ($p<0.001$)

Hydroxycitronellal (6)	
Design	double blinded, randomised
Test subjects	13 patients with a positive patch test to hydroxycitronellal
Controls	/
Substance	purity: unknown
Patch test	confirmatory
-dilution steps	
-control/vehicle	
-definition of threshold	
ROAT	finger immersion in fragrance solution in 10% ethanol
area	/
applications/day	Once per day for 10 min
dose	Step 1: 10 ppm Step 2: 250 ppm
dose/application/cm ²	Not applicable
control substance	10% alcohol
definition of positive	clinical grading scale and laser doppler comparison between active and control
period	two weeks with each concentration: maximum four weeks
Results	
PT ED10% (95% CI)	Not relevant
PT ED50% (95% CI)	Not relevant
PT no effect level(observed)	Not relevant
ROAT	Cumulative responses
Step 1 (10 ppm)	1/13
Step 2 (250 ppm)	5/13
Vehicle control	4/13
Other information	No difference between active substance and control application was found.

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC)

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) (7)	
Design	blinded, randomised and controlled
Test subjects	18 patients with a positive patch test to HICC
Controls	7 healthy controls
Substance	Purity: >99%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	6% to 0.0006%
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions
ROAT	volar aspect of lower arm
area	3 x 3 cm ²
applications/day	two with droplet bottle (theoretical:30 mg per application of solution)
dose	Step 1: 0.5% Step 2: 3%
µg/application/cm ²	Step 1: 15.3 (3.4-22.2) Step 2: 126.2 (40.5-226.2)
control substance	ethanol
definition of positive	erythema in at least 25% and at least one papule
period	two weeks for each step; total maximum 4 weeks
Results	
PT ED10% (95% CI)	0.9 µg/cm ² 29 (7-69) ppm
PT ED50% (95% CI)	20 µg/cm ² 662 (350-1250)ppm
PT no effect level (observed)	/
ROAT	Cumulative responses
Step 1 (0.5%)	11/18 (61%)
Step 2 (3%)	16/18 (89%)
Controls	No reactions were seen
Other information	Difference between test and control group statistically significant

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) (8)	
Design	blinded, randomised and controlled
Test subjects	15 patients with a positive patch test to HICC
Controls	10 healthy controls
Substance	Purity: > 98.8%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	6% to 0.0006% (5 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions
ROAT	Axilla
area	76 cm ² (template)
applications/day	two with roll on deodorant
dose	Step 1: 200 ppm Step 2: 600 ppm Step 3: 1800 ppm
dose/application/cm ²	median 0.79 µg HICC
control substance	deodorant matrix
definition of positive	spotty erythema involving at least 25% of the exposed area and infiltration represented by at least one papule.
period	two weeks for each step; total maximum 6 weeks
Results	
PT ED10% (95% CI)	0.75 µg/cm ² 25 ppm (0.69-120)
PT ED50% (95% CI)	18.3 µg/cm ² 610 ppm (120-2800)
PT no effect level (observed)	< 0.0006%
ROAT	Cumulative responses
Step 1 (200 ppm)	9/14* (64%)
Step 2 (600 ppm)	12/14* (86%)
Step 3 (1800 ppm)	14/14* (100%)
Controls	No reactions were seen
Other information	*14 patients completed the use test study Difference between HICC deodorant and matrix deodorant in cases ($p=0.0001$). Difference between controls and patients ($p=0.004$).

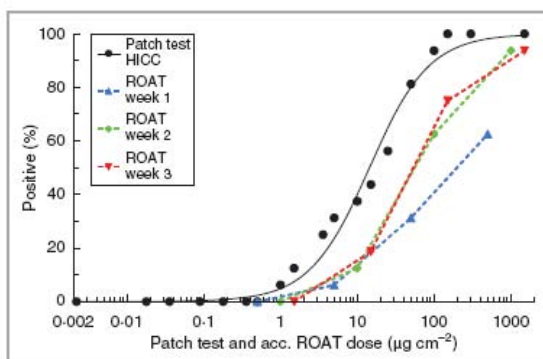
Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) (9)	
Design	blinded, randomised and controlled
Test subjects	17 patients with a positive patch test to HICC
Controls	15 healthy controls
Substance	IFF lot SM/8059062
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	1500 to 0.0022 µg/cm ² HICC (19 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions to higher concentrations
ROAT	volar aspect of forearms
area	3 x 3 cm (5 areas)
applications/day	two with micropipette (20 µl per application)
dose	Simultaneous application to 5 areas, four doses each and vehicle
µg /application/cm ²	Dose 1:0.0357 Dose 2: 0.357 Dose 3: 3.57 Dose 4: 35.7
control substance	ethanol
definition of positive	at least 5 points on a clinical scale, corresponding to erythema in 25% of test area and at least 1 papule
period	Three weeks. All concentrations applied simultaneously (randomised)
Results	
PT ED10% (95% CI)	0.662 µg/ cm ² (0.052-2.35)
PT ED50% (95% CI)	11.1 µg/ cm ² (3.41- 33.1)
PT no effect level(observed)	<0.0022 µg/ cm ²
ROAT	Cumulative responses
Dose 1 (0.0357)	0/16*
Dose 2 (0.357)	3/16 (19%)
Dose 3 (3.57)	12/16 (75%)
Dose 4 (35.7)	15/16 (94%)
Controls	No reactions were seen
Other information	*16 patients completed the use test study The evaporation rate of HICC was calculated to 72% over a 24-h period. ED10% ROAT: 0.064 µg/cm ² (more info see below)

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Table 2 The dose per application and accumulated dose after 1, 2 and 3 weeks in the ROAT

ROAT, dose per application ($\mu\text{g HICC cm}^{-2}$)	Number of applications after 1 week	Total accumulated dose after 1 week ($\mu\text{g HICC cm}^{-2}$)	Number of applications after 2 weeks	Total accumulated dose after 2 weeks ($\mu\text{g HICC cm}^{-2}$)	Number of applications after 3 weeks	Total accumulated dose after 3 weeks ($\mu\text{g HICC cm}^{-2}$)
35.7	14	500	28	1000	42	1500
3.57	14	50	28	100	42	150
0.357	14	5	28	10	42	15
0.0357	14	0.5	28	1	42	1.5

ROAT, repeated open application test; HICC, hydroxyisohexyl-3-cyclohexene carboxaldehyde.

Fig 3. The fitted dose-response curve for the patch test ($n = 16$) and the 1-week, the 2-week 3-week accumulated ROAT doses.

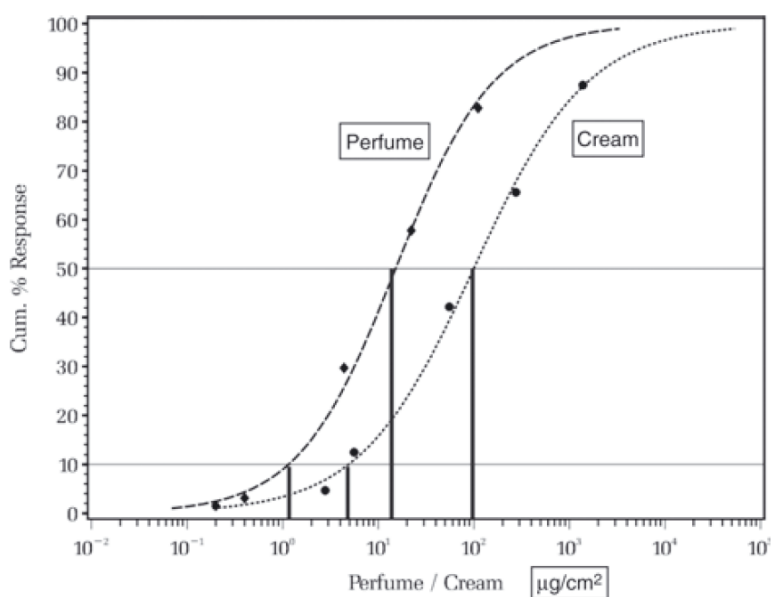
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

In a study by the German Contact Dermatitis Group, 64 persons previously diagnosed with HICC contact allergy were exposed to increasing doses of HICC in 2 different formulations, a hydrophilic cream and an ethanol solution, to mimic everyday exposures, following a standardised ROAT protocol (10). The concentration of HICC tolerated by 90% of the sensitised was estimated as 1.2 µg/cm² for perfume and 4.9 µg/cm² for cream. The dose-response curve is shown in Fig. 4.3 – 1 below.

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (10)			
Design	randomised and vehicle controlled		
Test subjects	67 patients with a previous positive patch test to HICC		
Controls	None		
Substance	Provided by International Flavor & Fragrances Inc, Hilversum, NL		
Patch test			
-dilution steps	2.5% and 5%		
-control/vehicle	petrolatum		
-definition of threshold	lowest concentration giving a positive skin reaction in a continuous line to next higher concentration.		
ROAT	Volar forearms (both sides)		
area	3 x 3 cm (4 areas: one test and one control each for alcoholic solution and cream, respectively)		
applications/day	two		
dose	2.8 µg/cm ² in cream	0.2 µg/cm ² in ethanol	
	5.6 µg/cm ² in cream	0.4 µg/cm ² in ethanol	
	55.6 µg/cm ² in cream	4.4 µg/cm ² in ethanol	
	277.8 µg/cm ² in cream	22.2 µg/cm ² in ethanol	
	1388.9 µg/cm ² in cream	111.1 µg/cm ² in ethanol	
µg /application/cm ²	See above		
control substance	Ethanol 96% and glyceryl stearate 15% in water, resp.		
definition of positive	(spotty) erythema of at least 25% of the test area along with homogeneous infiltration or papules regardless of the number		
period	Two weeks for each step until positive reaction or end of study, whichever occurred first		
Results			
PT ED10% (95% CI)	Not calculable; 52 of 60 Patients patch tested positive to 2.5% HICC, 57 / 60 to 5% HICC		
PT ED50% (95% CI)	Not calculable		
PT no effect level (observed)	Not calculable		
ROAT	Cumulative responses:		
	Cream preparation: 2.8 µg/cm ² : 4.7%	Ethanol preparation: 0.2 µg/cm ² : 1.6%	

	5.6 $\mu\text{g}/\text{cm}^2$: 12.5% 55.6 $\mu\text{g}/\text{cm}^2$: 42.2% 277.8 $\mu\text{g}/\text{cm}^2$: 65.6% 1388.9 $\mu\text{g}/\text{cm}^2$: 87.5%	0.4 $\mu\text{g}/\text{cm}^2$: 3.1% 4.4 $\mu\text{g}/\text{cm}^2$: 29.7% 22.2 $\mu\text{g}/\text{cm}^2$: 57.8% 111.1 $\mu\text{g}/\text{cm}^2$: 82.8%
Controls	No reactions to vehicle in the patients included into analysis	
Other information	See figure below. Three patients were excluded from the study, so results are based on 64 patients.	

Figure 4.3 – 1: Dose-response curve of 64 patients sensitised to HICC, according to a previous PT, regarding two preparations: perfume and cream, the rhomboid and dot symbol, respectively, indicating the observed response. The curve was fitted by a logistic function (10).



Isoeugenol

Isoeugenol (11)	
Design	blinded, randomised doses and controlled
Test subjects	20 patients with a positive patch test to isoeugenol
Controls	20 healthy controls
Substance	purity: 98%
Patch test	20 mg solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.01% (8 steps)
-control/vehicle	petrolatum
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line
ROAT	outer aspect of upper arms
area	5 x 5 cm (2 areas: one test and one control)
applications/day	two with roll-on
dose	0.2% in ethanol
μg /application/cm ²	Doses measured to 0.14 -0.13 mg/application the first 14 days = 5.6 $\mu\text{g}/\text{cm}^2$
control substance	ethanol
definition of positive	any degree of reaction
period	Two weeks at upper arm and if negative another two weeks including application to base of neck
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	0.08% 32 $\mu\text{g}/\text{cm}^2$
PT no effect level (observed)	< 0.01% = 0.4 $\mu\text{g}/\text{cm}^2$
ROAT	
Dose: 0.2%	12/19 (63%)
Controls	No reactions were seen
Other information	

Isoeugenol (12)	
Design	blinded, randomised
Test subjects	27 patients with a positive patch test to isoeugenol
Controls	20 healthy controls
Substance	purity: 98%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.00006% (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions to higher concentrations
ROAT	volar aspect of lower arm
area	3 x 3 cm (2 areas)
applications/day	two with droplet bottle (30 mg per application)
dose	0.05% in ethanol and 0.2%
µg /application/cm ²	Doses were calculated as mean 2.2 µg/cm ² (low conc.) and 9 µg/cm ² (high conc.)
control substance	ethanol
definition of positive	clear visible erythema
period	28 days
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level (observed)	< 0.0005% (5 ppm)
ROAT	Cumulative responses
Dose 1: 0.05%	10/24 (42%)
Dose 2: 0.2%	16/24 (67%)
Controls	No reactions were seen
Other information	Response to the low concentration in the ROAT appeared after median 15 days and to the high concentration after median 7 days.

Isoeugenol (13)	
Design	blinded, randomised and controlled
Test subjects	13 patients with a positive patch test to isoeugenol and 4 in part 1 (pre-test)
Controls	10 healthy controls (dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.00006% (w/v) (16 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting at least + reaction
ROAT	Axilla
area	10 x 10 cm ² (estimated)
applications/day	two with roll-on deodorant (117-586 mg per application of solution) average cases: 266 mg/application controls: only range given
dose	Part 1: Step 1:0.02% Step 2: 0.063% Step 3:0.2% Part 2: Step1:0.0063% Step 2:0.02% Step 3: 0.063%
dose/application/cm ²	Part 2: Step 1: 0.167 Step 2: 0.53 Step 3: 1.67 µg/application/cm ² (calculated based on data)
control substance	deodorant matrix
definition of positive	eczematous response covering 25% of test area
period	Part one: one week with each concentration: maximum three weeks Part two: two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level (observed)	<0.0005% (0.15 µg/cm ²)
ROAT	
Step 1 (0.0063%)	3/13 (23%)
Step 2 (0.02%)	9/13 (69%)
Step 3 (0.063%)	10/13 (77%)
Controls	No reactions were seen
Other information	Deodorants containing cinnamal were responsible for all reactions in cinnamal sensitized individuals ($p<0.001$) and all control persons were negative ($p<0.001$)

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 20, Revision 4 (FGE.20Rev4): Benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters from chemical groups 23 and 30¹

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)^{2,3}

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ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 45 flavouring substances in the Flavouring Group Evaluation 20, Revision 4 (FGE.20Rev4), using the Procedure in Commission Regulation (EC) No 1565/2000. This revision 4 is made due to inclusion of four additional substances, *o*-, *m*- and *p*-tolualdehyde [FL no: 05.026, 05.028 and 05.029] and phenylmethyl 2-methyl-2-butenolate [FL no: 09.858]. None of the substances were considered to have genotoxic potential. The substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that all the substances do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for all 45 candidate substances.

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KEYWORDS

Flavourings, safety, benzyl alcohols, benzaldehydes, benzoic acids, esters, acetals, FGE.20, revision.

1 On request from the European Commission, Question No (EFSA-Q-2012-00621, EFSA-Q-2012-00620, EFSA-Q-2012-00619, EFSA-Q-2012-00618), adopted on 20 November 2012.

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SUMMARY

The European Food Safety Authority (EFSA) asked the Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate 45 flavouring substances in the Flavouring Group Evaluation 20, Revision 4 (FGE.20Rev4), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These flavouring substances belong to chemical groups 23 and 30, Annex I of the Commission Regulation (EC) No 1565/2000.

The present revision of FGE.20, FGE.20Rev4, includes the evaluation of four additional substances, *o*- *m*- and *p*-tolualdehyde [FL no: 05.026, 05.028 and 05.029] and phenylmethyl 2-methyl-2-butenate [09.858].

Four flavouring substances can exist as optical isomers [FL-no: 06.104, 09.313, 09.317 and 09.852] and four substances can exist as geometrical isomers [FL-no: 09.314, 09.560, 09.570 and 09.858].

Forty-one candidate substances are classified into structural class I and four [FL-no: 02.205, 05.066, 05.221 and 06.104] are classified into structural class II according to the decision tree approach presented by Cramer et al., 1978.

Twenty-five flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the “Maximised Survey-derived Daily Intake” (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a “modified Theoretical Added Maximum Daily Intake” (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach, the 41 flavouring substances allocated to structural class I have intakes in Europe from 0.001 to 610 microgram/*capita*/day, which are below the threshold of concern value for structural class I (1800 microgram/person/day). The four substances in structural class II [FL-no: 02.205, 05.066, 05.221 and 06.104] have estimated intakes of 0.011, 1.2, 0.61 and 100 microgram/*capita*/day, respectively. These intakes are below the threshold values of 540 microgram/person/day for structural class II.

On the basis of the reported annual production in Europe (MSDI approach), the combined intake of the 41 of the candidate substances belonging to structural class I is approximately 1400 microgram/*capita*/day and the combined intake of the four candidate substances belonging to structural class II is approximately 100 microgram/*capita*/day. These values are lower than the threshold of concern for structural class I and II substances. Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for 76 of the 77 supporting substances. The total combined intakes of the candidate and supporting substances are approximately 75000 and 7100

microgram/capita/day for structural class I and II, respectively, which exceed the thresholds of concern. However, the substances are expected to be efficiently metabolised and are not expected to saturate the metabolic pathways.

For the substances in this group the available genotoxicity data do not preclude the evaluation of the candidate substances using the Procedure.

It is anticipated that the candidate substances in FGE.20Rev4 would be metabolised to innocuous products.

It was noted that where toxicity data were available they were consistent with the conclusions in the present FGE using the Procedure.

It is considered that on the basis of the default MSDI approach the 45 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI approach they ranged from 770 to 120000 microgram/person/day for 41 flavouring substances from structural class I. The intakes were all above the threshold of concern for structural class I of 1800 microgram/person/day, except for six flavouring substances [FL-no: 05.129, 05.142, 05.153, 05.158, 08.080 and 09.858]. The estimated intakes, based on the mTAMDI, of the four flavouring substances [FL-no: 02.205, 05.066, 05.221 and 06.104] assigned to structural class II were 3900, 1600, 7000 and 3900 microgram/person/day, respectively, which are all above the threshold of concern for the structural class (540 microgram/person/day for structural class II). The six substances which have mTAMDI intake estimates below the threshold of concern for structural class I are also expected to be metabolised to innocuous products. Thus, on the basis of the mTAMDI, the estimated intakes for 39 flavouring substances considered in this Opinion, exceed the relevant threshold for their structural class to which the flavouring substance has been assigned. Therefore, for these 39 substances more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be re-evaluated using the Procedure. Subsequently, additional toxicological data might become necessary.

In order to determine whether the conclusion for the 45 candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for each of the 45 flavouring substances.

For these 45 flavouring substances the Panel concluded that they would present no safety concern at their estimated levels of intake based of the MSDI approach.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) lays down a Procedure for the establishment of a list of flavouring substances the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2008/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999a). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

The FGE is revised to include substances for which data were submitted after the deadline as laid down in Commission Regulation (EC) No 622/2002 and to take into account additional information that has been made available since the previous Opinion on this FGE.

The Union list of flavourings and source materials is established in Commission Regulation (EC) No 872/2012 (EC, 2012a).

HISTORY OF THE EVALUATION

The Flavouring Group Evaluation 20 (FGE.20) dealt with 35 benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters.

The Revision 1 of FGE.20, FGE.20Rev1, included the assessment of one additional candidate substance, vanillin propylene glycol acetal [FL-no: 06.104]. For this substance there are hydrolysis data and for a related substance [FL-no: 02.248] *in vitro* genotoxicity data. Additional information for three substances [FL-no: 09.313, 09.317 and 09.852] was made available since FGE.20 was published. Toxicity and metabolism data on a substance, vanillin 3-l-menthoxypropane-1,2-diol acetal [FL-no: 02.248], related to the candidate substance vanillin propylene glycol acetal [FL-no:06.104], are included.

The Revision 2 of FGE.20, FGE.20Rev2, included the assessment of five additional candidate substances [FL-no: 05.221, 08.132, 08.133, 09.693 and 09.696]. Toxicity data are available for four of the five substances. *In vitro* genotoxicity data are available for [FL-no: 05.221 and 08.133], long-term toxicity data are available for [FL-no: 08.133] and acute toxicity data are available for [FL-no: 08.133, 09.693 and 09.696]. Two of the substances [FL-no: 09.693 and 09.696] were considered with respect to genotoxicity in FGE.202 in which the Panel concluded that the genotoxicity data available do not preclude their evaluation through the Procedure.

The Revision 3 of FGE.20, FGE.20Rev3, included the consideration of the SCF Opinion on benzoic acid (SCF, 2002c). Furthermore, the Industry has for two substances [FL-no: 06.104 and 09.570] submitted information on the stereoisomeric composition (EFFA, 2010a), which was missing in the previous version of the FGE. Finally, the Industry has submitted new information to support the re-allocation of structural class to the candidate substance piperonyl alcohol [FL-no: 02.205]. For piperonyl alcohol [FL-no: 02.205], the Flavouring Industry has submitted new information, since the publication of FGE.20Rev2, that suggests natural occurrence in several food sources of closely structurally related substances, which are most likely metabolised to piperonyl alcohol. Therefore Flavouring Industry considered it correct to answer the question whether the substance occur naturally

with a yes, and therefore the substance should be allocated to structural class II, instead of class III. The Panel agreed in this consideration and allocated [FL-no: 02.205] to structural class II.

FGE	Opinion adopted by EFSA	Link	No. Of candidate substances
FGE.20	7 December 2005	http://www.efsa.europa.eu/en/efsajournal/doc/296.pdf	35
FGE.20Rev1	29 November 2007	http://www.efsa.europa.eu/en/efsajournal/doc/976.pdf	36
FGE.20Rev2	26 November 2009	http://www.efsa.europa.eu/en/scdocs/doc/1405.pdf	41
FGE.20Rev3	17 May 2011	http://www.efsa.europa.eu/en/efsajournal/pub/2176.htm	41
FGE.20Rev4	20 November 2012		45

The present Revision of FGE.20, FGE.20Rev4, includes the assessment of four additional substances *o*-, *m*- and *p*-tolualdehyde [FL-no: 05.026, 05.028 and 05.029] and phenylmethyl 2-methyl-2-butenolate [FL no: 09.858].

No toxicity or metabolism data were submitted for the new substances. A search in the open literature for these substances provided four additional studies on the biotransformation of tolualdehydes. Additional data on toxicity were not found. Some genotoxicity studies on the tolualdehyde isomers were already included in the previous versions of this FGE for a supporting substance (tolualdehyde, mixture of isomers; [FL no: 05.027]). These studies have been relocated in the tables with genotoxicity data, but no new information was included. No additional data were found for substance [FL no: 09.858].

Furthermore, additional information on composition has been submitted for [FL-no: 05.221] (EFFA, 2012k) and [FL-no: 06.104] (EFFA, 2012u).

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the register (Commission decision 1999/217/EC), according to Commission Regulation (EC) No 1565/2000 (EC, 2000a), prior to their authorisation and inclusion in the Union list (Regulation (EC) No 1334/2008). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme. The evaluation programme was finalised at the end of 2009.

In addition, the Commission has asked EFSA to reflect newly submitted information on specifications in the revisions of FGEs.

ASSESSMENT

1. PRESENTATION OF THE SUBSTANCES IN FLAVOURING GROUP EVALUATION 20, REVISION 4

1.1. Description

The present Flavouring Group Evaluation 20, Revision 4 (FGE.20Rev4), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000a) (The Procedure – shown in schematic form in Annex I of this FGE), deals with 45 benzyl alcohols, benzaldehydes, a related acetal, benzoic acids and related esters. These flavouring substances belong to chemical groups 23 and 30, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a).

The 45 candidate substances under consideration with their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structure and specifications, are listed in Table 1.

This group of candidate substances includes 19 benzyl derivatives (subgroup 1), 25 hydroxy- and alkoxy-ringsubstituted benzyl derivatives (subgroup 2) and one hydroxy- and alkoxy-substituted biphenyl derivative (subgroup 3).

- Subgroup 1: Benzyl derivatives

This subgroup comprises three tolualdehyde isomers (*o*-, *m*- and *p*-tolualdehyde [FL no: 05.026, 05.028 and 05.029] and 15 alkyl esters, ten of which are benzyl esters [FL-no: 09.152, 09.313, 09.314, 09.315, 09.316, 09.317, 09.318, 09.611, 09.835 and 09.858] and five are benzoic acid esters [FL-no: 09.631, 09.656, 09.693, 09.779 and 09.825]. Four of these candidate esters contain a double-bond in the alkyl side chain [FL-no: 09.314, 09.656, 09.693 and 09.858] and two contain an alkyl substituent at the aromatic ring [FL-no: 09.631 and 09.611]. The remaining substance [FL-no: 06.017] is an acetal of benzaldehyde.

- Subgroup 2: Hydroxy- and alkoxy-ringsubstituted benzyl derivatives

This subgroup includes two benzyl alcohols [FL-no: 02.164, and the derivative piperonyl alcohol FL-no: 02.205], six benzaldehyde derivatives [FL-no: 05.066, 05.129, 05.142, 05.153, 05.158 and 06.104], four benzoic acids [FL-no: 08.080, 08.087, 08.132 and 08.133] and 13 related esters [FL-no: 09.362, 09.363, 09.367, 09.560, 09.570, 09.581, 09.623, 09.696, 09.762, 09.798, 09.799, 09.852 and 09.895]. One of the esters is a benzyl ester [FL-no: 09.895], all the others are benzoic acid esters. Three of the esters contain a double-bond in the alkyl side chain [FL-no: 09.560, 09.570 and 09.696].

- Subgroup 3: Hydroxy- and alkoxy-substituted biphenyl derivative

This subgroup contains one candidate substance, a hydroxy- and alkoxy-substituted biphenyl derivative [FL-no: 05.221].

The 45 flavouring substances (candidate substances) are closely related structurally to 77 flavouring substances (supporting substances) evaluated at the 46th and 57th JECFA meeting (JECFA, 1997a; JECFA, 2002b). The names and structures of the 77 supporting substances are listed in Table 3, together with their evaluation status (CoE, 1992; JECFA, 1997a; JECFA, 2002b; SCF, 1995).

The 77 supporting substances include 34 benzyl derivatives (subgroup 1) and 43 hydroxy- and alkoxy-substituted benzyl derivatives (subgroup 2).

The hydrolysis products of the candidate esters are listed in Table 2b.

1.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different, they may have different chemical properties resulting in possible variability in their absorption, distribution, metabolism, elimination and toxicity. Thus, information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number etc.).

Three flavouring substances possess one chiral centre [FL-no: 09.313, 09.317 and 09.852] and one flavouring substance possesses two chiral centres [FL-no: 06.104]. Due to the presence and the position of double bonds, four candidate substances can exist as geometrical isomers [FL-no: 09.314, 09.560, 09.570 and 09.858]. For all eight substances, the stereoisomeric composition has been specified (see Table 1).

1.3. Natural Occurrence in Food

Twenty-five candidate substances have been reported to occur in fruit (cherry, mango, papaya, bilberry, black currants, mulberry, sapodilla, cloudberry, pineapple, grape), cocoa, potato, coffee, tea, beer, rum, sherry, whisky, wine, honey, spices, soybean, peanut, wort and pork. Quantitative data on the natural occurrence of these substances have been reported for the occurrence of 16 of these substances in food.

Table 1.3.1 Candidate Substances Reported to Occur in Food (TNO, 2000; EFA, 2010a; TNO, 2012)

FL-no:	Name:	Quantitative data reported:
05.026	o-Tolualdehyde	0.6 mg/kg in allium species.
05.029	p-Tolualdehyde	Up to 0.3 mg/kg in tea, up to 0.004 mg/kg in honey.
05.129	2-Methoxybenzaldehyde	7000 mg/kg in cassia leaf (oil), up to 1500 mg/kg in cinnamon bark (oil).
05.142	3,4-Dihydroxybenzaldehyde	Up to 20 mg/kg in coffee, 313 mg/kg in bourbon vanilla.
05.153	4-Hydroxy-3,5-dimethoxybenzaldehyde	Up to 0.7 mg/kg in beer, up to 9.2 mg/kg in grape, up to 0.014 mg/kg in mango, 0.08 mg/kg in pineapple, 8.3 mg/kg in pork, up to 19.9 mg/kg in rum, 0.035 mg/kg in sherry, 1.9 mg/kg in bourbon vanilla, up to 8.7 mg/kg in whisky, up to 0.86 mg/kg in red wine, up to 0.04 mg/kg in wort.
05.158	3-Methoxybenzaldehyde	3900 mg/kg in clove bud (oil).
08.080	Gallic acid	Up to 0.6 mg/kg in beer, up to 7 mg/kg in cherry, up to 11 mg/kg in grape, up to 6.1 mg/kg in whisky, up to 35 mg/kg in wine.
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid	Up to 1.1 mg/kg in beer, 1.3 mg/kg in grape, up to 0.096 mg/kg in mango, up to 18 mg/kg in rum, up to 34 mg/kg in soybean, up to 1.4 mg/kg in whisky, up to 10 mg/kg in wine.
08.132	3-Hydroxybenzoic acid	Up to 2.7 mg/kg in honey.

Table 1.3.1 Candidate Substances Reported to Occur in Food (TNO, 2000; EFFA, 2010a; TNO, 2012)

FL-no:	Name:	Quantitative data reported:
08.133	3,4-Dihydroxybenzoic acid	Up to 1.4 mg/kg in brandy, up to 0.4 mg/kg in beer, up to 52 mg/kg in black currants, up to 6.8 mg/kg in honey, 4.3 mg/kg in mulberry, 0.15 mg/kg in rum, 10 mg/kg in soybean, up to 0.3 mg/kg in whisky, up to 10 mg/kg in wine.
09.152	Benzyl valerate	0.11 mg/kg in sea buckthorn.
09.314	Benzyl crotonate	0.0001 mg/kg in papaya.
09.779	Butyl benzoate	200 mg/kg in galanga (oil), 2 mg/kg in hog plum, up to 0.05 mg/kg in papaya.
09.798	Ethyl vanillate	0.3 mg/kg in rum, up to 113 mg/kg in red wine.
09.799	Methyl vanillate	0.05 mg/kg in cloudberry, up to 214 mg/kg in red wine.
09.825	Pentyl benzoate	0.001 mg/kg in bilberry, trace amounts in sapodilla fruit.

According to TNO the remaining 20 substances have not been reported to occur naturally in any food items.

Table 1.3.2 Candidate Substances Not Reported to Occur in Food (TNO, 2000; TNO, 2012)

FL-no:	Name:
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol
09.858	Phenylmethyl 2-methyl-2-butenate
09.315	Benzyl dodecanoate
09.317	Benzyl lactate
09.318	Benzyl octanoate
09.362	Ethyl 2-hydroxy-4-methylbenzoate
09.560	Hex-3(cis)-enyl anisate
09.581	Hexyl salicylate
09.611	4-Isopropylbenzyl acetate
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate
09.656	3-Methylbut-3-enyl benzoate
09.693	Prenyl benzoate
09.696	Prenyl salicylate
09.762	Pentyl salicylate
09.835	Benzyl decanoate
09.852	2-Methylbutyl 2-hydroxybenzoate
09.895	4-Methoxybenzyl-2-methylpropionate
05.066	4-Ethoxy-3-methoxybenzaldehyde
05.221	6,6'-Dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde
06.104	Vanillin propylene glycol acetal

2. SPECIFICATIONS

Purity criteria for the 45 candidate substances have been provided by the Flavour Industry (EFFA, 2003u; EFFA, 2004c; EFFA, 2007d; EFFA, 2011e; Flavour Industry, 2008c).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), this information is adequate for all the 45 substances (see Section 1.2 and Table 1).

3. INTAKE DATA

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the “Maximised Survey-derived Daily Intake” (MSDI) by assuming that the production figure only represents 60 % of the use in food due to underreporting and that 10 % of the total EU population are consumers (SCF, 1999a).

However, the Panel noted that due to year-to-year variability in production volumes, to uncertainties in the underreporting correction factor and to uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that in contrast to the generally low *per capita* intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of products flavoured at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds below which exposures are not considered to present a safety concern might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the SCF recommended also taking into account the results of other intake assessments (SCF, 1999a).

One of the alternatives is the “Theoretical Added Maximum Daily Intake” (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g., it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported) (EC, 2000a). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004a).

3.1. Estimated Daily *per Capita* Intake (MSDI Approach)

The intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999a). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995a). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population⁴ (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999a).

⁴ EU figure 375 millions. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

The total annual volume of production of the candidate substances in the present Flavouring Group Evaluation (FGE.20Rev4) from use as flavouring substances in Europe has been reported to be approximately 12500 kg (EFFA, 2003u; EFFA, 2004d; EFFA, 2007d; EFFA, 2011e; EFFA, 2012m; Flavour Industry, 2008c). For 76 of the 77 supporting substances the total annual volume of production is approximately 660000 kg in Europe (vanillin [FL-no: 05.018] accounts for 390000 kg) (JECFA, 2002a). The annual volume of production in Europe for one of the supporting substances [FL-no: 09.754] has not been reported.

On the basis of the annual volumes of production reported for the 45 candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated. Approximately 97 % of the annual volume of production for the candidate substances is accounted for by four substances [FL-no: 05.029; 06.104, 08.132 and 08.133]. The estimated daily *per capita* intake of these four candidate substances from use as flavouring substances is 160, 100, 610 and 610 microgram, respectively. For each of the remaining substances the estimated daily *per capita* intake is 10 microgram or less (Table 2a).

3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For all candidate substances information on food categories and normal and maximum use levels^{5,6,7} were submitted by the Flavour Industry (EFFA, 2003u; EFFA, 2004c; EFFA, 2007a; EFFA, 2007d; EFFA, 2012m; EFFA, 2012o; EFFA, 2012q; Flavour Industry, 2008c). The candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

⁵ "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).

⁶ The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

⁷ The use levels from food category 5 "Confectionery" have been inserted as default values for food category 14.2 "Alcoholic beverages" for substances for which no data have been given for food category 14.2 (EFFA, 2007a).

Table 3.1 Use of Candidate Substances in Various Food Categories

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All except [FL-no: 08.132, 08.133, 09.858]
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All except [FL-no: 05.026, 05.028, 05.029, 08.132, 08.133, 09.858]
03.0	Edible ices, including sherbet and sorbet	All except [FL-no: 05.026, 05.028, 05.029, 08.132, 08.133, 09.858]
04.1	Processed fruits	All except [FL-no: 05.026, 05.028, 05.029, 05.221, 08.132, 08.133, 09.858]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
05.0	Confectionery	All
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All except [FL-no: 05.026, 05.028, 05.029, 05.221, 08.132, 08.133, 09.858]
07..0	Bakery wares	All except [FL-no 05.129, 08.132, 08.133]
08.0	Meat and meat products, including poultry and game	All except [FL-no: 05.026, 05.028, 05.029, 05.221, 08.132, 08.133, 09.858]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All except [FL-no: 05.026, 05.028, 05.029, 05.221, 08.132, 08.133, 09.825]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products etc.	All except [FL-no: 05.026, 05.028, 05.029, 08.132, 08.133]
13.0	Foodstuffs intended for particular nutritional uses	All except [FL-no: 05.026, 05.028, 05.029, 05.221, 08.132, 08.133, 09.858]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All except [FL-no: 09.858]
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All except [FL-no: 09.858]
15.0	Ready-to-eat savouries	All except [FL-no: 05.026, 05.028, 05.029, 08.132, 08.133, 09.693, 09.858]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All except [FL-no: 05.026, 05.028, 05.029, 08.132, 08.133, 09.858]

According to the Flavour Industry the normal use levels for the candidate substances are in the range of 1 - 500 mg/kg food, and the maximum use levels are in the range of 5 - 2000 mg/kg (EFFA, 2003u; EFFA, 2004c; EFFA, 2007a; EFFA, 2007d; EFFA, 2012m; EFFA, 2012o; EFFA, 2012q; Flavour Industry, 2008c).

The mTAMDI value is 770 - 120000 microgram/person/day for the 41 candidate substances from structural class I (see Section 5). For the candidate substances [FL-no: 02.205, 05.066, 05.221, and 06.104] from structural class II (see Section 5) the mTAMDI are 3900, 1600, 7000 and 3900 microgram/person/day, respectively.

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 6 and Annex II.

4. ABSORPTION, DISTRIBUTION, METABOLISM AND ELIMINATION

The 45 candidate substances are subdivided into three subgroups. Subgroup 1 includes three tolualdehyde isomers and 16 benzyl derivatives of which 15 are benzyl esters or benzoic acid esters and one is an acetal, [FL-no: 06.017] (diethoxymethyl)benzene. Subgroup 2 includes 25 hydroxy- and alkoxy-substituted benzyl derivatives of which 12 are benzyl alcohols, benzaldehydes or benzoic acids and 13 are related esters. Subgroup 3 contains one derivative of biphenyl [FL-no: 05.221] (6,6'-dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde).

Subgroup 1

Nine of the 15 esters from subgroup 1, benzyl valerate [FL-no: 09.152], benzyl 2-methylbutyrate [FL-no: 09.313], benzyl crotonate [FL-no: 09.314], benzyl dodecanoate [FL-no: 09.315], benzyl hexanoate [FL-no: 09.316], benzyl lactate [FL-no: 09.317], benzyl octanoate [FL-no: 09.318], benzyl decanoate [FL-no: 09.835] and phenylmethyl 2-methyl-2-butenate [FL-no: 09.858], will yield benzyl alcohol, which has previously been evaluated by the JECFA (JECFA, 1996b) and SCF (SCF, 2002b). One candidate ester, 4-isopropylbenzyl acetate [FL-no: 09.611], will yield 4-isopropylbenzyl alcohol, previously evaluated by the JECFA (JECFA, 2002a). The benzyl alcohols are expected to be oxidised to corresponding benzoic acids, which will be conjugated with glycine and excreted as hippuric acids. Of the remaining five candidate esters in subgroup 1, four are expected to yield benzoic acid and simple aliphatic alcohols upon hydrolysis, 3-methylbut-3-enyl benzoate [FL-no: 09.656], butyl benzoate [FL-no: 09.779], pentyl benzoate [FL-no: 09.825] and prenyl benzoate [FL-no: 09.693]. One ester, methyl 4-methylbenzoate [FL-no: 09.631], will yield 4-methylbenzoic acid upon hydrolysis. Benzoic acid will mainly be conjugated with glycine and excreted as hippuric acid. Conjugation with glycine may be a saturable process with increasing levels of exposure and glucuronide conjugation may become relatively more important.

One of the substances in subgroup 1 is an acetal, (diethoxymethyl)benzene [FL-no: 06.017]. This substance would be expected to yield benzaldehyde and ethanol upon hydrolysis. Benzaldehyde has been evaluated by the JECFA (JECFA, 1996b). Benzaldehyde is expected to be oxidized to benzoic acid and subsequently conjugated with glycine or glucuronic acid and eliminated via the urine. The same biotransformations will occur with the three tolualdehyde isomers ([FL no: 05.026, 05.028 and 05.029]). Additionally, for the tolualdehyde isomers, reduction of the aldehyde function to yield the corresponding alcohol has also been demonstrated. These alcohols can be converted into the corresponding sulphate esters, which in their turn can further react with glutathione to give benzylmercapturic acids. This metabolic pathway is more important for *o*-toluadehyde than for the other two isomers, but at any rate, only a limited fraction of the dose (< 10 %) will be eliminated via this route.

Subgroup 2

Subgroup 2 includes 13 esters of which one, 4-methoxybenzyl-2-methylpropionate [FL-no: 09.895], will yield 4-methoxybenzyl alcohol (*p*-anisyl alcohol) (supporting substance [FL-no: 02.128]) upon hydrolysis. This substance has been evaluated by the JECFA (JECFA, 2002a). 4-Methoxybenzyl alcohol is expected to be excreted in the urine either unchanged or as glucuronic acid, glycine or sulphate conjugate. The same metabolic pathway is proposed for the candidate benzyl alcohol derivative, 4-hydroxy-3,5-dimethoxybenzyl alcohol [FL-no: 02.164].

The remaining 12 esters in subgroup 2, ethyl 2-hydroxy-4-methylbenzoate [FL-no: 09.362], ethyl 2-methoxybenzoate [FL-no: 09.363], ethyl 4-hydroxybenzoate [FL-no: 09.367], hex-3(cis)-enyl anisate [FL-no: 09.560] (hex-3(cis)-enyl 4-methoxybenzoate), hex-3-enyl salicylate [FL-no: 09.570] (hex-3-enyl 2-hydroxybenzoate), hexyl salicylate [FL-no: 09.581] (hexyl 2-hydroxybenzoate), methyl 2,4-dihydroxy-3,6-dimethylbenzoate [FL-no: 09.623], prenyl salicylate [FL-no: 09.696] (3-methylbut-2-enyl 2-hydroxybenzoate), pentyl salicylate [FL-no: 09.762] (pentyl 2-hydroxybenzoate), ethyl vanillate [FL-no: 09.798] (ethyl 3-methoxy-4-hydroxybenzoate), methyl vanillate [FL-no: 09.799]

(methyl 3-methoxy-4-hydroxybenzoate), 2-methylbutyl 2-hydroxybenzoate [FL-no: 09.852] (2-methylbutyl salicylate) will yield alkoxy- and/or hydroxy-substituted benzoic acids upon hydrolysis. The substituted benzoic acids that are hydrolysis products of candidate esters are expected to be excreted in the urine unchanged or as the glucuronic acid, glycine or sulphate conjugates. The same metabolic route is proposed for the candidate acids, 4-hydroxy-3,5-dimethoxybenzoic acid [FL-no: 08.087], 3-hydroxybenzoic acid [FL-no: 08.132] and 3,4-dihydroxybenzoic acid [FL-no: 08.133].

The main metabolic pathway for the acetal, vanillin propylene glycol acetal [FL-no: 06.104], after hydrolysis to the aldehyde, and for the five candidate aldehydes in subgroup 2, 4-ethoxy-3-methoxybenzaldehyde [FL-no: 05.066], 2-methoxybenzaldehyde [FL-no: 05.129], 3,4-dihydroxybenzaldehyde [FL-no: 05.142], 4-hydroxy-3,5-dimethoxybenzaldehyde [FL-no: 05.153], 3-methoxybenzaldehyde [FL-no: 05.158], is presumed to be oxidation of the aldehyde to the corresponding acids, followed by conjugation and excretion. The reduction to alcohols is a minor metabolic route and the oxidative pathway dominates clearly. For 2-methoxybenzaldehyde it has been shown that this reductive metabolic pathway leads to the formation of sulphate conjugates, which are converted into glutathione conjugates. The latter are eliminated via the urine as mercapturic acids. To a minor extent O-demethylation followed by conjugation may occur.

The candidate substance piperonyl alcohol [FL-no: 02.205] (3,4-methylenedioxybenzylalcohol) is expected to mainly undergo oxidation and conjugation of the side chain, and be excreted as glycine conjugate. Demethylenation of the methylenedioxy moiety is a very minor metabolic path for this compound.

The main metabolite of gallic acid [FL-no: 08.080] (3,4,5-trihydroxybenzoic acid) is expected to be 4-O-methyl gallic acid (3,5-dihydroxy-4-methoxybenzoic acid), the product of O-methylation. Decarboxylation to pyrogallol (1,2,3-trihydroxybenzene) may occur as a very minor pathway, but no further dehydroxylation to catechol has been observed.

Subgroup 3

The biphenyl substance in subgroup 3 [FL-no: 05.221] is expected to be metabolised in a similar way to the benzaldehyde derivatives in subgroup 2. It is expected that the aldehyde group(s) will undergo oxidation to form the corresponding carboxylic acid which is likely to be conjugated and excreted. The reduction to alcohol may again be a minor pathway, but some steric hindrance may occur making this less likely than for the benzaldehyde derivatives in subgroup 2.

Based on experimental evidence and general knowledge of toxicokinetics of structurally related compounds, it is expected, that at the reported levels of intake as flavouring substances, the candidate substances are metabolised to innocuous products.

For more detailed information, see Annex III.

5. APPLICATION OF THE PROCEDURE FOR THE SAFETY EVALUATION OF FLAVOURING SUBSTANCES

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

For the safety evaluation of the 45 candidate substances from chemical groups 23 and 30 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the substances are summarised in Table 2a.

Step 1

Forty-one of the flavouring substances are classified according to the decision tree approach by Cramer et al. (Cramer et al., 1978) into structural class I, four are classified into structural class II [FL-no: 02.205, 05.066, 05.221 and 06.104].

Step 2

Step 2 requires consideration of the metabolism of the candidate substances. It can be anticipated that at the estimated levels of intake all candidate substances are expected to be metabolised to innocuous products. Accordingly, the evaluation of these substances proceeds via the A-side of the Procedure scheme.

Step A3

The estimated levels of the European daily *per capita* intake (MSDI) for the 41 candidate substances classified into structural class I are in the range of 0.0012 to 610 micrograms. For the four candidate substances [FL-no: 02.205, 05.066, 05.221 and 06.104] classified into structural class II, the intakes are 0.011, 1.2, 0.61 and 100 micrograms, respectively (Table 2a). These intakes are below the thresholds of concern of 1800 and 540 microgram/person/day for structural class I and II, respectively.

Based on results of the safety evaluation sequence of the Procedure, these 45 candidate substances do not pose a safety concern when used as flavouring substances at the estimated levels of intake, based on the MSDI approach.

6. COMPARISON OF THE INTAKE ESTIMATIONS BASED ON THE MSDI APPROACH AND THE mTAMDI APPROACH

The estimated intakes for the 41 candidate substances in structural class I, based on the mTAMDI approach, range from 770 to 120000 microgram/person/day. For six of the substances [FL-no: 05.129, 05.142, 05.153, 05.158, 08.080 and 09.858] the mTAMDI values are below the threshold of concern of 1800 microgram/person/day for structural class I. For the remaining 35 substances in class I, the mTAMDI is above the threshold of concern.

The estimated intake of the four candidate substances [FL-no: 02.205, 05.066, 05.221 and 06.104] assigned to structural class II, based on the mTAMDI, are 3900, 1600, 7000 and 3900 microgram/person/day, respectively. These intakes are above the threshold of concern of 540 microgram/person/day for structural classes II.

Thus, for 39 candidate substances, for which the mTAMDI is above the threshold of concern, further information is required. This would include more reliable intake data and subsequently, if required, additional toxicological data.

For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach, see Table 6.1.

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/capita/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol	0.037	3900	Class I	1800
05.026	o-Tolualdehyde	1.0	9100	Class I	1800
05.028	m-Tolualdehyde	0.85	9100	Class I	1800
05.029	p-Tolualdehyde	160	9100	Class I	1800

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/capita/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
05.129	2-Methoxybenzaldehyde	0.16	1400	Class I	1800
05.142	3,4-Dihydroxybenzaldehyde	8.5	1600	Class I	1800
05.153	4-Hydroxy-3,5-dimethoxybenzaldehyde	0.74	1600	Class I	1800
05.158	3-Methoxybenzaldehyde	0.011	1600	Class I	1800
06.017	(Diethoxymethyl)benzene	1.7	3900	Class I	1800
08.080	Gallic acid	0.011	1600	Class I	1800
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid	1.2	3200	Class I	1800
08.132	3-Hydroxybenzoic acid	610	120000	Class I	1800
08.133	3,4-Dihydroxybenzoic acid	610	120000	Class I	1800
09.152	Benzyl valerate	1.7	3900	Class I	1800
09.313	Benzyl 2-methylbutyrate	7.3	3900	Class I	1800
09.314	Benzyl crotonate	0.37	3900	Class I	1800
09.315	Benzyl dodecanoate	0.13	3900	Class I	1800
09.316	Benzyl hexanoate	0.75	3900	Class I	1800
09.317	Benzyl lactate	0.91	3900	Class I	1800
09.318	Benzyl octanoate	0.12	3900	Class I	1800
09.362	Ethyl 2-hydroxy-4-methylbenzoate	0.0012	3900	Class I	1800
09.363	Ethyl 2-methoxybenzoate	5.5	3900	Class I	1800
09.367	Ethyl 4-hydroxybenzoate	10	3900	Class I	1800
09.560	Hex-3(cis)-enyl anisate	0.12	3900	Class I	1800
09.570	Hex-3-enyl salicylate	0.13	3900	Class I	1800
09.581	Hexyl salicylate	0.018	3900	Class I	1800
09.611	4-Isopropylbenzyl acetate	0.012	3900	Class I	1800
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate	0.012	3900	Class I	1800
09.631	Methyl 4-methylbenzoate	0.0012	3900	Class I	1800
09.656	3-Methylbut-3-enyl benzoate	0.12	3900	Class I	1800
09.693	Prenyl benzoate	0.012	4900	Class I	1800
09.696	Prenyl salicylate	0.011	3900	Class I	1800
09.762	Pentyl salicylate	0.24	3900	Class I	1800
09.779	Butyl benzoate	3.7	3900	Class I	1800
09.798	Ethyl vanillate	0.024	3900	Class I	1800
09.799	Methyl vanillate	0.011	3900	Class I	1800
09.825	Pentyl benzoate	1.1	3900	Class I	1800
09.835	Benzyl decanoate	0.35	3900	Class I	1800
09.852	2-Methylbutyl 2-hydroxybenzoate	0.011	3900	Class I	1800
09.858	Phenylmethyl 2-methyl-2-butenate	0.037	770	Class I	1800
09.895	4-Methoxybenzyl-2-methylpropionate	0.37	3900	Class I	1800
02.205	Piperonyl alcohol	0.011	3900	Class II	540
05.066	4-Ethoxy-3-methoxybenzaldehyde	1.2	1600	Class II	540
05.221	6,6'-Dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde	0.61	7000	Class II	540
06.104	Vanillin propylene glycol acetal	100	3900	Class II	540

7. CONSIDERATIONS OF COMBINED INTAKES FROM USE AS FLAVOURING SUBSTANCES

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this FGE may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The total estimated combined daily *per capita* intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2003u; EFFA, 2004d; EFFA, 2007d; EFFA, 2011e; EFFA, 2012m; Flavour Industry, 2008c) the combined estimated daily *per capita* intake as flavourings of the 41 candidate flavouring substances assigned to structural class I is 1400 microgram. This value does not exceed the threshold of concern for a substance belonging to structural class I of 1800 microgram/person/day. The combined estimated daily *per capita* intake as

flavourings of the four candidate flavouring substances assigned to structural class II is 100 microgram. This value does not exceed the threshold of concern for a substance belonging to structural class II of 540 microgram/person/day.

The candidate substances are structurally related to 77 supporting substances evaluated by the JECFA at its 46th and 57th meeting (JECFA, 1996b; JECFA, 2002a). Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for 76 of the 77 supporting substances. Production volumes in Europe were not reported for one of the supporting substances [FL-no: 09.754].

The total combined intakes of the candidate and supporting substances are approximately 75000 and 7100 microgram/*capita*/day for structural class I and II, respectively, which exceed the thresholds of concern of 1800 and 540 microgram/*capita*/day for structural classes I and II, respectively. However, the supporting substances were evaluated by the JECFA at the 46th and 57th meeting, where it was noted that although the combined intakes exceed the thresholds for the structural classes, the substances are expected to be efficiently detoxicated and the available detoxication pathways would not be saturated.

The Panel agreed with this view and concluded that the contributions to the total combined intakes of the candidate substances of about 1400 and 100 microgram/*capita*/day for structural class I and II, respectively, would not alter the JECFA conclusion based on combined intakes of approximately 75000 and 7100 microgram/*capita*/day for the two classes. The Panel noted that a considerable proportion of this combined intake is accounted for by the supporting substance vanillin [FL-no: 05.018] and for this compound the JECFA has allocated an Acceptable Daily Intake (ADI) of 0 - 10 mg/kg body weight (bw) (JECFA, 1967a; JECFA, 2002b).

8. TOXICITY

8.1. Acute Toxicity

Data are available for 13 candidate substances and for 63 structurally related supporting substances evaluated by the JECFA (JECFA, 2002a). The LD₅₀ values range from 500 to more than 5000 mg/kg body weight (bw) in four different animal species.

The acute toxicity data are summarised in Annex IV, Table IV.1.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Benzyl Derivatives (Subgroup 1)

There are no data available on short-term and long-term toxicity of candidate substances from subgroup 1 (benzyl derivatives). Data on benzyl derivatives are available for 10 supporting substances, which have been tested for subacute oral toxicity [FL-no: 05.110], for subchronic oral toxicity [FL-no: 09.051, 09.725, 09.812, 09.803 and 05.027] and for chronic toxicity and carcinogenicity [FL-no: 02.010, 09.014, 05.013 and 08.021].

Results from carcinogenicity studies on benzyl alcohol, benzyl acetate and benzaldehyde by administration via gavage showed that in some of the studies, in mice benign squamous cell hyperplasia and papillomas of the forestomach could be induced without progression into malignant carcinomas. In male mice benzyl acetate also induced adenomas (no carcinomas) in the liver. No tumorigenic effects were observed in rats. These substances are not genotoxic. The Panel considered the observed pathological changes in mouse forestomach and livers to be of no toxicological relevance for humans following dietary exposure at the indicated levels of use. This consideration is based on the observation that the spontaneous incidence of tumors is that high that the mouse carcinogenicity

study does not contribute to human risk assessment (Billington et al., 2010; Carmichael et al., 1997; EFSA, 2012). These substances have been evaluated by the JECFA (JECFA, 1996b). The JECFA concluded that “the data reviewed for compounds in this group were sufficient to demonstrate the lack of teratogenic, reproductive or carcinogenic potential”. A group ADI of 0 - 5 mg/kg bw was allocated to these compounds. The SCF evaluated data on benzyl alcohol (SCF, 2002b) and concluded that it did not show compound-related effects with respect to carcinogenicity.

Four candidate esters in subgroup 1, 3-methylbut-3-enyl benzoate [FL-no: 09.656], butyl benzoate [FL-no: 09.779], pentyl benzoate [FL-no: 09.825], prenyl benzoate [FL-no: 09.693], yield benzoic acid and simple aliphatic alcohols upon hydrolysis. One of the substances in subgroup 1 is an acetal, (diethoxymethyl)benzene [FL-no: 06.017], which would be expected to yield benzaldehyde and ethanol upon hydrolysis, and in turn benzaldehyde is expected to be oxidized to benzoic acid. The SCF (2002) has established a group ADI of 5 mg/kg bw for benzoic acid and its salts including benzyl alcohol and related benzyl derivatives used as flavourings, based on a developmental toxicity study in rats (SCF, 2002c)⁸.

From two studies with the supporting substance [FL no: 05.027], a mixture of tolualdehyde isomers, a NOAEL of 250 mg/kg bw could be derived, based on the study by Brantom et al., 1972 (Brantom et al., 1972). The second study (Oser et al., 1965) only provide information that no toxicity was observed at one exposure level of ca. 40 mg/kg bw/day when administered to male and female rats for 90 days.

Hydroxy-/Alkoxy- Substituted Benzyl Derivatives (Subgroup 2)

Short- and long-term toxicity data on hydroxy- and alkoxy-substituted benzyl derivatives (subgroup 2) are available for five candidate substances and eight supporting substances. The candidate substances have been tested for subacute oral toxicity [FL-no: 05.142, 08.080, 08.087 and 08.133] and for subchronic oral toxicity [FL-no: 08.080, 08.133 and 09.367]. There are data available on chronic toxicity and carcinogenicity for one candidate substance [FL-no: 08.133] in a study designed to evaluate incidences of lesions (hyperplasia, papillomas, squamous cell carcinoma and sarcoma incidence) in the forestomach in rats. No lesions developed in the forestomach of the rats. Other organs (oesophagus, stomach, intestines, liver and kidney) were inspected grossly. Mean body weight was not different from the control, but relative liver and kidney weight was significantly increased, but not further evaluated.

For the supporting substances, data are available on subacute oral toxicity [FL-no: 09.796], on subchronic oral toxicity [FL-no: 05.015 and 09.751] and on chronic toxicity and carcinogenicity [FL-no: 09.754, 05.018, 09.749, 05.019 and 05.016].

Ethyl 4-hydroxybenzoate and other parabens were evaluated by SCF in 1994 (SCF, 1996). From subchronic and chronic toxicity tests conducted in rats, dogs and mice, an overall NOAEL of 1000 mg/kg bw/day was derived. This NOAEL value has been confirmed for ethyl- and methyl paraben by EFSA (EFSA, 2004b).

Repeated dose toxicity data are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

There are data available for one candidate substance [FL-no: 09.367] (subgroup 2) and for 12 supporting substances of which four belong to subgroup 1 [FL-no: 02.010, 05.013, 08.021 and 09.014] and eight to subgroup 2 [FL-no: 05.016, 05.017, 05.018, 05.019, 08.076, 08.112, 09.749 and 09.754].

⁸ The CEF panel is aware that the benzoic acid is currently under reviewing in the ANS Panel.

For the candidate substance ethyl 4-hydroxybenzoate (ethyl paraben) [FL-no: 09.367], a NOAEL of 2600 mg/kg bw/day has been reported for developmental toxicity in rats (Moriyama et al, 1975), while a NOAEL of 460 mg/kg bw/day was found in the same study for maternal toxicity. From another study a NOAEL of 1043 mg/kg bw/day is available for reproductive toxicity in male rats (Oishi, 2004). Ethyl paraben has been evaluated as a food additive by the AFC panel, and the Panel considered 1000 mg/kg bw/day as the overall NOAEL, based on the absence of effects on sex hormones and on the male reproductive organs in juvenile rats at doses up to 1000 mg/kg bw/day in the above study (EFSA, 2004b).

As there are valid and sufficient studies available on the candidate substance ethyl paraben, the data on the supporting substance butyl paraben [FL-no: 09.754] were not considered for the evaluation of ethyl paraben.

Developmental/reproductive toxicity data are summarised in Annex IV, Table IV.3.

8.4. Genotoxicity Studies

Data from *in vitro* tests are available for 12 candidate substances [subgroup 1: FL-no: 05.026, 05.028, 05.029, 09.631; subgroup 2: FL-no: 09.367, 05.129, 05.158, 08.080, 05.153, 08.087 and 08.133; subgroup 3: FL-no: 05.221], for 28 supporting substances (12 from subgroup 1 and 16 from subgroup 2) and for one related substance (vanillin 3-(1-menthoxy)propane-1,2-diol acetal [FL-no: 02.248] related to subgroup 2). Data from *in vivo* tests are available for two candidate substances from subgroup 2 [FL-no: 09.367 and 08.080] and for 10 supporting substances (three from subgroup 1 and seven from subgroup 2).

All the candidate substances [FL-no: 05.026, 05.028, 05.029, 05.129, 05.153, 05.221, 08.080, 08.087, 09.367 and 09.631] tested for bacterial gene mutations gave negative results. For six candidate substances [FL-no: 09.367, 05.129, 05.158, 08.080, 08.087 and 08.133] both positive and/or negative results were reported in various other *in vitro* test systems (Rec assay, chromosomal aberration test, sister chromatid exchange (SCE) and mammalian cell gene mutation assays (mouse lymphoma tests and silk worm)) for most of which the validity cannot be evaluated or are known to be of very limited relevance.

The same situation was observed for the supporting substances. All the available bacterial gene mutation assays on supporting substances gave negative results. Both positive and negative results were reported in other *in vitro* test systems (Rec assay, chromosomal aberration test, sister chromatid exchange (SCE) and mammalian cell gene mutation assay) for most of which, however, the validity cannot be evaluated.

The available *in vivo* studies on candidate substances reported negative results for ethyl 4-hydroxybenzoate [FL-no: 09.367] in a chromosome aberration assay in rat bone marrow cells and for gallic acid [FL-no: 08.080] in a bioassay in the rat liver. However, due to very limited details on method and results, the validity of these studies cannot be evaluated.

The Panel noted that the supporting substance benzyl acetate [FL-no: 09.014] was positive in an *in vivo* Comet assay, which may indicate a genotoxic activity at high dose levels. The study was considered of limited validity. However, all other *in vivo* studies with benzyl acetate were negative and several of these studies, among which an unscheduled DNA synthesis (UDS) test in the liver and a mouse bone marrow micronucleus test, were considered to be of good quality (NTP, 1993d). Additionally, in the long term carcinogenicity studies with benzyl acetate (Table IV.2), no carcinogenic effects were observed in mice and rats after administration via the diet (NTP, 1993d). In a previous study by NTP (1986) in which this substance was administered by gavage in corn oil, concern was raised, in particular about pancreatic tumours in rats, but for these tumours a confounding influence of the vehicle was suspected. In two other genotoxicity studies, specifically aiming at the

determination of benzyl acetate-induced DNA damage (UDS test and alkaline elution assay) in rat pancreas, no indications of a genotoxic effect were obtained, although these studies were of limited or inaccessible validity. Taking all this information into account, the Panel considered the positive result from the *in vivo* Comet assay as insufficient grounds to preclude the evaluation of benzyl acetate via the Procedure.

Furthermore, all the studies carried out with 10 different supporting substances, among which were benzyl alcohol, benzyl acetate and benzaldehyde, give no indication of a genotoxic potential *in vivo* in several studies for different genetic endpoints and by different routes of administration.

Conclusion on genotoxicity:

While some of the *in vitro* studies indicated equivocal weak positive or positive results, considering the weight of evidence from candidate and supporting substances and the *in vivo* studies, the Panel concluded there was no safety concern with respect to genotoxicity of the substances in the present flavouring group.

Genotoxicity data are summaries in Annex IV, Table IV.4 and Table IV.5.

CONCLUSIONS

The present revision of FGE.20, FGE.20Rev4, includes the assessment of four additional substances, *o*- *m*- and *p*-tolualdehyde [FL no: 05.026, 05.028 and 05.029] and phenylmethyl 2-methyl-2-butenate [09.858].

So, the present FGE.20Rev4 deals in total with 45 benzyl alcohols, benzaldehydes, related acetals, benzoic acids and related esters and a hydroxy- and alkoxy-substituted biphenyl derivative. These flavouring substances belong to chemical groups 23 and 30 of Annex I of Regulation (EC) No 1565/2000.

Four flavouring substances can exist as optical isomers [FL-no: 06.104, 09.313, 09.317 and 09.852] and four substances can exist as geometrical isomers [FL-no: 09.314, 09.560, 09.570 and 09.858].

Forty-one candidate substances are classified into structural class I and four [FL-no: 02.205, 05.066, 05.221 and 06.104] are classified into structural class II according to the decision tree approach presented by Cramer et al., 1978.

Twenty-five of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach, the 41 flavouring substances allocated to structural class I have intakes in Europe from 0.001 to 610 microgram/*capita*/day, which are below the threshold of concern value for structural class I (1800 microgram/person/day). The four substances in structural class II [FL-no: 02.205, 05.066, 05.221 and 06.104] have estimated intakes of 0.011, 1.2, 0.61 and 100 microgram/*capita*/day, respectively. These intakes are below the threshold values of 540 microgram/person/day for structural class II.

On the basis of the reported annual production in Europe (MSDI approach), the combined intake of the 41 of the candidate substances belonging to structural class I is approximately 1400 microgram/*capita*/day and the combined intake of the four candidate substances belonging to structural class II is approximately 100 microgram/*capita*/day. These values are lower than the threshold of concern for structural class I and II substances. Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for 76 of the 77 supporting substances. The total combined intakes of the candidate and supporting substances are approximately 75000 and 7100

microgram/capita/day for structural class I and II, respectively, which exceed the thresholds of concern. However, the substances are expected to be efficiently metabolised and are not expected to saturate the metabolic pathways.

For the substances in this group the available genotoxicity data do not preclude the evaluation of the candidate substances using the Procedure.

It is anticipated that the candidate substances in FGE.20Rev4 would be metabolised to innocuous products.

It was noted that where toxicity data were available they were consistent with the conclusions in the present FGE using the Procedure.

It is considered that on the basis of the default MSDI approach the 45 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI approach they ranged from 770 to 120000 microgram/person/day for 41 flavouring substances from structural class I. The intakes were all above the threshold of concern for structural class I of 1800 microgram/person/day, except for six flavouring substances [FL-no: 05.129, 05.142, 05.153, 05.158, 08.080 and 09.858]. The estimated intakes, based on the mTAMDI, of the four flavouring substances [FL-no: 02.205, 05.066, 05.221 and 06.104] assigned to structural class II were 3900, 1600, 7000 and 3900 microgram/person/day, respectively, which are above the threshold of concern for the structural class (540 microgram/person/day). The six substances which have mTAMDI intake estimates below the threshold of concern for structural class I are also expected to be metabolised to innocuous products. Thus, on the basis of the mTAMDI, the estimated intakes for 39 flavouring substances considered in this Opinion, exceed the relevant threshold for their structural class to which the flavouring substance has been assigned. Therefore, for these 39 substances more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be re-evaluated using the Procedure. Subsequently, additional toxicological data might become necessary.

In order to determine whether the conclusion for the 45 candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for each of the 45 flavouring substances.

For these 45 flavouring substances the Panel concluded that they would present no safety concern at their estimated levels of intake based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 20, REVISION 4

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 20, Revision4

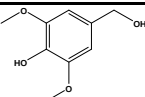
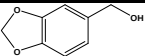
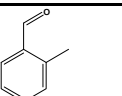
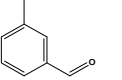
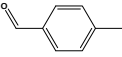
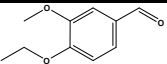
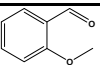
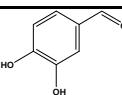
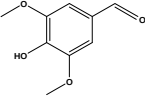
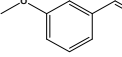
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol		530-56-3	Solid C ₉ H ₁₂ O ₄ 184.19	Practically insoluble or insoluble Freely soluble	387 133 MS 95 %	n.a. n.a.	
02.205	Piperonyl alcohol		10306 495-76-1	Solid C ₈ H ₈ O ₃ 152.15	Very slightly soluble Freely soluble	161 (26 hPa) 55 MS 95 %	n.a. n.a.	
05.026	o-Tolualdehyde		3068 529-20-4	Liquid C ₈ H ₈ O 120.15	Soluble Soluble	200 MS 95 %	1.540-1.547 1.013-1.029	
05.028	m-Tolualdehyde		3068 620-23-5	Liquid C ₈ H ₈ O 120.15	Soluble Soluble	199 MS 95 %	1.540-1.549 1.013-1.029	
05.029	p-Tolualdehyde		3068 104-87-0	Liquid C ₈ H ₈ O 120.15	Soluble Soluble	204 MS 95 %	1.540-1.547 1.013-1.027	
05.066	4-Ethoxy-3-methoxybenzaldehyde		703 120-25-2	Solid C ₁₀ H ₁₂ O ₃ 180.20	Practically insoluble or insoluble Freely soluble	168 (17 hPa) 63 MS 95 %	n.a. n.a.	
05.129	2-Methoxybenzaldehyde		4077 10350 135-02-4	Solid C ₈ H ₈ O ₂ 136.15	Practically insoluble or insoluble Freely soluble	238 38 MS 97 %	1.556-1.562 1.128-1.136	
05.142	3,4-Dihydroxybenzaldehyde		10328 139-85-5	Solid C ₇ H ₆ O ₃ 138.12	Slightly soluble Freely soluble	323 154 MS 98 %	n.a. n.a.	
05.153 1878	4-Hydroxy-3,5-dimethoxybenzaldehyde		10340 134-96-3	Solid C ₉ H ₁₀ O ₄ 182.18	Practically insoluble or insoluble Freely soluble	192 (19 hPa) 113 MS 95 %	n.a. n.a.	
05.158	3-Methoxybenzaldehyde		10351 591-31-1	Liquid C ₈ H ₈ O ₂ 136.15	Practically insoluble or insoluble Freely soluble	230 MS	1.549-1.555 1.116-1.122	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 20, Revision 4

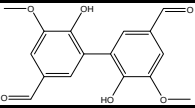
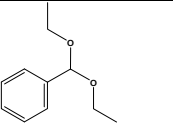
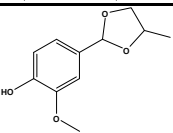
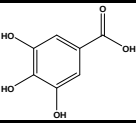
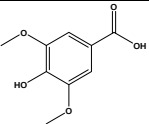
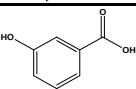
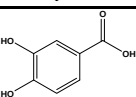
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
05.221 1881	6,6'-Dihydroxy-5,5'-dimethoxy- biphenyl-3,3'-dicarbaldehyde		2092-49-1	Solid C ₁₆ H ₁₄ O ₆ 302.28	Practically insoluble or insoluble Soluble	315 MS 91.4 %	n.a. n.a.	Secondary component is Vanillin at 5-7 % (EFFA, 2012k).
06.017	(Diethoxymethyl)benzene		517 774-48-1	Liquid C ₁₁ H ₁₆ O ₂ 180.25	Practically insoluble or insoluble Freely soluble	222 MS 95 %	1.475-1.481 0.903-0.909	
06.104 1882	Vanillin propylene glycol acetal		3905 68527-74-2	Liquid C ₁₁ H ₁₄ O ₄ 210.23	Practically insoluble or insoluble Freely soluble	154 (0.1 hPa) NMR 97 %	1.537-1.543 1.190-1.206	Commercial compound: Vanillin propylene glycol acetal up to 80 % and with 18-20 % vanillin (EFFA, 2010a). Four diastereoisomers (RR, RS, SS & SR - or two trans forms (RR & SS) & two cis forms (RS & SR)). The composition will be 50-70 % trans (50/50 for RR/SS) and 30-50 % cis (50/50 for RS/SR)(EFFA, 2012u).
08.080	Gallic acid		10170 149-91-7	Solid C ₇ H ₆ O ₅ 170.12	Sparingly soluble Freely soluble	501 242 MS 95 %	n.a. n.a.	
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid		10111 530-57-4	Solid C ₉ H ₁₀ O ₅ 198.18	Sparingly soluble Freely soluble	440 206 MS 95 %	n.a. n.a.	
08.132	3-Hydroxybenzoic acid		99-06-9	Solid C ₇ H ₆ O ₃ 138.12	Soluble Soluble	202 IR NMR MS >99%	n.a. n.a.	
08.133	3,4-Dihydroxybenzoic acid		99-50-3	Solid C ₇ H ₆ O ₄ 154.12	Soluble Soluble	221 IR NMR MS >99%	n.a. n.a.	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 20, Revision4

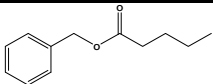
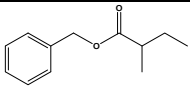
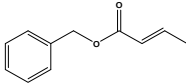
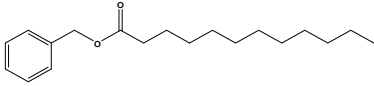
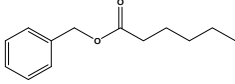
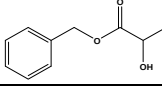
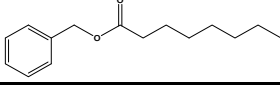
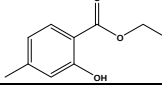
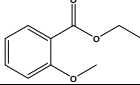
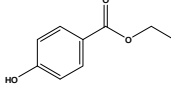
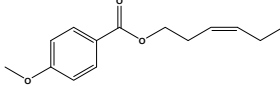
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.152	Benzyl valerate		470 10361-39-4	Liquid C ₁₂ H ₁₆ O ₂ 192.26	Practically insoluble or insoluble Freely soluble	236 MS 95 %	1.487-1.493 0.990-0.996	
09.313	Benzyl 2-methylbutyrate		10523 56423-40-6	Liquid C ₁₃ H ₁₆ O ₂ 192.26	Practically insoluble or insoluble Freely soluble	248 MS 99 %	1.486-1.495 0.982-0.994	Racemate.
09.314	Benzyl crotonate		65416-24-2	Liquid C ₁₁ H ₁₂ O ₂ 176.21	Practically insoluble or insoluble Freely soluble	138 (16 hPa) MS 95 %	1.515-1.521 1.029-1.035	
09.315	Benzyl dodecanoate		140-25-0	Liquid C ₁₉ H ₃₀ O ₂ 290.44	Practically insoluble or insoluble Freely soluble	210 (16 hPa) MS 95 %	1.479-1.485 0.937-0.943	
09.316	Benzyl hexanoate		10521 6938-45-0	Liquid C ₁₃ H ₁₈ O ₂ 206.28	Practically insoluble or insoluble Freely soluble	270 MS 99 %	1.486-1.492 0.978-0.985	
09.317	Benzyl lactate		2051-96-9	Liquid C ₁₀ H ₁₂ O ₃ 180.20	Practically insoluble or insoluble Freely soluble	134 (13 hPa) MS 95 %	1.512-1.518 1.120-1.144	Racemate.
09.318	Benzyl octanoate		10276-85-4	Liquid C ₁₅ H ₂₂ O ₂ 234.34	Practically insoluble or insoluble Freely soluble	153 (8 hPa) MS 95 %	1.484-1.490 0.960-0.966	
09.362	Ethyl 2-hydroxy-4-methylbenzoate		60770-00-5	Liquid C ₁₀ H ₁₂ O ₃ 180.20	Practically insoluble or insoluble Freely soluble	254 MS 95 %	1.514-1.520 1.088-1.094	
09.363	Ethyl 2-methoxybenzoate		7335-26-4	Liquid C ₁₀ H ₁₂ O ₃ 180.20	Practically insoluble or insoluble Freely soluble	235 MS 95 %	1.519-1.525 1.109-1.115	
09.367	Ethyl 4-hydroxybenzoate		120-47-8	Solid C ₉ H ₁₀ O ₃ 166.18	Slightly soluble Freely soluble	298 118 MS 95 %	n.a. n.a.	
09.560	Hex-3(cis)-enyl anisate		121432-33-5	Solid C ₁₄ H ₁₈ O ₃ 234.29	Practically insoluble or insoluble Freely soluble	363 73 NMR 95 %	n.a. n.a.	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 20, Revision4

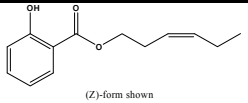
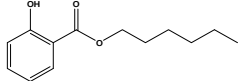
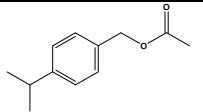
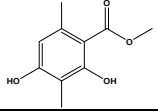
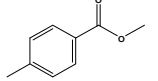
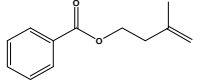
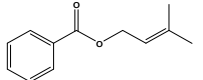
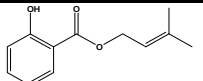
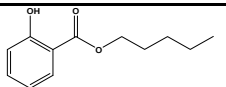
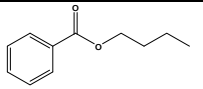
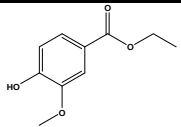
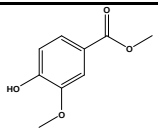
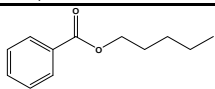
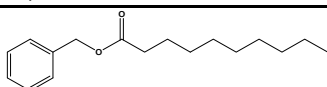
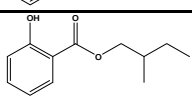
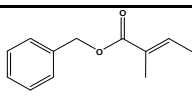
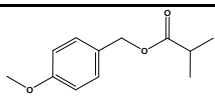
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.570	Hex-3-enyl salicylate	 (Z)-form shown	10685 65405-77-8	Solid C ₁₃ H ₁₆ O ₃ 220.26	Practically insoluble or insoluble Freely soluble	394 139 MS 98 %	1.518-1.522 1.057-1.065	Register name to be changed to (Z)-Hex-3-enyl salicylate (EFFA, 2010a).
09.581	Hexyl salicylate		10695 6259-76-3	Liquid C ₁₃ H ₁₈ O ₃ 222.28	Practically insoluble or insoluble Freely soluble	290 MS 99 %	1.501-1.507 1.029-1.040	
09.611	4-Isopropylbenzyl acetate		59230-57-8	Liquid C ₁₂ H ₁₆ O ₂ 192.26	Practically insoluble or insoluble Freely soluble	250 MS 95 %	1.494-1.500 0.998-1.004	
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate		4707-47-5	Solid C ₁₀ H ₁₂ O ₄ 196.20	Slightly soluble Freely soluble	246 143 MS 95 %	n.a. n.a.	
09.631	Methyl 4-methylbenzoate		99-75-2	Solid C ₉ H ₁₀ O ₂ 150.18	Practically insoluble or insoluble Freely soluble	421 33 MS 95 %	n.a. n.a.	
09.656	3-Methylbut-3-enyl benzoate		5205-12-9	Liquid C ₁₂ H ₁₄ O ₂ 190.24	Practically insoluble or insoluble Freely soluble	60 (0.1 hPa) MS 95 %	1.499-1.505 0.986-0.992	
09.693	Prenyl benzoate		4203 5205-11-8	Liquid C ₁₂ H ₁₄ O ₂ 190.24	Practically insoluble or insoluble Freely soluble	60 (0.1 hPa) MS 95 %	1.505-1.511 0.982-0.988	
09.696	Prenyl salicylate		68555-58-8	Solid C ₁₂ H ₁₄ O ₃ 206.24	Practically insoluble or insoluble Freely soluble	370 113 MS 95 %	n.a. n.a.	
09.762	Pentyl salicylate		613 2050-08-0	Liquid C ₁₂ H ₁₆ O ₃ 208.26	Practically insoluble or insoluble Freely soluble	268 MS 95 %	1.533-1.539 1.062-1.068	
09.779	Butyl benzoate		740 136-60-7	Liquid C ₁₁ H ₁₄ O ₂ 178.23	Practically insoluble or insoluble Freely soluble	249 MS 95 %	1.493-1.499 1.003-1.009	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 20, Revision4

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.798	Ethyl vanillate		2302 617-05-0	Solid C ₁₀ H ₁₂ O ₄ 196.20	Practically insoluble or insoluble Freely soluble	292 44 MS 95 %	n.a. n.a.	
09.799	Methyl vanillate		2305 3943-74-6	Solid C ₉ H ₁₀ O ₄ 182.18	Sparingly soluble Freely soluble	286 63 MS 95 %	n.a. n.a.	
09.825	Pentyl benzoate		2307 2049-96-9	Liquid C ₁₂ H ₁₆ O ₂ 192.26	Practically insoluble or insoluble Freely soluble	260 MS 95 %	1.482-1.493 0.989-0.993	
09.835	Benzyl decanoate		42175-41-7	Solid C ₁₇ H ₂₆ O ₂ 262.39	Practically insoluble or insoluble Freely soluble	400 76 MS 95 %	n.a. n.a.	
09.852	2-Methylbutyl 2-hydroxybenzoate		51115-63-0	Solid C ₁₂ H ₁₆ O ₃ 208.26	Practically insoluble or insoluble Freely soluble	366 117 MS 95 %	n.a. n.a.	Racemate.
09.858	Phenylmethyl 2-methyl-2-butenolate		3330 2184 67674-41-3	Liquid C ₁₂ H ₁₄ O ₂ 190.24	Insoluble Soluble	250 IR 95 %	1.515-1.526 1.029-1.040	Mixture of (Z)- and (E)-isomer, 60-90 % E-form and 10-40 % Z-form (EFFA, 2012k).
09.895	4-Methoxybenzyl-2-methylpropionate			Solid C ₁₂ H ₁₆ O ₃ 208.26	Practically insoluble or insoluble Freely soluble	287 40 MS 95 %	1.499-1.505 1.057-1.063	CASrn is missing. CASrn in Register to be introduced 71172-26-4. Register name to be changed to 4-methoxybenzyl 2-methylpropionate.

1) Solubility in water, if not otherwise stated.

2) Solubility in 95 % ethanol, if not otherwise stated.

3) At 1013.25 hPa, if not otherwise stated.

4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

TABLE 2A: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

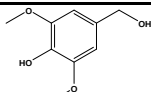
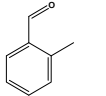
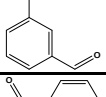
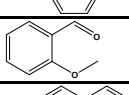
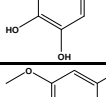
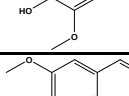
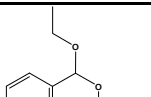
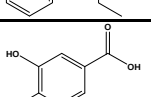
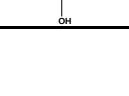
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol		0.037	Class I A3: Intake below threshold	4)	6)	
05.026	o-Tolualdehyde		1.0	Class I A3: Intake below threshold	4)	6)	
05.028	m-Tolualdehyde		0.85	Class I A3: Intake below threshold	4)	6)	
05.029	p-Tolualdehyde		160	Class I A3: Intake below threshold	4)	6)	
05.129	2-Methoxybenzaldehyde		0.16	Class I A3: Intake below threshold	4)	6)	
05.142	3,4-Dihydroxybenzaldehyde		8.5	Class I A3: Intake below threshold	4)	6)	
05.153 1878	4-Hydroxy-3,5-dimethoxybenzaldehyde		0.74	Class I A3: Intake below threshold	4)	6)	
05.158	3-Methoxybenzaldehyde		0.011	Class I A3: Intake below threshold	4)	6)	
06.017	(Diethoxymethyl)benzene		1.7	Class I A3: Intake below threshold	4)	6)	
08.080	Gallic acid		0.011	Class I A3: Intake below threshold	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

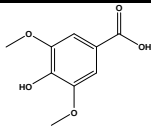
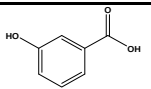
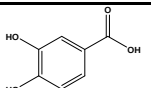
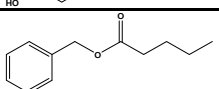
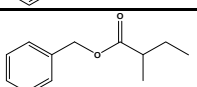
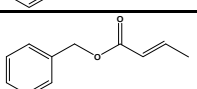
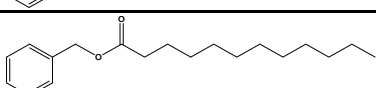
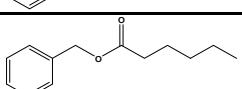
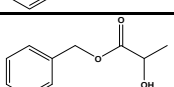
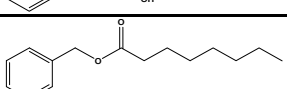
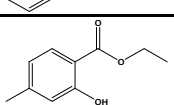
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid		1.2	Class I A3: Intake below threshold	4)	6)	
08.132	3-Hydroxybenzoic acid		610	Class I A3: Intake below threshold	4)	6)	
08.133	3,4-Dihydroxybenzoic acid		610	Class I A3: Intake below threshold	4)	6)	
09.152	Benzyl valerate		1.7	Class I A3: Intake below threshold	4)	6)	
09.313	Benzyl 2-methylbutyrate		7.3	Class I A3: Intake below threshold	4)	6)	
09.314	Benzyl crotonate		0.37	Class I A3: Intake below threshold	4)	6)	
09.315	Benzyl dodecanoate		0.13	Class I A3: Intake below threshold	4)	6)	
09.316	Benzyl hexanoate		0.75	Class I A3: Intake below threshold	4)	6)	
09.317	Benzyl lactate		0.91	Class I A3: Intake below threshold	4)	6)	
09.318	Benzyl octanoate		0.12	Class I A3: Intake below threshold	4)	6)	
09.362	Ethyl 2-hydroxy-4-methylbenzoate		0.0012	Class I A3: Intake below threshold	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

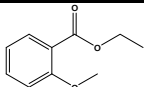
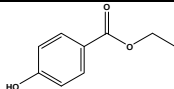
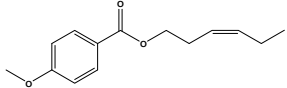
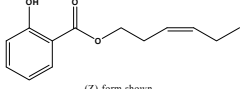
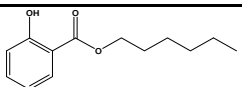
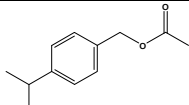
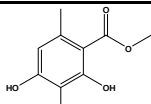
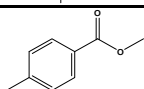
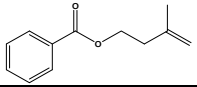
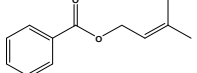
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.363	Ethyl 2-methoxybenzoate		5.5	Class I A3: Intake below threshold	4)	6)	
09.367	Ethyl 4-hydroxybenzoate		10	Class I A3: Intake below threshold	4)	6)	
09.560	Hex-3(cis)-enyl anisate		0.12	Class I A3: Intake below threshold	4)	6)	
09.570	Hex-3-enyl salicylate		0.13	Class I A3: Intake below threshold	4)	6)	
09.581	Hexyl salicylate		0.018	Class I A3: Intake below threshold	4)	6)	
09.611	4-Isopropylbenzyl acetate		0.012	Class I A3: Intake below threshold	4)	6)	
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate		0.012	Class I A3: Intake below threshold	4)	6)	
09.631	Methyl 4-methylbenzoate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.656	3-Methylbut-3-enyl benzoate		0.12	Class I A3: Intake below threshold	4)	6)	
09.693	Prenyl benzoate		0.012	Class I A3: Intake below threshold	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

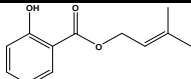
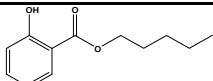
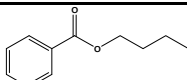
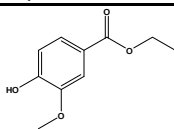
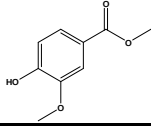
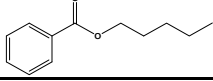
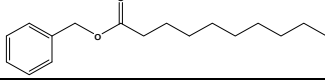
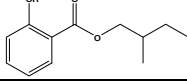
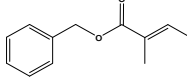
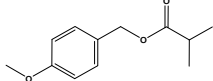
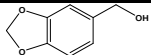
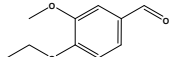
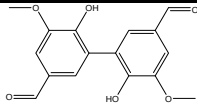
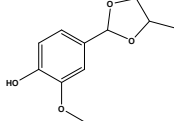
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.696	Prenyl salicylate		0.011	Class I A3: Intake below threshold	4)	6)	
09.762	Pentyl salicylate		0.24	Class I A3: Intake below threshold	4)	6)	
09.779	Butyl benzoate		3.7	Class I A3: Intake below threshold	4)	6)	
09.798	Ethyl vanillate		0.024	Class I A3: Intake below threshold	4)	6)	
09.799	Methyl vanillate		0.011	Class I A3: Intake below threshold	4)	6)	
09.825	Pentyl benzoate		1.1	Class I A3: Intake below threshold	4)	6)	
09.835	Benzyl decanoate		0.35	Class I A3: Intake below threshold	4)	6)	
09.852	2-Methylbutyl 2-hydroxybenzoate		0.011	Class I A3: Intake below threshold	4)	6)	
09.858	Phenylmethyl 2-methyl-2-butenate		0.037	Class I A3: Intake below threshold	4)	6)	
09.895	4-Methoxybenzyl-2-methylpropionate		0.37	Class I A3: Intake below threshold	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.205	Piperonyl alcohol		0.011	Class II A3: Intake below threshold	4)	6)	
05.066	4-Ethoxy-3-methoxybenzaldehyde		1.2	Class II A3: Intake below threshold	4)	6)	
05.221 1881	6,6'-Dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde		0.61	Class II A3: Intake below threshold	4)	6)	
06.104 1882	Vanillin propylene glycol acetal		100	Class II A3: Intake below threshold	4)	6)	

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

4) No safety concern based on intake calculated by the MSDI approach of the named compound.

5) Data must be available on the substance or closely related substances to perform a safety evaluation.

6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).

7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.

8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.

TABLE 2B: EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

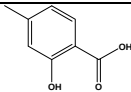
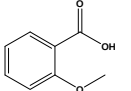
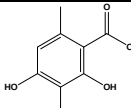
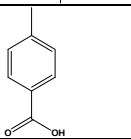
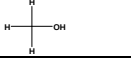
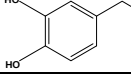
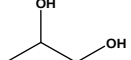
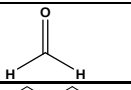
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	4-Methylsalicylic acid		Not evaluated as flavouring substance		Not in EU-Register
	<i>o</i> -Methoxybenzoic Acid		Evaluated as flavouring substance by JECFA (881)		Not in EU-Register
	2,4-Dihydroxy-3,6-dimethylbenzoic acid		Not evaluated as flavouring substance		Not in EU-Register
	<i>p</i> -Toluic acid		Not evaluated as flavouring substance		Not in EU-Register
	Methanol		Not evaluated as flavouring substance		Not in EU-Register
	3,4-Dihydroxybenzyl alcohol		Not evaluated as flavouring substance		Not in EU-Register
	Propylene glycol 925		No evaluation Pending definition of "flavouring agent"		Not in EU-Register
	Formaldehyde		Not evaluated as flavouring substance		Not in EU-Register
02.004	Butan-1-ol 85		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.005	Hexan-1-ol 91		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

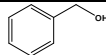
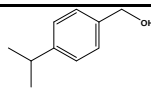
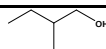
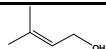
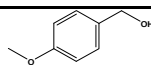
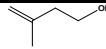
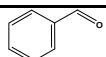
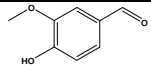
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
02.010	Benzyl alcohol 25		No safety concern d) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.039	4-Isopropylbenzyl alcohol 864		No safety concern d) Category B c)	Class I A3: Intake below threshold	
02.040	Pentan-1-ol 88		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
02.056	Hex-3(cis)-en-1-ol 315		Category 1 a) No safety concern e) Category A c)	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	
02.076	2-Methylbutan-1-ol 1199		Category 1 a) No safety concern f) Category B c)	Class I A3: Intake below threshold	
02.078	Ethanol 41		Category 1 a) No safety concern g)	No evaluation	At the forty-sixth JECFA meeting (JECFA, 1997a), the Committee concluded that ethanol posed no safety concern at its current level of intake when ethyl esters are used as flavouring agents.
02.109	3-Methylbut-2-en-1-ol 1200		No safety concern f)	Class I A3: Intake below threshold	This substance has been evaluated in FGE.202. it was concluded that there would be no safety concern with respect to genotoxicity or carcinogenicity.
02.128	p-Anisyl alcohol 871		No safety concern d) Category A c)	Class I A3: Intake below threshold	
02.176	3-Methylbut-3-en-1-ol			Class I A3: Intake below threshold	
FGE.06					
05.013	Benzaldehyde 22		No safety concern d) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
05.018	Vanillin 889		No safety concern d) Category A c)	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

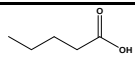
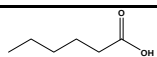
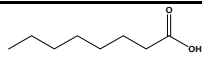
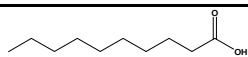
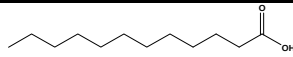
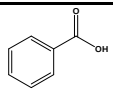
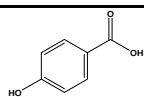
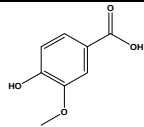
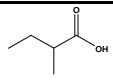
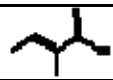
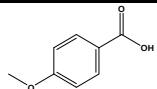
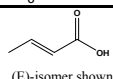
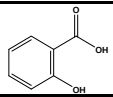
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
08.002	Acetic acid 81		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.004	Lactic acid 930		No safety concern d) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.006	2-Methylpropionic acid 253		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.007	Valeric acid 90		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.009	Hexanoic acid 93		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.010	Octanoic acid 99		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.011	Decanoic acid 105		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.012	Dodecanoic acid 111		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.021	Benzoic acid 850		No safety concern h) Deleted c)	Class I A3: Intake below threshold	Substances for which CoE Committee of Experts had no information as to real use in foodstuffs and/or for which insufficient technological and/or toxicological information was available (CoE, 1992).
08.040	4-Hydroxybenzoic acid 957		No safety concern d) Category A c)	Class I A3: Intake below threshold	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
08.043	Vanillic acid 959		No safety concern d) Category A c)	Class I A3: Intake below threshold	
08.046	2-Methylbutyric acid 255		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.064	2-Methylcrotonic acid 1205		No safety concern f)	Class I A3: Intake below threshold	
08.071	p-Anisic acid 883		No safety concern d)	Class I A3: Intake below threshold	
08.072	But-2-enoic acid (cis and trans)	 (E)-isomer shown	FGE.05	Class I A3: Intake below threshold	
08.112	Salicylic acid 958		No safety concern d)	Class I A3: Intake below threshold	

1) Category 1: Considered safe in use Category 2: Temporarily considered safe in use Category 3: Insufficient data to provide assurance of safety in use Category 4): Not acceptable due to evidence of toxicity.

2) No safety concern at estimated levels of intake.

3) Category A: Flavouring substance, which may be used in foodstuffs Category B: Flavouring substance which can be used provisionally in foodstuffs.

4) Threshold of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.

5) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

a) (SCF, 1995).

b) (JECFA, 1999b).

c) (CoE, 1992).

d) (JECFA, 2002b).

e) (JECFA, 2000a).

f) (JECFA, 2004a).

g) (JECFA, 1997a).

h) (JECFA, 2002c).

ND: Not detected.

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

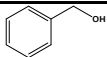
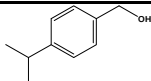
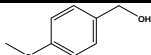
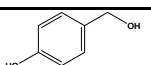
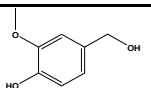
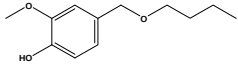
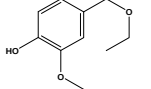
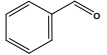
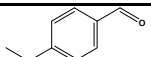
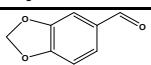
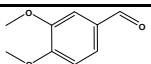
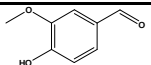
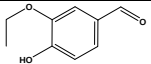
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
02.010	Benzyl alcohol		2137 58 100-51-6	25 JECFA specification (JECFA, 2001c).	13000	No safety concern a) Category A b)	GrADI: 0-5 (JECFA, 1997a).
02.039	4-Isopropylbenzyl alcohol		2933 88 536-60-7	864 JECFA specification (JECFA, 2001c).	0.24	No safety concern a) Category B b)	
02.128	p-Anisyl alcohol		2099 66 105-13-5	871 JECFA specification (JECFA, 2001c).	130	No safety concern a) Category A b)	
02.165	4-Hydroxybenzyl alcohol		3987 623-05-2	955 JECFA specification (JECFA, 2002d).	5.2	No safety concern a)	
02.213	Vanillyl alcohol		3737 690 498-00-0	886 JECFA specification (JECFA, 2001c).	5.4	No safety concern a) Category A b)	
04.093	Butyl vanillyl ether		3796 82654-98-6	888 JECFA specification (JECFA, 2001c).	1.4	No safety concern a)	
04.094	Ethyl 4-hydroxy-3-methoxybenzyl ether		3815 13184-86-6	887 JECFA specification (JECFA, 2001c).	20	No safety concern a)	
05.013	Benzaldehyde		2127 101 100-52-7	22 JECFA specification (JECFA, 2001c).	7900	No safety concern a) Category A b)	ADI: 0-5 (JECFA, 1997a).
05.015	4-Methoxybenzaldehyde		2670 103 123-11-5	878 JECFA specification (JECFA, 2001c).	370	No safety concern a) Category A b)	
05.016	Piperonal		2911 104 120-57-0	896 JECFA specification (JECFA, 2001c).	1500	No safety concern a) Category A b)	ADI: 0-2.5 (JECFA, 1968).
05.017	Veratraldehyde		3109 106 120-14-9	877 JECFA specification (JECFA, 2001c).	120	No safety concern a) Category A b)	
05.018	Vanillin		3107 107 121-33-5	889 JECFA specification (JECFA, 2001c).	47000	No safety concern a) Category A b)	ADI: 0-10 (JECFA, 1968).
05.019	Ethyl vanillin		2464 108 121-32-4	893 JECFA specification (JECFA, 2001c).	5400	No safety concern a) Category A b)	ADI: 0-3 (JECFA, 1995).

Table 3: Supporting Substances Summary

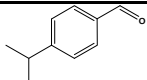
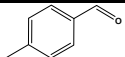
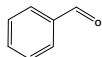
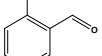
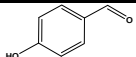
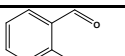
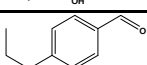
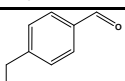
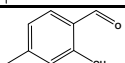
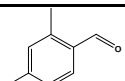
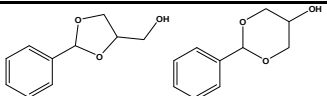
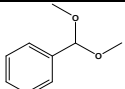
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1 (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
05.022	4-Isopropylbenzaldehyde		2341 111 122-03-2	868 JECFA specification (JECFA, 2001c).	110	No safety concern a) Category B b)	
05.027	Tolualdehyde	  	3068 115 1334-78-7	866 JECFA specification (JECFA, 2002d).	7.5	No safety concern a) Category A b)	CASrn does not specify position of methyl substituent, "Incompletely Defined Substance".
05.047	4-Hydroxybenzaldehyde		3984 558 123-08-0	956 JECFA specification (JECFA, 2002d).	55	No safety concern a) Category B b)	
05.055	Salicylaldehyde		3004 605 90-02-8	897 JECFA specification (JECFA, 2001c).	84	No safety concern a) Category B b)	
05.056	4-Ethoxybenzaldehyde		2413 626 10031-82-0	879 JECFA specification (JECFA, 2001c).	0.073	No safety concern a) Category B b)	
05.068	4-Ethylbenzaldehyde		3756 705 4748-78-1	865 JECFA specification (JECFA, 2001c).	0.37	No safety concern a) Category A b)	
05.091	2-Hydroxy-4-methylbenzaldehyde		3697 2130 698-27-1	898 JECFA specification (JECFA, 2001c).	0.61	No safety concern a) Category B b)	
05.110	2,4-Dimethylbenzaldehyde		3427 15764-16-6	869 JECFA specification (JECFA, 2001c).	0.37	No safety concern a)	
06.002	5-Hydroxy-2-phenyl-1,3-dioxane		2129 36 1319-88-6	838 JECFA specification (JECFA, 2001c).	13	No safety concern a) Category A b)	CASrn refers to benzaldehyde glyceryl acetate.
06.003	alpha,alpha-Dimethoxytoluene		2128 37 1125-88-8	837 JECFA specification (JECFA, 2001c).	0.12	No safety concern a) Category A b)	

Table 3: Supporting Substances Summary

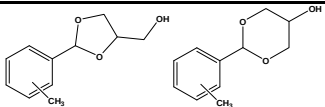
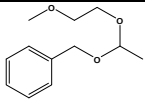
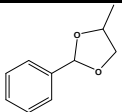
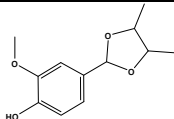
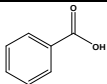
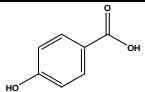
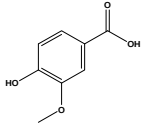
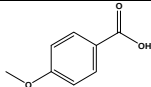
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06.012	Tolualdehyde glyceryl acetal		3067 46 1333-09-1	867 JECFA specification (JECFA, 2001c).	0.012	No safety concern a) Category A b)	CASrn refers to named substance.
06.019	1-Benzyloxy-1-(2-methoxyethoxy)ethane		2148 523 7492-39-9	840 JECFA specification (JECFA, 2001c).	1.2	No safety concern a) Category B b)	Racemate. Min. assay value: 98% (sum of parent compound and starting materials). The "starting materials" are methoxyethanol, acetaldehyde and benzyl alcohol which make up less than 10% combined of the mixture under anhydrous conditions (EFA, 2010a).
06.032	4-Methyl-2-phenyl-1,3-dioxolane		2130 2226 2568-25-4	839 JECFA specification (JECFA, 2001c).	0.037	No safety concern a) Category A b)	
06.132	Vanillin butan-2,3-diol acetal (mixture of stereo isomers)		4023 63253-24-7	960 JECFA specification (JECFA, 2002d).	3.4	No safety concern a)	CASrn does not specify stereoisomers. Stereoisomeric composition to be specified.
08.021	Benzoic acid		2131 21 65-85-0	850 JECFA specification (JECFA, 2001c).	34	No safety concern c) Deleted b)	GrADI: 0-5 (JECFA, 1997a).
08.040	4-Hydroxybenzoic acid		3986 693 99-96-7	957 JECFA specification (JECFA, 2002d).	16	No safety concern a) Category A b)	
08.043	Vanillic acid		3988 697 121-34-6	959 JECFA specification (JECFA, 2002d).	24	No safety concern a) Category A b)	
08.071	p-Anisic acid		3945 10077 100-09-4	883 JECFA specification (JECFA, 2001c).	1.7	No safety concern a)	

Table 3: Supporting Substances Summary

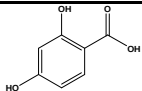
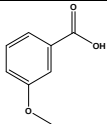
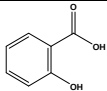
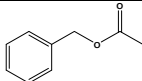
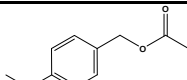
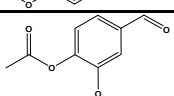
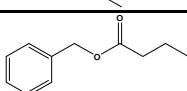
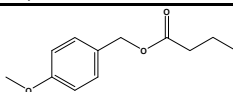
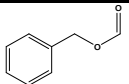
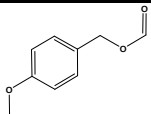
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1 (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
08.076	2,4-Dihydroxybenzoic acid		3798 89-86-1	908 JECFA specification (JECFA, 2001c).	5.5	No safety concern a)	
08.092	3-Methoxybenzoic acid		3944 586-38-9	882 JECFA specification (JECFA, 2001c).	0.012	No safety concern a)	
08.112	Salicylic acid		3985 10165 69-72-7	958 JECFA specification (JECFA, 2002d).	0.024	No safety concern a)	
09.014	Benzyl acetate		2135 204 140-11-4	23 JECFA specification (JECFA, 2001c).	1200	No safety concern a) Category B b)	GrADI: 0-5 (JECFA, 1997a).
09.019	p-Anisyl acetate		2098 209 104-21-2	873 JECFA specification (JECFA, 2001c).	50	No safety concern a) Category B b)	
09.035	Vanillyl acetate		3108 225 881-68-5	890 JECFA specification (JECFA, 2001c).	1.8	No safety concern a) Category B b)	
09.051	Benzyl butyrate		2140 277 103-37-7	843 JECFA specification (JECFA, 2001c).	100	No safety concern a) Category A b)	
09.058	p-Anisyl butyrate		2100 286 6963-56-0	875 JECFA specification (JECFA, 2001c).	29	No safety concern a) Category B b)	
09.077	Benzyl formate		2145 344 104-57-4	841 JECFA specification (JECFA, 2001c).	35	No safety concern a) Category A b)	
09.087	p-Anisyl formate		2101 354 122-91-8	872 JECFA specification (JECFA, 2002d).	39	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

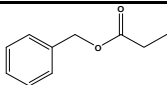
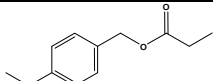
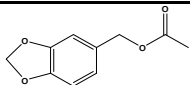
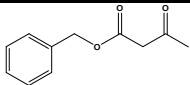
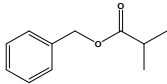
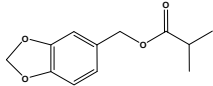
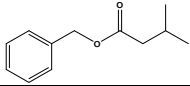
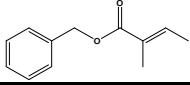
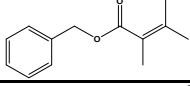
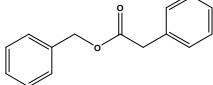
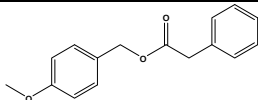
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1 (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.132	Benzyl propionate		2150 413 122-63-4	842 JECFA specification (JECFA, 2001c).	41	No safety concern a) Category A b)	
09.145	p-Anisyl propionate		2102 426 7549-33-9	874 JECFA specification (JECFA, 2001c).	0.42	No safety concern a) Category B b)	
09.220	Piperonyl acetate		2912 2068 326-61-4	894 JECFA specification (JECFA, 2001c).	34	No safety concern a) Category B b)	
09.406	Benzyl 3-oxobutyrate		2136 244 5396-89-4	848 JECFA specification (JECFA, 2001c).	0.24	No safety concern a) Category B b)	
09.426	Benzyl isobutyrate		2141 301 103-28-6	844 JECFA specification (JECFA, 2001c).	13	No safety concern a) Category B b)	
09.430	Piperonyl isobutyrate		2913 305 5461-08-5	895 JECFA specification (JECFA, 2001c).	0.085	No safety concern a) Category B b)	
09.458	Benzyl isovalerate		2152 453 103-38-8	845 JECFA specification (JECFA, 2001c).	12	No safety concern a) Category B b)	
09.494	Benzyl 2-methylcrotonate		3330 2184 37526-88-8	846 JECFA specification (JECFA, 2001c).	0.012	No safety concern a) Category B b)	
09.508	Benzyl 2,3-dimethylcrotonate		2143 11868 7492-69-5	847 JECFA specification (JECFA, 2002d).	0.012	No safety concern a)	
09.705	Benzyl phenylacetate		2149 232 102-16-9	849 JECFA specification (JECFA, 2001c).	4.3	No safety concern a) Category B b)	
09.706	Anisyl phenylacetate		3740 233 102-17-0	876 JECFA specification (JECFA, 2001c).	0.0024	No safety concern a) Category B b)	

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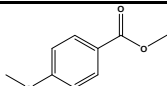
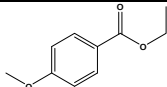
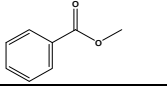
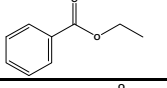
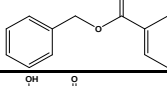
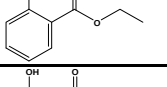
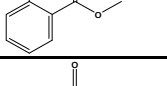
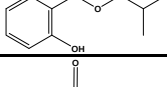
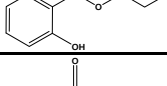
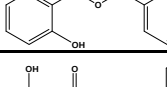
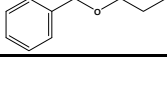
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1 (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.713	Methyl 4-methoxybenzoate		2679 248 121-98-2	884 JECFA specification (JECFA, 2001c).	0.97	No safety concern a) Category B b)	
09.714	Ethyl 4-methoxybenzoate		2420 249 94-30-4	885 JECFA specification (JECFA, 2001c).	9.1	No safety concern a) Category B b)	
09.725	Methyl benzoate		2683 260 93-58-3	851 JECFA specification (JECFA, 2001c).	40	No safety concern a) Category B b)	
09.726	Ethyl benzoate		2422 261 93-89-0	852 JECFA specification (JECFA, 2001c).	96	No safety concern a) Category B b)	
09.727	Benzyl benzoate		2138 262 120-51-4	24 JECFA specification (JECFA, 2001c).	1600	No safety concern a) Category A b)	GrADI: 0-5 (JECFA, 1980a).
09.748	Ethyl salicylate		2458 432 118-61-6	900 JECFA specification (JECFA, 2001c).	27	No safety concern a) Category B b)	
09.749	Methyl salicylate		2745 433 119-36-8	899 JECFA specification (JECFA, 2001c).	410	No safety concern a) Category A b)	ADI: 0-0.5 (JECFA, 1968).
09.750	Isobutyl salicylate		2213 434 87-19-4	902 JECFA specification (JECFA, 2001c).	0.97	No safety concern a) Category B b)	
09.751	Isopentyl salicylate		2084 435 87-20-7	903 JECFA specification (JECFA, 2001c).	41	No safety concern a) Category B b)	
09.752	Benzyl salicylate		2151 436 118-58-1	904 JECFA specification (JECFA, 2001c).	26	No safety concern a) Category B b)	
09.753	Phenethyl salicylate		2868 437 87-22-9	905 JECFA specification (JECFA, 2001c).	0.12	No safety concern a) Category B b)	

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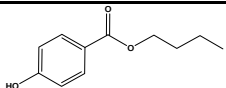
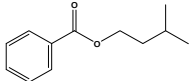
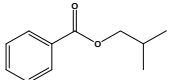
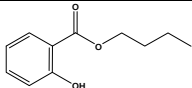
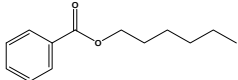
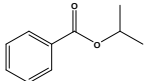
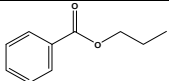
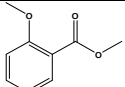
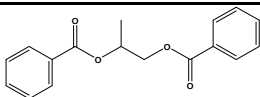
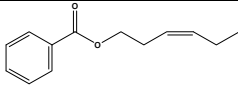
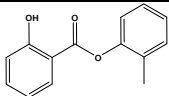
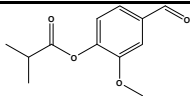
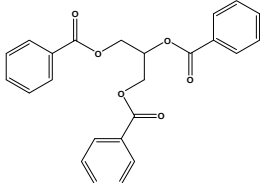
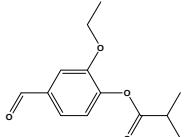
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1 (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.754	Butyl 4-hydroxybenzoate		2203 525 94-26-8	870 JECFA specification (JECFA, 2002d).	ND	No safety concern c) Deleted b)	
09.755	Isopentyl benzoate		2058 562 94-46-2	857 JECFA specification (JECFA, 2001c).	96	No safety concern a) Category B b)	
09.757	Isobutyl benzoate		2185 567 120-50-3	856 JECFA specification (JECFA, 2001c).	0.37	No safety concern a) Category B b)	
09.763	Butyl salicylate		3650 614 2052-14-4	901 JECFA specification (JECFA, 2001c).	0.012	No safety concern a) Category B b)	
09.768	Hexyl benzoate		3691 645 6789-88-4	854 JECFA specification (JECFA, 2001c).	320	No safety concern a) Category B b)	
09.770	Isopropyl benzoate		2932 652 939-48-0	855 JECFA specification (JECFA, 2001c).	0.0037	No safety concern a) Category B b)	
09.776	Propyl benzoate		2931 677 2315-68-6	853 JECFA specification (JECFA, 2001c).	0.012	No safety concern a) Category B b)	
09.796	Methyl 2-methoxybenzoate		2717 2192 606-45-1	880 JECFA specification (JECFA, 2001c).	49	No safety concern a) Deleted b)	
09.803	Propylene glycol dibenzoate		3419 10890 19224-26-1	862 JECFA specification (JECFA, 2002d).	13	No safety concern c)	CASrn refers to the racemate.
09.806	Hex-3-enyl benzoate	 (Z)-isomer shown	3688 11778 25152-85-6	858 JECFA specification (JECFA, 2001c).	6.7	No safety concern a)	CASrn refers to (Z)-isomer.
09.807	o-Tolyl salicylate		3734 617-01-6	907 JECFA specification (JECFA, 2001c).	28	No safety concern a)	

Table 3: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.811	Vanillin isobutyrate		3754 20665-85-4	891 JECFA specification (JECFA, 2001c).	55	No safety concern a)	
09.812	Glyceryl tribenzoate		3398 10656 614-33-5	861 JECFA specification (JECFA, 2002d).	45	No safety concern c)	
09.933	Ethyl vanillin isobutyrate		3837 188417-26-7	953 JECFA specification (JECFA, 2001c).	0.61	No safety concern a)	

1) EU MSDI: Amount added to food as flavouring substance in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.

3) No safety concern at estimated levels of intake.

4) Category A: Flavouring substance, which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.

a) (JECFA, 2002b).

b) (CoE, 1992).

c) (JECFA, 2002c).

ND) No intake data reported.

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), named the "Procedure", is shown in schematic form in Figure I.1. The Procedure is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999a), which is derived from the evaluation Procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 microgram/person/day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996a).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- can the flavourings be predicted to be metabolised to innocuous products⁹ (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous¹⁰ (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

⁹ "Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).

¹⁰ "Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).

Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

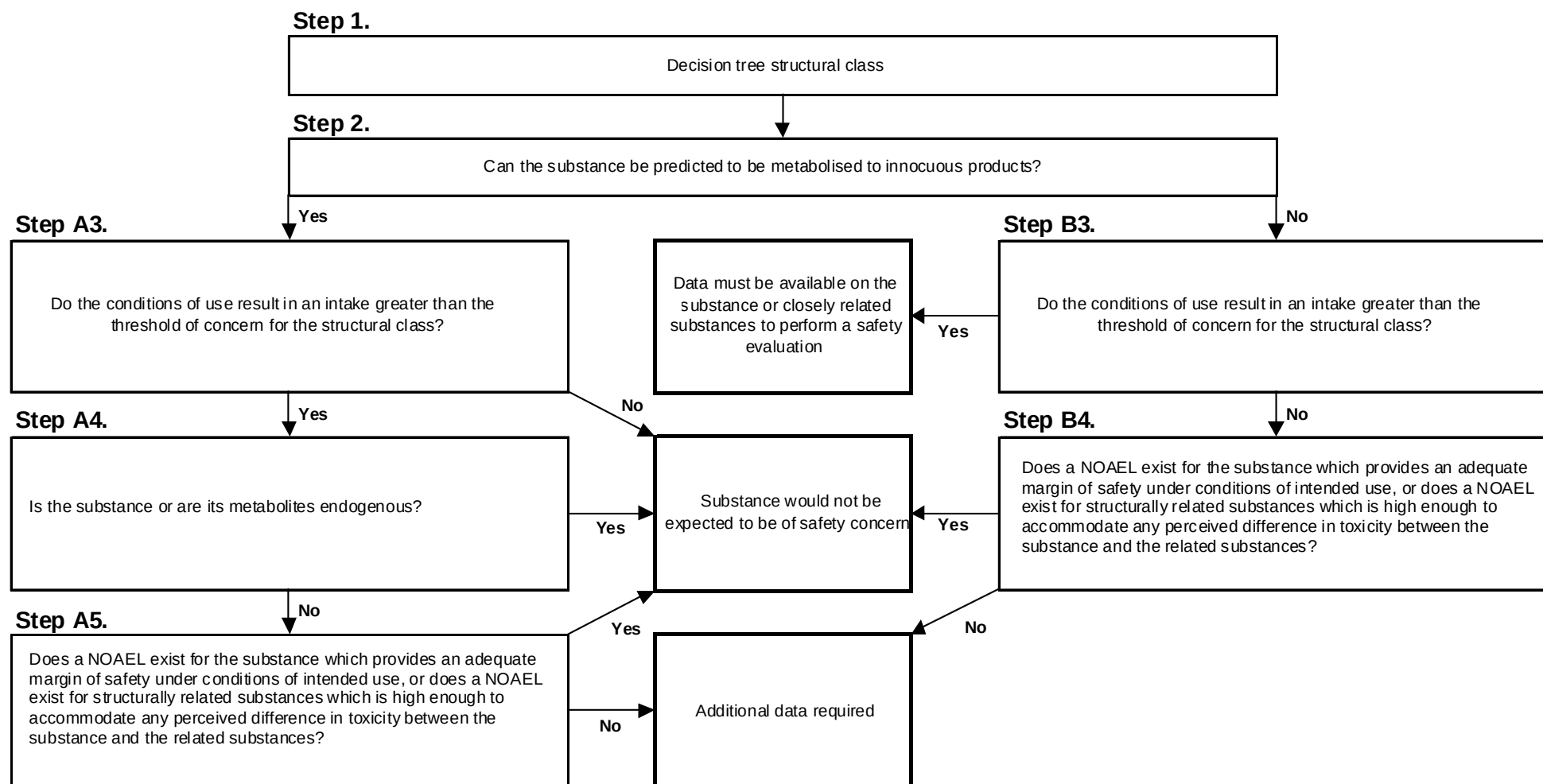


Figure I.1 Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

ANNEX II: USE LEVELS / mTAMDI

II.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table II.1.1) in which the candidate substances are used, Flavour Industry reports a “normal use level” and a “maximum use level” (EC, 2000a). According to the Industry the “normal use” is defined as the average of reported usages and “maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002i). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

Table II.1.1 Food categories according to Commission Regulation (EC) No 1565/2000 (EC, 2000a)

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds
05.0	Confectionery
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic ("soft") beverages, excl. dairy products
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0

The “normal and maximum use levels” are provided by Industry for 45 candidate substances in the present flavouring group (Table II.1.2).

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.20Rev4 (EFFA, 2003u; EFFA, 2004c; EFFA, 2007a; EFFA, 2007d; EFFA, 2012m; EFFA, 2012o; EFFA, 2012q; Flavour Industry, 2008c).

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
02.164	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.205	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
05.026	15,0	-	-	-	-	56,2	-	25,8	-	-	-	-	-	-	9,91	6,23	-	-
	3	-	-	-	-	6	-	2	-	-	-	-	-	-	13,2	12,7	-	-
	17,7	-	-	-	-	111,	-	33,5	-	-	-	-	-	-	9	-	-	-
	4	-	-	-	-	2	-	6	-	-	-	-	-	-	-	-	-	-
05.028	15,0	-	-	-	-	56,2	-	25,8	-	-	-	-	-	-	9,91	6,23	-	-
	3	-	-	-	-	6	-	2	-	-	-	-	-	-	13,2	12,7	-	-
	17,7	-	-	-	-	111,	-	33,5	-	-	-	-	-	-	9	-	-	-
	4	-	-	-	-	2	-	6	-	-	-	-	-	-	-	-	-	-
05.029	15,0	-	-	-	-	56,2	-	25,8	-	-	-	-	-	-	9,91	6,23	-	-
	3	-	-	-	-	6	-	2	-	-	-	-	-	-	13,2	12,7	-	-
	17,7	-	-	-	-	111,	-	33,5	-	-	-	-	-	-	9	-	-	-
	4	-	-	-	-	2	-	6	-	-	-	-	-	-	-	-	-	-
05.066	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.20Rev4 (EFSA, 2003u; EFSA, 2004c; EFSA, 2007a; EFSA, 2007d; EFSA, 2012m; EFSA, 2012o; EFSA, 2012q; Flavour Industry, 2008c).

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
05.129	3	2	3	2	-	5	2	-	1	1	-	-	2	3	2	5	5	2
	15	10	15	10	-	25	10	-	5	5	-	-	10	15	10	25	25	10
05.142	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
05.153	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
05.158	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
05.221	10	15	10	-	-	10	-	30	-	-	-	-	10	-	5	15	30	10
	40	20	20	-	-	20	-	50	-	-	-	-	30	-	15	30	50	20
06.017	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
06.104	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
08.080	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
08.087	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
08.132	-	-	-	-	-	500	-	-	-	-	-	-	-	-	300	300	-	-
	-	-	-	-	-	2000	-	-	-	-	-	-	-	-	500	500	-	-
08.133	-	-	-	-	-	500	-	-	-	-	-	-	-	-	300	300	-	-
	-	-	-	-	-	2000	-	-	-	-	-	-	-	-	500	500	-	-
09.152	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.313	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.314	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.315	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.316	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.317	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.318	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.362	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.363	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.367	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.560	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.570	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.581	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.611	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.623	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.631	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	34	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.656	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.693	7	5	10	7	-	10	5	10	2	2	-	-	5	20	5	10	-	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	100	25	50	-	25
09.696	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.762	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.779	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.20Rev4 (EFFA, 2003u; EFFA, 2004c; EFFA, 2007a; EFFA, 2007d; EFFA, 2012m; EFFA, 2012o; EFFA, 2012q; Flavour Industry, 2008c).

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
09.798	35	25	50	35	-	50	25	50	11	10	-	-	25	50	25	50	100	25
	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.799	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.825	7	5	10	7	-	10	5	10	2	-	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	-	-	-	25	50	25	50	100	25
09.835	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.852	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.858	-	-	-	-	-	-	-	5	-	-	-	-	5	-	-	-	-	-
	-	-	-	-	-	-	-	8	-	-	-	-	8	-	-	-	-	-
09.895	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25

Three candidate substances [FL-no. 5.026, 05.028 and 05.029] are also used in chewing gum, which is not covered by any of the above food categories. The normal/maximum use levels for chewing gum are reported to be 387/657.60 mg/kg for all three substances. Under the assumptions that all of the flavouring substances are released from the chewing gum and that the intake estimate is 2 g chewing gum/day, the calculation of the mTAMDI of the candidate substance based on the food categories and the use of chewing gum sum up to 9100 µg/person/day. These figures are presented in tables II.2.3 and 6.1

II.2 mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000a) and reported by the Flavour Industry in the following way (see Table II.2.2):

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000a)

- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16 (EC, 2000a)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000a)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000a)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000a)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000a)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000a) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

Food categories according to Commission Regulation 1565/2000		Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food		

The mTAMDI values (see Table II.2.3) are presented for each of the 45 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2003u; EFFA, 2004c; EFFA, 2007a; EFFA, 2007d; EFFA, 2012m; EFFA, 2012o; EFFA, 2012q; Flavour Industry, 2008c). The mTAMDI values are only given for the highest reported normal use levels.

Table II.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol	3900	Class I	1800
05.026	o-Tolualdehyde	9100	Class I	1800
05.028	m-Tolualdehyde	9100	Class I	1800
05.029	p-Tolualdehyde	9100	Class I	1800
05.129	2-Methoxybenzaldehyde	1400	Class I	1800
05.142	3,4-Dihydroxybenzaldehyde	1600	Class I	1800
05.153	4-Hydroxy-3,5-dimethoxybenzaldehyde	1600	Class I	1800
05.158	3-Methoxybenzaldehyde	1600	Class I	1800
06.017	(Diethoxymethyl)benzene	3900	Class I	1800
08.080	Gallic acid	1600	Class I	1800

Table II.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid	3200	Class I	1800
08.132	3-Hydroxybenzoic acid	120000	Class I	1800
08.133	3,4-Dihydroxybenzoic acid	120000	Class I	1800
09.152	Benzyl valerate	3900	Class I	1800
09.313	Benzyl 2-methylbutyrate	3900	Class I	1800
09.314	Benzyl crotonate	3900	Class I	1800
09.315	Benzyl dodecanoate	3900	Class I	1800
09.316	Benzyl hexanoate	3900	Class I	1800
09.317	Benzyl lactate	3900	Class I	1800
09.318	Benzyl octanoate	3900	Class I	1800
09.362	Ethyl 2-hydroxy-4-methylbenzoate	3900	Class I	1800
09.363	Ethyl 2-methoxybenzoate	3900	Class I	1800
09.367	Ethyl 4-hydroxybenzoate	3900	Class I	1800
09.560	Hex-3(cis)-enyl anisate	3900	Class I	1800
09.570	Hex-3-enyl salicylate	3900	Class I	1800
09.581	Hexyl salicylate	3900	Class I	1800
09.611	4-Isopropylbenzyl acetate	3900	Class I	1800
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate	3900	Class I	1800
09.631	Methyl 4-methylbenzoate	3900	Class I	1800
09.656	3-Methylbut-3-enyl benzoate	3900	Class I	1800
09.693	Prenyl benzoate	4900	Class I	1800
09.696	Prenyl salicylate	3900	Class I	1800
09.762	Pentyl salicylate	3900	Class I	1800
09.779	Butyl benzoate	3900	Class I	1800
09.798	Ethyl vanillate	3900	Class I	1800
09.799	Methyl vanillate	3900	Class I	1800
09.825	Pentyl benzoate	3900	Class I	1800
09.835	Benzyl decanoate	3900	Class I	1800
09.852	2-Methylbutyl 2-hydroxybenzoate	3900	Class I	1800
09.858	Phenylmethyl 2-methyl-2-butenate	770	Class I	1800
09.895	4-Methoxybenzyl-2-methylpropionate	3900	Class I	1800
02.205	Piperonyl alcohol	3900	Class II	540
05.066	4-Ethoxy-3-methoxybenzaldehyde	1600	Class II	540
05.221	6,6'-Dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde	7000	Class II	540
06.104	Vanillin propylene glycol acetal	3900	Class II	540

ANNEX III: METABOLISM

III.1. Introduction

The flavouring group consists of 45 substances out of which 19 are benzyl derivatives (subgroup 1) and 25 are hydroxy- and alkoxy-ringsubstituted benzyl derivatives (subgroup 2) and one is a hydroxy- and alkoxy-substituted biphenyl derivative (subgroup 3).

Subgroup 1 (Benzyl derivatives)

Comprises three tolualdehyde isomers (*o*-, *m*- and *p*-tolualdehyde [FL no: 05.026, 05.028 and 05.029] and 15 alkyl esters, ten of which contain benzyl alcohol in the alcohol moiety and straight or branched carboxylic acids as acid moiety [FL-no: 09.152 (benzyl valerate); 09.313 (benzyl 2-methylbutyrate); 09.314 (benzyl crotonate); 09.315 (benzyl dodecanoate); 09.316 (benzyl hexanoate); 09.317 (benzyl lactate); 09.318 (benzyl octanoate); 09.611 (4-isopropylbenzyl acetate), 09.835 (benzyl decanoate); 09.858 (phenylmethyl 2-methyl-2-butenolate)]. Five of the esters contain benzoic acid in the acid moiety [FL-no: 09.631 (methyl 4-methylbenzoate); 09.656 (3-methylbut-3-enyl benzoate); 09.693 (prenyl benzoate); 09.779 (butyl benzoate); 09.825 (pentyl benzoate)].

One substance in subgroup 1 is an acetal [FL-no: 06.017 ((diethoxymethyl)benzene)].

Two of the substances in subgroup 1 contain an alkyl substituent on the aromatic ring [FL-no: 09.611 (4-isopropylbenzyl acetate); 09.631 (methyl 4-methylbenzoate)]. Four compounds contain a double bond in an alkyl chain [FL-no: 09.314 (benzyl crotonate); 09.656 (3-methylbut-3-enyl benzoate); 09.693 (prenyl benzoate); 09.858 (phenylmethyl-2-methyl-2-butenolate)].

Subgroup 2 (Hydroxy- and alkoxy-substituted benzyl derivatives)

Includes two derivatives of benzyl alcohol [FL-no: 02.164 (4-hydroxy-3,5-dimethoxybenzyl alcohol) and 02.205 (piperonyl alcohol (3,4-methylenedioxybenzyl alcohol))]. Piperonyl alcohol may also be considered as a cyclic acetal.

Six substances are derivatives of benzaldehyde [FL-no: 05.066 (4-ethoxy-3-methoxybenzaldehyde); 05.129 (2-methoxybenzaldehyde); 05.142 (3,4-dihydroxybenzaldehyde); 05.153 (4-hydroxy-3,5-dimethoxybenzaldehyde); 05.158 (3-methoxybenzaldehyde) and 06.104 (vanillin propylene glycol acetal)].

Four are derivatives of benzoic acid [FL-no: 08.080 (gallic acid (3,4,5-trihydroxybenzoic acid)); 08.087 (4-hydroxy-3,5-dimethoxybenzoic acid); 08.132 (3-hydroxybenzoic acid); 08.133 (3,4-dihydroxybenzoic acid)].

The remaining 13 substances are esters of which one is an ester with alkoxy substituted benzyl alcohol as the alcohol moiety [FL-no: 09.895 (4-methoxybenzyl-2-methylpropionate)] and 12 are esters with substituted benzoic acid as the acid moiety [FL-no: 09.362 (ethyl 2-hydroxy-4-methylbenzoate); 09.363 (ethyl 2-methoxybenzoate); 09.367 (ethyl 4-hydroxybenzoate); 09.560 (hex-3(cis)-enyl anisate (hex-3(cis)-enyl 4-methoxybenzoate)); 09.570 (hex-3-enyl salicylate (hex-3-enyl 2-hydroxybenzoate)); 09.581 (hexyl salicylate (hexyl 2-hydroxybenzoate)); 09.623 (methyl 2,4-dihydroxy-3,6-dimethylbenzoate); 09.696 (prenyl salicylate (3-methyl-but-2-enyl 2-hydroxybenzoate)); 09.762 (pentyl salicylate (pentyl 2-hydroxybenzoate)); 09.798 (ethyl vanillate (ethyl 3-methoxy-4-hydroxybenzoate)); 09.799 (methyl vanillate (methyl 3-methoxy-4-hydroxybenzoate)); 09.852 (2-methylbutyl 2-hydroxybenzoate (2-methylbutyl salicylate))].

Three of the esters in subgroup 2 contain a double bond in an alkyl chain [FL-no: 09.560 (hex-3(cis)-enyl anisate); 09.570 (hex-3-enyl salicylate); 09.696 (prenyl salicylate)].

Subgroup 3 (Biphenyl)

Contains one derivative of biphenyl [FL-no: 05.221 (6,6'-dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde)].

III.2. Absorption, Distribution and Elimination

Subgroup 1 (Benzyl derivatives)

Candidate substances from subgroup 1

There are no studies submitted on the candidate substances from subgroup 1.

Supporting substances from subgroup 1

Several studies have been submitted demonstrating efficient absorption, metabolism and excretion of the supporting substances benzyl alcohol [FL-no: 02.010], benzaldehyde [FL-no: 05.013], benzoic acid [FL-no: 08.021] and benzyl acetate [FL-no: 09.014].

Benzyl alcohol [FL-no: 02.010]

As already stated, the benzyl alcohol is expected to be oxidised to benzoic acid, which will be conjugated with glycine and excreted as hippuric acid (Bridges et al., 1970). This has been demonstrated in a study by LeBel et al. (1988) in which similar intravenous doses (range 0.036 to 0.222 micromole/kg bw) of benzyl alcohol were given via medications to 14 full-term and nine pre-term infants, in order to estimate plasma levels of benzoic and hippuric acids. In the urine of full-term new-borns a larger proportion of benzyl alcohol was found as hippuric acid and a smaller proportion benzoic acid, which is in line with what Bridges et al suggest. However, the mean peak concentrations of benzoic acid in the plasma of pre-term babies were almost 10 times higher than in full-term new-borns and in the urine of pre-term babies a larger proportion of benzyl alcohol was found as benzoic acid and a smaller proportion as hippuric acid in contrary to what was found in full-term new-borns. The results suggest that hippuric acid formation is deficient in pre-term newborns (LeBel et al., 1988). Overall, the results suggest that humans metabolise benzyl alcohol to both benzoic acid and hippuric acid but hippuric acid formation is deficient in pre-term new-borns.

Five minutes after single intraperitoneal doses of 500 - 1100 mg/kg bw of benzyl alcohol administered to CD1 mice, benzyl alcohol was detected in plasma (McCloskey et al., 1986).

Benzaldehyde [FL-no: 05.013]

In the rabbit, approximately 83 % of given single oral doses of 350 or 750 mg/kg bw benzaldehyde was absorbed; it was found in the urine of both dose groups. The aldehyde was oxidised mainly to benzoic acid and excreted predominantly as hippuric acid (approximately 68 %). Other urinary metabolites detected were benzoylglucuronic acid (10 %), benzoyl glucuronide (3 %), free benzoic acid (1.5 %) and trace amounts of benzyl mercapturic acid (Laham et al., 1988).

Benzoic acid [FL-no: 08.021]

Following administration of 375 mg [carboxyl-14C]-benzoic acid/kg bw to rats (orally) and mice intraperitoneally (i.p.), 88 - 89 % of the radioactivity was recovered in the urine of rats within 24 hours with 91 - 94 % recovery after 72 hours, and only 1 - 6 % was present in the faeces. In mice, 92 - 98 % of the radioactivity was recovered in the urine of rats within 24 hours and only 1 - 10 % was present in the faeces. It was possible to conclude that after both route of administration more than 95 % of benzoic acid is absorbed, metabolised and rapidly excreted. The following metabolites were identified: hippuric acid (70.2 -

84.2 %), benzoyl glucuronide (0.7 - 1.8 %), benzoic acid (0.4 - 12.8 %) and 3-hydroxy-3-phenyl propionic acid (0.1 - 0.2 %) (Nutley, 1990).

Benzyl acetate [FL-no: 09.014]

A study on benzyl acetate metabolism in male Fischer 344 rats and male B6C3F₁ mice was performed. Ring-labelled ¹⁴C-benzyl acetate was used for single dose studies and unlabelled benzyl acetate was used for repeated dose studies. For intravenous administration of single doses, three rats were injected with 5 mg ¹⁴C-benzyl acetate in the tail vein and three mice were similarly injected with 10 mg. For the single oral dose study, groups of three rats were given 5, 50 or 500 mg/kg bw and groups of three mice were given 10, 100 or 1000 mg/kg bw in corn oil by gavage. For the repeated dose studies, three rats and three mice were given unlabelled benzyl acetate in corn oil by gavage at 500 or 1000 mg/kg bw, respectively, once a day, 5 days a week for 2 weeks. Metabolites in urine were determined by High Performance Liquid Chromatography (HPLC). After administration of the radioactive compound, rats and mice were housed in metabolism cages and urine and faeces were collected during 24 hours. After intravenous administration, CO₂ and volatiles were collected.

Benzyl acetate was rapidly and almost completely absorbed, based on the high recovery of radioactivity (nearly 90 % of the dose) in the urine in 24 hours, following both intravenous or oral dosing in rats and mice. Little radioactivity (0.3 - 1.3 % of the dose) was recovered in the faeces. Elimination as CO₂ or volatiles was minimal following intravenous administration and was not determined after oral dosing. This clearance pattern was not affected by repeated oral dosing, indicating no potential for bioaccumulation, as supported also by the absence of radioactivity in tissues analysed at 24 hours after dosing. The major metabolite of benzyl acetate in the urine of rats and mice was hippuric acid, accounting for more than 90 % of the total metabolites excreted in urine of all dose groups. Mercapturic acid was detected as a minor metabolite in the urine of rats and mice (less than 1 %), but was not found in all dose groups and not in all animals of the dose groups where it was detected. Small amounts of unidentified metabolites were also present. The absorption, routes of metabolism and excretion of benzyl acetate were apparently unaffected by the size or number of doses administered in the metabolism study. There was no evidence to indicate a reduction or saturation of the metabolic capacity in tested animals in the tested dose range (Abdo et al., 1985).

Subgroup 2 (Hydroxy- and alkoxy-substituted benzyl derivatives)

Candidate substances from subgroup 2

Piperonyl alcohol (1-hydroxymethyl-3,4-methylenedioxybenzene) [FL-no: 02.205]

In a study of several methylenedioxyphenyl compounds, male Swiss-Webster mice were administered a dimethyl sulphoxide (DMSO) solution of radiolabelled piperonyl alcohol (1-hydroxymethyl-3,4-methylene-¹⁴C-dioxybenzene) by oral gavage at a dose of 0.76 mg/kg bw. Total radiocarbon determinations were made on expired ¹⁴CO₂ at 0.5, 1, 2, 4 and 6 hours after dosing and at each six-hours interval thereafter, on urine and faeces samples taken at 12, 24 and 48 hours after treatment and on selected organs removed from the animals and the remaining carcass at 48 hours after treatment. Only the 12-hours samples were used for separation and characterisation of metabolites. Forty-eight hours after treatment, the distribution of radioactivity was as follows (averages of four experiments): CO₂, 3.0 %; urine, 93.3 %; faeces, 8.5 %; intestine, 0.2 %; liver, 0.1 %; carcass, 0.3 %. These data indicate that piperonyl alcohol is almost completely absorbed in the gastrointestinal (GI) tract, then metabolised and rapidly and almost completely excreted, mostly via the urine. Less than 10 % was excreted in the faeces. In all cases, the major metabolite was the glycine conjugate of piperonylic acid. Free piperonylic acid was not detected. Minor amounts of two unidentified metabolites were also present (Klungsoeyr and Scheline, 1984).

Gallic acid (3,4,5-trihydroxybenzoic acid) [FL-no: 08.080]

Gallic acid [FL-no: 08.080] was given orally to six week old male Wistar rats in order to determine the metabolic fate of the substance. After oral administration of 100 mg/kg bw, gallic acid was absorbed fairly quickly and reached the maximum concentration at 15 minutes in portal blood. The concentration was halved by 30 minutes and gallic acid had almost disappeared after six hours. The metabolite 4-O-methyl gallic acid also reached peak values within 15 minutes, and then decreased slowly. In the inferior vena cava, gallic acid and its metabolite were detected in approximately equal proportions and both reached peak values at 30 minutes after oral administration and decreased gradually until six hours after administration. The main metabolite in urine was 4-O-methyl gallic acid, but unchanged gallic acid was also found in urine. The ratio of 4-O-methyl gallic acid to total gallic acid metabolites in urine ranged from 0.55 to 0.76, indicating that a significant amount of gallic acid was excreted without being metabolised (Zong et al., 1999).

Ethyl 4-hydroxybenzoate (ethyl paraben) [FL-no: 09.367]

Ethyl paraben [FL-no: 09.367] was given orally in capsules at a dose of 1 g/kg bw to groups of three fasted dogs and blood and urine were analysed at frequent predetermined intervals until 48 hours after dosing. Metabolites were detectable in the blood up to 24 hours post-ingestion. Recovery as urine metabolites was 66 % of the administered dose at 48 hours. Dogs were also administered a 100 mg/kg bw dose of ethyl paraben intravenously and then killed to determine the distribution of the parent material and its metabolites. The ester was detected only in the brain and pancreas, whereas high concentrations of metabolites were detected in the liver and kidneys (Jones et al., 1956).

Absorption, distribution, metabolism and excretion of ethyl paraben [FL-no: 09.367] were investigated in Wistar rats administered 100 mg by oral gavage. Animals were held in metabolism cages for the collection of urine (at approximately 15, 30, 60, 75, 90, 120, 150 and 210 minutes) and blood (at approximately 30, 60, 90, 120, 180, 240 and 360 minutes), and samples were analysed to establish the excretion kinetics. Metabolites were detected in the urine starting at 30 minutes after dosing, and their concentration increased steadily during the next three to six hours. Absorption of ethyl paraben was followed by metabolism and excretion of mainly free 4-hydroxybenzoic acid and its glucuronic and glycine conjugates. A small portion of the dose was excreted as sulphate conjugate (Derache and Gourdon, 1963).

¹⁴C-labelled ethyl 4-hydroxybenzoate was orally given to four male cats in the diet at a single dose of 156 mg/kg bw. Essentially all (mean = 96.0 %) of the radioactivity was excreted in urine within 72 hours as *p*-hydroxyhippuric acid and 4-hydroxybenzoic acid (Phillips et al., 1978).

Supporting substances from subgroup 2

Piperonal (3,4-methylenedioxybenzaldehyde) [FL-no: 05.016]

In male rats a 150 mg/kg bw dose of piperonal in propylene glycol was administered by gavage. Urine samples were collected at 24 and 48 hours. Recovery of urine metabolites made up 90 % of the given dose, and metabolite excretion occurred mainly within 24 hours. No unchanged compound was detected in the urine (Klungsoeyr and Scheline, 1984).

Veratraldehyde (3,4-dimethoxybenzaldehyde) [FL-no: 05.017]

A 1 g/kg bw oral dose of veratraldehyde was administered to rabbits by gavage and urine was collected for 24 hours. At least approximately 70 % of the aldehyde was absorbed as it was present in the urine, mainly as the corresponding acid and its conjugates (Scheline, 1972).

Vanillin (4-hydroxy-3-methoxybenzaldehyde) [FL-no: 05.018]

Oral dosage of 100 mg/kg bw of vanillin to male albino rats resulted in an urinary excretion of most metabolites within 24 hours, mainly as glucuronide and sulphate conjugates, although vanillic acid was also

excreted as free acid and as glycine conjugate. After 48 hours, 94 % of the dose was excreted as different metabolites (Strand and Sheline, 1975).

A 100 mg dose of vanillin dissolved in water was given to an adult human and the urine was collected for 24 hours. During this period, an increase, from a background level, in the vanillic acid output in the urine level was measured, accounting for approximately 94 % of the vanillin dose (Dirscherl and Wirtzfeld, 1964).

4-Hydroxybenzoic acid [FL-no: 08.040]

Groups of four to eight rabbits were administered 100, 250, 500, 1000 or 1500 mg 4-hydroxybenzoic acid/kg bw by gavage. Urine was collected continuously and analysed for metabolites. Based on the total urinary recovery of the test material (84 to 104 %), the compound was almost completely absorbed, metabolised and excreted (Bray et al., 1947).

Concluding remarks on absorption, distribution and excretion

The results of these studies indicate that the benzyl derivatives in subgroup 1 as well as the hydroxy- and alkoxy-substituted benzyl derivatives in subgroup 2 are expected to be rapidly absorbed, metabolised and excreted, mainly in the urine.

III.3. Metabolism

III.3.1. Hydrolysis of Esters and Acetals

In general, esters containing an aromatic ring system are expected to be hydrolysed *in vivo* to the component acid and alcohol through the catalytic activity of carboxylesterases or esterases. In mammals, esterases occur in most tissues throughout the body but predominate in the hepatocytes (Heymann, 1980).

Subgroup 1 (Benzyl derivatives)

Candidate substances from subgroup 1

Benzyl 2-methylbutyrate (benzyl 2-methylbutanoate) [FL-no: 09.313]

Benzyl 2-methylbutyrate at a concentration of 40 microlitre/l (0.21 mM) was incubated in 0.5 M phosphate buffer at pH 7.5 and 37°C with a preparation of pancreatin for two hours. The extent of hydrolysis was 100 % as determined by gas-liquid chromatography. The supporting substance benzyl acetate [FL-no: 09.014] at a concentration of 70 microlitre/l (0.49 mM) was 50 % hydrolysed after 2 hours (Grundschober, 1977).

Supporting substances from subgroup 1

Benzyl acetate [FL-no: 09.014]

Neat benzyl acetate was spiked into control rat plasma (1 microlitre/0.5 ml), vortexed and incubated at room temperature for 0.5 - 36 minutes. Incubation was terminated by addition of acetonitrile. The plasma was centrifuged to precipitate plasma proteins and the clear plasma was analysed by (High Performance Liquid Chromatography) HPLC to determine benzyl acetate and benzyl alcohol. Benzyl acetate was found to be rapidly hydrolysed to benzyl alcohol. The half-life of benzyl acetate was about 4 minutes and 24 minutes after spiking virtually all benzyl acetate was hydrolysed to benzyl alcohol. Hydrolysis was partially inhibited by the esterase inhibitor sodium fluoride, which suggests that plasma esterases contribute to the rapid

hydrolysis. When benzyl acetate was administered to rats and mice in gavage and dosed feed studies, benzyl acetate was not detected in any plasma samples collected (Yuan et al., 1995).

In vivo metabolism studies in mice and rats clearly indicate that radiolabelled benzyl acetate is readily hydrolysed since more than 90 % of the radioactivity is demonstrated in the urine as benzoic or hippuric acid (Abdo et al., 1985).

Benzyl acetate was hydrolysed in pig liver homogenate. At pH 7.4 and 25° C the velocity was calculated to 27 micromole/min/mg, K_m 0.55 mM. (Greenzaid & Jenks, 1971 referred in (Heymann, 1980)).

Alkyl- and aryl-benzoates

The plasma half-lives ($t_{1/2}$) for the *in vitro* hydrolysis by plasma enzymes of a series of four alkyl benzoates (including supporting chemicals methyl benzoate [FL-no: 09.725], ethyl benzoate [FL-no: 09.726] and propyl benzoate [FL-no: 09.776]) and two aryl benzoates (including supporting substance benzyl benzoate [FL-no: 09.727]) in 80 % human blood plasma ranged from 24 to 210 minutes for the alkyl benzoates. By increasing chain length, an increasing enzymatic degradation was seen, except when going from methyl to ethyl. The butyl ester was the least resistant ($t_{1/2}$ 24 minutes), while the ethyl ester was the most resistant to hydrolysis ($t_{1/2}$ 210 minutes). The plasma half-lives were 19 and 15 minutes for phenyl benzoate and benzyl benzoate, respectively (Nielsen and Bundgaard, 1987).

An *in vitro* hydrolysis study demonstrated that benzyl phenylacetate was 100 % hydrolysed within two hours of incubation with a pancreatin solution, whereas the supporting substance benzyl acetate [FL-no: 09.014] was only 50 % hydrolysed after 2 hours incubation. Benzyl cinnamate and methyl phenylacetate were 80 and 70 % hydrolysed, respectively (Leegwater and Straten, 1974a).

Other related substances:

4-Methyl-2-phenyl-1,3-dioxolane (benzaldehyde propylene glycol acetal)

Benzaldehyde-related acetals readily hydrolyse to their component alcohols and benzaldehyde under acidic conditions. Hydrolysis of acetals in simulated gastric juice (pH 1.2) and simulated intestinal fluid (pH 7.5) was monitored by the formation rate of aldehyde liberated during treatment. Data show that non-cyclic acetals are completely hydrolysed at pH 1.2 but that hardly any hydrolysis occurs at pH 7.5. Benzaldehyde-propylene-glycol acetal (4-methyl-2-phenyl-1,3-dioxolane, MPD), a cyclic acetal, was hydrolysed to an extent of around 50 % after one hour in simulated gastric juice and no further hydrolysis was observed after five hours. Reflux of MPD for five hours in 0.1 N HCl also resulted in hydrolysis to an extent of 50 % of the theoretical maximum. Due to the same poor hydrolysis of MPD (to around 50 %), even after five hours reflux in 0.1 N HCl, the author questioned the chemical identity of the substance (Morgareidge, 1962a). The result of this study on hydrolysis of a cyclic benzaldehyde acetal is inconclusive.

Subgroup 2 (Hydroxy- and alkoxy-substituted benzylderivatives)

Candidate substances from subgroup 2

Ethyl 4-hydroxybenzoate (ethyl paraben) [FL-no: 09.367]

An *in vitro* assay demonstrated that ethyl paraben is efficiently hydrolysed by the liver and kidney esterases as 96 % hydrolysis was measured after three minutes in dog liver tissue suspension and 100 % hydrolysis after 30 minutes in dog kidney suspension (Jones et al., 1956).

Ethyl paraben [FL-no: 09.367] was 80 % hydrolysed to free 4-hydroxybenzoic acid within 60 minutes in perfused mouse liver, only 2.3 % intact ester was recovered. Ethyl paraben was not detected in the blood of six humans 1 - 4½ hours after oral intake of 10 to 20 mg/kg bw. When given orally to dogs at doses between

25 and 500 mg/kg bw, high serum concentrations of 4-hydroxybenzoic acid were reported and no ethyl paraben was detected in the blood except for the 500 mg/kg bw dose (Heim et al., 1957).

Studies were conducted with methyl, ethyl [FL-no: 09.367], propyl and butyl 4-hydroxybenzoate [FL-no: 09.754] (supporting substance) in dogs. The results showed significantly higher rates of test material recovery in the urine of dogs dosed orally, 1 g/kg bw orally or 50 mg/kg bw by the intravenously route for the methyl, ethyl and propyl esters (% of dose excreted within 48 hours, oral: 89.0, 66.0 and 57.6 %, respectively; i.v. 85, 70 and 94 %, respectively) as compared to the butyl ester (oral 48.2 %; i.v. 40.1 %). The methyl, ethyl and propyl esters showed 100 % hydrolysis within 3 minutes when incubated with liver homogenate, whereas the butyl ester was completely hydrolysed only after 30 - 60 minutes. This finding suggests that an increase in the alkyl chain length in the homologous series of alkyl esters make the esters more resistant to hydrolysis (Jones et al., 1956).

Vanillin propylene glycol acetal [FL-no: 06.104]

Under acidic conditions, pH 2.6, vanillin propylene glycol acetal [FL-no: 06.104] began to hydrolyse immediately with approximately 3 % of the acetal disappearing and 92 % hydrolysed within two hours. At pH 1.8, approximately 90 % of vanillin propylene glycol acetal hydrolysed immediately and 93 % hydrolysed within five minutes (Bennett, 1997).

Supporting substances from subgroup 2

Methyl salicylate (methyl 2-hydroxybenzoate) [FL-no: 09.749]

An oral dose of methyl salicylate equivalent to 500 mg/kg bw of salicylic acid was dissolved in 2 % methyl cellulose and administered to male rats. The plasma levels measured within 20 minutes of dosing showed complete hydrolysis of methyl salicylate. A similar experiment was conducted with male dogs. Capsules containing 320 mg methyl salicylate/kg bw were given orally to three fasted dogs in five repeated experiments. Blood drawn 1 and 4 hours after dosing showed 95 % hydrolysis of methyl salicylate to salicylic acid at both time intervals. Six humans were given a 0.42 ml dose of methyl salicylate administered in ginger ale. Blood was drawn by venipuncture 15 and 90 minutes later. In contrast to the other two species an appreciable portion of unhydrolysed methyl salicylate was found, 39 % after 15 minutes and 21 % after 90 minutes (Davison et al., 1961).

Other related substances

At low pH similar to that found in the stomach, a structurally related substance, vanillin 3-(1-menthoxy)propane-1,2-diol acetal [FL-no: 02.248], is readily hydrolysed. In a hydrolysis study, 12 - 39 mM vanillin 3-(1-menthoxy)propane-1,2-diol acetal underwent 91 % hydrolysis at pH 2 within 45 minutes. At pH 3, approximately 86 % of vanillin 3-(1-menthoxy)propane-1,2-diol acetal was hydrolysed within 90 minutes. At pH 4, approximately 92 % of the acetal was hydrolysed within eight hours. At pH 5, approximately 12 % of the flavouring substance was hydrolysed within eight hours (Reitz, 1995).

Concluding remarks on hydrolysis

There is some information about hydrolysis of esters in *in vivo* as well as *in vitro* systems for some candidate and supporting substances in subgroups 1 and 2.

It is expected that esters in subgroup 1 and 2 will be hydrolysed *in vivo*.

Nine of the candidate substances in subgroup 1 [FL-no: 09.152 (benzyl valerate); 09.313 (benzyl 2-methylbutyrate); 09.314 (benzyl crotonate); 09.315 (benzyl dodecanoate); 09.316 (benzyl hexanoate); 09.317 (benzyl lactate); 09.318 (benzyl octanoate); 09.835 (benzyl decanoate) and 09.858 (phenylmethyl 2-

methyl-2-butenolate)] will yield benzyl alcohol and simple aliphatic carboxylic acids upon hydrolysis. One ester, 4-isopropylbenzyl acetate [FL-no: 09.611], will yield 4-isopropylbenzyl alcohol and acetic acid.

The acetal in subgroup 1, (diethoxymethyl)benzene [FL-no: 06.017], is expected to be efficiently hydrolysed to yield benzaldehyde and ethanol.

Four of the remaining esters in subgroup 1 are expected to yield benzoic acid and simple aliphatic alcohols upon hydrolysis, [FL-no: 09.656 (3-methylbut-3-enyl benzoate); 09.693 (prenyl benzoate); 09.779 (butyl benzoate); 09.825 (pentyl benzoate)]. One ester, [FL-no: 09.631] (methyl 4-methylbenzoate), will yield 4-methylbenzoic acid upon hydrolysis. The alcohol part of the candidate substance [FL-no: 09.656] (3-methylbut-3-enyl benzoate) includes a terminal double bond.

Of the 13 esters in subgroup 2, one ester, [FL-no: 09.895] (4-methoxybenzyl-2-methylpropionate)], will yield 4-methoxybenzyl alcohol (*p*-anisyl alcohol) [FL-no: 02.128] upon hydrolysis. The remaining 12 esters in subgroup 2, [FL-no: 09.362 (ethyl 2-hydroxy-4-methylbenzoate); 09.363 (ethyl 2-methoxybenzoate); 09.367 (ethyl 4-hydroxybenzoate); 09.560 (hex-3(*cis*)-enyl anisate); 09.570 (hex-3-enyl salicylate); 09.581 (hexyl salicylate); 09.623 (methyl 2,4-dihydroxy-3,6-dimethylbenzoate); 09.696 (prenyl salicylate); 09.762 (pentyl salicylate); 09.798 (ethyl vanillate); 09.799 (methyl vanillate); 09.852 (2-methylbutyl 2-hydroxybenzoate)], will yield hydroxy and/or alkoxy-substituted benzoic acid upon hydrolysis.

III.3.2 Metabolism Studies

Subgroup 1 (Benzyl derivatives)

Candidate substances from subgroup 1

In a series of *in vitro* studies with purified microsomal rat liver aldehyde dehydrogenase, Martini and Murray (1996) (Martini and Murray, 1996) demonstrated that *m*- and *p*-tolualdehyde [FL no: 05.028 and 05.029] are substrates for this enzyme, which converts these aldehydes into the corresponding toluic acid derivatives using NAD as a co-factor. The affinity (K_m) was much lower than for aliphatic aldehydes ($\sim 200 - 500$ higher K_m), but V_{max} values were in the same order of magnitude. The *o*-tolualdehyde is a relatively poor substrate for which the V_{max} was at least 5 times lower than for the other two isomers. It was not studied to what extent this enzyme contributes to the overall oxidation of these three aldehydes *in vivo*.

The overall conversion of the three tolualdehyde isomers into the corresponding toluic acids was studied by Watanabe et al (1995) (Watanabe et al., 1995) in mouse liver microsomes. The oxidation rates were predominantly depending on enzymes using NADPH, rather than NAD, as co-factors with reaction rate ratios of 3, 1.1 and 1.4 (rate NADPH/NAD) for *o*-, *m*- and *p*-tolualdehyde, respectively. This would indicate that cytochrome P450 enzymes contribute for 50 to 75 % to the overall conversion rate. Using a reconstituted metabolic system and antibodies against cyp2C29, it was demonstrated that this enzyme contributed most. The enzymatic nature of the NAD-driven oxidation reaction was not further investigated but could have been a non-P450-related aldehyde dehydrogenase. The contribution of cyp2C29 to the overall tolualdehyde oxidation *in vivo* was not further investigated.

Seutter-Berlage et al. (1982) (Seutter-Berlage et al., 1982) studied the influence of various *o*- and *p*-ring substituents of benzaldehyde and benzyl alcohol derivatives on the excretion of urinary thioethers in rats after a single intraperitoneal injection with approximately 400 to 440 micromol per substance/animal. No increase in thioether elimination was observed with the candidate substance *p*-tolualdehyde [FL no: 05.029] and with the supporting substances benzaldehyde, *p*-hydroxybenzaldehyde and *p*-methoxybenzaldehyde [FL no: 05.013, 05.047 and 05.015]. However, with the candidate substances *o*-methoxybenzaldehyde and *o*-tolualdehyde [FL no: 05.129 and 05.026] increases in urinary thioester excretion were observed. It was

estimated that from the two candidate substances, approximately 2 and 7 % of the dose was eliminated as a mercapturic acid conjugate after reduction of the aldehyde group via a benzyl alcohol sulphate ester as intermediate. By comparison of the influence of the various ring substituents, it was speculated that a larger substituent at the ortho position would result in a larger part of the dose to participate in aldehyde reduction and subsequent sulphate conjugation followed by conjugation with glutathione (Seutter-Berlage et al., 1982). Similarly, conversion of *o*-tolualdehyde into mercapturic acid conjugates has been reported by Van Doorn et al. (1981). After intraperitoneal dosing of rats with 1 mmol/kg bw (roughly equivalent to 250 micromol/animal) ca. 2 % of the dose was excreted as thioether conjugate via the urine (van Doorn et al., 1981).

Supporting substances from subgroup 1

Benzyl alcohol [FL-no: 02.010]

Five minutes after single intraperitoneal doses of 500 - 1100 mg/kg bw of benzyl alcohol administered to CD1 mice, benzyl alcohol was detected in plasma. At doses of 700 - 1100 mg/kg bw, plasma also contained measurable concentrations of benzaldehyde. Animals pre-treated with an alcohol dehydrogenase inhibitor (pyrazole) showed a 200 % increase in plasma benzyl alcohol levels, whereas pre-treatment with an aldehyde dehydrogenase inhibitor (disulphiram) resulted in a 368 % increase in plasma benzaldehyde levels as compared to control values (McCloskey et al., 1986).

Conjugation of benzyl alcohol with glutathione has been observed by Van Doorn et al (1981) (van Doorn et al., 1981), who estimated that after an intraperitoneal dose of 1 mmol/kg bw to rats, approximately 0.8 % of the dose was eliminated as mercapturic acid via the urine. From additional studies it was anticipated that the sulphate ester of the alcohol would participate as an intermediate in the ultimate conjugation to glutathione.

Benzaldehyde [FL-no: 05.013]

The metabolism of benzaldehyde was investigated in Sprague Dawley rats (5/group/sex) which were administered single oral doses of 400, 750 or 1000 mg/kg bw of pure benzaldehyde by gavage once daily for 13 consecutive days. Urine was collected for 24 hours after the 2nd, the 8th and the 13th dose and analysed for the presence of metabolites. The major metabolites were benzoic acid conjugates and benzylmercapturic acid. Although females in the mid- and high-dose groups exhibited a slight decrease in excretion of benzylmercapturic acid after the 8th dose, all groups showed increased urinary levels after the 13th doses. An increase in dose from 400 to 1000 mg/kg bw/day resulted in a 7- to 8-fold increase in benzylmercapturic acid excretion. The amount of benzylmercapturic acid excreted in urine collected for 24 hours ranged between 0.13 - 2.05 mg/rat, the higher amounts collected from the rats in the highest dose groups. Benzaldehyde is reduced to benzyl alcohol only to a minor extent; the alcohol sulphate conjugate may further react with glutathione to form benzyl mercapturic acid (Laham and Potvin, 1987).

In the rabbits orally dosed with 350 or 750 mg/kg bw, the aldehyde was oxidised mainly to benzoic acid and excreted predominantly as hippuric acid (approximately 68 % of the administered dose). Other urinary metabolites detected were benzoylglucuronic acid (10 %), benzoyl glucuronide (3 %), free benzoic acid (1.5 %), and trace amounts of benzylmercapturic acid (Laham et al., 1988).

4-Isopropylbenzaldehyde (cuminaldehyde) [FL-no: 05.022]

High doses (2000 mg) of *p*-isopropylbenzaldehyde were given orally to male rabbits. Urine was collected for three days post-treatment. The yield of urinary oxidation metabolites was higher than that of reduction metabolites. This was in contrast to *o*-isopropylbenzaldehyde, the reduction of which was more extensive and the corresponding acids were not found. *p*-Isopropylbenzaldehyde mainly undergoes a combination of oxidation of the aldehyde function and the oxidation of the alkyl-side chain to yield 9-hydroxycuminic acid and 8-hydroxycuminic acid. Cumyl alcohol (cumyl alcohol, 4-isopropylbenzyl alcohol) and 2-carboxyphenylpropionic acid were minor urinary metabolites. It was concluded that oxidation or reduction

was controlled by the position of substituents, in that oxidation occurs with the *p*-isomer and reduction occurs with the *o*-isomer. In addition, stereoselective oxidation was found in the aromatic isopropyl group of the *p*-isomer (Ishida et al., 1989b).

Benzoic acid [FL-no: 08.021]

Ring labelled ^{14}C -benzoic acid was given orally at doses in the range of 1 - 400 mg/kg bw to various species including primates, pigs, rabbits, rodents, cats, dogs, hedgehogs, bats, birds and reptiles. Hippuric acid was the primary urinary metabolite in most species. The ornithine conjugate of benzoic acid, ornithic acid, was the major urinary metabolite excreted within 24 hours in chickens and reptiles. Benzoyl glucuronide was predominant in bats. In humans, more than 99 % of ^{14}C was excreted as hippuric acid within 24 hours (Bridges et al., 1970).

Following oral administration of 375 mg [^{14}C]-benzoic acid/kg bw to rats, 91 - 94 % of the radioactivity was recovered in the urine of rats after 72 hours, whereas only 1 - 6 % was present in the faeces. The following metabolites were identified: hippuric acid (70.2 - 84.2 %), benzoyl glucuronide (0.7 - 1.8 %), benzoic acid (0.4 - 12.8 %) and 3-hydroxy-3-phenyl propionic acid (0.1 - 0.2 %) (Nutley, 1990).

Urinary hippuric acid is used as a biological marker of toluene exposure. In order to investigate the types and quantities of beverages that increase urinary hippuric acid excretion, 137 healthy students were recruited and divided into quintiles based on their consumption of non-alcoholic beverages containing benzoic acid. HPLC was used to determine benzoic acid intake from beverages and urinary hippuric acid before, and 1.5 and 3 hours after consumption of various beverages. The range of benzoic acid in 13 beverages was 0 - 1.02 mg/ml and the benzoic acid intakes from the beverages for groups 1 - 5, respectively, were: 0.4 mg $\pm$ 0.5; 23.4 mg $\pm$ 9.8; 55.2 mg $\pm$ 2.3; 76.3 mg $\pm$ 4.0; 116.5 mg $\pm$ 16.5. Urinary hippuric acid geometric mean concentrations before consuming beverages in the five groups, respectively, were 0.276, 0.270, 0.207, 0.262 and 0.316 g/l; 1.5 hours after beverage consumption they were 0.210, 0.603, 1.026, 1.066 and 1.688 g/l and significantly increased ($p < 0.001$) after adjustment for urinary hippuric acid before ingestion. Three hours after beverage consumption, urinary hippuric acid geometric mean concentrations in the five groups, respectively, were 0.160, 0.232, 0.306, 0.287 and 0.337 g/l ($p < 0.001$). The authors concluded that beverages containing more than 100 mg benzoic acid may increase urinary hippuric acid significantly (Chang et al., 2000).

Benzyl acetate [FL-no: 09.014]

Following gavage administration of [methylene- ^{14}C]-benzyl acetate to groups of three or more male Fischer 344 rats at a dose of 5, 250 or 500 mg/kg bw as the substance alone, in corn oil, or in propylene glycol, 70 - 89 % of the dose was excreted in the urine within 24 hours. Approximately 4 % of the radioactivity was detected in the faeces after 72 hours and about 1 % in the carcass after 72 hours. The elimination of benzyl acetate and metabolites, regardless of vehicle, was largely complete after three days. Urine was collected and urinary metabolites were assayed by Thin Layer Chromatography (TLC) and HPLC. In other animals, ^{14}C plasma levels were measured, and variation of metabolites in plasma were assayed. No benzyl acetate was detected in the plasma or urine at any time point. Small amounts of benzyl alcohol were detected in the plasma at early time points after administration of the neat substance or dissolved in propylene glycol. After administration of 500 and 250 mg/kg bw, unconjugated benzoic acid was the major plasma metabolite. After the 5 mg/kg bw dose, hippuric acid was the major plasma metabolite. At the higher dose levels, small amounts of radioactivity (< 5 % of total plasma ^{14}C) was present as unknown metabolites of high and moderate polarity, but not in all samples. At the 5 mg/kg bw dose, 20 % of plasma ^{14}C was present as the unknown polar metabolite, although this became less important with time. When propylene glycol was used as vehicle, benzylmercapturic acid was detected in plasma, but only at the 5 mg/kg bw dose. Hippuric acid was always the major urinary metabolite but the proportion of dose present as benzoyl glucuronide increased with dose. Low levels (1.0 - 3.6 %) of benzoic acid and benzylmercapturic acid (1.0 - 1.9 %) excreted in urine were not significantly affected by dose or vehicle (Chidgey and Caldwell, 1986).

Chidgey et al. (1986) suggest that formation of benzylmercapturic acid occurs via formation of benzyl sulphate. In a study designed to define the route of metabolism of benzyl acetate leading to the formation of benzylmercapturic acid, male Fischer 344 rats were dosed by gavage with [methylene-¹⁴C]benzyl acetate (500 mg/kg bw) alone or together with pyrazole (200 mg/kg), pentachlorophenol (10 mg/kg bw) or both. Urine and faeces were collected and urinary metabolites were assayed by radio-TLC and HPLC. The excretion of ¹⁴C was rapid in all cases, with most of the dose being excreted in urine within 24 hours. Co-administration of benzyl acetate with pyrazole, an inhibitor of alcohol dehydrogenase, caused an 11-fold increase in the excretion of benzylmercapturic acid and halved the percentage of the dose excreted as benzoyl glucuronide. Pretreatment with pentachlorophenol, an inhibitor of sulphotransferase activity *in vivo*, abolished the excretion of benzylmercapturic acid, while excretion of the mercapturate following treatment with both pyrazole and pentachlorophenol was higher than in control or pentachlorophenol treated rats, but much lower than in the animals given pyrazole alone. Taken together, these results suggest that the formation of benzylmercapturic acid involves the sulphate ester of benzyl alcohol as an obligatory intermediate and that formation of reactive metabolites of toxicological significance is unlikely (Chidgey et al., 1986).

Fischer 344 rats and C57BL/6N mice were administered [ring-UL-¹⁴C]benzyl acetate at single oral doses of 5 or 500 mg/kg bw in rats or 10 mg/kg bw in mice, and urine and faeces were collected for 96 hours to determine the effects of age on disposition of benzyl acetate. Age groups studied were 3 to 4, 9 and 25 month-old rats and 2, 13 and 25 month-old mice. In rats, approximately 80 % of radioactivity was recovered in the urine in the first 24 hours for all age groups. The major urinary metabolite was hippuric acid (> 90 % of total urinary radioactivity) and benzylmercapturic acid (1 - 2 %) was the only other metabolite detected in the urine of rats. There were no age differences in the percentage of [¹⁴C]benzyl acetate excreted as hippuric acid, but the amount of excreted benzyl mercapturic acid increased slightly in the 25 month-old rats as compared to younger rats. The percentage of radioactivity excreted in the faeces was slightly decreased in the 25 months old group. In mice, hippuric acid was the major urinary metabolite, constituting 93 - 96 % of the total dose after 96 hours. Less radioactivity was excreted in the urine of 25 month-old mice than in the younger groups. Faecal excretion was a minor route and the amount was similar for all age groups. The authors concluded that formation of hippuric acid is not affected by age, but aging does affect the minor routes of metabolism and excretion of benzyl acetate in rats and mice (McMahon et al., 1989).

Benzyl acetate was administered to rats and mice in gavage and dosed feed studies. Gavage study groups of six male F344/N rats and twelve male B6C3F₁ mice were administered benzyl acetate in corn oil at 500 mg/kg (rat) and 1000 mg/kg (mouse). Blood samples were collected 5 min - 24 hours after dosing. Dosed feed studies groups of ten rats and ten mice, of the same strains as in the gavage study, were dosed with benzyl acetate in feed (10,800 ppm for rats and 2700 ppm for mice) *ad libitum* during the study. The concentrations in feed were estimated to provide a daily benzyl acetate dose of 648 mg/kg for rats and 900 mg/kg for mice. At day 7 and 8 blood samples were collected at five time points during 15 hours, with two animals from each species sampled at each time point. Benzyl acetate was not detected in any plasma samples collected in the studies. Except for the 5 and 10 minutes rat plasma samples and the 5 minutes mice plasma samples in the gavage study, no benzyl alcohol was detected in plasma. Concentrations of benzoic acid and hippuric acid in plasma rapidly increased to peak concentrations within 3 hours after gavage with the peak benzoic acid concentrations being much higher (about 10- to 20-fold) than the peak hippuric acid concentrations. Plasma concentrations of benzoic acid in the dosed feed studies were much lower (more than 100-fold) than the concentrations in the gavage studies, consistently with the mode of administration (bolus dose with gavage). Plasma concentrations of hippuric acid were comparable in both studies. The absence of benzyl acetate in plasma shows that benzyl acetate is rapidly hydrolysed to benzyl alcohol. The major metabolite of benzyl acetate, benzoic acid, is mainly dependent on the conjugation pathway involving Coenzyme A (CoA). This pathway would be saturated when plasma concentrations of benzoic acid are very high or when the CoA is depleted. Such conditions appear to have occurred after a bolus gavage dose of benzyl acetate to result in a brief peak in the plasma concentration of benzoic acid. When benzyl acetate was administered to rats and mice in dosed feed, it appears that the CoA conjugation pathway was never saturated and plasma concentration of benzoic acid remained low (Yuan et al., 1995).

Other related substances

Sodium benzoate

Male volunteers were given oral doses of 2000 to 5000 mg sodium benzoate. The 5000 mg dose group was given a 5000 mg dose of glycine one hour later and 2000 mg doses every two hours thereafter. Benzoate was excreted mainly as hippuric acid. No free benzoic acid was detected. Minor amounts of benzoyl glucuronide were detected at both doses. Co-administration of glycine with benzoate increased the rate of hippuric acid excretion, indicating that at high dose levels, glycine is rate limiting for formation of hippuric acid (Amsel and Levy, 1969).

After administration of oral doses of 40, 80 and 160 mg/kg bw of sodium benzoate to humans, the mean plasma Area Under Curves (AUCs) of benzoic acid increased disproportionately to the dose, 3.7 and 12.0 times greater, respectively, for the higher dosages than for the lowest dose, while the mean AUCs for hippuric acid was proportional to dose. Peak plasma concentrations of benzoic acid increased with increasing dose, while peak hippuric acid concentrations did not change. The data suggest that the conjugation with glycine to form hippuric acid is a saturable process in humans (Kubota et al., 1988; Kubota and Ishizaki, 1991).

Subgroup 2 (Hydroxy- and alkoxy-substituted benzyl derivatives)

Candidate substances from subgroup 2

Piperonyl alcohol (3,4-methylenedioxybenzyl alcohol) [FL-no: 02.205]

The metabolism of piperonyl alcohol [FL-no: 02.205] and piperonal (3,4-methylenedioxybenzaldehyde) [FL-no: 05.016] (supporting substance) was studied in male Wistar rats. Piperonyl alcohol dissolved in propylene glycol was administered by oral gavage at a dose of 1 mmol/kg bw (corresponding to 152 mg/kg bw) and urine samples were taken at 24 and 48 hours. Recovery of urinary metabolites were 90 %, and metabolite excretion occurred mainly within 24 hours. Piperonyl glycine was identified as the major metabolite (70 ± 5 %; 24-hour analysis expressed as a percent of administered dose) and piperonylic acid (17 ± 3 %) was the other important metabolite. Demethylenation of the methylenedioxy moiety led to the excretion of three catechol derivatives, which accounted for 0.7 % of the dose, protocatechuic acid (0.4 ± 0.1 %), protocatechuyal alcohol (0.3 ± 0.1 %). Other minor metabolites were piperonyl alcohol (1.4 ± 0.5 %) and vanillyl alcohol (0.05 ± 0.03 %) (Klungsoeyr and Scheline, 1984).

In a study of several methylenedioxyphenyl (MDP) compounds, radiolabelled piperonyl alcohol (1-hydroxymethyl-3,4-methylene- ^{14}C -dioxybenzene) was administered to male Swiss-Webster mice in a DMSO solution by oral gavage at a dose of 0.76 mg/kg bw. Total radiocarbon determinations were made on expired $^{14}\text{CO}_2$ at 0.5, 1, 2, 4 and 6 hours after dosing and at each 6-hour interval thereafter. Urine and faeces samples were taken at 12, 24 and 48 hours after treatment. The 12-hour urine samples were used for separation and characterisation of metabolites. At the end of the experiment, $^{14}\text{CO}_2$ excretion amounted to 3 % of the dose, indicating that demethylenation of piperonyl alcohol only occurs as a minor metabolic pathway. The major part of radioactivity was retrieved in urine, 93.3 %, and less than 10 % in faeces. The major urinary metabolite after administration of piperonyl alcohol was piperonyl glycine. Other MDP substances studied were safrole, dihydrosafrole, myristicin, Tropital, piperonyl butoxide, piperonal and piperonylic acid. The major metabolic pathway for piperonyl butoxide, safrole, dihydrosafrole and myristicin was demethylenation of the methylenedioxy moiety. As for piperonyl alcohol, oxidation and conjugation of the side chain is the major metabolic pathway for Tropital, piperonal and piperonylic acid. The authors discussed that the polar nature of these compounds or their ease of conversion to polar products may minimise their entrance to the lipid components of the microsomal enzymes so that no extensive demethylenation would occur (Kamienski and Casida, 1970).

Gallic acid (3,4,5-trihydroxybenzoic acid) [FL-no: 08.080]

Following administration of gallic acid [FL-no: 08.080] to rats, either in the diet at a concentration of 0.5 % or in single doses of 100 mg/rat via oral gavage, the major urinary excretion products were the unchanged parent substance and one metabolite which was concluded to be 4-O-methyl gallic acid. A minor metabolite, 2-O-methylpyrogallol, was excreted mainly as an acid-labile conjugate. When the same compound was given at 100 mg/rat as intraperitoneal injection, the results were similar to those obtained when the substance was given orally, however, a minor metabolite identified as pyrogallol was also present along with a trace of 2-O-methylpyrogallol. Rabbits administered a diet containing 0.5 % gallic acid also excreted 4-O-methyl gallic acid, pyrogallol, and possibly also 2-O-methyl pyrogallol. The data indicated that mostly free benzoic acid derivatives were excreted, although rabbits excreted an acid-labile conjugate of 4-O-methyl gallic acid. The results indicate that O-methylation and decarboxylation are the reactions involved in the metabolic conversion of gallic acid. The authors stated that this selective O-methylation would prevent the formation of the catechol configurations (Booth et al., 1959).

Scheline (1966a) reported that rats that were administered 100 mg/kg bw of gallic acid [FL-no: 08.080] by oral gavage excreted the parent substance and the free and acid-labile conjugates of its 4-O-methyl ether. Pyrogallol and 2-O-methylpyrogallol, the decarboxylated metabolites, were excreted in their conjugated forms. Dosing with 30 and 300 mg gallic acid/kg bw showed that excretion of decarboxylated metabolites increased with increasing dose. Intraperitoneal injection of gallic acid in four rats resulted in the urinary excretion of gallic acid and 3,5-dihydroxy-4-methoxybenzoic acid, but neither pyrogallol or 2-O-methylpyrogallol were detected in these urines. A study of the ability of rat intestinal contents to metabolise gallic acid showed that it was decarboxylated to pyrogallol when test substance was added to medium containing extracts of caecal or colon contents. Test substance was recovered essentially unchanged when small intestine contents were used (Scheline, 1966a).

In order to examine decarboxylation and demethylation of some phenolic benzoic acid derivatives by rat caecal contents, test substances were incubated for 22 hours in medium containing caecal contents. Solutions together with appropriate standards were then examined by TLC. Gallic acid [FL-no: 08.080] gave rise to pyrogallol which was present in five out of eight samples. When pyrogallol was absent after incubation, large amounts of resorcinol were observed on the chromatograms. Dehydroxylation to resorcinol was also seen when pyrogallol itself was incubated with caecal extract. Unchanged gallic acid was found in 4 out of 8 samples. Pyrogallol was not dehydroxylated to catechol in these experiments. The main findings of the study that covered 27 phenolic benzoic acid derivatives was that decarboxylation only occurred when a free hydroxyl group was present in the *para* position (Scheline, 1966b).

The metabolic fate of gallic acid [FL-no: 08.080] in peripheral blood, liver and urine after oral administration was studied in six-week-old male Wistar rats in order to determine the most appropriate route of administration for the treatment of liver cancer, i.e. the route that gives the highest concentration of gallic acid in liver. Gallic acid was given orally to the rats at 50, 100 or 500 mg/kg bw (The number of animals is not reported, but results from the 100 mg/kg bw group are shown as the mean of 4 - 6 animals). Blood samples were taken from the portal vein and the inferior vena cava at 5, 15, 30, 60, 180 and 360 minutes after administration and urine was collected at the same time points. Intestinal contents were collected and, in order to avoid contamination due to enterohepatic circulation, bile duct was ligated before oral administration of 100 mg/kg bw. Animals were sacrificed and the entire intestinal contents were collected. The liver was removed from terminated animals after perfusion with saline to eliminate blood contamination. For analysis, samples from serum, urine and liver were processed and then analysed by HPLC. Gallic acid reached its peak concentration in the portal vein 15 minutes after oral administration of 100 mg/kg bw. After 30 minutes it had decreased to half the concentration and had almost disappeared after 6 hours. The only metabolite detected in the blood and urine was identified as 4-O-methyl gallic acid. 4-O-Methyl gallic acid also reached peak concentration in the portal vein after 15 minutes, and then decreased slowly. In the inferior vena cava both gallic acid and 4-O-methyl gallic acid reached peak concentration at 30 minutes after oral administration of 100 mg gallic acid/kg bw. In the portal vein, gallic acid was detected at about twice the concentration of 4-O-methyl gallic acid. In the inferior vena cava, 4-O-methyl gallic acid and gallic acid

were detected in approximately equal proportions and in about the same amount as 4-O-methyl gallic acid in the portal vein. 4-O-Methyl gallic acid, but not gallic acid, was found in the liver homogenate prepared after thorough perfusion with saline. The main metabolite of gallic acid in urine was 4-O-methyl gallic acid and its concentration was about 100 times higher than in the inferior vena cava. Gallic acid was also found in urine at a higher concentration than in the inferior vena cava, but at lower concentration than 4-O-methyl gallic acid in urine. In contrast to previously published studies (Booth et al., 1959; Scheline, 1966a), this study (Zong et al., 1999) did not detect pyrogallol as a metabolite in blood or urine. The authors attribute this discrepancy to the earlier studies using TLC for determination of metabolites, but without proper determination of structures, and also comment that the time for collecting urine under unstable conditions may have led to the decomposition of gallic acid to pyrogallol (Zong et al., 1999).

Ethyl 4-hydroxybenzoate (ethyl paraben) [FL-no: 09.367]

Absorption, distribution, metabolism and excretion of ethyl paraben [FL-no: 09.367] were investigated in Wistar rats administered 100 mg by oral gavage. Animals were held in metabolism cages for the collection of urine (at approximately 15, 30, 60, 75, 90, 120, 150 and 210 minutes) and blood (at approximately 30, 60, 90, 120, 180, 240 and 360 minutes), and samples were analysed to establish the excretion kinetics. Metabolites were detected in the urine starting at 30 minutes after dosing, but no unchanged ethyl paraben was identified. *p*-Hydroxyhippuric acid appeared in the urine 30 minutes after dosing and its concentration increased steadily during the next three hours. The glucuronide and ethereal sulphate metabolites only appeared between 30 and 75 minutes post-ingestion. A continuous increase of free 4-hydroxybenzoic acid in the blood occurred during the first hour post-dosing, but its concentration then decreased over the next hour and plateaued for the remaining four hours of sample collection. Maximum urinary excretion of free 4-hydroxybenzoic acid occurred at 90 minutes post-dosing, whereas excretion of the glucuronic and glycine conjugates increased until the end of the collection period at 210 minutes post-dosing. In summary, absorption of ethyl paraben was followed by metabolism and excretion of mainly free 4-hydroxybenzoic acid and its glucuronic and glycine conjugates. A small portion of the dose was excreted as sulphate conjugate (Derache and Gourdon, 1963).

The ¹⁴C-labelled ethyl 4-hydroxybenzoate was orally given to four male cats in the diet at a concentration that provided a single dose of 156 mg/kg bw, equivalent to 130 mg/kg bw of the parent acid. Urine was collected at 24-, 48- and 72-hour intervals, total faeces were collected at 72-hour, and all samples were assayed for total radioactivity. The radioactive metabolites present in the 24-hour samples were isolated and identified. Essentially, all (mean = 96.0 %) of the radioactivity was excreted within 72 hour with the breakdown expressed as mean values for the four animals as follows: 24-hour urine, 85.8 %; 48-hour urine, 3.6 %; 72-hour urine, 0.8 %; 72-hour faeces, 5.8 %. The 24-hour urine samples revealed two metabolites. Metabolite I contained between 54 and 69 % of the administered radioactivity and had a similar retention volume as *p*-hydroxyhippuric acid. Metabolite II contained between 31 and 46 % of the administered radioactivity and had a similar retention volume as 4-hydroxybenzoic acid. Additional evaluations confirmed the identity of the suggested metabolites (Phillips et al., 1978).

Ethyl 4-hydroxybenzoate (ethyl paraben) [FL-no: 09.367] and supporting substances 4-hydroxybenzoic acid [FL-no 08.040] and butyl 4-hydroxybenzoate (butyl paraben) [FL-no: 09.754]

Groups of three fasted dogs were administered single doses of 1 g/kg bw of 4-hydroxybenzoic acid ([FL-no 08.040], supporting substance) or its methyl, ethyl ([FL-no: 09.367], candidate substance), propyl and butyl ([FL-no: 09.754], supporting substance) esters orally or 50 mg/kg bw by intravenous injection. Blood and urine samples were collected at fixed intervals until 48 hours. Recovery of total test material as metabolites in urine after the oral and intravenous doses was 60 - 95 % for the acid and the methyl, ethyl and propyl esters. For the candidate substance ethyl 4-hydroxybenzoate [FL-no: 09.367], recovery of total material was 66 % of the oral and 70 % of the intravenous dose. Metabolites were detectable in the blood up to 24 hours post-ingestion. Of the dose, 12.3 % was excreted as free acid and 32.5 % as the glucuronic acid conjugate. Recovery of the butyl ester was 48 % after oral and 40 % after intravenous dosing. After oral dosing about 5

% of the butyl ester was excreted as free 4-hydroxybenzoic acid and 27.5 % as the glucuronic acid conjugate. Other conjugates were not determined. After intravenous dosing, 11.3 % of the given dose was recovered as free acid in urine and 20.1 % as the glucuronic acid conjugate. The test material was mainly excreted within 24 hours after dosing. The low rate of recovery seen with both dosing methods was attributed to incomplete hydrolysis of the butyl ester in the body. *In vitro* incubation of the butyl ester with freshly prepared liver homogenate showed complete hydrolysis within 30 - 60 minutes. Studies conducted with related benzoate esters, methyl and ethyl *p*-hydroxybenzoate, showed significantly higher rates of test material recovery when given to dogs by the oral and intravenous route, and showed 100 % hydrolysis within 3 minutes when incubated with liver homogenate. This finding suggests that an increase in the length of the alkyl rest in the homologous series of alkyl esters make the esters more resistant to hydrolysis and may result in the activation of other metabolic and excretion pathways (Jones et al., 1956).

Supporting substances from subgroup 2

p-Anisyl alcohol (4-methoxybenzyl alcohol) [FL-no: 02.128]

In an *in vitro* study, *p*-anisyl alcohol (4-methoxybenzyl alcohol) [FL-no: 02.128] was incubated with rat caecal extract. Analysis after approximately 46 hours showed the presence of unchanged compound and anisic acid. No observation of O-demethylation was observed (Scheline, 1972).

Vanillyl alcohol (4-hydroxy-3-methoxybenzyl alcohol) [FL-no: 02.213] and vanillin (4-hydroxy-3-methoxybenzaldehyde) [FL-no: 05.018]

In an *in vivo* study conducted in male albino rats, vanillyl alcohol [FL-no: 02.213] and vanillin [FL-no: 05.018] were administered by gavage in doses of 100 or 300 mg/kg bw. Urinary metabolites were collected over the first 24 - 48 hours period and analysed qualitatively. Vanillyl alcohol was mainly excreted as vanillyl alcohol or vanillic acid and related conjugates. The aldehyde intermediate was also detected. Conjugated fractions of vanillin, guaiacol, catechol, 4-methylguaiacol and 4-methylcatechol were also identified in smaller quantities. Oral dosage of 100 mg/kg bw of vanillin resulted in an urinary excretion of most metabolites within 24 hours, mainly as glucuronide and sulphate conjugates, although vanillic acid was also excreted as free acid and as glycine conjugate. After 48 hours, 94 % of the dose was accounted for as follows: vanillin 7 %, vanillyl alcohol 19 %, vanillic acid 47 %, vanilloylglycine 10 %, catechol 8 %, 4-methyl catechol 2 %, guaiacol 0.5 % and 4-methyl guaiacol 0.6 %. Vanillin and its primary reduction and oxidation metabolites were also excreted in appreciable amounts in the bile. Bile collected five hours after two rats were given 100 and 300 mg/kg bw oral doses of vanillin contained glucuronide conjugates of vanillin (6 %), vanillyl alcohol (8 %) and vanillic acid (9 %). The results show that both oxidative and reductive pathways exist for the metabolism of vanillin, although the oxidative metabolism dominates. At a dose level of 100 mg/kg bw, 57 % of the dose of vanillin was excreted as free vanillic acid or its conjugates and in total oxidation products amounted to approximately 65 - 70 % of the dose. The reduction pathway accounted for a little more than 20 % of the dose (Strand and Sheline, 1975).

In Sprague-Dawley albino rats, 100 mg vanillin [FL-no: 05.018]/kg bw was given by intraperitoneal injection and 24-hour urine was collected and analysed. The main urinary metabolite was conjugated vanillic acid which accounted for 41 % of the administered dose, while free vanillic acid accounted for 6 %. In addition, there was a trace of catechol. Vanillyl alcohol, a reductive product, represented 10 % of the administered dose. The presence of the urinary glycine conjugate of vanillic acid was not reported in this study. The oxidative path of metabolism was found to predominate, however, the importance of the minor reductive pathway may be magnified by inhibition of the oxidative process, as was achieved by administration of disulphiram in the study (Wong and Sourkes, 1966).

An experiment was conducted with the aim to determine whether man is capable of oxidising vanillin to vanillic acid. A 100 mg dose of vanillin dissolved in water was given to an adult human and the urine collected for 24 hours. Examination revealed an increase in the vanillic acid output in the urine from a

background level of 0.3 mg/24 hours to 96 mg/24 hours. The observed increase accounted for approximately 94 % of the vanillin dose (Dirscherl and Wirtzfeld, 1964).

Veratraldehyde (3,4-dimethoxybenzaldehyde) [FL-no: 05.017]

A 1 g/kg bw oral dose of veratraldehyde (3,4-dimethoxybenzaldehyde) was administered to rabbits by gavage and urine was collected for 24 hours. Approximately 70 % of the aldehyde was accounted for in urine, mainly as the corresponding acid, veratric acid (28 %), and its glucuronic acid (38 %) or sulphate (3 - 7 %) conjugate. To a small extent, veratric acid was decarboxylated and O-demethylated to yield catechol (Sammons and Williams, 1941). Presumably, veratric acid may enter the enterohepatic circulation where gut microflora decarboxylate the acid to yield catechol (o-hydroxyphenol). The observation that catechol was formed as a minor metabolite when veratraldehyde was incubated with rat caecal extract illustrates this decarboxylation pathway in gut bacteria (Scheline, 1972).

4-Hydroxybenzaldehyde [FL-no: 05.047] and salicylaldehyde (2-hydroxybenzaldehyde) [FL-no: 05.055]

In rabbits, 96 % of a single oral dose of 400 mg/kg bw 4-hydroxybenzaldehyde was excreted in the urine within 24 hours as 4-hydroxybenzoic acid and its glycine, glucuronic acid and sulphate conjugates (Bray et al., 1952b).

A single dose of 400 mg/kg bw salicylaldehyde (was administered to a fasted rabbit in three or six experiments. Approximately 75 % of the dose was excreted as ether soluble acids in the urine collected over 24 hours, and 27 % and 3 % accounted for as glucuronic acid and sulphate conjugates, respectively (Bray et al., 1952b).

In a corresponding study, approximately 94 % of a single oral dose of 250 or 500 mg/kg bw salicylaldehyde administered to two groups of four rabbits was excreted unchanged or as the glucuronic acid and sulphate conjugates, while the major part was excreted as the unchanged acid (Bray et al., 1948).

Veratraldehyde (3,4-dimethoxybenzaldehyde) [FL-no: 05.017], vanillin (4-hydroxy-3-methoxybenzaldehyde) [FL-no: 05.018] and vanillic acid (4-hydroxy-3-methoxy benzoic acid) [FL-no: 08.043]

Veratraldehyde [FL-no: 05.017], vanillin [FL-no: 05.018] or vanillic acid [FL-no: 08.043] were given orally by gavage to six rabbits at a dose of approximately 1 g/kg bw. Urine was collected for 5 hours. After administration of veratraldehyde, approximately 70 % of the material was recovered in the urine as free corresponding acid (28 %) and its glucuronic acid (38 %) or sulphate (3 - 7 %) conjugate. Approximately 69 % of vanillin was oxidised to vanillic acid, of which 44 % was recovered as free acid and 25 % conjugated acid. About 14 % of the dose was excreted as the glucuronic acid conjugate of vanillin. In the case of vanillic acid, 56 % was excreted as free vanillic acid and 27 % as conjugated, as glucuronide conjugate or ethereal sulphate. Less than 5 % was demethylated (Sammons and Williams, 1941).

4-Hydroxybenzoic acid [FL-no: 08.040]

Groups of four to eight rabbits were administered doses of 100, 250, 500, 1000 or 1500 mg/kg bw 4-hydroxybenzoic acid by gavage. Urine was collected continuously and analysed for metabolites. Total urinary recovery of the test material was in the range of 84 to 104 %, with ether soluble acids comprising 64 to 75 % of the total. Glucuronic acid and sulphate conjugates were also detected in the urine at 10 to 35 % and 4 to 7 %, respectively. The levels of all the metabolites returned to background levels within 24 hours after dosing (Bray et al., 1947).

Results from four experiments showed that between 2.2 and 5.4 % was excreted in the urine within 24 hours as the corresponding hippurate of a 0.41 mmole 4-hydroxybenzoic acid dose administered by intraperitoneal injection to female albino rats (Teuchy et al., 1971).

Other related substances

Methyl 4-hydroxybenzoate (methyl paraben)

Methyl 4-hydroxybenzoate (methyl paraben) was administered to three male rabbits by oral gavage at 800 mg/kg bw as a 12 % sodium salt solution, and the 24-hours urine was analysed. Three major metabolites, 4-hydroxybenzoic acid, *p*-hydroxyhippuric acid and *p*-carboxyphenyl glucuronide, as well as two minor metabolites, *p*-hydroxybenzoyl glucuronide and *p*-carboxyphenyl sulphate, were identified (Tsukamoto and Terada, 1962).

Concluding remarks on metabolism

The esters in subgroup 1, benzyl derivatives, will upon hydrolysis yield benzyl alcohol or benzoic acid along with alkyl carboxylic acids or alcohols. The metabolic fate of alkyl carboxylic acids and alcohols has been discussed in previous FGEs, and will not be discussed further in this evaluation. Benzyl alcohol, benzaldehyde and benzoic acid have been evaluated by the JECFA (JECFA, 1996b), as has 4-isopropylbenzyl alcohol (JECFA, 2002a), which is the alcohol moiety of candidate substance [FL-no: 09.611] (4-isopropylbenzyl acetate). Benzaldehyde and the benzyl alcohols are expected to be oxidized to corresponding benzoic acids, which will be conjugated with glycine and excreted as hippuric acids.

The candidate substance [FL-no: 09.314 (benzyl crotonate)] will yield crotonic acid as acid moiety, this substance has been discussed in the previous FGE.05Rev2 (EFSA, 2010f). In addition, crotonic acid has been evaluated by the SCF (SCF, 2002a).

The alcohol part of the candidate substance [FL-no: 09.656] (3-methylbut-3-enyl benzoate) includes a terminal double bond, a structure that has been discussed in FGE.06Rev3 (EFSA, 2011w) and will not be further discussed in this FGE.

The candidate substance (diethoxymethyl)benzene [FL-no: 06.017] is an acetal. This substance would be expected to yield benzaldehyde and ethanol upon hydrolysis. Benzaldehyde is expected to be oxidized to benzoic acid and subsequently conjugated with glycine or glucuronic acid and eliminated via the urine. The same biotransformations will occur with the three tolualdehyde isomers ([FL no: 05.026, 05.028 and 05.029]).

Subgroup 2, hydroxy- and alkoxy-substituted benzyl derivatives, includes 13 esters of which one [FL-no: 09.895] (4-methoxybenzyl-2-methylpropionate) will yield 4-methoxybenzyl alcohol (*p*-anisyl alcohol) [FL-no: 02.128] upon hydrolysis. This substance has been evaluated by the JECFA (JECFA, 2002a). 4-Methoxybenzyl alcohol is expected to be excreted in the urine either unchanged or as glucuronic acid, glycine or sulphate conjugate. The same metabolic pathway is proposed for the candidate benzyl alcohol derivative [FL-no: 02.164] (4-hydroxy-3,5-dimethoxybenzyl alcohol).

The remaining 12 esters in subgroup 2 will yield hydroxy- and/or alkoxy-substituted benzoic acids upon hydrolysis. The substituted benzoic acids that are hydrolysis products of candidate esters are expected to be excreted in the urine as the glucuronic acid, glycine or sulphate conjugate or at a minor extent unchanged. The same metabolic route is proposed for the candidate acids [FL-no: 08.087] (4-hydroxy-3,5-dimethoxybenzoic acid), [FL-no: 08.132] (3-hydroxybenzoic acid) and [FL-no: 08.133] (3,4-dihydroxybenzoic acid).

The candidate substance piperonyl alcohol [FL-no: 02.205] (3,4-methylenedioxybenzyl alcohol) is expected to mainly undergo oxidation and conjugation of the side chain, and be excreted as a glycine conjugate. Demethylenation of the methylenedioxy moiety does seem to be only a very minor metabolic path for this compound.

For the six candidate aldehydes in subgroup 2, the main metabolic pathway is presumed to be oxidation to the corresponding acids, followed by glycine and glucuronic acid conjugation and excretion. The reduction

to alcohols is a minor metabolic route and the oxidative pathway clearly dominates. To a minor extent O-demethylation followed by conjugation may occur.

The main metabolite of gallic acid (3,4,5-trihydroxy-benzoic acid) [FL-no: 08.080] is expected to be 4-O-methyl gallic acid, i.e. the product of O-methylation. Decarboxylation to pyrogallol (1,2,3-trihydroxybenzene) may occur as a very minor pathway, but no further dehydroxylation to catechol has been observed.

The biphenyl substance in subgroup 3 is expected to be metabolised in a similar way to the benzaldehyde derivatives in subgroup 2.

III.4. Summary and Conclusions

It is expected that esters in subgroup 1 and 2 will be hydrolysed *in vivo* to their component alcohols and acids. Nine of the 15 esters from subgroup 1 (benzyl derivatives) will yield benzyl alcohol which has previously been evaluated by the JECFA (JECFA, 1996b) and SCF (SCF, 2002b). One candidate ester [FL-no: 09.611] (4-isopropylbenzyl acetate) will yield 4-isopropylbenzyl alcohol. This substance has been previously evaluated by the JECFA (JECFA, 2002a). The benzyl alcohols are expected to be oxidized to corresponding benzoic acids, which will be conjugated with glycine and excreted as hippuric acids.

Subgroup 1

Nine of the 15 esters from subgroup 1 will yield benzyl alcohol, which has previously been evaluated by the JECFA (JECFA, 1996b) and SCF (SCF, 2002b). One candidate ester, 4-isopropylbenzyl acetate [FL-no: 09.611], will yield 4-isopropylbenzyl alcohol, previously evaluated by the JECFA (JECFA, 2002a). The benzyl alcohols are expected to be oxidised to corresponding benzoic acids, which will be conjugated with glycine and excreted as hippuric acids. Of the remaining five candidate esters in subgroup 1, four are expected to yield benzoic acid and simple aliphatic alcohols upon hydrolysis, 3-methylbut-3-enyl benzoate [FL-no: 09.656], butyl benzoate [FL-no: 09.779], pentyl benzoate [FL-no: 09.825] and prenyl benzoate [FL-no: 09.693]. One ester, methyl 4-methylbenzoate [FL-no: 09.631], will yield 4-methylbenzoic acid upon hydrolysis. Benzoic acid will mainly be conjugated with glycine and excreted as hippuric acid. Conjugation with glycine may be a saturable process and with increasing levels of exposure glucuronide conjugation may become relatively more important.

One of the substances, (diethoxymethyl)benzene [FL-no: 06.017], in subgroup 1 is an acetal. This substance would be expected to yield benzaldehyde and ethanol upon hydrolysis. Benzaldehyde has been evaluated by the JECFA (JECFA, 1996b). Benzaldehyde is expected to be oxidized to benzoic acid and subsequently conjugated with glycine or glucuronic acid and eliminated via the urine. The same biotransformations will occur with the three tolualdehyde isomers ([FL no: 05.026, 05.028 and 05.029]). Additionally, for the tolualdehyde isomers reduction of the aldehyde function to yield the corresponding alcohol has also been demonstrated. These alcohols can be converted into the corresponding sulphate esters which in their turn can further react with glutathione to give benzylmercapturic acids. This metabolic pathway is more important for *o*-tolualdehyde than for the other two isomers, but at any rate only a limited fraction of the dose (< 10 %) will be eliminated via this route.

Subgroup 2

Subgroup 2 (hydroxy- and alkoxy-substituted benzyl derivatives) includes 13 esters of which one, 4-methoxybenzyl-2-methylpropionate [FL-no: 09.895], will yield 4-methoxybenzyl alcohol (*p*-anisyl alcohol) (supporting substance [FL-no: 02.128]) upon hydrolysis. This substance has been evaluated by the JECFA (JECFA, 2002a). 4-Methoxybenzyl alcohol is expected to be excreted in the urine either unchanged or as a

glucuronic acid, glycine or sulphate conjugate. The same metabolic pathway is proposed for the candidate benzyl alcohol derivative 4-hydroxy-3,5-dimethoxybenzyl alcohol [FL-no: 02.164].

The remaining 12 esters in subgroup 2 [FL-no: 09.362 (ethyl 2-hydroxy-4-methylbenzoate); 09.363 (ethyl 2-methoxybenzoate); 09.367 (ethyl 4-hydroxybenzoate); 09.560 (hex-3(cis)-enyl anisate; hex-3(cis)-enyl-4-methoxybenzoate); 09.570 (hex-3-enyl salicylate; hex-3-enyl-2-hydroxybenzoate); 09.581 (hexyl salicylate; hexyl 2-hydroxybenzoate); 09.623 (methyl 2,4-dihydroxy-3,6-dimethylbenzoate); 09.696 (prenyl salicylate; 3-methyl-but-2-enyl 2-hydroxybenzoate); 09.762 (pentyl salicylate; pentyl 2-hydroxybenzoate); 09.798 (ethyl vanillate; ethyl 3-methoxy-4-hydroxybenzoate); 09.799 (methyl vanillate; methyl 3-methoxy-4-hydroxybenzoate); 09.852 (2-methylbutyl 2-hydroxybenzoate; 2-methylbutyl salicylate)] will yield hydroxy- and/or alkoxy-substituted benzoic acids upon hydrolysis. The substituted benzoic acids that are hydrolysis products of candidate esters are expected to be excreted in the urine unchanged or as the glucuronic acid, glycine or sulphate conjugate. The same metabolic route is proposed for the candidate acids [FL-no: 08.087] (4-hydroxy-3,5-dimethoxybenzoic acid), [FL-no: 08.132 (3-hydroxybenzoic acid)] and [FL-no: 08.133] (3,4-dihydroxybenzoic acid).

The main metabolic pathway for the acetal [FL-no: 06.104 (vanillin propylene glycol acetal)], after hydrolysis to the aldehyde and for the five candidate aldehydes in subgroup 2 [FL-no: 05.066 (4-ethoxy-3-methoxybenzaldehyde); 05.129 (2-methoxybenzaldehyde); 05.142 (3,4-dihydroxybenzaldehyde); 05.153 (4-hydroxy-3,5-dimethoxybenzaldehyde); 05.158 (3-methoxybenzaldehyde)] is presumed to be oxidation to the corresponding acids, followed by conjugation and excretion. The reduction to alcohols is a minor metabolic route and the oxidative pathway dominates clearly. For 2-methoxybenzaldehyde it has been shown that this reductive metabolic pathway leads to the formation of sulphate conjugates, which are converted into glutathione conjugates. The latter are eliminated via the urine as mercapturic acids. To a minor extent O-demethylation followed by conjugation may occur.

The candidate substance piperonyl alcohol (3,4-methylenedioxybenzyl alcohol) [FL-no: 02.205] is expected to mainly undergo oxidation and conjugation of the side chain, and be excreted as glycine conjugate. Demethylenation of the methylenedioxy moiety is a very minor metabolic path for this compound, according to published literature.

The main metabolite of gallic acid (3,4,5-trihydroxy-benzoic acid) [FL-no: 08.080] is expected to be 4-O-methyl gallic acid the product of O-methylation. Decarboxilation to pyrogallol (1,2,3-trihydroxybenzene) may occur as a very minor pathway, but no further dehydroxylation to catechol has been observed.

Subgroup 3

The biphenyl substance in subgroup 3, [FL-no: 05.221 (6,6'-dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde)], is expected to be metabolised in a similar way to the benzaldehyde derivatives in subgroup 2. It is expected that the aldehyde group(s) will undergo oxidation to form the corresponding carboxylic acid which is likely to be conjugated and excreted. The reduction of the alcohol groups may again be a minor pathway, but some steric hindrance may occur making this less likely than for the benzaldehyde derivatives in subgroup 2.

Based on experimental evidence and general knowledge of toxicokinetics of structurally related compounds it is expected that at the reported levels of intake as flavouring substances, the candidate substances in FGE.20Rev4 would be rapidly and efficiently absorbed, metabolised to innocuous products and excreted.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for 13 candidate substances of the present flavouring group evaluation from chemical groups 23 and 30, and for 63 supporting substances evaluated by the JECFA at the 57th meeting (JECFA, 2002a). The supporting substances are listed in brackets.

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
(Benzyl alcohol [02.010])	Rabbit	NR	Oral	1040	(Graham and Kuizenga, 1945)	
	Rat	NR	Oral	2979	(Ciba-Geigy Corp., 1945)	
	Rat	NR	Oral	2080	(Graham and Kuizenga, 1945)	
	Rat	M, F	Gavage	1230	(Jenner et al., 1964)	
	Rat	M, F	Oral	1570	(Damment, 1980)	
	Rat	NR	Oral	3100	(Smyth et al., 1951a)	
	Mouse	NR	Gavage	1580	(Jenner et al., 1964)	
	Mouse	NR	Oral	1150	(Carter et al., 1958)	
(Benzyl formate [09.077])	Rat	M, F	Gavage	1.7 (1.4-2.1) ml/kg bw (1840; 1510-2270) ¹⁰	(Shelanski and Moldovan, 1971d)	
(Benzyl acetate [09.014])	Rabbit	NR	Oral	2640	(Graham and Kuizenga, 1945)	
	Rat	M, F	Gavage	2490	(Jenner et al., 1964)	
	Rat	NR	Oral	3690	(Graham and Kuizenga, 1945)	
(Benzyl propionate [09.132])	Rat	NR	Oral	3300	(Moreno, 1973u)	
(Benzyl butyrate [09.051])	Rat	NR	Oral	1850	(Moreno, 1973v)	
	Rat	M, F	Gavage	2330	(Jenner et al., 1964)	
(Benzyl isobutyrate [09.426])	Rat	M, F	Oral	2850	(Owen, 1971)	
(Benzyl isovalerate [09.458])	Rat	NR	Oral	5000	(Moreno, 1974j)	
(Benzyl dodecanoate [09.315])	Rat	NR	Oral	> 5000	(Moreno, 1975m)	
(Benzyl 2-methylcrotonate [09.494])	Rat	NR	Oral	> 5000	(Moreno, 1979d)	
(Benzyl benzoate [09.727])	Cat	NR	Oral	2240	(Graham and Kuizenga, 1945)	
	Rabbit	NR	Oral	2016	(Draize et al., 1948)	
	Rabbit	NR	Oral	1680	(Graham and Kuizenga, 1945)	
	Rabbit	NR	Oral	1800	(Lehman, 1955)	
	Guinea pig	NR	Oral	1120	(Draize et al., 1948)	
	Guinea pig	NR	Oral	1000	(Lehman, 1955)	
	Rat	NR	Oral	1904	(Draize et al., 1948)	
	Rat	NR	Oral	2800	(Graham and Kuizenga, 1945)	
	Rat	NR	Oral	1700	(Lehman, 1955)	
	Mouse	NR	Oral	1568	(Draize et al., 1948)	
	Mouse	NR	Oral	1400	(Lehman, 1955)	

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
(Benzyl phenylacetate [09.705])	Rat	M, F	Oral	> 5000	(Owen, 1971)	
(Benzaldehyde [05.013])	Guinea pig	M, F	Gavage	1000	(Jenner et al., 1964)	
	Rat	M, F	Gavage	1300	(Jenner et al., 1964)	
	Rat	NR	Oral	2850	(Sporn et al., 1967)	
	Rat	M, F	Gavage	1300	(Taylor et al., 1964)	
	Mouse	NR	Diet	1250	(Schafer and Bowles, 1985)	
(alpha, alpha- Dimethoxytoluene [06.003])	Rat	NR	Oral	1220	(Moreno, 1977z)	
(5-Hydroxy-2-phenyl-1,3-dioxane [06.002])	Rat	NR	Oral	3749	(Levenstein, 1974g)	
	Rat	NR	Oral	2750	(Moreno, 1980k)	
(4-Methyl-2-phenyl-1,3-dioxolane [06.032])	Rat	M, F	Gavage	3000	(Lewis and Palanker, 1979b)	
(Benzoic acid [08.021])	Mouse	NR	Oral	1250	(Schafer and Bowles, 1985)	
	Mouse	NR	Oral	1996	(Sado, 1973)	
	Mouse	NR	Gavage	1950	(Shell Oil Company, 1982)	
(Methyl benzoate [09.725])	Rabbit	NR	Oral	2170	(Graham and Kuizenga, 1945)	
	Guinea pig	NR	Gavage	4100	(Kravets-Bekker and Ivanova, 1970)	
	Rat	NR	Oral	2170	(Graham and Kuizenga, 1945)	
	Rat	M, F	Gavage	1350	(Jenner et al., 1964)	
	Rat	NR	Gavage	3500	(Kravets-Bekker and Ivanova, 1970)	
	Rat	M, F	Oral	3420	(Smyth et al., 1954)	
	Mouse	NR	Gavage	3330	(Jenner et al., 1964)	
	Mouse	NR	Gavage	3000	(Kravets-Bekker and Ivanova, 1970)	
	Rat	M	Gavage	2987	(Dashiehl and Hinckle, 1981)	
(Ethyl benzoate [09.726])	Rat	NR	Oral	3300	(Moreno, 1977aa)	
	Rabbit	NR	Oral	2630	(Graham and Kuizenga, 1945)	
	Rat	NR	Oral	2100	(Graham and Kuizenga, 1945)	
Butyl benzoate [09.779]	Rat	M, F	Oral	6480	(Smyth et al., 1954)	
	Mouse	M, F	Gavage	3450	(Bier, 1979)	
	Rat	M, F	Gavage	5140 ¹	(Smyth et al., 1954)	
(Hexyl benzoate [09.768])	Rat	NR	Oral	12300	(Smyth et al., 1951a)	
(Isopropyl benzoate [09.770])	Rat	NR	Oral	3730	(Smyth et al., 1951a)	
(Isobutyl benzoate [09.757])	Rat	M, F	Gavage	3685	(Levenstein, 1973e)	
(Isopentyl benzoate [09.755])	Rat	NR	Oral	6330	(Weir and Wong, 1971b)	
(Hex-3-enyl benzoate [09.806])	Rat	NR	Oral	> 5000	(Moreno, 1976u)	
(4-Isopropylbenzyl alcohol [02.039])	Rat	NR	Oral	1020	(Moreno, 1973z)	

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
4-Isopropylbenzyl acetate [09.611]	Rat	NR	Oral	1450	(Moreno, 1978i)	
(4-Ethylbenzaldehyde [05.068])	Rat	M, F	Oral	1970	(Costello, 1984)	
(Tolualdehydes (mixed <i>o</i> , <i>m</i> , <i>p</i>) [05.027])	Rat	NR	Oral	2250	(Moreno, 1973w)	
(Tolualdehyde glyceryl acetal [06.012])	Rat	NR	Oral	3400	(Moreno, 1972i)	
(4-Isopropylbenzaldehyde [05.022])	Rat	M, F	Gavage	1390	(Jenner et al., 1964)	
(2,4-Dimethylbenzaldehyde [05.110])	Rat	M, F	Gavage	between 1750 and 5000	(deGroot et al., 1974)	Death of 3/5 male and 3/5 female rats after single dose of 5000 mg/kg bw. No death after repeated doses of 1750 mg/kg bw in 5 male and 5 female rats.
(4-Hydroxybenzaldehyde [05.047])	Rat	NR	Oral	3980	(Dow Chemical Company, 1992b)	
(4-Hydroxybenzoic acid [08.040])	Mouse	NR	Oral	2200	(Sokol, 1952)	
(Salicylic acid [08.112])	Mouse	NR	Oral	908	(Sado, 1973)	
	Rat	NR	Gavage	1050	(Hasegawa et al., 1989)	
Ethyl 4-hydroxybenzoate [09.367]	Rat	F	Gavage	4300	(CTFA, 1980b)	
	Mouse	NR	Oral	8000	(Sokol, 1952)	
	Mouse	NR	Oral	6008	(Sado, 1973)	
	Rabbit	NR ²	Oral	5000	(Sabalitschka and Neufeld-Crzellitzer, 1954)	
	Dog	NR ²	Oral	5000	(Sabalitschka and Neufeld-Crzellitzer, 1954)	
(Butyl 4-hydroxybenzoate [09.754])	Mouse	NR	Oral	13200	(Sado, 1973)	
	Mouse	NR	Oral	> 5000	(Sokol, 1952)	
(<i>p</i> -Anisyl alcohol [02.128])	Mouse	NR	Oral	1780	(Draize et al., 1948)	
	Rat	NR	Oral	1340	(Draize et al., 1948)	
(Anisyl formate [09.087])	Rat	NR	Oral	1770	(Levenstein, 1975j)	
(Anisyl acetate [09.019])	Rat	M, F	Oral	2250	(Weir and Wong, 1971b)	
(<i>p</i> -Anisyl propionate [09.145])	Rat	NR	Oral	3330	(Wohl, 1974d)	
(<i>p</i> -Anisyl butyrate [09.058])	Rat	NR	Oral	3400	(Moreno, 1976v)	
(Anisyl phenylacetate [09.706])	Rat	M, F	Gavage	M: 5417 F: 4641	(Reagan and Becci, 1984d)	
	Rat	NR	Oral	> 5000	(Moreno, 1977ab)	
(Veratraldehyde [05.017])	Rat	NR	Oral	2000	(Moreno, 1974k)	
	Rat	M	Oral	2040	(Field, 1979a)	
	Mouse	M	Oral	3200	(Field, 1979b)	
(4-Methoxybenzaldehyde [05.015])	Rat	NR	Oral	3210	(BASF, 1981)	
	Rat	M, F	Gavage	1510	(Jenner et al., 1964)	
	Guinea pig	M, F	Gavage	1260	(Jenner et al., 1964)	
	Rat	M, F	Gavage	1510	(Taylor et al., 1964)	
2-Methoxybenzaldehyde [05.129]	Rat	M	Gavage	2.4 –2.8 ml/kg (2705 –3156) ³	(Field, 1979b)	
	Rat	NR	Oral	2500	(Moreno, 1977ac)	
	Mouse	M	Gavage	2.4 ml/kg (2705) ³	(Field, 1979b)	

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
(4-Ethoxybenzaldehyde [05.056])	Rat	NR	Oral	2100	(Moreno, 1977ad)	
(Methyl 2-methoxybenzoate [09.796])	Rat	NR	Oral	3800	(Moreno, 1982l)	
(Methyl 4-methoxybenzoate [09.713])	Rat	NR	Oral	> 5000	(Levenstein, 1975k)	
(Ethyl 4-methoxybenzoate [09.714])	Rat	NR	Oral	2240	(Levenstein, 1975l)	
Gallic acid [08.080]	Mouse	M, F	Oral	> 5000	(Rajalakshmi et al., 2001)	
(Vanillin [05.018])	Rabbit	NR	Gavage	5000 ⁴	(Dollahite et al., 1962)	
	Mouse	M	Gavage	1000	(Inouye et al., 1988)	
	Rabbit	NR	Gavage	2600	(Deichmann and Kitzmiller, 1940)	
	Rat	M, F	Gavage	1580	(Taylor et al., 1964)	
	Rat	M, F	Gavage	1580	(Jenner et al., 1964)	
	Rat	M, F	Gavage	3978 ⁵ 3925 ⁶	(Lheritier, 1992)	
	Rat	M	Gavage	3830	(Monsanto Co., 1955b)	
	Rat	M, F	Oral	3300	(Monsanto Co., 1976)	
	Rat	NR	Oral	4370	(Makaruk, 1980)	
	Guinea pig	M, F	Gavage	1400	(Jenner et al., 1964)	
(Vanillin isobutyrate [09.811])	Rat	M, F	Gavage	4755	(Mallory et al., 1983)	
(Salicylaldehyde [05.055])	Rat	NR	Oral	520	(Moreno, 1977af)	
	Rat	M	Gavage	566	(Eastman Kodak Co., 1991b)	
	Mouse	M	Gavage	504	(Eastman Kodak Co., 1991b)	
(2-Hydroxy-4-methylbenzaldehyde [05.091])	Rat	M, F	Gavage	1520	(Mondino, 1982)	
	Rat	M, F	Oral	1520	(Peano and Berruto, 1982)	
(Methyl salicylate [09.749])	Mouse	M	Gavage	1390	(Ohsumi et al., 1984)	
	Rat	NR	Gavage	1250	(Giroux et al., 1954b)	
	Rat	M, F	Oral	M: 3049 F: 2642	(Hazleton Laboratories, 1982c)	
	Rat	M, F	Gavage	887	(Jenner et al., 1964)	
	Rat	NR	Oral	1220	(Nivikov et al., 1994)	
	Mouse	M	Oral	1110	(Davison et al., 1961)	
	Guinea pig	M, F	Gavage	1060	(Jenner et al., 1964)	
	Mouse	M, F	Gavage	1440	(NTP, 1984a)	
	Rat	NR	Oral	1320	(Moreno, 1976x)	
(Ethyl salicylate [09.748])	Rat	NR	Oral	1836	(Levenstein, 1975m)	
(Butyl salicylate [09.763])	Rat	NR	Oral	1560	(Moreno, 1973aa)	
(Isobutyl salicylate [09.750])	Rat	NR	Oral	4100	(Moreno, 1982m)	
(Isopentyl salicylate [09.751])	Rat	NR	Oral	> 5000	(Hazleton Laboratories, 1982c)	
	Rat	M, F	Oral	> 5000	(Moreno, 1975n)	
Hexyl salicylate [09.581]	Rat	NR	Oral	5000	(Moreno, 1975o)	
Hex-3-enyl salicylate [09.570]	Rat	NR	Oral	3200	(Moreno, 1978k)	
Prenyl salicylate [09.696]	Rat	NR	Oral			

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference	Comments
(Benzyl salicylate [09.752])	Rat	M	Gavage	2227	(Fogleman and Margolin, 1970)	
(Phenethyl salicylate [09.753])	Rat	NR	Oral	> 5000	(Moreno, 1973ab)	
(o-Tolyl salicylate [09.807])	Rat	M, F	Oral	1.81 ml/kg (1810) ⁷	(Sterner and Chibanguza, 1983)	
(2,4-Dihydroxybenzoic acid [08.076])	Mouse	NR	Intraperitoneal	> 800	(Grady et al., 1976)	Highest dose applied was not lethal.
(Ethyl 4-hydroxy-3-methoxybenzyl ether [04.094])	Rat	M, F	Oral	> 2000	(Dufour, 1994)	
(Butyl vanillyl ether [04.093])	Rat	M, F	Gavage	M: 5104 F: 4734	(Buch, 1989)	
(Ethyl vanillin [05.019])	Rat	M, F	Gavage	> 2000	(Jenner et al., 1964)	
	Rat	M	Gavage	4470	(Rhône-Poulenc Inc., 1992b)	
	Rat	M, F	Oral	3500 ⁸	(Monsanto Co., 1991a)	
	Rat	M, F	Oral	3500 ⁹	(Monsanto Co., 1991b)	
	Rabbit	NR	Gavage	2000	(Deichmann and Kitzmiller, 1940)	
(Ethyl vanillin isobutyrate [09.933])	Rat	M, F	Oral	> 2000	(Sanders and Crowther, 1997)	
(Piperonyl acetate [09.220])	Rat	NR	Oral	2100	(Moreno, 1973ac)	
(Piperonal [05.016])	Rat	M, F	Gavage	2700	(Jenner et al., 1964)	
	Rat	M, F	Gavage	2700	(Taylor et al., 1964)	
	Rat	M, F	Gavage	2700	(Hagan et al., 1965)	
Prenyl benzoate [09.693]	Rat	NR	Oral	4700	(Moreno, 1978j)	
Prenyl salicylate [09.696]	Rat	NR	Oral	3200	(Moreno, 1978k)	
3,4-Dihydroxybenzoic acid [08.133]	Mice	NR	Oral	> 800	(Grady et al., 1976)	

¹ NR: Not Reported.

¹ Dose range-finding study.

² Article published in German. Data point not verified.

³ Calculation based on a specific gravity of 1.127 g/ml.

⁴ Dosed as a 10 % solution.

⁵ Calculated using Bliss' method.

⁶ Calculated using Litchfield and Wilcox's method.

⁷ Calculation based on an assumed specific gravity of 1.0 g/ml.

⁸ Administered as a 10 % solution in corn oil.

⁹ Administered as a 20 % solution-suspension in corn oil.

¹⁰ Calculated based on a specific gravity of 1.081 g/ml.

Subacute / Subchronic / Chronic / Carcinogenic toxicity data are available for five candidate substances of the present flavouring group evaluation from chemical group 23 and 30, and for 18 supporting substances evaluated by the JECFA at the 46th and 57th meetings (JECFA, 1996b; JECFA, 2002a). The supporting substances are listed in brackets.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
(Benzyl alcohol [02.010])	Rat; M, F 20	Gavage	0, 50, 100, 200, 400, 800 mg/kg bw/day	13 weeks	100	(NTP, 1989a)	Fully described NTP study. Reduced relative weight gain in females at 200 mg/kg bw/day and more, and at 800 mg/kg bw/day in males.
	Rat; M, F 100	Gavage	0, 200, 400 mg/kg bw/day	103 weeks	ND ⁷	(NTP, 1989a)	Fully described NTP study. Survival in both dose group of females was 50 % that of controls. This was, as concluded by NTP, primarily due to an increased number of gavage-related deaths. NTP conclusion on carcinogenicity: No evidence of carcinogenic activity.
	Mouse; M, F 20	Gavage	0, 50, 100, 200, 400, 800 mg/kg bw/day	13 weeks	100	(NTP, 1989a)	Fully described NTP study. Reduced relative weight gain in females at 200 mg/kg bw/day and more, and at 400 and 800 mg/kg bw/day in males.
	Mouse; M, F 100	Gavage	0, 100, 200 mg/kg bw/day	103 weeks	200	(NTP, 1989a)	Fully described NTP study. NTP conclusion on carcinogenicity: No evidence of carcinogenic activity.
(Benzyl acetate [09.014])	Rat; M, F 20	Gavage	0, 62.5, 125, 250, 500, 1000 mg/kg bw/day	13 weeks	250	(NTP, 1986c)	Fully described NTP study. Clinical signs of toxicity in females at 500 mg/kg and in males and females at 1000 mg/kg. Decreased body weight in males at 1000 mg/kg. Deaths at highest dose (1 F/2 M). At necropsy thickened stomach walls in surviving animals (2/8 M, 4/9 F). Hippocampal necrosis in both sexes at 1000 mg/kg (8/8 M, 4/9 F).
	Rat; M 30	Oral	0, 20000, 35000, 50000 mg/kg in the diet (0, 1500, 2700, 3800 mg/kg bw/day) ^{25, 26}	13 weeks	ND ⁷	(Abdo et al., 1998)	Published non-GLP study of good quality. Benzyl acetate caused an increase in mortality, incidence of abnormal neural behavioral signs along with astrocyte hypertrophy and neuronal necrosis in the cerebellum, hippocampus and pyriform cortex of the brain at 35000 mg/kg feed and more. Body weight was statistically significant reduced from 20000 mg/kg feed. These effects were reduced significantly by glycine but not by L-alanine.
	Rat; M, F 20	Oral	0, 3130, 6250, 12500, 25000, 50000 mg/kg in the diet (equivalent to 0, 230, 460, 900, 1750, 3900 mg/kg bw/day for males	13 weeks	460	(NTP, 1993d)	Fully described NTP study. High mortality at highest dose (9/10 F, 9/10 M). Statistically significant decreases in final body weights (over 10 %) observed at 25000 mg/kg feed. Clinical signs of intoxication at 50000 mg/kg feed. At the highest dose level degeneration and necrosis of neurons and glial cells in the cerebellum and hippocampus, renal tubular degeneration and

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
			0, 240, 480, 930, 1870, 4500 mg/kg bw/day for females)				histopathological changes in skeletal thigh muscles. Testicular tubular atrophy in a few males at 12500 mg/kg feed.
	Rat; M, F 120	Oral	0, 3000, 6000, 12000 mg/kg in the diet (equal to 0, 130, 260, 510 mg/kg bw/day in males and 0, 145, 290, 575 mg/kg bw/day in females)	103 weeks	260	(NTP, 1993d)	Fully described NTP study. Slightly reduced mean body weight and feed consumption at the highest dose. NTP conclusion on carcinogenicity: No evidence of carcinogenic activity in male and female Fischer 344/N rats.
	Rat; M, F 100	Gavage	0, 250, 500 mg/kg bw/day	103 weeks	ND ⁷	(NTP, 1986c)	Fully described NTP study. No observable adverse effects on mean body weight gain and survival. NTP conclusion on carcinogenicity: Benzyl acetate increased the incidence of acinar-cell adenomas of the endocrine pancreas in male F344/N rats; the gavage vehicle may have been a contributing factor. No evidence of carcinogenic activity for female rats.
	Mouse; M, F 20	Gavage	0, 125, 250, 500, 1000, 2000 mg/kg bw/day for females and 0, 62.5, 125, 250, 500, 1000 mg/kg bw/day for males	13 weeks	500	(NTP, 1986c)	Fully described NTP study. High mortality (8/10) in females at 2000 mg/kg due to gavage error. Clinical signs of toxicity were observed at 1000 mg/kg bw/day. Hippocampal necrosis in one female at 1000 mg/kg.
	Mouse; M, F 20	Oral	0, 3130, 6250, 12500, 25000, 50000 mg/kg in the diet (equal to 0, 425, 1000, 2000, 3700, 7900 mg/kg bw/day for males and 0, 650, 1280, 2980, 4300, 9400 mg/kg bw/day for females)	13 weeks	ND ⁷	(NTP, 1993d)	Fully described NTP study. Statistically significant, dose-related decreases in final body weights (over 10 %) observed in all treated animals. Hippocampal necrosis in one male and three females of highest dose group.
	Mouse; M, F 120	Oral	0, 330, 1000, 3000 mg/kg in the diet (equal to 0, 37, 112, 346 mg/kg bw/day in males and 0, 42, 132, 382 mg/kg bw/day in	103 weeks	ND ⁷	(NTP, 1993d)	Fully described NTP study. Decreased mean body weights (9-13 %) in all treated mice except for females at 330 mg/kg feed (statistics not reported). Statistically significant, dose-related incidence and severity of non-neoplastic lesions of the nasal mucosa and glands in all treated animals. NTP conclusion on carcinogenicity: No evidence of carcinogenic activity in male and

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
	Mouse; M, F 100	Gavage	females) 0, 500, 1000 mg/kg bw/day	103 weeks	ND ⁷	(NTP, 1986c)	female B6C3F ₁ mice. Fully described NTP study. No observable adverse effects on mean body weight gain and survival. NTP conclusion on carcinogenicity: For male and female B6C3F ₁ mice there was evidence of carcinogenicity, in that benzyl acetate caused an increased incidence of hepatocellular neoplasms particularly adenoma, and squamous cell neoplasms (papillomas; no progression into carcinomas) of the forestomach.
(Benzyl butyrate [09.051])	Rat; M, F 12	Oral ¹	0, 26.5 mg/kg bw/day ²⁴	12 weeks	26.5	(Oser, 1957)	Unpublished non-GLP study with limited details on study protocol and results.
(Benzaldehyde [05.013])	Rat; M, F 20	Gavage	0, 50, 100, 200, 400, 800 mg/kg bw/day	13 weeks	200	(Kluwe et al., 1983; NTP, 1990c)	Fully described NTP study. High mortality in males (6/10) and death of three females in the highest group. Death of one female at 400 mg/kg. Reduced terminal body weights (26 %) in males at highest dose. Treatment-related lesions in the brain, forestomach, liver and kidney in both sexes at 800 mg/kg including necrosis and degeneration of cerebellum, necrosis of neurons of the hippocampus, hyperplasia and/or hyperkeratosis of squamous epithelium of the forestomach, degeneration and/or necrosis of liver and kidney.
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day) ²¹	16 weeks	500 ²	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 10	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day) ²¹	27 - 28 weeks	50 ²	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 100	Gavage	0, 200, 400 mg/kg bw/day	103 weeks	ND ⁷	(NTP, 1990c)	Fully described NTP study. Significantly reduced survival of the high dose group rats after one year and significant dose-related trend to reduced survival in the treated groups of males. Body weight not affected. NTP conclusion on carcinogenicity: No evidence of carcinogenic activity for male or female F344/N rats.
	Mouse; M, F 20	Gavage	0, 75, 150, 300, 600, 1200 mg/kg bw/day	13 weeks	300	(Kluwe et al., 1983; NTP, 1990c)	Fully described NTP study. High mortality in males (9/10) and death of one female in the highest group. Reduced mean body weight at 600 mg/kg in males, but not in females. Mild to moderate renal tubular degeneration in all males at 1200 mg/kg and in one male at 600 mg/kg.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
	Mouse; M, F	Gavage	0, 200, 400 mg/kg bw/day for males, 0, 300, 600 mg/kg bw/day for females	103 weeks	ND ⁷	(NTP, 1990c)	Fully described NTP study. No significant effects on body weight and survival observed in any group. Increased incidences of squamous cell papillomas of the forestomach in both exposure groups with dose-related increased incidences in forestomach hyperplasia. No progression into malignant carcinomas was observed. NTP conclusion on carcinogenicity: Some evidence of carcinogenic activity for male or female B6C3F ₁ mice.
(Methyl benzoate [09.725])	Rat; NR 13	Gavage	0, 0.005, 0.05 mg/kg bw/day	6 months	0.005	(Kravets-Bekker and Ivanova, 1970)	Published non-GLP study of limited quality. Unusual study design and parameters analysed. Limited report of experimental details and results.
(Benzoic acid [08.021])	Mouse; M, F 100	Gavage	0, 80 mg/kg bw/day	3 months	ND ⁷	(Shtenberg and Ignat'ev, 1970)	Published non-GLP study of limited quality. Insufficient details on methods and results provided. Reduced weight gain with normal food intake in treated animals.
	Mouse; M, F 50	oral (paste)	0, 40 mg/kg bw/day	17 months	40 ²	(Shtenberg and Ignat'ev, 1970)	Published non-GLP study of limited quality. Insufficient details on methods and results provided.
(Glyceryl tribenzoate [09.812])	Rat; M, F 30	Oral	0, 120, 600, 2600 mg/kg bw/day	90 days	600	(Carson, 1972a)	Unpublished non-GLP study carried out in accordance with OECD Guideline 408. Decreased body weight gain (by 23 %) in high dose males with normal food intake.
(Propylene glycol dibenzoate [09.803])	Rat; M, F 30	Oral	0, 130, 630, 2500 mg/kg bw/day	90 days	2500 ²	(Carson, 1972b)	Unpublished non-GLP study carried out in accordance with OECD Guideline 408.
(Tolualdehydes (mixed o, m, p) [05.027])	Rat; M, F 30	Oral	36 mg/kg bw/day for males, 43 mg/kg bw/day for females	90 days	36 ²	(Oser et al., 1965)	Published non-GLP study. Very limited details provided. The test substance is only indicated as "tolualdehydes", without any indication of isomeric composition
	Rat; M, F 30	Gavage	0, 50, 250, 500 mg/kg bw/day	13 weeks	250	(Brantom et al., 1972)	Published non-GLP study of good quality, carried out in accordance to OECD Guideline 408. Decreased relative pituitary weight in females at 500 mg/kg bw/day. Reduced weight and relative weight of small intestine in all treated groups. However, this effect was not dose-related and not reproduced in a second study. The test substance was an approx. equimolar mixture of the three isomers.
(2,4-Dimethylbenzaldehyde [05.110])	Rat; M, F 10	Gavage	0, 0.175, 1.75 mg/kg bw/day	2 weeks	1.75 ²	(deGroot et al., 1974)	Unpublished non-GLP study with limited parameters analysed and limited report of results. Increased relative liver weight in high-dose males without histopathological changes.
Ethyl 4-hydroxybenzoate [09.367]	Rat; M, F 24	Oral	0, 2, 8 % in the diet (0, 1050, 5700 mg/kg bw/day)	12 weeks	1050	(Matthews et al., 1956)	Published non-GLP study of very limited quality. Insufficient endpoints analysed. High mortality at highest dose.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
	Rat; NR 10 or 11	Oral	0, 0.2, 1, 2 % in the diet (0, 100, 500, 1000 mg/kg bw/day) ¹⁸	25 weeks	1000	(Sado, 1973)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported.
(Butyl 4-hydroxybenzoate [09.754])	Rat; NR 10 or 18 ³	Gavage	0, 0.25, 50 mg/kg bw/day	13 - 15 weeks	50 ²	(Ikeda and Yokoi, 1950)	Published non-GLP study in Japanese with English translation. Validity cannot be evaluated due to incomplete report of data.
	Rat; M, F 24	Oral	0, 2, 8 % in the diet (0, 1050, 5700 mg/kg bw/day)	12 weeks	1050	(Matthews et al., 1956)	Published non-GLP study of very limited quality. Insufficient endpoints analysed. High mortality (100 % in males) at highest dose.
	Mouse; M, F 20	Oral	0, 0.6, 1.25, 2.5, 5, 10 % in the diet (0, 900, 1850, 3750, 7500, 15000 mg/kg bw/day)	6 weeks	900	(Inai et al., 1985)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported. Copy partly unreadable.
	Mouse; M, F 100	Oral	0, 0.15, 0.3, 0.6 % in the diet (0, 225, 450, 900 mg/kg bw/day)	102 weeks	900 ²	(Inai et al., 1985)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported. Copy partly unreadable.
(4-Methoxybenzaldehyde [05.015])	Rat; M, F 20	Oral	0, 7.3 mg/kg bw/day	12 weeks	7.3 ^{4, 2}	(Trubek Laboratories Inc., 1958f)	Unpublished study of poor quality with insufficient study protocol and report of data; Eugenol: JECFA evaluation; NOAEL 250 mg/kg bw/day in rat (diet), ADI 2.5 mg/kg bw (See footnote 4).
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day) ²¹	16 weeks	500 ²	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; M, F 10	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day) ²¹	28 weeks	50 ²	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day) ²¹	15 weeks	500 ²	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 10	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day) ²¹	27 - 28 weeks	50 ²	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
(Methyl 2-methoxybenzoate [09.796])	Rat; M, F 10	Oral	0, 94 mg/kg bw/day	14 days	94 ²	(Van Miller and Weaver, 1987)	Unpublished GLP-study.
3,4-Dihydroxybenzaldehyde [05.142]	Rat; M, F 10	Oral	0, 1.5 % in the diet	4 weeks	1500 ¹⁵	(Shibata et al., 1990)	Published non-GLP study of limited quality. Experimental details and results insufficiently

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
			(0, 1500 mg/kg bw/day) ¹⁶				reported.
Gallic acid [08.080]	Rat; M, F 20	Oral	0, 0.2, 0.6, 1.7, 5 % in the diet	13 weeks	0.6 % in the diet (M: 119 F: 128) ¹⁷	(Niho et al., 2001)	Published non-GLP study of good quality. Centrilobular liver cell hypertrophy, reflected in a significant increase in liver weight, which was observed in animals of both sexes from 1.7 %.
	Rat; M 5	Oral	0, 2 % in the diet (0, 2000 mg/kg bw/day) ¹⁶	4 weeks	2000 ^{2,6}	(Hirose et al., 1987)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported.
	Mouse; M, F 12	Oral	0, 1000 mg/kg bw/day	28 days	1000 ²	(Rajalakshmi et al., 2001)	Published non-GLP study of acceptable quality.
(Vanillin [05.018])	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day) ²¹	16 weeks	500 ²	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; M, F 10	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day) ²¹	27 - 28 weeks	50 ²	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M 5	Oral	0, 2, 5 % in the diet (0, 1000, 2500 mg/kg bw/day) ²¹	1 year	2500 ²	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; NR 8	Oral	64 mg/kg bw/day	70 days	ND	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls. No NOAEL could be derived.
	Rat; NR 8	Oral	20 mg/kg bw/day	126 days	20 ²	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls.
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day) ²¹	16 weeks	500 ²	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 24	Oral	0, 0.5, 1, 2 % in the diet (0, 250, 500, 1000 mg/kg bw/day) ²¹	2 years	1000 ²	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 10	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day) ²¹	28 weeks	50 ²	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; NR 12	Gavage	300 mg/kg bw twice a week	14 weeks	300 ^{2,5}	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
	Rat; M 10	Oral	0, 0.1, 0.5, 1.0 % in the diet (0, 40, 214, 437 mg/kg bw/day) ²⁰	26 weeks	437 ²	(Monsanto Co., 1955a)	No adequate controls. Unpublished non-GLP study of limited quality. Insufficient analysis of clinical-chemical parameters. Results of microscopic examination not reported.
	Rabbit; NR 3	Oral	240 mg/kg bw/day	56 or 126 days ¹⁹	240 ²	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls.
	Rabbit; NR 1	Oral	83 mg/kg bw/day for 14 days 103 mg/kg bw/day for 61 days	14 or 61 days	ND	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls. The animal treated with 83 mg/kg bw/day died due to glycerol poisoning (solvent), the animal treated with 103 mg/kg bw/day suffered from anemia, diarrhea and showed a reduced wt gain.
	Dog; M, F 2	Capsule	0, 25, 100 mg/kg bw/day	26 weeks	100	(Monsanto Co., 1955a)	Unpublished non-GLP study of limited quality. Insufficient analysis of clinical-chemical parameters. Results of microscopic examination not reported.
4-Hydroxy-3,5-dimethoxybenzoic acid [08.087]	Rat; M 5	Oral	0, 2 % in the diet (0, 2000 mg/kg bw/day) ¹⁶	4 weeks	2000 ^{3,6}	(Hirose et al., 1987)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported.
(Methyl salicylate [09.749])	Rat; M, F 20	Oral	0, 0.1, 1.0 % in the diet (0, 50, 500 mg/kg bw/day) ²¹	17 weeks	500 ²	(Webb and Hansen, 1963)	Published non-GLP FDA-study of good quality. Preliminary study in extensive toxicological evaluation. Study protocols fully described, results not reported in detail but only summarised in text.
	Rat; M, F 6	Oral	0, 2 % in the diet (0, 1000 mg/kg bw/day) ²¹	Up to 71 days	1000 ⁷	(Webb and Hansen, 1963)	Published non-GLP FDA-study of good quality. Supplemental study in extensive toxicological evaluation to analyse bone effects. Study protocols fully described, results not reported in detail but only summarised in text.
	Rat; NR	Oral	1.12, 2 % in the diet (560, 1000 mg/kg bw/day) ^{21,23}	10 weeks	< 560	(Harrison et al., 1963)	Only abstract available. No details reported. Study carried out to further investigate the increase of cancellous bone reported by Webb & Hansen, 1963). Effects confirmed at levels of 2 % and 1.12 % but not at lower (unspecified) levels.
	Rat; M, F 10	Oral	0, 0.2, 0.36, 0.63, 1.13, 2.0 % in the diet (0, 100, 180, 320, 560, 1000 mg/kg bw/day) ²¹	11 weeks	180	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. Decreased weight gain at 320 mg/kg, decreased weight gain and increased bone density from 560 mg/kg.
	Rat; M 5	Oral	0.6, 2.0 % in the diet (300, 1000 mg/kg	12 weeks	300	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
			bw/day) ²¹				parameters. No control animals used. Hundred % mortality in high dose group after 6 weeks with bone lesions. No such effects at 300 mg/kg.
	Rat; M, F 15	Oral	0, 2.0 % in the diet (0, 1000 mg/kg bw/day) ²¹	11 weeks	ND ⁷	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. Changes in bone density and 20 % mortality in treated animals. No such effects in controls.
	Rat; M 10 ⁸	Oral	0, 0.6, 2.0 % in the diet (0, 300, 1000 mg/kg bw/day) ²¹	6 weeks	ND ⁹	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. Deaths occurred among the rats at the high dose ad libitum and in all members of the pair-fed groups (incl. controls).
	Rat; M, F 20	Oral	0, 0.6, 0.9, 1.2, 2.0 % in the diet (0, 300, 450, 600, 1000 mg/kg bw/day) ²¹	11 weeks	450	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. Weekly X-ray evaluation of animals for progression of bone changes. Bone changes at 1000 mg/kg from week 2, at 600 mg/kg from week 5.
	Dog; M, F 2	Oral	50, 100, 250, 500, 800, 1200 mg/kg bw	Up to 59 days	250	(Webb and Hansen, 1963)	Published non-GLP FDA-study of good quality. Preliminary study in extensive toxicological evaluation. Study protocols fully described, results not reported in detail but only summarised in text.
	Dog; M, F 6	Oral	0, 150, 300, 500, 800 mg/kg bw/day ²²	7.5 months ¹⁰	ND ⁷	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. Hundred % mortality at highest dose, only 2 surviving animals at 500 mg/kg. At lower doses no effects on body weight and hematological parameters. Increased liver and kidney weight at all doses but not after recovery diet. No NOEL could be derived.
	Dog; M, F 8, 12 ¹¹	Oral	0, 50, 100, 170 mg/kg bw/day	6 months ¹²	170 ²	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. No adverse effects at any dose.
	Rat; M, F 50	Oral	0, 0.1, 0.5, 1.0, 2.0 % in the diet (0, 50, 250, 500, 1000 mg/kg bw/day) ²¹	2 years	50	(Webb and Hansen, 1963)	Published non-GLP FDA-study of good quality. Study protocols fully described, results not reported in detail but only summarised in text.
	Rat; M, F 50	Oral	0.07, 0.21 % in the diet (0, 35 100) ²¹	2 years	100 ²	(Packman et al., 1961)	Only abstract available with very limited report of study details and results.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
(Isopentyl salicylate [09.751])	Dog; M, F 4	Oral	0, 50, 150, 350 mg/kg bw/day	2 years	50	(Webb and Hansen, 1963)	Published non-GLP FDA-study of good quality. Study protocols fully described, results not reported in detail but only summarised in text.
	Rat; M, F 30	Oral	0, 0.005, 0.05, 0.5 % in the diet (0, 4.7, 46, 420 mg/kg bw/day in males and 0, 4.8, 47, 480 mg/kg bw/day in females)	13 weeks	5	(Drake et al., 1975)	Published non-GLP study of good quality. Reduced body weight gain at 0.5 % associated with reduced food intake. Increased relative kidney weight without any histopathological changes at 0.05 and 0.5 % in the diet.
	Rat; M, F 10	Oral	0. 0.5 % in the diet (0, 420 mg/kg bw/day in males and 0, 480 mg/kg bw/day in females) ¹³	98 days	420	(Drake et al., 1975)	Published non-GLP study of good quality. Reduced body weight gain at 0.5 % associated with reduced food intake. Only body weight gain analysed.
(Ethyl vanillin [05.019])	Rat; M 5	Oral	0, 2, 5 % in the diet (0, 1000, 2500 mg/kg bw/day) ²¹	1 year	2500 ²	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; NR 8	Oral	64 mg/kg bw/day	70 days	64 ²	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls. No NOAEL could be derived.
	Rat; NR 8	Oral	20 mg/kg bw/day	126 days	20 ²	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls.
	Rat; NR 12	Gavage	300 mg/kg bw twice a week	14 weeks	300 ^{2,5}	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls.
	Rat; M, F 40	Oral	0, 500, 1000, 2000 mg/kg bw/day	13 weeks	500	(Hooks et al., 1992)	Unpublished report summarised by the JECFA (1996a). Study has been reported to be designed in accordance with toxicological principles for the safety assessment of food additives established by the US FDA in 1982. Limited report of experimental details and results.
	Rat; M, F 24	Oral	0, 0.5, 1, 2 % in the diet (0, 250, 500, 1000 mg/kg bw/day) ²¹	2 years	1000 ²	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rabbit; NR 3	Oral	240 mg/kg bw/day	56 or 126 days ¹⁹	240 ²	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
(Piperonal [05.016])	Rabbit; NR 1	Oral	15-49 mg/kg bw/day (15, 15, 32, 41, 49 mg/kg day for 15, 31, 17, 31, 49 days, respectively)	15 - 49 days	41 ²	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls. The animal treated with 15 mg/kg bw/day for 15 days died due to glycerol poisoning (solvent). The animal treated with 49 mg/kg bw/day for 49 days suffered from anemia, diarrhea and showed a reduced weight gain.
	Rat; M, F 20	Oral	0, 16 mg/kg bw	12 weeks	16 ^{14, 2}	(Trubek Laboratories Inc., 1958f)	Unpublished study of poor quality with insufficient study protocol and report of data; Eugenol: JECFA evaluation; NOAEL 250 mg/kg bw/day in rat (diet), ADI 2.5 mg/kg bw (See footnote 4).
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day) ²¹	16 weeks	500 ²	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; M, F 10	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day) ²¹	28 weeks	50 ²	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day) ²¹	15 weeks	500 ²	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 10	Oral	0, 0.1 % (0, 50 mg/kg bw/day) ²¹	27 - 28 weeks	50 ³	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F NR	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day) ²¹	28 weeks	50 ³	(Hagan et al., 1965)	Published report of subchronic and/or chronic toxicity studies on 7 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. No detailed results reported.
	Rat; M, F NR	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day) ²¹	16 weeks	500 ²	(Hagan et al., 1965)	Published report of subchronic and/or chronic toxicity studies on 7 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. No detailed results reported.
	Rat; M, F 20 - 60	Oral	0, 0.1, 0.5 % in the diet (0, 50, 250 mg/kg bw/day) ²¹	1.5 - 2 years	250 ²	(Bär and Griepentrog, 1967)	Published study with incomplete report of experimental details and results.
	Rat; M, F 10	Oral	0, 1.5 % in the diet (0, 750 mg/kg bw/day)	4 weeks	750	(Shibata et al., 1990)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported.
3,4-Dihydroxybenzoic acid [08.133]	Rat, M 10	Oral	0, 50 mg/kg bw/day	2 weeks	50	(Guglielmi et al., 2003)	
	Rat, M	Oral	0, 1000 ppm (0,	28 weeks	50	(Tanaka et al.,	

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
	11		50 mg/kg bw/day)			1993a; Tanaka et al., 1993b)	
	Rat, M 16	Oral	0, 2000 ppm (0, 100 mg/kg bw/day)	32 weeks	100	(Tanaka et al., 1994)	
	Rat, M 15	Oral	0, 2000 ppm (0, 100 mg/kg bw/day)	41 weeks	100	(Hirose et al., 1995)	
	Rat, M 15	Oral	0, 1.5 % in the diet (0, 530 mg/kg bw/day)	51 weeks	< 530	(Hirose et al., 1992)	It was noted that the relative liver and kidney weight was significantly increased but this was not further evaluated.

¹ Six aromatic esters (ethyl benzoate, 0.15 ppm; isobutyl benzoate, 25 ppm; benzyl acetate, 18.7 ppm; benzyl butyrate, 25 ppm; ethyl methylphenyl glycidate, 25 ppm; and glycidate M-116, 25 ppm) were blended in the diet.

² This study was performed at either a single dose level or multiple dose levels that produced no adverse effects. Therefore, this dose level is the highest dose tested that produced no adverse effects.

³ Five rats were used per group for low dose. Nine rats were used per group for high dose.

⁴ Rats were fed a test mixture containing 123 ppm of eugenol, 10 ppm of 4-methoxybenzaldehyde and 22 ppm of piperonal.

⁵ Compound was administered two times per week.

⁶ Study evaluated histological changes in the rat forestomach.

⁷ This study was performed at a single dose level or at multiple dose levels that produced adverse effects. No NOEL could be derived from that study.

⁸ Two groups fed ad libitum (2.0 and 0.6 %) and one group (0.6 %) pair-fed.

⁹ The 0.6 % pair-fed rats showed adverse effects; however, those fed ad libitum did not.

¹⁰ Two animals from the 150 mg/kg/day group and three animals from the 300 mg/kg/day group were sacrificed after 6.5 months. Additionally, three animals from the 300 mg/kg/day group discontinued feeding at 6.5 months and recovered for 1.5 months, before being sacrificed.

¹¹ There were 8 dogs used for the 50 and 100 mg/kg bw doses and 12 dogs used for 167 mg/kg bw.

¹² All animals were fed the substance for six months and then sacrificed, with the exception of two dogs from the high dose group. These animals were fed the substance for four months, placed on control diets for two months, and then sacrificed with the other animals at six months.

¹³ Pair-fed study.

¹⁴ Rats were fed a test mixture containing 123 ppm of eugenol, 10 ppm of 4-methoxybenzaldehyde and 22 ppm of piperonal.

¹⁵ This study evaluated the cell proliferation in the rat forestomach at a single concentration, 1.5 %. There was a statistically significant weight reduction in the females; however, it was deemed to be associated with a palatability problem.

¹⁶ Calculated based on general assumptions for bw and food intake in weanling rats (e.g. 2 % in diet resulting in 2000 mg/kg bw/day).

¹⁷ Calculated by using mean values of body weight and food intake in the 0.2 % group.

¹⁸ Estimated based on FDA (1993). Priority-based assessment of food additives (PAFA) database. Center for food safety and applied nutrition. page 58.

¹⁹ One animal was treated for 56 days and two animals for 126 days.

²⁰ Calculated based on a final bw of 453, 444, 427 g and a total consumption of test compound of 18.2, 95.0 and 186.8 mg/animal/day, respectively, in the three dose groups.

²¹ Calculated based on general assumptions for bw and food intake (e.g. 1 % in diet resulting in 500 mg/kg bw/day). As estimated by JECFA (2002a).

²² The dogs were given one-half of the dose in the morning and the other half in the afternoon for six days/week.

²³ Also lower dose levels applied, however, not reported in more detail.

²⁴ Benzyl butyrate administered in the diet in a mixture of six aromatic esters (ethyl benzoate 0.15 mg/kg, isobutyl benzoate 25 mg/kg, benzyl acetate 18.7 mg/kg, benzyl butyrate 25 mg/kg, ethyl methylphenylglycidate 25 mg/kg, glycidate 25 mg/kg) providing intakes of 26.5 mg benzylbutyrate/kg bw/day and a total of 126 mg/kg bw/day of the mixture.

²⁵ Calculated based on a final bw of 200 g and a daily intake of 15.2 g/rat, as reported for the 20000 mg/kg feed group, for all groups.

²⁶ Additional groups with either 50000 mg benzyl acetate/kg feed plus 27000 mg glycine/kg feed or 32000 mg *L*-alanine/kg feed (supplemental *L*-alanine and glycine were equimolar). The *L*-alanine group served as amino-nitrogen control.

Developmental and reproductive toxicity data are available for one candidate substance of the present flavouring group evaluation from chemical group 23 and for twelve supporting substances evaluated by the JECFA at the 46th and 57th meetings (JECFA, 1996b; JECFA, 2002a). Supporting substance listed in brackets.

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical Name	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), Including information of possible maternal toxicity	Reference	Comments
(Benzyl alcohol [02.010])	Developmental toxicity: Gestation days 6 - 15	Mouse; F 50	Gavage	0, 550 mg/kg bw/day	Maternal: 550 Foetal: 550	(JECFA, 1996a)	Unpublished study carried out by NIOSH of assumed good quality. Only summary available. In a preliminary experiment clinical signs of maternal toxicity were observed at 1320 mg/kg and reduced number of viable fetuses at 720 mg/kg.
	Developmental toxicity: Gestation days 6 - 13	Mouse; F 50	Gavage	0, 750 mg/kg bw/day	Maternal: ND ¹ Foetal: ND ¹	(Hardin et al., 1987)	Published study carried out by NIOSH of assumed good quality. Increased mortality, clinical signs of maternal toxicity, reduced maternal body weight-gain and reduced pup weight in treated group.
(Benzyl acetate [09.014])	Developmental toxicity: Gestation days 6 - 15	Rat; F 20	Gavage	0, 10, 100, 500, 1000 mg/kg bw/day	Maternal: 1000 Foetal: 500	(Ishiguro et al., 1993)	Published non-GLP study. At highest dose, slight, but not significant maternal toxicity observed with slightly reduced maternal weight-gain. Also significantly reduced fetal body weight at highest dose and increased incidence of skeletal internal variations.
	Reproductive toxicity: Sperm morphology and vaginal cytology: 13 weeks	Mouse; M, F 20	Oral	0, 3130, 6250, 12500, 25000, 50000 mg/kg in the diet (equivalent to 0, 425, 1000, 2000, 3700, 7900 mg/kg bw/day for males and 0, 650, 1280, 2980, 4300, 9400 mg/kg bw/day for females)	Reproductive toxicity: Females: 3700 Males: 7900	(Morrissey et al., 1988; NTP, 1993d)	Published report of screening test (SMVCE assay) carried out at end of fully described NTP-study. Statistically significant, dose-related decreases in final body weights (over 10 %) observed in all treated animals. Mean length of estrous cycle significantly greater in high dose than in controls. No effects on male reproductive endpoints.
	Reproductive toxicity: Sperm morphology and vaginal cytology: 13 weeks	Rat; M, F 20	Oral	0, 3130, 6250, 12500, 25000, 50000 mg/kg in the diet (equivalent to 0, 230, 460, 900, 1750, 3900 mg/kg bw/day for males 0, 240, 480, 930, 1870, 4500 mg/kg bw/day for females)	Reproductive toxicity: Females: 4500 Males: 3900	(Morrissey et al., 1988; NTP, 1993d)	Published report of screening test (SMVCE assay) carried out at end of fully described NTP-study. Statistically significant decreases in final body weights (over 10 %) observed at 25000 mg/kg feed. Clinical signs of intoxication at 50000 mg/kg feed. No effects on male and female reproductive endpoints.
(Benzaldehyde [05.013])	Reproductive toxicity 32 weeks (every other day)	Rat; M, F 10	Gavage	2 mg/animal (equivalent to 5 mg/kg bw/day)	ND ¹	(Sporn et al., 1967)	Published non-GLP study of limited quality. Study in Romanian with English summary only. Reduced number of pregnant females among treated animals (no statistics presented).

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical Name	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), Including information of possible maternal toxicity	Reference	Comments
(Benzoic acid [08.021])	Developmental toxicity Gestation day: 9	Rat; F 7	Gavage	510 mg/kg bw/day ²	Maternal: NR Foetal: 510	(Kimmel et al., 1971)	Published non-GLP study of limited validity due to inadequate study design. Study evaluated the influence of benzoic acid on the teratogenic effects of acetylsalicylic acid. No teratogenicity was observed with a single dose of benzoic acid. Pretreatment with benzoic acid increased the teratogenicity of acetylsalicylic acid by increasing the salicylate concentration in the embryo and serum.
	Four generation reproduction study: Continuously in diet	Rat; M, F 40	Oral	0, 0.5, 1.0 % in the diet (0, 275, 550 mg/kg bw/day) ³	550	(Kieckebusch and Lang, 1960)	Published non-GLP study of acceptable quality. Limited male reproduction parameters analysed.
(Salicylic acid [08.112])	Developmental toxicity Gestation days: 8 - 14	Rat; F 20	Oral	0, 0.06, 0.1, 0.2, 0.4 % in the diet (0, 46.4, 77.4, 165.4, 330 mg/kg bw/day) (0, 50.7, 77.4, 165.4, 205.9 mg/kg bw/day)	Maternal: 165.4 Foetal: 77.4 Postnatal: 77.4	(Tanaka et al., 1973a)	Published non-GLP study of acceptable quality. No maternal mortality. At highest dose reduced maternal body weight, signs of clinical toxicity (salivation, piloerection), no alive fetuses in 9/15 dams, reduced litter size. External and skeletal anomalies in fetuses at 0.2 % and more. Internal anomalies at 0.4 %. Skeletal anomalies in postnatal animals at 0.4 %.
	Developmental toxicity Gestation days: 8 - 14	Rat; F 20	Gavage	0, 75, 150, 300 mg/kg bw/day	Maternal: 75 Foetal: 75 Postnatal: 75	(Tanaka et al., 1973b)	Published non-GLP study of acceptable quality. At highest dose 3 animals died, reduced maternal body weight, signs of clinical toxicity (salivation, piloerection), At 150 mg/kg and more significantly reduced uterus weight. No live fetuses at highest dose. At 150 mg/kg and more reduced litter size and fetal weight, internal, skeletal and external anomalies.
Ethyl 4-hydroxybenzoate [09.367]	Developmental toxicity Gestation days: 8 - 15	Rat; F 5 to 12	Oral	0, 0.1, 1, 10 % in the diet (0, 50, 460, 2600 mg/kg bw/day) ⁴	Maternal: 460 Foetal: 2600	(Moriyama et al., 1975)	Published non-GLP study of good quality. Sufficient parameters analysed and detailed report of experimental design and results. Reduced terminal maternal body weight at high dose.
	Postnatal development Gestation days: 8 - 15	Rat; F 5 to 12 (46 to 73 fetuses nursed for 1 month)	Oral	0, 0.1, 1, 10 % in the diet (0, 50, 460, 2600 mg/kg bw/day) ⁴	Maternal: NR Neonatal: 2600	(Moriyama et al., 1975)	Published non-GLP study of good quality. Sufficient parameters analysed and detailed report of experimental design and results.
	Reproductive toxicity 8 weeks	Rat; M 8	Oral	0, 0.1, 1.0 % in the diet (0, 103, 1043 mg/kg bw/day)	1043	(Oishi, 2004)	Published study of good quality. No effects on weights of reproductive

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical Name	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), Including information of possible maternal toxicity	Reference	Comments
							organs, on sperm counts in the testes and epididymides and sperm morphology. No effect on serum testosterone, LH, FSH
	Uterotrophic assay 3 days	Mouse; F (immature) 7-10	SC	0, 100 mg/kg bw/day	100	(Hossaiani et al., 2000)	Published non-GLP study of good quality. No estrogenic response observed at the dose levels tested.
	Uterotrophic assay 3 days	Rat; F (immature) 11-16	SC	0, 6, 18, 60, 180 mg/kg bw/day	60	(Lemini et al., 2003)	Published non-GLP study of good quality. Significantly increased uterine weight (wet and dry) at the highest dose (ED ₅₀ for uterotrophic effect: uterine wet weight 68 mg/kg, uterine dry weight 94 mg/kg).
	Uterotrophic assay 3 days	Mouse; F (immature and ovariectomized adult) 10-25	SC	0, 0.6, 6.0, 18, 60, 180 mg/kg bw/day ⁵	0.6	(Lemini et al., 2003)	Published non-GLP study of good quality. Significantly increased uterine weight in immature mice at 6 mg/kg and more and in ovariectomized mice at 18 mg/kg and more (ED ₅₀ 74 mg/kg and 25 mg/kg, respectively)
	Uterotrophic assay 3 days	Mouse; F (adult ovariectomized) 6	SC	0, 60, 180 mg/kg bw/day	ND ¹	(Lemini et al., 2004)	Published study of good quality with full report of experimental details and results. Uterotrophic effect with estrogenic histological changes in uteri in both dose groups.
(Butyl <i>p</i> -hydroxybenzoate [09.754])	Reproductive toxicity 8 weeks	Rat; M 8	Oral	0, 0.01, 0.10, 1.0 % in the diet (0, 10, 100, 1000 mg/kg bw/day)	ND ¹	(Oishi, 2001)	Published non-GLP study of good quality. Reduced cauda epididymal sperm reserve and daily sperm production in testis significantly reduced in all treated groups. Dose-dependently reduced serum testosterone concentration and absolute and relative epididymis weight (significant at 0.1 % or more).
	Reproductive toxicity 10 weeks	Mouse; M 8	Oral	0, 0.01, 0.10, 1.0 % in the diet (0, 14, 146, 1500 mg/kg bw/day)	ND ¹	(Oishi, 2002)	Published non-GLP study of good quality. Absolute and relative weight of epididymides significantly increased at 1.0 %. Dose-dependent decrease of both round and elongated spermatid counts. Significantly decreased elongated spermatid counts in all treated groups. Dose-dependant decrease of serum testosterone concentrations (significant at 1.0 %).
	Uterotrophic assay 3 days	Rat; F (ovarectomized) 5	Oral	4, 40, 400, 800, 1200 mg/kg bw ⁶	1200	(Routledge et al., 1998)	Published study of good quality. No estrogenic activity found after oral administration (small, statistically insignificant increase in uterus wet weight) in immature rats. Positive
			SC	40, 200, 400, 600, 800, 1000, 1200 mg/kg bw/day ⁶	40		

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical Name	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), Including information of possible maternal toxicity	Reference	Comments
							response after s.c. administration. In the same study also an <i>in vitro</i> estrogenic activity test in yeast was carried out in which butyl paraben was found to be weakly estrogenic.
	Uterotrophic assay 3 days	Mouse; F (immature) 10	SC	0, 100 mg/kg bw/day	100	(Hossaiani et al., 2000)	Published non-GLP study of good quality. No estrogenic response observed at the dose level tested.
	Uterotrophic assay 3 days	Rat; F (immature) 10	SC	0, 100, 400, 600 mg/kg bw/day	400	(Hossaiani et al., 2000)	Published non-GLP study of good quality. A weak estrogenic response with a significantly increased relative uterus weight was observed at 600 mg/kg. At 100 and 400 mg/kg the uterus wet weight was significantly increased, but not the relative uterus weight.
	Uterotrophic assay 3 days	Rat; F (immature) 11-16	SC	0, 7, 21, 70, 210 mg/kg bw/day	21	(Lemini et al., 2003)	Published non-GLP study of good quality. Significantly increased uterine wet weight at 70 mg/kg and more, and uterine dry weight at 210 mg/kg (ED ₅₀ for uterotrophic effect: uterine wet weight 87 mg/kg, 338 mg/kg uterine dry weight).
	Uterotrophic assay 3 days	Mouse; F (immature and ovariectomized adult) 6-16	SC	0, 0.7, 7.0, 21, 70, 210 mg/kg bw/day ⁵	0.7	(Lemini et al., 2003)	Published non-GLP study of good quality. Significantly increased uterine weight in immature mice at 7 mg/kg and more and in ovariectomized mice at 21 mg/kg and more (ED ₅₀ 65 mg/kg and 22 mg/kg, respectively).
	Uterotrophic assay 3 days	Mouse; F (adult ovariectomized) 6	SC	0, 70, 210 mg/kg bw/day	ND ¹	(Lemini et al., 2004)	Published study of good quality with full report of experimental details and results. Uterotrophic effect with estrogenic histological changes in uteri in both dose groups.
	Developmental toxicity Gestation days: 6 - 19	Rat; F 25	Gavage	0, 10, 100, 1000 mg/kg bw/day	Maternal: 100 Foetal: 1000	(Daston, 2004)	Published study of good quality with full report of experimental details and results. Maternal food consumption and weight gain significantly decreased at highest dose.
(Methyl salicylate [09.749])	Reproductive toxicity: continuous breeding (RACB) 18 weeks ⁷	Mouse; M, F 40	Gavage	0, 100, 250, 500 mg/kg bw/day	250	(NTP, 1984a)	Fully described NTP-study. Significant slight decrease in mean number of litters, number of pups per litter, mean number of pups born alive per litter and mean live pup weight at highest dose. The experiment was unable to discriminate which sex was affected in reproduction.
(Veratraldehyde [05.017])	Reproductive and	Rat; F	Gavage	0, 80, 400, 800 mg/kg bw/day	Maternal: ND ¹	(Vollmuth et al., 1990)	Unpublished study with limited report of

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical Name	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), Including information of possible maternal toxicity	Reference	Comments
	developmental toxicity: 1 week before mating until 4 days post parturition	10			Foetal: 800		results. Summary of study published as abstract. Validity of the study cannot be evaluated.
(Vanillin [05.018])	Reproductive and developmental toxicity: 1 week before mating until 4 days post parturition	Rat; F 10	Gavage	0, 125, 250, 500 mg/kg bw/day	Maternal: 250 Foetal: 500	(Vollmuth et al., 1990)	Unpublished study with limited report of results. Summary of study published as abstract. Validity of the study cannot be evaluated.
(2,4-Dihydroxybenzoic acid [08.076])	Teratogenicity: Gestation day 9	Rat; F 10	SC	0, 380 mg/kg bw/day	380	(Koshajki and Schulert, 1973)	Published study of limited quality. Limited report of experimental details and results. Insufficient endpoints analysed.
	Teratogenicity Gestation day 11	Rat; F Not Reported	SC	0, 428 mg/kg bw/day (plus 214 mg/kg bw/day after 2 hours)	ND ¹	(Saito et al., 1982)	Published study of limited quality. Unusual study design and limited report of experimental details and results. No effect on plasma Ca level in dams after a single dose. Reduced plasma Ca levels in dams and malformations and foetotoxicity after the additional dose. No effects on maternal reproductive parameters.
(Ethyl vanillin [05.019])	Reproductive and developmental toxicity: 1 week before mating until 4 days post parturition	Rat; F 10	Gavage	0, 200, 1000, 2000 mg/kg bw/day	Maternal: ND ¹ Foetal: 2000	(Vollmuth et al., 1990)	Unpublished study with limited report of results. Summary of study published as abstract. Validity of the study cannot be evaluated.
(Piperonal [05.016])	Reproductive and developmental toxicity: 1 week before mating until 4 days post parturition	Rat; F 10	Gavage	0, 250, 500, 1000 mg/kg bw/day	Maternal: 500 Foetal: 500	(Vollmuth et al., 1990)	Unpublished study with limited report of results. Summary of study published as abstract. Validity of the study cannot be evaluated.

¹ This study was performed at a single dose level or at multiple dose levels that produced adverse effects. No NOEL could be derived from that study.

² Study evaluated the effects of Aspirin (acetylsalicylic acid) when administered to rats during gestation; however, two dose groups used benzoic acid (510 mg/kg) as a pre-treatment and one dose group was administered only benzoic acid. Benzoic acid alone had no effect.

³ Calculated based on the reported daily intake of 150 mg per animal, equal to 0.45 mMol per 100 g bw, in animals of the high dose group and a molecular weight of 122.12.

⁴ Calculated from data on bw and food consumption presented in the article.

⁵ The lowest dose was used in immature mice only.

⁶ The number of dose groups was comprised from two separate experiments. In the first experiment dose levels of 40 and 400 mg/kg/day were investigated, while in the second experiment dose levels of 800 and 1200 mg/kg/day were explored.

⁷ Reproductive assessment by continuous breeding (RACB) consisted of a 7-day premating phase, a 98-day cohabitation period and a 21-day segregation period.

In vitro mutagenicity/genotoxicity data are available for 12 candidate substances of the present flavouring group evaluation from chemical groups 23 and 30 and for 28 supporting substances evaluated by the JECFA at the 46th and 57th meeting (JECFA, 1996b; JECFA, 2002a) and for one related substance. Supporting substances are listed in brackets.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [EL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Benzyl alcohol [02.010])	Ames test (preincubation method)	<i>S. typhimurium</i> TA92; TA94; TA98; TA100; TA1535; TA1537	Up to 10,000 µg/plate (6 concentrations)	Negative ¹	(Ishidate et al., 1984)	Published study in accordance with OECD Guideline 471. Although some details of results are not reported, the study is considered valid.
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA100	1000 µg/plate	Negative ²	(Ball et al., 1984)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	Not reported	Negative ²	(Rogan et al., 1986)	
	Ames test (pre-incubation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	6666 µg/plate	Negative ¹	(Mortelmans et al., 1986)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	3 µmole/plate	Negative ¹	(Florin et al., 1980)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	50,000 µg/plate ⁴	Negative ¹	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	5 µl/plate	Negative ²	(Milvy and Garro, 1976)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	0, 100, 333, 1000, 3333, 6666 µg/plate	Negative ¹	(NTP, 1989a)	Valid study in accordance with OECD Guideline 471 (except that only four strains were used). Cytotoxicity was reported at the highest concentration tested.
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA97; TA102	1000 µg/plate	Negative ¹	(Fujita et al., 1992)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA1535	5 µM/plate	Negative ¹	(Wiessler et al., 1983)	
	Mutation assay	<i>Escherichia coli</i> WP2 <i>uvrA</i>	1000 to 8000 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
	Rec assay	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	10 µg/disc	Positive	(Kuroda et al., 1984b)	Study published in Japanese with English abstract. Data extracted from figure. Validity of the study cannot be evaluated. Inhibition of growth was reported.
	Rec assay	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	20 µl/disc	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. A weak positive result (i.e. 4 mm ≤ D < 8 mm) was reported (D=5 mm). No information on the use of metabolic activation.
	Chromosomal aberration test	Chinese hamster fibroblast cells	1000 µg/ml ⁴ (three	Negative ²	(Ishidate et al., 1984)	Published study carried out only in the absence of

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
			concentrations, max. concentration inducing 50 % cell-growth inhibition)			metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Chromosomal aberration test	Chinese hamster ovary cells	50 to 5000 µg/ml	Equivocal ¹	(Anderson et al., 1990)	Published summary report including detailed results from studies on 42 compounds tested in various laboratories within the NTP in accordance with OECD Guideline 473. Lowest effective dose was 4000 µg/ml with and without S9. No dose-response observed. Positive results were not reproducible in all trials. Absence of cytotoxicity reported up to the highest dose.
	Chromosomal aberration test	Chinese hamster ovary cells	50 to 5000 µg/ml	Negative ² Positive ³	(NTP, 1989a)	Valid study in accordance with OECD Guideline 473. A positive result was reported only in the presence of S9 at relatively high concentrations of 4000 µg/ml in 3 of 4 tests carried out with harvest times between 12 and 18 hours. No data on cytotoxicity reported.
	Sister chromatid exchange assay	Chinese hamster ovary cells	16 to 5000 µg/ml	Positive	(NTP, 1989a)	Valid study in accordance with OECD Guideline 479. Dose-related increase in frequency of SCE at concentrations from 500 - 1250 µg/ml (without metabolic activation) and 500 - 4000 µg/ml (with metabolic activation). No data on cytotoxicity reported. Number of chromosomes per cell reduced at 4000 µg/ml with S9.
	Sister chromatid exchange assay	Chinese hamster ovary cells	16 to 1250 µg/ml ² 16 to 4000 µg/ml ³	Positive ¹	(Anderson et al., 1990)	Published summary report including detailed results from studies on 42 compounds tested in various laboratories within the NTP in accordance with OECD Guideline 479. Significant increase (20 %) in SCE only at the highest doses. No dose-response observed. No second trial using high concentrations to reproduce the positive effects performed. Absence of cytotoxicity reported up to the highest dose.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	Up to 5000 µg/ml	Questionable	(McGregor et al., 1988a; Myhr et al., 1990)	Published summary report including detailed method and results from study on 72 compounds tested in various laboratories within the NTP in accordance with OECD Guideline 476 (however, no colony sizing performed). Positive responses observed in some experiments at concentrations of 3500 and higher. No dose-response was observed. The highest concentration was lethal in some experiments. Positive and negative responses could not be reproduced in all experiments.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	150 to 5000 µg/ml	Negative ³ Positive ²	(NTP, 1989a)	Valid study in accordance with OECD Guideline 476. In one of three trials without S9 a positive result (relative mutant fraction ≥ 1.6) was reported at 4500 µg/ml with relative total growth of 20 %. The concentration of 5000 µg/ml was lethal in this trial, whereas in another one of three trials without S9 3500 µg/ml was lethal.
	Mutation assay	<i>E. coli</i> WP2 <i>uvrA</i>	Not reported	Negative	(Kuroda et al., 1984a)	Only abstract available. Methods, test concentrations and detailed results not reported.
	Cytotoxicity assay	Human alveolar tumour cells	0.5 mM	Negative	(Waters et al., 1982)	
	DNA damage assay	Human alveolar tumour cells	0.5 mM	Negative	(Waters et al., 1982)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Benzyl formate [09.077])	DNA damage assay	Rat hepatocytes	10 mM	Negative	(Storer et al., 1996)	Cytotoxicity was reported at the highest concentration tested.
	DNA damage assay	<i>E. coli</i> P3478	50 µl/disc	Negative ¹	(Fluck et al., 1976)	
	Rec assay	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	20 µl/disc	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. A weak positive result (i.e. 4 mm ≤ D < 8 mm) was reported (D=4 mm). No information on the use of metabolic activation.
	Mutation assay	<i>E. coli</i> WP2 <i>uvrA</i>	500 to 4000 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
(Benzyl acetate [09.014])	Ames test (pre-incubation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative ¹	(Mortelmans et al., 1986)	
	Ames test (pre-incubation and plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	5000 µg/plate	Negative ¹	(Schunk et al., 1986)	Cytotoxicity was observed at the three highest doses tested.
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	3 µM/plate	Negative ¹	(Florin et al., 1980)	
	Rec assay	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	20 µl/disc	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. A weak positive result (i.e. 4 ≤ D < 8) was reported (D could not clearly be determined). No information on the use of metabolic activation.
	Mutation assay	<i>E. coli</i> WP2 <i>uvrA</i>	250 to 2000 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells; Human lymphoblast TK6 cells	Mouse cells 0, 250, 500, 1000 µg/ml; Human cells 0, 500, 1000, 1250, 1500 µg/ml	Negative ² Positive ³	(Caspary et al., 1988)	Published non-GLP study in accordance with OECD Guideline 476 (except that no colony sizing was performed). Thus, the study is considered not fully valid. The lowest significantly effective doses in the presence of S9 were 500 µg/ml in mouse cells and 1500 µg/ml in human cells. Cytotoxicity was reported above 500 µg/ml with and without S9.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	0-1600 µl/ml (6 concentrations)	Positive ²	(McGregor et al., 1988a)	Published summary report including detailed method and results from study on 72 compounds tested in various laboratories within the NTP. The study was not in accordance with OECD Guideline 476 (no colony sizing performed, only in the absence of metabolic activation) and thus not considered valid. The lowest significantly effective doses was 900 µg/ml at which the relative total growth was 50 %. The highest dose was lethal. A positive response was observed in two of three experiments. No dose-response was observed.
	Mammalian cell gene	Mouse lymphoma L5178Y cells	Not reported	Negative ²	(Rudd et al., 1983)	Study carried out within a larger NTP project. Only abstract

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	mutation test			Positive ³		available. Validity of the study cannot be evaluated.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y TK+/- cells	Not reported	Negative ² Inconclusive ³	(Honma et al., 1999a)	Published collaborative study on 40 chemicals. Protocol was in accordance with OECD Guideline 476, except that no colony sizing was performed. As the results are insufficiently reported, their validity cannot be evaluated. In the presence of S9 metabolic activation one laboratory achieved a statistically significant dose-dependant result, but did not induce mutations greater than three times the spontaneous response. The second laboratory did not obtain a positive response.
	Chromosomal aberration test	Chinese hamster ovary cells	160 - 1600 µg/ml ² ; 500 - 5000 µg/ml ³	Negative ¹	(Galloway et al., 1987a)	Published non-GLP study. Doses were selected based on preliminary assay. Although some details of results are not reported the study is considered valid.
	Chromosomal aberration test	Chinese hamster lung fibroblast cells	2400 µg/ml	Negative ¹	(Matsuoka et al., 1996)	Cytotoxicity was reported at the highest concentration tested.
	Sister chromatid exchange assay	Chinese hamster ovary cells	50 - 500 µg/ml ² ; 500 - 5000 µg/ml ³	Negative ¹	(Galloway et al., 1987a)	Published non-GLP study. Doses were selected based on preliminary assay. Although some details of results are not reported the study is considered valid.
	Unscheduled DNA synthesis test	Rat hepatocytes	Not reported	Negative	(Mirsalis et al., 1983)	Only abstract available. Methods, test concentrations and detailed results not reported.
	Micronucleus test	Human lymphocytes and hepatoma cell line Hep G2	500 µM	Negative ¹	(Kevekordes et al., 2001)	
(Benzyl propionate [09.132])	Rec assay	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
(Benzyl benzoate [09.727])	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	3 µM/plate	Negative ¹	(Florin et al., 1980)	
	Ames test (pre-incubation and plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	Up to 5000 µg/plate	Negative ¹	(Schunk et al., 1986)	Cytotoxicity was observed at the three highest doses tested.
(Benzaldehyde [05.013])	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	37,500 nl/plate ⁴	Negative ¹	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	50 to 300 µl/plate	Negative ¹	(Rockwell and Raw, 1979)	Assay of urine samples from rats given benzaldehyde by oral gavage.
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	100 µl/plate	Negative ³	(Rockwell and Raw, 1979)	Samples assayed prior to administration to rats.
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA2637	2000 mg/plate	Negative ¹	(Nohmi et al., 1985)	Article published in Japanese. Data reported from English summary.
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	3 µM/plate	Negative ¹	(Florin et al., 1980)	
	Ames test (pre-incubation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	0, 10, 33, 100, 333, 1000 µg/plate	Negative ¹	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD Guideline 471.
	Ames test	<i>S. typhimurium</i> TA100; TA102; TA104	3333 µg/plate	Negative ¹	(NTP, 1990c)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test	<i>S. typhimurium</i> TA100	1000 µg/plate	Negative	(Rapson et al., 1980)	The use of metabolic activation was not reported.
	Ames test (pre-incubation method)	<i>S. typhimurium</i> TA98; TA100	Not reported	Negative ¹	(Sasaki and Endo, 1978)	
	Ames test (pre-incubation method)	<i>S. typhimurium</i> TA100; TA102; TA104	Not reported	Negative ¹	(Dillon et al., 1992)	
	Ames test (pre-incubation method)	<i>S. typhimurium</i> TA100	2000 nM/plate	Negative ¹	(Vamvakas et al., 1989)	
	Ames test (pre-incubation method)	<i>S. typhimurium</i> TA97; TA102	1000 µg/plate	Negative ¹	(Fujita et al., 1992)	
	Ames test	<i>S. typhimurium</i> TA98; TA100	0.05 to 500 µg/plate	Negative ¹	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test (pre-incubation method)	<i>S. typhimurium</i> TA98; TA1535	5 µM/plate	Negative ¹	(Wiessler et al., 1983)	
	Ames test (pre-incubation method)	<i>S. typhimurium</i> TA97a; TA100; TA102; TA104	Not reported	Negative ¹	(Dillon et al., 1998)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA1537; TA7001; TA7002; TA7003; TA7004; TA7006; Mix of TA7001–7006 TA7005	1000 µg/ml	Negative ¹ Negative ² ; Positive ³	(Gee et al., 1998)	
	Rec assay	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	Not reported	Negative ² Positive ³	(Matsui et al., 1989)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Unscheduled DNA synthesis test	Rat hepatocytes	251 nl/ml	Negative	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	12.5 to 800 nl/ml	Negative ² Positive ³	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Different concentration ranges (12.5-800, 25-600, 400-600 nl/ml) were used in three independent experiments within which positive responses were observed. A 2.8 to 5.2-fold increase in mutant frequency was observed in the presence of S9.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	0 to 800 µg/ml (6 concentrations)	Positive ²	(McGregor et al., 1991)	Published summary report including detailed method and results from study on 27 compounds tested in various laboratories within the NTP in accordance with OECD Guideline 476 (however, no colony sizing performed). Statistically significant increase in mutant fraction at the highest non-lethal concentration (400 µg/ml) in two experiments. Concentration of 640 and 800 µg/ml were lethal. Thus, significant increases in mutant fraction were close to toxic doses. No dose-response was observed. Since a positive response was observed without S9, no experiment was carried out with S9.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y +/- cells	600 µg/ml	Negative ²	(Bigger and Clarke, 1991)	
	Chromosomal aberration test	Chinese hamster cells	0, 800, 1000, 1200 µg/ml	Positive ² Weak positive ³	(Sofuni et al., 1985)	Article published in Japanese. Data extracted from English summary and tables. Validity of the study cannot be evaluated. Cytotoxicity was observed at the two maximum concentrations tested. In the presence and in the absence of S9 a positive response was only observed at cytotoxic concentrations. Polyploidization (11 %) was reported at non-cytotoxic concentrations.
	Chromosomal aberration test	Chinese hamster ovary cells	50 - 500 µg/ml ² ; 160-1600 µg/ml ³	Negative ¹	(Galloway et al., 1987a)	Published non-GLP study. Doses were selected based on preliminary assay. Although some details of results are not reported the study is considered valid.
	Chromosomal aberration test	Chinese hamster cell line B241	50 nM (0.0053 µg/ml)	Positive ¹	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Information is only given for the final concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. Dose-dependent increase of total aberrations (chromatid gaps, chromatid breaks, chromosome breaks observed, no ring or dicentric aberrations or chromatic exchanges).
	Sister chromatid exchange assay	Chinese hamster ovary cells	5-160 µg/ml ² ; 160-1600 µg/ml ³	Positive ² Positive ³	(Galloway et al., 1987a)	Published non-GLP study. Doses were selected based on a preliminary assay. Although some details of results are not reported the study is considered valid. Weakly positive results with metabolic activation were observed at the highest concentration which was cytotoxic and resulted in 50 % growth reduction.
	Sister chromatid exchange assay	Chinese hamster ovary cells	Up to 1000 µM (up to 106 µg/ml)	Negative ³	(Sasaki et al., 1989)	Published non-GLP study of limited quality. Study designed to investigate the influence on spontaneous as well as on mitomycin-induced SCEs. The substance did not influence cell cycle (data not shown) and spontaneous SCEs at the concentrations used. Cytotoxicity was reported at the highest concentration tested.
	Sister chromatid exchange assay	Human lymphocytes	0 - 2 mM (0-212 µg/ml)	Positive ²	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD Guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1538	2500 µg/plate	Negative ¹	(Anderson and Styles, 1978)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1536	3.6 µg/plate	Negative ¹	(Cotruvo et al., 1977)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative ¹	(Zeiger et al., 1988)	
(Benzoic acid [08.021])	Ames test	<i>S. typhimurium</i> TA100	Up to 1000 µg/plate	Negative	(Rapson et al., 1980)	Cytotoxicity was reported at the highest concentration tested.
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	1000 µg/plate	Negative ³	(McCann et al., 1975)	
	Ames test (preincubation)	<i>S. typhimurium</i> TA92; TA94; TA98;	Up to 10,000 µg/plate (6	Negative ¹	(Ishidate et al., 1984)	Published study in accordance to OECD Guideline 471.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	method)	TA100; TA1535; TA1537	concentrations)			Although some details of results are not reported the study is considered valid.
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	100 µg/plate	Negative ²	(Milvy and Garro, 1976)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA1535; TA1537; TA1538	0.5 % (5 mg/ml)	Negative ¹	(FDA, 1975b)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100	100 to 10000 µg/plate	Negative ¹	(Kuboyama and Fujii, 1992)	Published non-GLP study deficient in the report of some details on method and results (no single doses, no data on cytotoxicity reported), however, of sufficient quality to be taken into account in the evaluation.
	<i>Umu</i> mutation assay	<i>S. typhimurium</i> TA1535/ pSK1002	1607 µg/ml	Negative ¹	(Nakamura et al., 1987)	
	Rec assay (liquid method)	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	Not reported	Positive	(Nonaka, 1989)	Only abstract available. Details on method and results not reported. Use of metabolic activation not reported. The validity of the study cannot be evaluated.
	Rec assay	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	0 to 5000 µg/disc	Positive	(Kuboyama and Fujii, 1992)	Well conducted published non-GLP study with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported) of sufficient quality to be taken into account in the evaluation. A weak positive result (D > 2 mm) was observed at concentrations of 4 mg/disc or more. At 5 mg/disc D = 2.9 mm.
	Mutation assay	<i>S. cerevisiae</i> D3	0.18 %	Negative ¹	(Cotruvo et al., 1977)	
	Mutation assay	<i>S. cerevisiae</i> D4	0.15 %	Negative ¹	(FDA, 1975b)	
	Indirect DNA repair test	<i>E. coli</i> PQ37	400 µg/ml	Negative	(Glosnicka and Dziadziuszko, 1986)	Genotoxicity measured as ability to induce β-galactosidase.
	SOS Chromotest	<i>E. coli</i> PQ37	50 µg	Negative ¹	(Kevekordes et al., 1999)	
	Chromosomal aberration test	Chinese hamster fibroblast cells	1500 µg/ml (three concentrations, max. concentration inducing 50 % cell-growth inhibition) ⁴	Equivocal ²	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Total incidence of cells with aberrations was 8 %. Negative response for polyploidization.
	Sister chromatid exchange assay	Human lymphocytes	0-2 mM (0-244 µg/ml)	Negative ²	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD Guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	<i>In vitro</i> Micronucleus assay	Mouse lymphoma L5178Y cells	1000 µg/ml	Negative ¹	(Nesslany and Marzin, 1999)	
(Methyl benzoate [09.725])	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535; TA1537	0 to 666 µg/plate (-S9); 0 to 6666 µg/plate (+S9) (6 concentrations)	Negative ¹	(Zeiger et al., 1992)	Published summary report including detailed results from NTP studies on 311 compounds in accordance with OECD Guideline 471.
	Mutation assay	<i>E. coli</i> Sd-4-73	Not reported	Negative ²	(Szybalski, 1958)	
Methyl 4-methylbenzoate [09.631]	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535; TA1537;	0 to 333 µg/plate (-S9); 0 to 3333 µg/plate (+S9) (6 concentrations)	Negative ¹	(Zeiger et al., 1992)	Published summary report including detailed results from NTP studies on 311 compounds in accordance with OECD Guideline 471.
(Isopentyl benzoate [09.755])	Mutation assay	<i>E. coli</i> Sd-4-73	Not reported	Negative ²	(Szybalski, 1958)	
(4-Isopropylbenzyl alcohol [02.039])	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	100 µl/plate	Negative ³	(Rockwell and Raw, 1979)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	300 µl/plate	Negative ¹	(Rockwell and Raw, 1979)	Assay of urine samples from rats given isopropylbenzyl alcohol by oral gavage.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
o-Tolualdehyde [05.026]	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	3 µmol/plate	Negative ¹	(Florin et al., 1980)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA100; TA1535; TA1537	10 - 666 µg/plate	Negative ¹	(Zeiger et al., 1988)	
m-Tolualdehyde [05.028]	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	3 µmol/plate	Negative ¹	(Florin et al., 1980)	
p-Tolualdehyde [05.029]	Ames test (preincubation method)	<i>S. typhimurium</i> TA104	0.8 µmol/plate	Negative ²	(Marnett et al., 1985a)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	3 µmol/plate	Negative ¹	(Florin et al., 1980)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100; TA102	0.8 nmol -0.8 mmol/plate	Negative ¹	(Aeschbacher et al., 1989)	
(Tolualdehydes (mixed o, m, p) [05.027])	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	18,750 µg/plate ⁴	Negative ¹	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. Test substance only indicated as "tolualdehyde" without specification of isomeric composition. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Unscheduled DNA synthesis test	Rat hepatocytes	1000 µg/ml ⁴	Negative ²	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Test substance only indicated as "tolualdehyde" without specification of isomeric composition. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	300 µg/ml (+S9), 600 µg/ml (-S9) ⁴	Negative ¹	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Test substance only indicated as "tolualdehyde" without specification of isomeric composition.
	Ames test (pre-incubation method)	<i>S. typhimurium</i> TA104	0.8 µM/plate	Negative ¹	(Marnett et al., 1985a)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	3 µM/plate	Negative ¹	(Florin et al., 1980)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA102	0.8 mM/plate	Negative ¹	(Aeschbacher et al., 1989)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA100; TA1535; TA1537	666 µg/plate	Negative ¹	(Zeiger et al., 1988)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	100 µl/plate	Negative ³	(Rockwell and Raw, 1979)	
		<i>S. typhimurium</i> TA98; TA100	300 µl/plate	Negative ¹	(Rockwell and Raw, 1979)	Assay of urine samples from rats given 4-isopropyl benzaldehyde (cuminaldehyde) by gavage.
	Umu test	<i>S. typhimurium</i> TA1535/ pSK1002	1 µmole/ml	Negative	(Miyazawa et al., 2000)	Results indicated that 4-isopropylbenzaldehyde (cuminaldehyde) was positive for antimutagenicity, but not genotoxic.
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 333 µM (up to 50	Negative ²	(Sasaki et al., 1989)	Published non-GLP study of limited quality. Study

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration µg/ml	Result	Reference	Comments
	assay					designed to investigate the influence on spontaneous as well as on mitomycin-induced SCEs. The substance did not influence cell cycle (data not shown) and spontaneous SCEs at the concentrations used. Cytotoxicity was reported at the highest concentration tested.
(4-Hydroxybenzoic acid [08.040])	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	5000 µg/plate	Negative ²	(Mikulasova and Bohovicova, 2000)	
	DNA Repair test	<i>E. coli</i> WP2, WP2uvrA, CM611; CM561	2000 µg/ml	Negative	(Mikulasova and Bohovicova, 2000)	
(Salicylic acid [08.112])	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100	100 to 10000 µg/plate	Negative ¹	(Kuboyama and Fujii, 1992)	Published non-GLP study deficient in the report of some details on method and results (no single doses, no data on cytotoxicity reported), however, of sufficient quality to be taken into account in the evaluation.
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	Not reported	Negative ²	(McCann et al., 1975)	
	Rec assay	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	0 to 5000 µg/disc	Positive	(Kuboyama and Fujii, 1992)	Well conducted published non-GLP study, with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported), however, of sufficient quality to be taken into account in the evaluation. A weak positive result (D > 2 mm) was observed at concentrations of 2 mg/disc or more. At 5 mg/disc D = 4.7 mm.
	Mitotic recombination assay	<i>S. cerevisiae</i> D7	10,000 µg/ml	Negative ²	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Negative response reported both at neutral and alkaline conditions.
	Mutation assay	<i>S. cerevisiae</i> rad18	Up to 0.1 mM (up to 13.8 µg/ml; 8 concentrations)	Positive	(Zetterberg, 1979)	Published non-GLP study with limited report of experimental details and result. Use of metabolic activation not reported. The validity of the study cannot be evaluated. The dose level tested was clearly cytotoxic. An increase in mutant frequency was not evident until 95 - 99 % of cells were killed.
Ethyl 4-hydroxybenzoate [09.367]	Ames test	<i>S. typhimurium</i> TA98; TA100	Not reported	Negative ¹	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Rec assay	<i>B. subtilis</i>	Not reported	Negative ¹	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration assay	Hamster lung fibroblast cells	Not reported	Positive ² Negative ³	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration assay	Human embryo fibroblasts	Not reported	Negative ²	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration assay	Chinese hamster fibroblast cells	Up to 250 µg/ml	Positive	(Ishidate et al., 1978)	Published non-GLP study in Japanese with English summary and tabulated results. Some important details of method and results are not available. There is no

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Sister chromatid exchange assay	Human embryo fibroblasts	Not reported	Negative ²	(Kawachi et al., 1980a)	information on the use of metabolic activation. The substance was tested up to the maximum dose tolerated. Thus, the validity of the study cannot be evaluated.
	Sister chromatid exchange assay	Human fibroblastic cells HE2144	0, 83, 166 µg/ml	Negative ²	(Sasaki et al., 1980)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Mutation assay	Silk worms	Not reported	Negative	(Kawachi et al., 1980a)	Published non-GLP study not in accordance with OECD Guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Mutation assay	Silk worms	Not reported	Negative	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot be evaluated.
(Butyl 4-hydroxybenzoate [09.754])	Ames test	<i>S. typhimurium</i> TA98; TA100	1000 µg/plate	Negative ¹	(Haresaku et al., 1985)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 1000 µg/plate (6 concentrations)	Negative ¹	(Ishidate et al., 1984)	Published study in accordance to OECD Guideline 471. Although some details of results are not reported the study is considered valid.
	Chromosomal aberration test	Chinese hamster fibroblast cells	60 µg/ml (three concentrations, max. concentration inducing 50 % cell-growth inhibition) ⁴	Negative ²	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Ames test (plate incorporation assay)	<i>S. typhimurium</i> TA100	500 µg/plate	Negative ²	(Ball et al., 1984)	
(Veratraldehyde [05.017])	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA15378	8000 µg/plate	Negative ¹	(Nestmann et al., 1980)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	8000 µg/plate	Negative ¹	(Douglas et al., 1979)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535; TTA1537	6666 µg/plate	Negative ¹	(Mortelmans et al., 1986)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	1000 µg/plate ⁴	Negative ¹	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test (preincubation method)	<i>S. typhimurium</i> TA100; TA102; TA104; TA982; TA1538	Not reported	Negative ¹	(Dillon et al., 1992)	
	Ames test (preincubation protocol)	<i>S. typhimurium</i> TA100; TA102; TA104	33 - 3333 µg/plate	Negative ¹	(Dillon et al., 1998)	
	Mutation assay	<i>S. cerevisiae</i> D7; XV185-14C	Not reported	Negative ²	(Nestmann and Lee, 1983)	
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	250 to 1800 µg/ml	Positive ¹	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Different concentration ranges (250, 1400 - 1600, 1400 - 1800 µg/ml) were used in three independent experiments within which positive responses were observed. A 2.3 to 6.2 fold increase in the mutation frequency was observed both with and without S9.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	5000 µg/plate	Negative ²	(Mikulasova and Bohovicova, 2000)	
	DNA Repair test	<i>E. coli</i> WP2; WP2uvrA; CM611; CM561	2000 µg/ml	Negative	(Mikulasova and Bohovicova, 2000)	
	Unscheduled DNA synthesis test	Rat hepatocytes	100 µg/ml ¹	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
(4-Methoxybenzaldehyde [05.015])	Ames test (preincubation method)	<i>S. typhimurium</i> TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 5000 µg/plate (6 concentrations)	Negative ¹	(Ishidate et al., 1984)	Published study in accordance to OECD Guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test	<i>S. typhimurium</i> TA98; TA100	0.05 to 500 µg/plate	Negative ¹	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test (preincubation method)	<i>S. typhimurium</i> TA1537	Up to 5000 µg/plate (6 concentrations)	Negative ¹	(Engelhardt, 1986)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	408 µg/plate	Negative ¹	(Florin et al., 1980)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA102	1000 µg/plate	Negative ¹	(Fujita and Sasaki, 1987)	
	Rec assay	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	22 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
	Ames test	<i>S. typhimurium</i> TA102	5000 µg/plate	Negative ¹	(Müller et al., 1993)	
	Ames test	<i>S. typhimurium</i> TA 100	1000 µg/plate	Negative	(Rapson et al., 1980)	
	Mutation assay	Phage PM2	1362 µg/ml	Negative	(Becker et al., 1996)	
	Chromosomal aberration test	Chinese hamster fibroblast cells	500 µg/ml (three concentrations, max. concentration inducing 50 % cell-growth inhibition) ⁴	Negative ²	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Chromosomal aberration test	Chinese hamster cell line B241	50 nM (0.0068 µg/ml)	Positive ¹	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Results are reported for the concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. Dose-dependent increase of total aberrations (chromatid gaps, chromatid breaks, chromosome breaks observed, ring and dicentric aberrations, chromatic exchanges).
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y TK+/- cells	0 -3.0 mM (0 - 408 µg/ml) 3.6 - 5.1 mM (484 - 691 µg/ml)	Negative ² Positive ²	(Wangenheim and Bolcsfoldi, 1988)	Published non-GLP study not in accordance with OECD Guideline 476 (no metabolic activation, no colony sizing). Important details of method and results are insufficiently reported. This study is not considered valid.
	Ames test	<i>S. typhimurium</i> TA102	5000 µg/plate	Negative ¹	(Jung et al., 1992)	Results confirmed at three separate contract laboratories.
	Sister chromatid exchange assay	Human lymphocytes	0-2 mM (0-273 µg/ml)	Positive ²	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD Guideline 479 (no metabolic activation). Insufficient report

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						of important details of method and results. This study is not considered valid.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	14 µg/ml	Negative	(Sasaki et al., 1987)	
	DNA alkaline unwinding assay	Mouse lymphoma L5178Y TK+/- cells	0, 4, 5, 6 mole/l (0, 544, 680, 816 µg/ml) 7, 8 mole/l (953, 1089 µg/ml)	Negative ² Positive ²	(Garberg et al., 1988)	Published study on 78 compounds not in accordance with standard guidelines. Test suitable for rapid screening only. Strand breaks or mutations observed only at cytotoxic concentrations.
2-Methoxybenzaldehyde [05.129]	Mutation assay	<i>E. coli</i> WP2uvrA, <i>trpE</i>	5000 µg/plate	Negative ²	(Watanabe et al., 1989)	Published non-GLP study with limited report of experimental details and results. Study evaluating the enhancing effect on <i>N'</i> -nitro- <i>N</i> -nitrosoguanidine (MNNG)-induced mutagenesis in pretreated cells and not on the mutagenicity of the substance itself. Absence of an enhancing effect reported.
	Sister chromatid exchange assay	Human lymphocytes	0 - 0.25 mM (0-34 µg/ml)	Positive ²	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD Guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
3-Methoxybenzaldehyde [05.158]	Sister chromatid exchange assay	Human lymphocytes	0-2.0 mM (0-273 µg/ml)	Positive ²	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD Guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y TK+/- cells	0 - 2.5 mM (0 - 340 µg/ml) 3 mM (408 µg/ml)	Negative ² Positive ²	(Wangenheim and Bolcsfoldi, 1988)	Published non-GLP study not in accordance with OECD Guideline 476 (no metabolic activation, no colony sizing). Important details of method and results are insufficiently reported. This study is not considered valid.
(4-Ethoxybenzaldehyde [05.056])	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative ²	(Wild et al., 1983)	
(Methyl 4-methoxybenzoate [09.713])	Paper disk mutation assay	<i>E. coli</i> Sd-4-73	Not reported	Negative ²	(Szybalski, 1958)	
Gallic acid [08.080]	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100	3000 µg/plate	Negative ¹	(Chen and Chung, 2000)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	0, 100, 333, 1000, 3333, 6666 µg/plate (solvent DMSO) 0, 100, 333, 1000, 3333, 10,000 µg/plate (solvent acetone)	Negative ¹ Equivocal ¹	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD Guideline 471. Results on gallic acid from two different laboratories using different solvent. A negative response was observed in both laboratories with TA98, TA1535, TA1537. A negative result was also reported with TA100 in the laboratory using DMSO as solvent. With acetone, a low-level response with a dose-related trend was found with TA100 both in the absence and in the presence of metabolic activation. The effect was reproducible in a second, not reproducible in a third experiment.
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100; TA1535	5000 µg/plate	Negative ¹	(Rashid et al., 1985)	Inhibition was noted at the 5000-µg/plate dose-level; however, this may have been due to toxicity. No mutagenicity was observed at the 1000-µg/plat dose-level.
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1537	15 µM/plate	Negative ¹	(Wang and Klemencic,	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Vanillin [05.018])	Ames test	<i>S. typhimurium</i> TA100	100 µg/plate	Positive ² Positive ³	(Yamaguchi, 1981)	Published non-GLP. Insufficient report of important details of method and results, thus the validity of the result cannot be evaluated.
	Ames test	<i>S. typhimurium</i> TA98; TA100	Not reported	Negative ¹	(Sugimura et al., 1976)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of the study cannot be evaluated. Results are reported for one concentration only which was half the dose inducing mitotic inhibition. The clastogenic activity was reported to be reduced by the addition of S9.
	Chromosomal aberration test	Chinese hamster ovary cells	50 µg/ml	Positive ¹	(Stich et al., 1981c)	
	Chromosomal aberration test	Chinese hamster ovary K1 cells	Up to 2 mM (up to 340 µg/ml)	Negative ¹	(Tayama and Nakagawa, 2001)	
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	0, 0.25, 0.5, 1.0, 1.5, 2.0 mM (0, 42.5, 85, 170, 255, 340 µg/ml)	Positive ²	(Tayama and Nakagawa, 2001)	Published non-GLP study. Well conducted part of the study, however with insufficient report of some important details of method and results (results with metabolic activation not reported).
	Mitotic gene conversion assay	<i>S. cerevisiae</i> D7	0, 100, 1000 µg/ml	Negative ² Positive ²	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Gallic acid did not induce a significant extent of gene conversions under acidic conditions. At neutral pH no convertogenic activity was reported at 100 µg/ml, however, gallic acid was considerably convertogenic at 1000 µg/ml. The presence of catalase completely inhibited the convertogenic activity gene conversions. Under alkaline conditions (pH 10), the concentration of 100 µg/ml was reported to induce a significant ($p < 0.01$) increase of Trp ⁺ convertants.
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate ⁴	Negative ¹	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	5000 µg/plate	Negative ¹	(Pool and Lin, 1982)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	21 µg/disc	Negative	(Oda et al., 1979)	
	Ames test (preincubation assay)	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative ¹	(Mortelmans et al., 1986)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test	<i>S. typhimurium</i> TA98; TA100	0.05 to 1000 µg/plate	Negative ¹	(Kasamaki et al., 1982)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	Not reported	Negative ¹	(Nagabhushan and Bhide, 1985)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test	<i>S. typhimurium</i> TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (6 concentrations)	Negative ¹	(Ishidate et al., 1984)	Published study in accordance with OECD Guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test	<i>S. typhimurium</i> TA100	1000 µg/plate	Negative	(Rapson et al., 1980)	
	Paper disk mutation assay	<i>E. coli</i> Sd-4-73	Not reported	Negative ²	(Szybalski, 1958)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	2500 µg/plate	Negative ²	(Mikulasova and Bohovicova, 2000)	
	DNA Repair test	<i>E. coli</i> WP2; WP2uvrA; CM611; CM561	2000 µg/ml	Negative	(Mikulasova and Bohovicova, 2000)	
	Mutation assay	<i>E. coli</i> CSH26/pYM3; CSH26/pSK 1002	15,215 µg/ml	Negative	(Takahashi et al., 1990)	
	Mitotic recombination assay	<i>S. cerevisiae</i> D7	10,000 µg/ml	Negative ²	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Negative response reported both at neutral and alkaline conditions.
	Chromosomal aberration test	Chinese hamster cell line B241	5, 20, 40 nM (0.0008, 0.003, 0.006 µg/ml)	Negative	(Kasamaki and Urasawa, 1985)	
	Chromosomal aberration test	Chinese hamster fibroblast cells	1000 µg/ml (three concentrations, max. concentration inducing 50 % cell-growth inhibition) ⁴	Negative ²	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Chromosomal aberration test	Chinese hamster V79 lung cells	15,215 - 152,150 µg	Negative ²	(Tamai et al., 1992)	
	Chromosomal aberration test	Human lymphocytes	0, 1, 2, 4 mM (0, 152, 304, 608 µg/ml)	Negative	(Jansson and Zech, 1987)	Published non-GLP study not in accordance with OECD Guideline 473 (no metabolic activation). Insufficient report of important details of method and results. No information on cytotoxicity. This study is not considered valid.
	Chromosomal aberration test	Chinese hamster cell line B241	20 nM (0.003 µg/ml)	Negative ¹	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Results are only reported for the final concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. No significant increase of single types of aberrations and of total aberrations.
	Sister chromatid exchange assay	Human lymphocyte cells	0 - 1.0 mM (0 - 152 µg/ml)	Positive ²	(Jansson et al., 1986)	Published non-GLP study not in accordance with OECD Guideline 479 (no metabolic activation). This study is not considered valid. Dose-dependent effect reported. Insufficient report of important details of method and results.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	15 µg/ml	Negative	(Sasaki et al., 1987)	
	Sister chromatid exchange assay	Human lymphocytes	0, 1, 2 mM (0, 152, 304 µg/ml)	Positive ²	(Jansson and Zech, 1987)	Published non-GLP study not in accordance with OECD Guideline 479 (no metabolic activation). Insufficient report of important details of method and results. Dose-dependent effect reported. This study is not considered valid.
	Mutation assay	Mouse lymphoma L5178Y cells	1000 µg/ml (-S9), 1500 µg/ml (+S9) ⁴	Negative ¹	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Unscheduled DNA synthesis test	Rat hepatocytes	500 µg/ml ¹	Negative	(Heck et al., 1989)	study cannot be evaluated. Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Micronucleus assay	Human hepatoma (Hep-G2) cells	5, 50 µg/ml 500 µg/ml	Negative ² Positive ²	(Sanyal et al., 1997)	Published non-GLP study carried out only in the absence of metabolic activation. Thus, the study is not considered valid. A statistically significant increase of spontaneous micronucleus frequency was reported at the highest concentration. Low concentrations of vanillin (0.25 - 5 µg/ml) but not higher (50, 500 µg/ml) showed an inhibitory effect on micronuclei induced by heterocyclic amines.
(Vanillic acid [08.043])	Chromosomal aberration test	Chinese hamster ovary cells	25,000 µg/ml	Positive ¹	(Stich et al., 1981c)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of the study cannot be evaluated. Data are only reported for one concentration which was half the dose inducing mitotic inhibition. The clastogenic activity was reported to be increased by the addition of S9.
	Mitotic recombination assay	<i>S. cerevisiae</i> D7	10,000 µg/ml	Negative ²	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Negative response reported both at neutral and alkaline conditions.
4-Hydroxy-3,5-dimethoxybenzaldehyde [05.153]	Ames test	<i>S. typhimurium</i> TA100	10,000 µg/plate	Negative	(Rapson et al., 1980)	The use of metabolic activation was not reported.
4-Hydroxy-3,5-dimethoxybenzoic acid [08.087]	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	366 µg/plate	Negative ¹	(Florin et al., 1980)	
	Chromosomal aberration test	Chinese hamster ovary cells	3000 µg/ml	Positive ¹	(Stich et al., 1981c)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of the study cannot be evaluated. Data are only reported for one concentration which was half the dose inducing mitotic inhibition. The clastogenic activity was reported to be reduced by the addition of S9.
	Mitotic recombination assay	<i>S. cerevisiae</i> D7	10,000 µg/ml	Negative ²	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid.
(Salicylaldehyde [05.055])	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	366 µg/plate	Negative ¹	(Florin et al., 1980)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100	Not reported	Negative ¹	(Sasaki and Endo, 1978)	
	Ames test	<i>S. typhimurium</i> TA98; TA100	16 µg/ml	Negative ¹	(Kono et al., 1995)	
	Mutation assay	<i>S. typhimurium</i> TA1535/ pSK1002	111 µg/ml	Negative ¹	(Nakamura et al., 1987)	
	Chromosomal aberration test	CHL/IU cells	Not reported (max. 5 mg/ml)	Positive ¹	(Kusakabe et al., 2002)	Published study in accordance with OECD Guideline 473. However, some details on method and results are insufficiently reported. Thus, the validity of the study cannot be evaluated. Positive result with minimum effective dose manifesting over 50 % cytotoxicity at short-

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Methyl salicylate [09.749])						term treatment (6 hours, less than 50 % cells with chromosomal aberrations without S9, less than 20 % cells with chromosomal aberrations with S9). Reduced effect at continuous treatment without S9 (24 hours less than 10 % cells with chromosomal aberrations). No chromosomal aberrations after 48 hours treatment without S9. After 48 hours treatment without S9 18 % polyploid cells.
	Sister chromatid exchange assay	Human lymphocyte cells	0-0.5 mM (0-61 µg/ml)	Negative ²	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD Guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Ames test	<i>S. typhimurium</i> TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (6 concentrations)	Negative ¹	(Ishidate et al., 1984)	Published study in accordance with OECD Guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535; TA1537	333.3 µg/plate	Negative ¹	(Mortelmans et al., 1986)	
	Ames test	<i>S. typhimurium</i> TA98; TA100	Not reported	Negative ¹	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration test	Hamster lung fibroblast cells	Not reported	Positive ² Negative ³	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration test	Chinese hamster fibroblasts	250 µg/ml ⁴ (three concentrations, max. concentration inducing 50 % cell-growth inhibition)	Negative ²	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100	100 to 10000 µg/plate	Positive ¹	(Kuboyama and Fujii, 1992)	Published non-GLP study deficient in the report of some details on method and results (no single doses, no data on cytotoxicity reported), however, of sufficient quality to be taken into account in the evaluation. At 100 µg/plate, a positive response was observed in strain TA98 in the presence of S9 mix obtained from hamsters, a negative response was observed in TA98 in the presence of S9 mix obtained from rat, mouse and guinea pig.
	Rec assay	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	23 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	<i>B. subtilis</i>	Not reported	Negative ¹	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Rec assay	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	0 to 5000 µg/disc	Negative	(Kuboyama and Fujii, 1992)	Well conducted published non-GLP study with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported), however, of sufficient quality to be taken into account in the evaluation.
	Mutation assay	Silkworm	Not reported	Negative	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Chromosomal aberration test	Human embryo fibroblast cells	Not reported	Negative ²	(Kawachi et al., 1980b; Kawachi et al., 1980a)	be evaluated. Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Sister chromatid exchange assay	Human embryo fibroblast cells	Not reported	Negative ²	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot be evaluated.
(Butyl vanillyl ether [04.093])	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	5000 µg/plate	Negative ¹	(Watanabe and Morimoto, 1989c)	
	Mutation assay	<i>E. coli</i> WP2 <i>uvrA</i>	5000 µg/plate	Negative ¹	(Watanabe and Morimoto, 1989c)	
(Ethyl vanillin [05.019])	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative ¹	(Wild et al., 1983)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535; TA1537	8000 µg/plate	Negative ¹	(Mortelmans et al., 1986)	
	Ames test	<i>S. typhimurium</i> TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (six concentrations)	Negative ¹	(Ishidate et al., 1984)	Published study in accordance with OECD Guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA102	1000 µg/plate	Negative ¹	(Fujita and Sasaki, 1987)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate ⁴	Negative ¹	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Rec assay	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Chromosomal aberration test	Chinese hamster fibroblast cells	250 µg/ml (three concentrations, maximal concentration inducing 50 % cell-growth inhibition) ⁴	Positive ²	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Polyploidization in 48 % of cells reported at 48 hours. Negative response for chromosomal aberrations.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	125-800 µg/ml	Negative ² Weak positive ³	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Different concentration ranges (125-500 µg/ml, 600 µg/ml, 800 µg/ml) were used in three independent experiments within which positive responses were observed. In the presence of S9 a 2.1 to 3-fold increase in the mutant frequency was reported.
	Unscheduled DNA synthesis test	Rat hepatocytes	199 µg/ml ⁴	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Sister chromatid exchange	Human lymphocytes	0-2.0 mM (0-332 µg/ml)	Negative ²	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	assay					Guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	17 µg/ml	Negative	(Sasaki et al., 1987)	
(Ethyl vanillin isobutyrate [09.933])	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	5000 µg/plate	Negative ¹	(King and Harnasch, 1997)	
(Piperonyl acetate [09.220])	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535; TA1537	3333 µg/plate	Negative ¹	(Mortelmans et al., 1986)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative ¹	(Wild et al., 1983)	
(Piperonal [05.016])	Ames test					
	Modified Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538 <i>E. coli</i> WP2uvrAtrp ⁻	0, 300, 600, 1200, 2400 µg/plate	Negative ¹	(Sekizawa and Shibamoto, 1982)	Valid study in accordance with OECD Guideline 471. The plate incorporation method was used -S9; the preincubation method +S9.
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate ⁴	Negative ¹	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test	<i>S. typhimurium</i> TA98; TA100	0.05 to 5000 µg/plate	Negative ¹	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1537; TA1538	5000 µg/plate	Negative ¹	(White et al., 1977)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	0, 10, 33, 100, 333, 1000 µg/plate	Negative ¹	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD Guideline 471.
	Rec assay	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	20 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	<i>B. subtilis</i> M45 (rec ⁻), H17 (rec ⁺)	5000 µg/disc	Positive ²	(Sekizawa and Shibamoto, 1982)	Well designed and reported study, however with some limitations with respect to results. DNA-repair tests in the presence of S9 were not successful (no data reported).
	Chromosomal aberration test	Chinese hamster cell line B241	50 nM (0.0075 µg/ml)	Positive ¹	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Data are only reported for the concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. Dose-dependent increase of total aberrations (chromatid gaps, chromatid breaks, chromosome breaks observed, no ring or dicentric aberrations or chromatic exchanges).
	Chromosomal aberration test	Chinese hamster cell line B241	0.15 µg/ml	Negative	(Kasamaki and Urasawa, 1985)	
	Mammalian cell gene	Mouse lymphoma L5178Y cells	1000 µg/ml ⁴	Negative ¹	(Heck et al., 1989)	Published non-GLP study. Some important details of study

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	mutation test					design and results are not reported. Thus, the validity of the study cannot be evaluated.
	Unscheduled DNA synthesis test	Rat hepatocytes	10 to 502 µg/ml	Positive	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
(Vanillin 3-(1-menthoxy)propane-1,2-diol acetal [02.248]) ⁵	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	Up to 5000 µg/plate	Negative ¹	(Kajiura, 1996b)	
	Mutation assay	<i>E. coli</i> WP2 uvrA	Up to 5000 µg/plate	Negative ¹	(Kajiura, 1996b)	
6,6'-Dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde [05.221]	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100; Ta102; TA1535; TA1537	0, 50, 150, 500, 1500, 5000 µg/plate	Negative ¹	(King and Harnasch, 2002c)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100; Ta102; TA1535; TA1537	0, 15, 50, 150, 500, 1500, 5000 µg/plate	Negative ¹	(King and Harnasch, 2002c)	
3,4-Dihydroxybenzoic acid [08.133]	Sister chromatid exchange assay	Chinese hamster ovary cells		Negative ¹	(Stich et al., 1981c)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of the study cannot be evaluated. Data are only reported for one concentration which was half the dose inducing mitotic inhibition. The clastogenic activity was reported to be increased by the addition of S9.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	0, 33, 100, 333, 1000, 3333 µg/ml	Negative ²	(McGregor et al., 1988c)	

NR = not reported.

¹ With and without S9 metabolic activation.

² Without S9 metabolic activation.

³ With S9 metabolic activation.

⁴ Concentration listed is either the highest tested if the result was negative or the concentration at which the maximum effect was observed for positive results.

⁵ Related substance.

In vivo mutagenicity/genotoxicity data are available for two candidate substances of the present flavouring group evaluation from chemical group 23 and for ten supporting substances evaluated by JECFA at the 46th and 57th meeting (JECFA, 1996b; JECFA, 2002a). Supporting substances are listed in brackets.

Table IV.5: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Benzyl alcohol [02.010])	<i>In vivo</i> Sex- linked recessive lethal mutations(SLRL)	<i>D. melanogaster</i>	Diet	5000 ppm	Negative	(Foureman et al., 1994)	
	<i>In vivo</i> SLRL	<i>D. melanogaster</i>	Injection	8000 ppm	Negative	(Foureman et al., 1994)	
	<i>In vivo</i> Micronucleus test	Mouse bone marrow cells	IP injection	200 mg/kg bw	Negative	(Hayashi et al., 1988)	
	<i>In vivo</i> Replicative DNA synthesis test	Mouse and rat hepatocytes	Not reported	Not reported	Negative	(Yoshikawa, 1996)	Screening test for the detection of non-genotoxic hepatocarcinogens. The substance was administered once at the maximum tolerated dose or at half the maximum tolerated dose to male mice and rats. Hepatocytes were prepared after 24, 39 and 48 hours.
	<i>In vivo</i> Replicative DNA synthesis test	Mouse hepatocytes	Oral gavage	800 mg/kg	Negative	(Miyagawa et al., 1995)	
	<i>In vivo</i> Replicative DNA synthesis test	Rat hepatocytes	Oral or SC injection	600 mg/kg	Negative	(Uno et al., 1994)	
(Benzyl acetate [09.014])	<i>In vivo</i> SLRL	<i>D. melanogaster</i>	Diet	300 ppm	Negative	(NTP, 1993d; Foureman et al., 1994)	
	<i>In vivo</i> SLRL	<i>D. melanogaster</i>	Injection	20,000 ppm	Negative	(NTP, 1993d; Foureman et al., 1994)	
	<i>In vivo</i> Sister chromatid exchange assay	Mouse bone marrow cells	IP injection	1700 mg/kg bw	Negative	(NTP, 1993d)	
	<i>In vivo</i> Chromosomal aberration test	Mouse bone marrow cells	IP injection	0 to 1700 mg/kg bw	Negative	(NTP, 1993d)	Test substance same batch as NTP chronic bioassays. The highest dose caused toxicity and cell cycle delay. Test not fully in compliance with the OECD guideline (insufficient cells per animal studied). GLP status not stated. The study is considered of limited validity.
	<i>In vivo</i> Micronucleus test	Mouse bone marrow cells	3 IP injection with 24 h intervals	0, 312, 625 and 1250 mg/kg bw	Negative	(NTP, 1993d; Shelby et al., 1993)	Test substance same batch as NTP chronic bioassays. Study in compliance with OECD guideline. GLP not stated. Micronuclei were determined at 24 hours after the last dose. A dose-related decrease in PCE/NCE ratio was observed. The study is considered valid.
	<i>In vivo</i> Micronucleus test	Mouse erythrocytes	Dietary exposure for 13 weeks.	0 to 50,000 ppm (equal to 0 to 7900 mg/kg bw/day for males and 0 to 9400 mg/kg bw/day for females)	Negative	(NTP, 1993d)	Test substance same batch as NTP chronic bioassays. In life phase under GLP; for determination of genotoxic effects. GLP not specified. Test in compliance with OECD guideline. The test is considered valid, but of limited relevance because no change in PCE/NCE ratio was observed.
	<i>In vivo</i> Unscheduled DNA synthesis test	Rat hepatocytes	Oral gavage	0, 50, 200 and 1000 mg/kg bw	Negative	(Mirsalis et al., 1989)	Test substance same batch as NTP chronic bioassays. Test in compliance with OECD guidelines. GLP not stated. The test is considered valid.
	<i>In vivo</i> Unscheduled DNA synthesis test	Rat pancreatic cells	Oral gavage	1000 mg/kg bw	Negative	(Steinmetz and Mirsalis, 1984)	Only abstract available. Non guideline test. Validity cannot be assessed.
	<i>In vivo</i> DNA damage	Rat pancreatic cells	IP injection	0, 150, 500 and	Negative	(Longnecker et al., 1990)	Alkaline elution assay. GLP status not specified.

Table IV.5: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
				1500 mg/kg bw			Limited number of animals/group; DNA damage monitored at 1 hour post dosing. The study is of limited validity.
	<i>In vivo</i> Comet assay	Mouse/ Rat	Oral	1600 mg/kg (mouse); 1200 mg/kg (rat)	Positive	(Sekihashi et al., 2002)	Non-GLP and non-guideline test; but in compliance with recommended protocols. Some important details of method and results insufficiently reported. No toxicity data reported. The administered dose was 0.5 x LD ₅₀ . Sampling time was 3, 8 and 24 hours after dosing. Positive result reported in mice for stomach, colon, kidney, urinary bladder and brain, in rats for stomach, colon, liver, kidney, urinary bladder, lung. After 24 hours no significant effect in mice, significant effects in rat only in lung and kidney. The study is of limited validity.
(Benzaldehyde [05.013])	<i>In vivo</i> SLRL	<i>D. melanogaster</i>	Diet	1150 ppm	Negative	(Woodruff et al., 1985)	
	<i>In vivo</i> SLRL	<i>D. melanogaster</i>	Injection	2500 ppm	Negative	(Woodruff et al., 1985)	
(Salicylic acid [08.112])	<i>In vivo</i> Chromosomal aberration assay	Mouse bone marrow cells	IP injection gavage	0, 50, 100, 200 mg/kg 0, 350 mg/kg	Negative Negative	(Giri et al., 1996)	Published study widely in accordance with OECD Guideline 475 and well reported (except that only males were tested, only one sampling time was chosen and signs of toxicity were not reported). Oral and i.p. dose were selected to be 1/3 and 1/5 of the reported oral LD ₅₀ .
	<i>In vivo</i> Sister chromatid exchange assay	Mouse bone marrow cells	IP injection gavage	0, 25, 50, 100 mg/kg 0, 350 mg/kg	Negative Negative	(Giri et al., 1996)	Well described published study of good quality. Oral and i.p. dose were selected to be 1/3 and 1/10 of the reported oral LD ₅₀ .
Ethyl 4-hydroxybenzoate [09.367]	<i>In vivo</i> Chromosomal aberration assay	Rat bone marrow cells	Not reported	Not reported	Negative	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
(4-Ethoxybenzaldehyde [05.056])	<i>In vivo</i> Basic test Micronucleus test	<i>D. melanogaster</i>	NR	751 µg/ml	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere. However, results sufficiently reported. Study is considered valid.
	<i>In vivo</i> Micronucleus test	NMRI mice	NR	1005 mg/ kg bw	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
Gallic acid [08.080]	<i>In vivo</i> Medium-term rat liver bioassay	Male rats initiated with IP injection of diethylnitrosamine	Not reported.	Not reported	Negative	(Shirai, 1997)	Published non-GLP study. Unusual study protocol not following OECD guidelines. Some important details of method missing and only summarised results of a large screening study reported. Thus, the validity of the study cannot be evaluated.
(Vanillin [05.018])	<i>In vivo</i> Micronucleus test	Male BDF ₁ mice	Oral gavage	500 mg/kg bw	Negative	(Inouye et al., 1988)	Published non-GLP study not in accordance with OECD Guideline 474 (smaller group size, only males tested, no toxicity data reported, single dose level used, no negative control, effect on PCE/NCE ratio not reported). Induction of micronuclei in mitomycin-treated mice was suppressed by post-treatment with vanillin due to an anticlastogenic

Table IV.5: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Salicylaldehyde [05.055])	<i>In vivo</i> Spot test	<i>D. melanogaster</i> BINS-C <i>D. melanogaster</i> Oregon-R	NR	1.05 to 1.40 ppm 0.09 to 0.35 ppm	Negative Negative	(Kono et al., 1995)	effect. Vanillin itself did not induce micronucleated PCEs (vanillin control group without mitomycin-treatment, six sampling times from 5 to 65 hours). Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
(Ethyl vanillin [05.019])	<i>In vivo</i> Basic test	<i>D. melanogaster</i>	NR	8309 µg/ml	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere. However, results sufficiently reported. Study is considered valid.
	<i>In vivo</i> Micronucleus test	Male BDF ₁ mice	IP injection	Not reported	Negative	(Furukawa et al., 1989)	Only abstract available. Insufficient report of experimental details and result to evaluate the validity of the study.
	<i>In vivo</i> Micronucleus test	NMRI mice	NR	1000 mg/kg bw	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
(Piperonyl acetate [09.220])	<i>In vivo</i> Basic test	<i>D. melanogaster</i>	NR	4855 µg/ml	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere. However, results sufficiently reported. Study is considered valid.
	<i>In vivo</i> Micronucleus test	NMRI mice	NR	970 mg/kg bw	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
(Piperonal [05.016])	<i>In vivo</i> Dominant lethal assay	ICR/Ha Swiss mice	IP injection	0, 124, 620 mg/kg bw	Negative	(Epstein et al., 1972)	Published non-GLP study evaluating 174 substances. Study protocol not fully in accordance with OECD Guideline 478 (lower number of animals and of dose levels used, limited report of experimental observations). However, due to the large body of control data available the results are considered valid. Doses were selected in preliminary acute toxicity tests. Parameters recorded were percent pregnancy, total implants and early and late fetal deaths.
	<i>In vivo</i> Dominant lethal assay	ICR/Ha Swiss mice	Oral gavage	0, 1000 mg/kg bw (repeated doses on 5 successive days)	Negative	(Epstein et al., 1972)	Published non-GLP study evaluating 174 substances. Study protocol not fully in accordance with OECD Guideline 478 (lower number of animals and of dose levels used, limited report of experimental observations). However, due to the large body of control data available the results are considered valid. Doses were selected in preliminary acute toxicity tests. Parameters recorded were percent pregnancy, total implants and early and late fetal deaths.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
AFC	Additives, Flavourings and Food Contact Materials
ANS	Food Additives and Nutrient Sources
AUC	Area Under Curve
BW	Body Weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoA	Coenzyme A
CoE	Council of Europe
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
EC	European Commission
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
FSH	Follicle-stimulating hormone
GI	Gastrointestinal
GLP	Good Laboratory Practice
GSH	Glutathione
HPLC	High Performance Liquid Chromatography
ID	Identity
IP	Intraperitoneal
IV	Intravenous
IOFI	International Organization of the Flavour Industry
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD ₅₀	Lethal Dose, 50 %; Median lethal dose
LH	Luteinizing hormone
MDP	Methylenedioxyphenyl

MNNG	<i>N'</i> -nitro- <i>N</i> -nitrosoguanidine
MPD	4-Methyl-2-phenyl-1,3-dioxolane
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PAFA	Priority-based assessment of food additives
RACB	Reproductive assessment by continuous breeding
SC	Subcutaneous
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food
SMART	Somatic Mutation and Recombination Test
TAMDI	Theoretical Added Maximum Daily Intake
TLC	Thin Layer Chromatography
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation

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 by the SCCNFP
during the plenary session of 8 December 1999**

Executive summary

1. Terms of Reference

1.1 Context of the question

Under the current Community legislation, fragrance ingredients as all ingredients which are part of cosmetic products, fall under the scope of the Cosmetics Directive 76/768/EEC of July 1976 and are submitted to all specified provisions. In relation with the labelling of the ingredients, the Article 6 of the Directive provides a specific provision for fragrance ingredients which states that “perfume and aromatic compositions and their raw materials shall be referred to by the word ‘perfume’ or ‘flavour’”. This general wording substitutes for the detailed list of fragrance ingredients.

Independently from the existing legal provisions, the fragrance industry is self-regulated through the Code of Practice of the International Fragrance Association (IFRA). IFRA issues recommendations for the safe use of fragrance ingredients, which are published in the IFRA Code of Practice and its guidelines. The most important reason for quantitative restrictions is skin sensitisation.

1.2 Request to the SCCNFP

In response to a question from a Member state and members of the European parliament, the SCCNFP has been asked by DG Enterprise to respond to the following mandate in relation to the safety of fragrance ingredients:

- It is proposed that all known fragrance allergens are labelled on cosmetics if used in the products. Does the SCCNFP agree to this proposal ? If so :
 - Which chemicals fall under this classification ?
 - Is there a maximum concentration of each chemical permissible without the requirement for labelling ?
- Does the SCCNFP agree to the inclusion of all IFRA restricted materials in the Annex III (List of substances which cosmetic products must not contain except subject to restrictions and conditions laid down) ?

Are the permitted levels recommended by IFRA suitable for use in the Cosmetics Directive 76/768/EEC ?
- Does the SCCNFP agree that all materials that IFRA recommend should not be used as fragrance compounds are included in Annex II (List of substances which must not form part of the composition of cosmetic products) ?

- Restrictions are proposed for the 3 most common fragrance allergens (cinnamic aldehyde, isoeugenol, hydroxycitronellal). Does the SCCNFP agree to restriction on the use of common fragrance allergens (Annex III listing)? If so :
 - Which fragrance materials should be subject to restrictions?
 - What are the conditions for restrictions (maximum concentration, fields of applications, etc) ?

1.3 Strategy of the SCCNFP

The SCCNFP has considered that this mandate can be usefully divided into two sections (Interim position on Fragrance allergy SCCNF/0202/99 adopted at the SCCNFP session of 23. June 99):

- I. Identification of those fragrance ingredients, which are of concern as allergens for the consumer. Recommendations on informing the consumer of the presence of important allergens to permit the consumer with a known fragrance allergy a means to avoid contact with an allergen. An opinion as to whether such identification can be related to concentrations present in a product when elicitation levels are known.
- II. An opinion on the adoption of industry prohibited substances into Annex 2 and adoption of industry restricted substances into Annex 3. Considerations as to whether the concentration limits or other restrictions suggested by industry can be supported or need to be changed if there is such inclusion in Annex 3. Whether there are additional substances which should be subject to inclusion in an Annex.

This opinion relates to the first section and consists of :

- a critical review of the problem of fragrance allergy in consumers;
- identification of those fragrance ingredients which are well-recognised as consumer allergens;
- an opinion as to whether such identification can be related to concentrations present in a product when elicitation levels are known.

Allergy to natural ingredients will be analysed separately.

2. Opinion of the SCCNFP

It is the opinion of the SCCNFP that:

- Fragrance ingredients have to be considered an important cause of contact allergy.
- Based on criteria restricted to dermatological data reflecting the clinical experience, it has been possible to identify 24 fragrance ingredients, which correspond to the most frequently

recognised allergens.

Thirteen of these have been reported more frequently, these are well-recognised contact allergens in consumers and are thus of most concern, eleven others are less well documented.

List A: Fragrance chemicals, which according to existing knowledge, are most frequently reported and well-recognised consumer allergens:

Common name	CAS no	Common name	CAS no
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-	31906-04-4
Isoeugenol	97-54-1	cyclohexenecarboxaldehyde	

List B: Fragrance chemicals, which are less frequently reported and thus less documented as consumer allergens. These are:

Common name	CAS no	Common name	CAS no
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

- There are at the present not sufficient scientific data to allow for determination of dose-response relationships and thresholds for these allergens.

Further, SCCNFP is of the opinion that information should be provided to consumers about the known presence in cosmetic products of fragrance ingredients with a well-recognised potential to cause contact allergy.

Information regarding these fragrance chemicals should be given to consumers if deliberately added to a fragrance formulation either in the form of a chemical or as an identified constituent of an ingredient.

In the future other fragrance chemicals may be included (or excluded) depending on the epidemiological and safety data available.

This is required to improve the protection of the consumer by ensuring that the correct diagnosis of contact allergy to well-recognised fragrance allergens can be made without undue

delay and by providing information that will help the consumer avoid specific substances that they may not tolerate.

Natural materials, such as oak moss, will according to the interim position statement given by the SCCNFP be considered in a separate document (SCCNFP/0202/99).

In the DG Enterprise's request the term "labelling" of cosmetic products with respect to fragrance ingredient is used. This term belongs to the sector of risk management which is beyond the remit of the SCCNFP. It is up to the legislator to consider the best way to provide the information to the consumers.

Full opinion

1. Introduction

1.1 Background

In recent years there has been a scientific debate on the safety of fragrance (perfumery) ingredients. Dermatologists have highlighted the risk of contact allergy from fragrance ingredients (9,25,64,65) and actions to prevent the disease have been requested (1,2,63).

1.2 Terms of Reference

1.2.1 Context of the question

Under the current Community legislation fragrance ingredients, as all ingredients which are part of cosmetic products, fall under the scope of the Cosmetics Directive 76/768/EEC of July 1976 and are submitted to all specified provisions. In relation with the labelling of the ingredients, the Article 6 of the Directive provides a specific provision for fragrance ingredients which states that “perfume and aromatic compositions and their raw materials shall be referred to by the word ‘perfume’ or ‘flavour’”. This general wording substitutes for the detailed list of fragrance ingredients.

Independently from the existing legal provisions, the fragrance industry is self-regulated through the Code of Practice of the International Fragrance Association (IFRA) (68). IFRA issues recommendations for the safe use of fragrance ingredients, which are published in the IFRA Code of Practice and its guidelines. The most important reason for quantitative restrictions is skin sensitisation (53, 54). Restrictions in use are recommended for 28 fragrance ingredients with sensitising potential (1995) (53).

1.2.2 Request to the SCCNFP

In response to a question from a Member state and members of the European parliament the SCCNFP has been asked by DG Enterprise to respond to the following mandate in relation to the safety of fragrance ingredients:

- It is proposed that all known fragrance allergens are labelled on cosmetics if used in the products. Does the SCCNFP agree to this proposal ? If so :
 - Which chemicals fall under this classification ?
 - Is there a maximum concentration of each chemical permissible without the requirement for labelling ?
- Does the SCCNFP agree to the inclusion of all IFRA restricted materials in the Annex

III (List of substances which cosmetic products must not contain except subject to restrictions and conditions laid down) ?

Are the permitted levels recommended by IFRA suitable for use in the Cosmetics Directive 76/768/EEC ?

- Does the SCCNFP agree that all materials that IFRA recommend should not be used as fragrance compounds are included in Annex II (List of substances which must not form part of the composition of cosmetic products) ?
- Restrictions are proposed for the 3 most common fragrance allergens (cinnamic aldehyde, isoeugenol, hydroxycitronellal). Does the SCCNFP agree to restriction on the use of common fragrance allergens (Annex III listing)? If so :
 - Which fragrance materials should be subject to restrictions?
 - What are the conditions for restrictions (maximum concentration, fields of applications, etc) ?

1.2.3 Strategy of the SCCNFP

The SCCNFP has considered that this mandate can be usefully divided into two sections (Interim position on Fragrance allergy SCCNF/0202/99 adopted at the SCCNFP session of 23. June 99):

- I. Identification of those fragrance ingredients, which are of concern as allergens for the consumer. Recommendations on informing the consumer of the presence of important allergens to permit the consumer with a known fragrance allergy a means to avoid contact with an allergen. An opinion as to whether such identification can be related to concentrations present in a product when elicitation levels are known.
- II. An opinion on the adoption of industry prohibited substances into Annex 2 and adoption of industry restricted substances into Annex 3. Considerations as to whether the concentration limits or other restrictions suggested by industry can be supported or need to be changed if there is such inclusion in Annex 3. Whether there are additional substances which should be subject to inclusion in an Annex.

Taking into account the importance and enormity of the mandate, the first section has been considered initially and is the subject of this opinion. It consists of :

- a critical review of the problem of fragrance allergy in consumers;
- identification of those fragrance ingredients which are well-recognised as consumer allergens;
- an opinion as to whether such identification can be related to concentrations present in a product when elicitation levels are known.

Allergy to natural ingredients will be analysed separately.

2. Critical review of the problem of fragrance allergy for the consumer

The following is a response to the first section of the mandate regarding identification of fragrance ingredients well recognised as consumer allergens.

2.1 Definitions of terms

2.1.1 Fragrances

A fragrance ingredient is defined by the International Fragrance Association (IFRA) as any basic substance used in the manufacture of fragrance materials for its odorous, odour-enhancing or blending properties. Fragrance ingredients may be obtained by chemical synthesis from synthetic, fossil or natural raw materials or by physical operations from natural sources. The class comprises aroma chemicals, essential oils, natural extracts, distillates and isolates, oleoresins etc.

A fragrance compound is a blend of fragrance ingredients, representing a specific fragrance formula.

A fragrance material is defined as fragrance ingredient or a fragrance formula (68).

2.1.2 Contact allergy to fragrances

Contact allergy is a type IV immunological reaction caused by low-molecular weight substances that come in close contact with the skin (3). The clinical manifestation of contact allergy is eczema, which is an inflammatory skin disease characterised by erythema, induration and in some cases vesicles. At a later stage scaling and fissures may develop. In case of contact allergy to fragrance ingredients the face, axillae or hands will often be affected (4,5,6), while generalised eczema is rare. Contact eczema may be a significant burden to the individual because of itch, changed appearances, discomfort and functional limitations. Medical consultations, treatment with corticosteroids and in some individuals sick-leave is a consequence.

Contact allergy is diagnosed by patch testing, where the patient under investigation is re-exposed to suspected allergens under controlled circumstances. International recommendations and standardisation for the patch test method, most common allergens and recording of results exist (7).

More than 2000 fragrance ingredients are available to the perfumer for compounding a fragrance formula, that may consist of 10 to 300 or more different ingredients. For this reason

a mixture of 7 fragrance chemicals and one natural extract is used for diagnosing fragrance contact allergy (table 1). This mixture is called the Fragrance Mix and is included in the standard patch test tray containing the most common allergens in Europe.

Table 1: Ingredients of the Fragrance Mix.

alfa-amyl cinnamic aldehyde (= amyl cinnamal)	1%
cinnamic alcohol (=cinnamyl alcohol)	1%
cinnamic aldehyde (=cinnamal)	1%
eugenol	1%
geraniol	1%
hydroxycitronellal	1%
isoeugenol	1%
oak moss (a natural extract)	1%
sorbitan sesquioleate (added as an emulsifier)	5%

2.2 Frequency of fragrance allergy – as measured by the Fragrance Mix

2.2.1 - in eczema patients

In two multicentre studies performed in Europe, 7.5-8.3% (8,9) of tested eczema patients were allergic to fragrances as determined by patch testing with the Fragrance Mix. In comparison contact allergy to preservatives like formaldehyde and Kathon CG* has been found in 2.6% and 3.0% respectively of tested patients on an European basis (10). Both these preservatives are restricted in the Cosmetic Directive as contact allergens and covered by the duty of ingredient labelling.

The frequency of fragrance contact allergy and the use of cosmetics vary between different countries as shown in table 2. (11).

In several studies an increase of fragrance contact allergy has been reported as measured by reactions to the Fragrance Mix (12-16).

In a German multicentre study it was found that an average of 10.2% of more than 36.000 patients tested reacted to the Fragrance Mix in the period of 1990-95 (17). It was argued that this figure could be regarded as representative for central Europe (17). A variation between different regions of Germany was found (17) and also a variation over years showing an increasing trend from 7.4% in 1992 to 10.3% Fragrance Mix positive patients in 1996 (12).

*: methylchloroisothiazolinone (and) methylisothiazolinone

Table 2: Frequency of fragrance allergy in consecutively tested eczema patients detected by the Fragrance Mix. Studies including data from 1990 and on.

Clinic	Country	Year	No. tested	% positive
Multicentre (17)	Germany	1990-95	36.552	10.2%
- (12)		1992	6.700	7.4%
- (12)		1996	9.600	10.3%
Multicentre (16)	USA	1992-94	3.509	11.4%
		1994-96	3.082	14.0%
Multicentre (57)	Netherlands	1991	677	9.0%
Multicentre (14)	Denmark	1985/86	1232	4.1%
		1997/98	1267	9.9%
Bologna (58)	Italy	1991/92	1.802	5.8%
Lisbon (59)	Portugal	1990-96	4.318	8.7%
Malmö (60)	Sweden	1992 males only	149	10.6%
Multicentre (18)	Italy	1984-88*	1518	6.0%
		1989-93	947	5.4%
London (19)	England	1980-96*	25.545	8.5% (females) 6.7% (males)

*: The test concentration of the Fragrance Mix was reduced from 16% to 8% in 1985 in Italy and from 1984 in London

In one dermatological clinic in Denmark a statistically significantly rising trend of fragrance allergy for women, as measured by reactions to the Fragrance Mix, was found over more than a decade (13). A result that recently has been confirmed by the Danish Contact Dermatitis Group, who has repeated an investigation done in 1985/86 (14). The study was performed again in 1997/98 in the same clinics using the same methods and for the same 6 months period as 12 years before. Results are presented in Figure 1. It is seen that a significant increase in fragrance allergy has occurred and that fragrance allergy is the most common cause of contact allergy, next to nickel (fig 1), which is in agreement with other investigations (12,15,16). The

increase in fragrance allergy affected all age groups also children of 0-18 years, where an increase from 2.1% to 5.8% was found (14).

Figure 1. Patch test results from 1985/86 compared with 1997/98.

Data from the Danish Contact Dermatitis Group

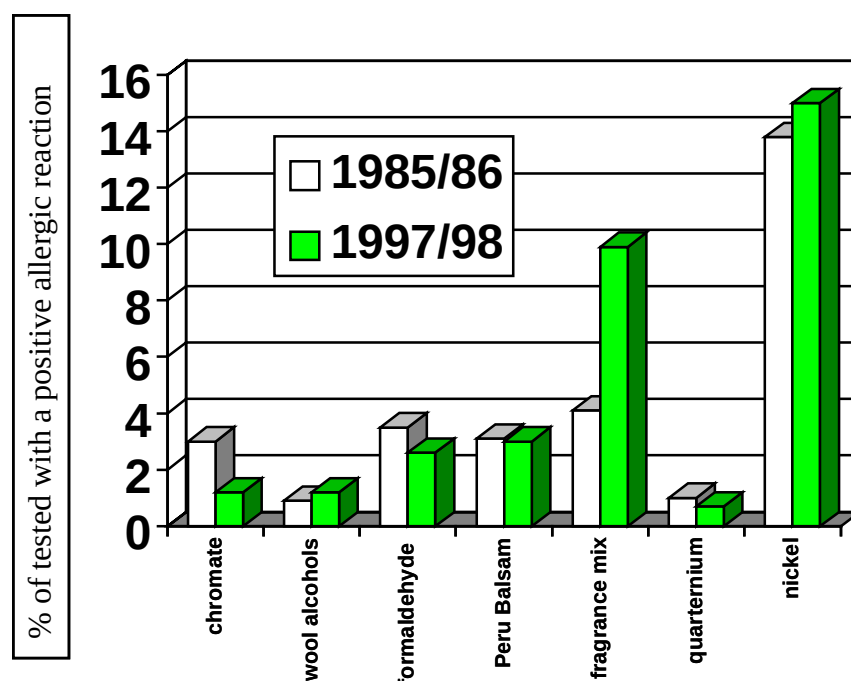


Figure 1. 1232 eczema patients were included in the study in 1985/86 and 1267 in 1997/98 (14). The sex distribution was similar in the two periods. Fragrance Mix was tested in 8% petrolatum in both periods. Quarternium -15 and formaldehyde: preservatives used in cosmetics.

The North American Contact Dermatitis Group has also recently provided results indicating an increase in fragrance allergy. They found that in 1992/94, 11.4% of 3509 patients were positive to Fragrance Mix and in 1994/96, a significant rise ($p=0.002$) to 14.3% of 3082 patients had occurred (16). In comparison the frequency of nickel allergy in this time period remained unchanged at 14.3%.

In other countries, such as Italy (1984-93), and UK (1980-96), no significant changes have been reported over years (18,19). Still fragrance ingredients were one of the most frequent causes of contact allergy in these countries (table 2).

2.2.2 -In population groups

Investigations of contact allergy in the general population are difficult to perform and for this reason few studies exist (cf. table 3). A Swedish study of hand eczema in an industrial city

showed that among 1087 individuals recruited from the general population with symptoms of present or previous hand eczema 5.8% were positive to the Fragrance Mix (20). 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 (21). In Italy female eczema patients with hand eczema due to contact with detergents were patch tested. 3.1% of 1100 women reacted to the Fragrance Mix (22). A control group of 619 female patients with no eczema disease were also patch tested, 1.3% were positive to the Fragrance Mix (22). Among Danish school children, 14-15 years of age, fragrance contact allergy was detected in 1.8% by patch testing with the Fragrance Mix (23). A study of 85 American student nurses showed that 15 (17.6%) had a positive reaction to the Fragrance Mix, 12 of the individuals also had a positive history of contact dermatitis (24). In this study the concentration of Fragrance Mix was 16% as opposed to the currently recommended 8% and the study included only young females which both may contribute to the high prevalence of fragrance sensitivity found.

**Table 3: Results from patch testing with the Fragrance Mix
in different population based groups.**

Country	Population	Year	No. tested	% positive
Italy (22)	Dermatological patients. -females without eczema	1988 ?	619	1.3%
Denmark (21)	population sample adults,15-69 years	1990-91	567	1.1%
Denmark (23)	school children 14-15 years old	1995/96	717	1.8%
USA (24)	student nurses, females	1980	85	17.6%*
Sweden (20)	population sample adults, 20-65 years reporting hand eczema	1983-84	1087	5.8%*

*testing performed with Fragrance Mix 16%. The current standard is Fragrance Mix 8%.

2.3 The role of cosmetic products in fragrance contact allergy.

2.3.1 Personal awareness

Two out of three patients with fragrance contact allergy are aware that they cannot tolerate fragranced products on their skin before the diagnosis is made (25,26). Most of them are also able to name specific product categories that started their skin problems (26). In an epidemiological study fragranced deodorants, colognes and lotions alone or in combination were significantly more often given as the primary cause of skin problems in fragrance allergic eczema patients compared with two control groups, one drawn as a sample from the general population and the other a group of eczema patients without fragrance contact allergy (26). The same 3 product categories have the most intense contact with the skin and contain the highest amount of perfume concentrate (27).

2.3.2 Provocation of allergic contact eczema by fragrance formulas from commercial products.

A. Patch tests studies

The 10 most sold fine fragrance brands on the European market were shown by patch testing to be able to provoke contact allergy in 6.9% of 335 consecutive female eczema patients (table 4). For comparison 8.1% of the females reacted to the diagnostic test, the Fragrance Mix, which means that the fine fragrances, that are commercially available consumer products intended for skin contact, were equally potent provokers of contact allergy as the diagnostic test for fragrance allergy (28).

Fragrance formulas from other marketed consumer products: toilet soaps, shampoos, lotions, deodorants, and after shaves have also been shown to provoke allergic contact eczema in eczema patients when used for patch testing (29-31) (table 4).

Table 4. Fragrance formulas from cosmetic products used for patch testing

	Patch test concentration	Number of patients tested	Result % positive	Ref.
perfumes from the 4 most common brands of toilet soap	5%-2.5% in pet.	1943	4%	29
perfume from an after shave lotion	3% in pet.	1823	3.6%	30
10 best selling fine fragrances	undiluted	335 (females only)	6.9%	28
perfumes from lower price cosmetics:				31
9 from wash-off products	5% in pet.	498	4.2%	
8 from stay-on products	5% in pet.	497	3.2%	

(pet. means petrolatum)

B. Use test studies

●Methodology

In a use test the normal contact with the allergen is simulated. Either a solution of the allergen or a product containing the allergen is applied openly and repeatedly to normal skin in subjects

sensitized to the substance under study. Usually 2-3 open applications are made a day on normal skin, either the upper arm or the cubital fossa, for a minimum of 7 days (7).

•Results from testing with single allergens

The fragrance allergens cinnamal and isoeugenol are some of the most common causes of fragrance contact allergy (27). They have been used as model substances in studies elucidating the risk of contact eczema from exposure to single fragrance allergens in cosmetic products (32,33). The allergens were tested openly in concentrations that could occur in consumer products either as the maximal or usual concentration according to Fragrance industry (34,35). 12/19 (63%) of subjects sensitised to isoeugenol developed eczema to an 0.2% isoeugenol solution in ethanol (33), which at the time of study was the maximal permitted concentration according to IFRA guideline (34). 13/18 (72%) of subjects sensitized to cinnamal developed eczema to a solution of 0.8% cinnamal in ethanol (32), which was the maximum used in perfumes according to industry (35). There is no limit to the use concentrations of cinnamal in IFRA guideline. Healthy control groups performed similar usage tests and none reacted (32,33).

Fragrance industry has studied hydroxycitronellal in usage tests performed by healthy individuals, who have been sensitised to hydroxycitronellal experimentally (36). It was found that none of 41 subjects reacted to a soap-bar or a lotion containing 0.03-0.05% hydroxycitronellal used for several months. When 31 of these subjects proceeded to use a cologne containing 0.05% and later 1% hydroxycitronellal a total of 4 subjects reacted (36). No documentation exist, that testing in experimentally sensitised individuals can predict the risk of contact eczema in subject sensitised by everyday exposure to cosmetic products.

• Results from testing with commercial products

A fine fragrance, containing 6 of the allergens from the Fragrance Mix, was used for patch testing in consecutive female eczema patients (28). 5/83 (6%) of the patients had a positive allergic reaction. All 83 patients also applied the fine fragrance openly to healthy skin 3 times a day for 7 days. 3 of the 5 patients, who had a positive patch test to the fine fragrance, developed an eczematous reaction on the skin from the open application of the fine fragrance. None of those with a negative patch test developed eczema in the usage test (28).

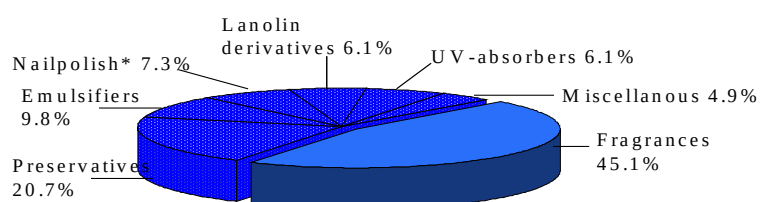
A group of 14 Fragrance Mix positive patients identified one or two deodorants each that within the past year had caused axillary dermatitis (37). The patients performed a 7 days use test with the relevant deodorants on normal axillary skin. Eczema was provoked within 7 days

in 60% of the cases. No controls were included. The deodorants were chemically analysed by gas chromatography mass spectrometry, and were all, except one, found to contain one or more of the allergens from the diagnostic tests, the Fragrance Mix (37).

2.3.3 The relative importance of fragrance ingredients in contact allergy to cosmetic products.

Fragrance ingredients account for 30-45% of the allergic reactions to cosmetics in eczema patients and is the most frequent cause of contact allergy to cosmetic products followed by preservatives (4,38,40). The results of a Dutch study is given in fig 2. It is seen that 45.1% of allergic reactions to ingredients of cosmetics were caused by fragrance ingredients. The results were based on the reviewing of 1781 patients seen in a period of 6 years (1981-86) in a private practice in a medium sized town in the Netherlands (38).

Figure 2:



Contact allergy to cosmetics-causative ingredients()

*: Toluenesulphonamide formaldehyde resin

2.4 Exposure to fragrance allergens in cosmetic products

2.4.1 General exposure

Exposure to nearly 400 fragrance substances used in major commercial products marketed around the world was studied. The results from fine fragrances, household products and soaps were published in 1989 (41). The 25 most frequently detected fragrance substances with a concentration exceeding 1% in the product were listed. Three of these were constituents of the diagnostic test the Fragrance Mix, that is:

Geraniol, which was found in 43% of the fine fragrance products in an average concentration of 3.2%.

Eugenol, which was found in 26% of the products in an average concentration of 2.0% and

Hydroxycitronellal, which was in 21% of the products in an average concentration of 3.0% (41).

Since 1987 The IFRA guideline has restricted the use of hydroxycitronellal to 1% in consumer products. Among the 25 most frequently found fragrance substances in fine fragrances (41) several other substances have been reported as allergens in cosmetics or toiletries (27).

In a series of studies more than 100 cosmetic products purchased on the European retail market have been analysed by gas chromatography-mass spectrometry for their contents of well-known fragrance allergens (28,31,37,42,43,44). Some of these results can be seen in table 5. One or more of the allergens from the diagnostic test, The Fragrance Mix, was found in 15%-100% of the examined cosmetic products depending on its type (table 5). The concentrations covered a large range, but the fine fragrances, generally had the highest amount of the examined fragrance allergens. The content of allergens in these products as well as in other products have been demonstrated to be sufficiently high to provoke eczema in sensitised individuals (cf. section 2.3.2). Cases, where the restrictions of the IFRA guideline were not respected are indicated for two substances, hydroxycitronellal and isoeugenol, in table 5.

A general finding was that 3 to 4 of the allergens from the Fragrance Mixture was found in the same cosmetic product (28,31,37). This increases the risk of provoking allergic contact eczema, as exposure to combinations of fragrance allergens have a synergistic effect on the inflammation and extent of eczematous reactions provoked in subjects sensitised to the fragrances in question (45). The effect of allergen combination on the induction of contact allergy is unknown.

Table 5: Detection of fragrance allergens from the diagnostic test, the Fragrance Mix, in cosmetic products by gas chromatography -mass spectrometry.

	Natural ingredient based perfumes, n=22 (42)		Other natural ingredient based cosmetics, n= 20 (42)		Women's best-selling fine fragrances,n=10 (28)		Deodorants on the European market,n=73 (43)	
	in no (%)	Concentration of products % (w/v)	in %	Concentration of products % (w/v)	in %	Concentration of products % (w/v)	in %	Concentration of products % (w/v)
a-amylcinnamal	8 (36%)	0.19-3.0	1(5%)	0.082	3(30%)	0.0-0.69	22(31%)	0.0001-0.062
cinnamyl alcohol	3 (14%)	0.089-2.1	1(5%)	0.004	6(60%)	0.03-0.79	27(39%)	0.0006-0.12
cinnamal	0		1(5%)	0.013	0		12(17%)	0.0001-0.04
eugenol	8 (36%)	0.035-2.3	1(5%)	0.019	9(90%)	0.04-0.89	40(57%)	0.0001-0.24
geraniol	14 (64%)	1.191§	1(5%)	0.016	9(90%)	0.08-0.48	53(76%)	0.0001-0.12
hydroxycitronellal	5 (23%)	0.135-6.04*	0		9(90%)	0.25-1.2*	35(50%)	0.0001-0.10
isoeugenol	2 (5%)	0.027,0.14	1(5%)	0.013	7(70%)	0.05-0.34*	20(29%)	0.0001-0.05
Number of products with one or more of the allergens	82%		15%		100%		NG	

Of the substances in this table only hydroxycitronellal and isoeugenol are quantitatively restricted in the IFRA guideline. The asterix * means that the recommendations of the IFRA guideline at the time of study was exceeded. 6% hydroxycitronellal was found in one product (42). 5% hydroxycitronellal have been reported to induce sensitisation in 36% of healthy volunteers (36).

§: quantitative analysis only possible for 1 product due to interferenc

2.4.2 Cosmetic products from fragrance allergic individuals

Cosmetic products identified as the cause of contact eczema in fragrance sensitised individuals have generally been shown to contain the fragrance allergens in question (37,44). It has also been demonstrated that there may be a quantitative difference between products that cause contact allergic reactions and those which do not. This is exemplified by hydroxycitronellal: its concentration on average was 5 times higher in cosmetics inducing positive patch tests in hydroxycitronellal-sensitised individuals, when compared with cosmetics tested in hydroxycitronellal negative patients (44). In accordance the average concentration of Fragrance Mix allergens was found to be 1.3-8.6 times higher in deodorants causing axillary eczema in a one week usage test performed by Fragrance Mix sensitive individuals than in deodorants negative after one week (37).

3. Information to the consumer regarding fragrance ingredients

3.1 Exposure information

3.1.1 In prevention of contact allergy to fragrance ingredients

Appropriate diagnostic procedures and patients information are cornerstones in secondary prevention of contact allergy (62). Lack of information on the ingredients of cosmetic products seriously interferes with the adequate diagnosis of patients with cosmetic allergy. In addition even when the offending allergens can be identified, such patients cannot be given proper advice about which products to avoid and which can be used without risking recurrence of dermatitis (1,2).

The Fragrance Mixture identifies 50-80% of cases with perfume allergy (28,50). It means that there are patients where the correct diagnosis cannot be made as the diagnostic test is negative, but no information is available of the content of other fragrance allergens in the products used by the patient. It has been the policy of the fragrance industry not to disclose the fragrance formula for many years (54). In the most recent edition of the International Fragrance Association (IFRA)'s Code of practice fragrance manufacturers are required to collaborate with dermatologists to discover the causative agents of clinical dermatitis in individual patients¹. However, the correspondence with industry delays the investigations of the patient's disease. Testing with coded fractions of the perfumes in question is the alternative to ingredient information. In two published cases it took 9 months to 2½ years to reach a final diagnosis, that could have been made in one week if relevant exposure information had been present

¹ It is also stated that fragrance manufacturers will respond to requests for information about fragrance formulations issued by customers or governmental bodies.

(51,52). Such delays in the diagnosis of contact allergy may affect the prognosis of the patient's disease and is a burden for the patient.

Fragrance ingredients are widely used. If the diagnosis of fragrance contact allergy fails to be made it will mean a risk of continuous exposure to the allergen(s) and recurrent or chronic eczema. It may also cause occupational problems for the patient as fragrance formulas are used in industrial products and also as preservatives or degreasing agents (66).

Once the diagnosis of contact allergy is made the patient is informed about the cause of his disease and advised to avoid further contact with the specified allergens. In case of contact allergy to fragrance ingredients no information can be provided to the consumer that makes it possible to avoid fragranced cosmetics that do not contain the offending allergen.

Further it has been a concern that fragrance industry does not receive feedback about their use of fragrance allergens and thus cannot take sufficient measure to improve consumer protection, if necessary (67).

3.1.2 Dermatological expert opinions

The European Society of Contact Dermatitis and the European Environmental and Contact Dermatitis Research Group have called attention to the need for a more useful identification of fragrance ingredients in products than the statement: "contains perfume" as presently required in the Cosmetics Directive (25,56). The American Academy of Dermatology recently sent a similar request to The Food and Drug Administration (63). The members of these organisations are mainly clinical working dermatologists that in their daily work see patients with side effects from exposure to fragrances. Full information (declaration) of fragrance ingredients has been mentioned as the optimal solution (56), however since a fragrance formula may consist of hundreds of ingredients it may not in practice be possible to supply this information. Instead it has been suggested that ingredients known to be allergens should be identified by the manufacturers (56).

3.2 Identification of fragrance ingredients recognised as consumer allergens.

3.2.1 Existing criteria for classification of contact allergens

The criteria for classification of sensitising substances in the European Union (Annex VI of Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances) consider that positive data from appropriate patch testing are sufficient to classify a substance as a skin sensitiser (contact allergen). Normally data from more than one dermatological clinic are required.

The criteria of the World Health Organisation (WHO) are similar in this respect (61) and regard sufficient evidence to classify a substance as a significant contact allergen to be epidemiological studies and/or studies in consecutive skin tested patients conducted in accordance with well established principles which demonstrate an association between exposure and the clinical evaluation of dermatitis/contact urticaria, including positive skin tests. Data from more than one patient in more than one independent centre are required.

In addition both the EU and WHO criteria consider positive results from animal tests to be sufficient evidence on its own to classify a substance as a skin sensitiser (contact allergen).

3.2.2 Criteria for selection of fragrance ingredients recognised as contact allergens in consumers.

The criteria for selection is restricted to dermatological data reflecting the clinical experience of fragrance allergens in accordance with the WHO/EU criteria. Results from animal sensitisation tests are thus not taken into consideration.

The following criteria have been applied:

- Positive patch test data from more than one patient in more than one independent centre should be present.
- The patch test should be conducted according to well-established principles.
- Relationship to clinical contact eczema should be established preferably by an exposure assessment.

However as data about fragrance ingredients in cosmetic products are not available, the following criteria are also used:

- a patient history of clinical contact reactions to fragrance ingredients/cosmetic products or
- a clinical presentation of disease in accordance with contact allergy to fragrance ingredients/cosmetic products.

3.2.3 Fragrance ingredients identified as contact allergens

Fragrance chemicals have been considered, which have been reported as contact allergens in cosmetics or toiletries according to a review paper (27). Natural materials, such as oak moss, have not been considered in the present document, as they constitute a complex group of compounds. They will be reviewed separately as stated in the interim position on Fragrance allergy SCCNFP/0202/99.

In table 6a and 6b twenty-four fragrance chemicals are listed which, based on dermatological data reflecting the clinical experience, correspond to the most recognised contact allergens.

The fragrance chemicals mentioned in table 6a meet the above criteria of selection (3.2.2), but have been more frequently reported than the allergens in table 6b, and thus according to existing knowledge of most concern.

Table 6a: Fragrance chemicals most frequently reported as contact allergens.

Ref *	Common name	CAS registry number	Comments
1	Amyl cinnamal	122-40-7	Ingredient of the Fragrance Mix
2	Amylcinnamyl alcohol	101-85-9	May crossreact with amyl cinnamal (Ref.1)
3	Benzyl alcohol	100-51-6	
4	Benzyl salicylate	118-58-1	
5	Cinnamyl alcohol	104-54-1	Ingredient of the Fragrance Mix
6	Cinnamal	104-55-2	Ingredient of the Fragrance Mix
7	Citral	5392-40-5	
8	Coumarin	91-64-5	
9	Eugenol	97-53-0	Ingredient of the Fragrance Mix
10	Geraniol	106-24-1	Ingredient of the Fragrance Mix
11	Hydroxycitronellal	107-75-5	Ingredient of the Fragrance Mix
12	Hydroxymethyl-pentylcyclo-hexenecarboxaldehyde	31906-04-4	
13	Isoeugenol	97-54-1	Ingredient of the Fragrance Mix

Fragrance chemicals mentioned in table 6b also meet the criteria of selection (3.2.2), but less documentation exist than for the ingredients mentioned in table 6a or other reservations exist as in the case of d-limonene.

Table 6b: Fragrance chemicals reported less frequently as contact allergens

Ref *	Common name	CAS registry number	Comments
14	Anisyl alcohol	105-13-5	
15	Benzyl benzoate	120-51-4	
16	Benzyl cinnamate	103-41-3	
17	Citronellol	106-22-9	
18	Farnesol	4602-84-0	
19	Hexyl cinnamaldehyde	101-86-0	used as positive control substance in animal sensitization tests
20	Lilial	80-54-6	=2-(4-tert-Butyl benzyl)propion aldehyde
21	d-Limonene	5989-27-5	oxidisation products are strong sensitisers. Mostly reported as an occupational allergen.
22	Linalool	78-70-6	
23	Methyl heptine carbonate	111-12-6	
24	3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	127-51-5	Synonym: γ -methylionone

(* refers to the number in the appendix)

3.3 Dose-response relationships in fragrance allergy

The risk of induction as well as provocation of contact allergy depends on a range of factors one of which is the concentration of allergen in the product. The product type and the individual composition of products will affect the concentration of allergen necessary to induce/provoke a reaction (46,47). In addition individual factors such as skin condition and the frequency of usage will be important (46). Few fragrance allergens have been investigated as regards to the no-effect level of provocation under experimental conditions (32,33,36,48). A great variation was found in sensitivity between individual patients (32,33) and none of the experiments take all the above mentioned factors into account. Therefore there is not sufficient scientific data to determine concentration levels of no concern for all fragrance allergic patients.

4. Summary

- Contact allergy is a type IV immunological reaction, which if due to fragrance ingredients, may cause facial, axillary and/or hand eczema and can be a compounding factor for other eczematous conditions. This is a burden to the individual because of discomfort, functional limitations, altered appearance, medical treatment and in some cases sick-leave.
- About 8% of eczema patients on a European basis 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 1-2%. A rising trend of fragrance allergy among eczema patients has been demonstrated in some clinics in Europe.
- Most individuals with contact allergy to fragrance ingredients are aware that they cannot tolerate fragranced products on their skin and are able to specifically name product categories that initiated their disease. In this context colognes, deodorants and lotions are named significantly more often by fragrance allergic eczema patients than by patients without fragrance contact allergy.
- Commercially available fragrance formulas are potent provokers of allergic contact dermatitis under patch test as well as simulated use conditions. Amongst individuals with eczema referred to dermatology clinics fragrance ingredients account for 30-45% of allergic reactions to cosmetic products and are thus the most frequent cause of cosmetic allergy. Well-known fragrance allergens are found in 15%-100% of cosmetic products and most often in combinations of 3 to 4 in the same product. It has been demonstrated by two model allergens, cinnamal and isoeugenol, that concentrations, which have been used by fragrance industry, will provoke contact eczema in 2 out of 3 individuals sensitised to the allergen in question.
- The fragrance industry is self-regulated. Recommendations for the safe use of fragrance ingredients are issued by the International Fragrance Association (IFRA) and published as a code of practice. The main focus has been on experimental evidence of sensitisation in healthy human volunteers, which means that secondary prevention of clinical disease in sensitised consumers is not considered in the code of practice.
- Appropriate diagnostic procedures and patient information are cornerstones in secondary prevention of contact allergy. The current standard diagnostic procedure detects only 50-80% of cases of contact allergy to fragrance ingredients.

No information about fragrance ingredients in cosmetic products is provided, which means that:

- an adequate diagnosis of fragrance contact allergy cannot be made in a significant proportion of cases without undue delay.
- consumers with known fragrance ingredient allergy have no information about the presence of specific fragrance allergens in cosmetic products, which they may not tolerate.
- industry does not receive feedback about their use of fragrance allergens and cannot take measures to improve consumer protection, if necessary.

The European Society of Contact Dermatitis and the European Environmental and Contact Dermatitis Research Group have called attention to the need for a more useful identification of fragrance ingredients in products than the statement: 'contains parfum' as presently required in the Cosmetics Directive. The American Academy of Dermatology recently sent a similar request to the Food and Drug Administration.

- The European Union as well as the World Health Organization (WHO) have defined general criteria to classify substances as skin sensitisers (contact allergens). On this basis a selection of fragrance ingredients recognised as contact allergens has been made. The selection is mainly based on dermatological data reflecting the clinical experience, without considering animal sensitisation tests. 24 fragrance chemicals have been identified: 13 of these have been reported more frequently, these are well-recognised contact allergens in consumers and are thus of most concern (Table 6a), 11 others are less well documented (Table 6b).
- There are insufficient scientific data available at the present time to determine dose- response relationships in contact allergy to these fragrance ingredients.

5. Opinion of the SCCNFP

In order to answer to the questions asked by DG Enterprise concerning the safety of fragrance ingredients the SCCNFP has decided to divide its mandate into two sections as indicated previously.

The opinion given here is related to the first section and includes the review of the fragrance ingredient allergy problem in consumers, the identification of fragrance chemicals well-recognised as consumer allergens, the scientific assessment of dose response relationships and thresholds in fragrance allergy, and finally the need for appropriate consumer information.

It is the opinion of the SCCNFP that:

- Fragrance ingredients have to be considered an important cause of contact allergy.
- Based on criteria restricted to dermatological data reflecting the clinical experience, it has been possible to identify 24 fragrance ingredients, which correspond to the most frequently

recognised allergens. Thirteen of these have been reported more frequently, these are well-recognised contact allergens in consumers and are thus of most concern, eleven others are less well documented.

List A: Fragrance chemicals, which according to existing knowledge, are most frequently reported and well-recognised consumer allergens:

Common name	CAS no	Common name	CAS no
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-	31906-04-4
Isoeugenol	97-54-1	cyclohexenecarboxaldehyde	

List B: Fragrance chemicals, which are less frequently reported and thus less documented as consumer allergens:

Common name	CAS no	Common name	CAS no
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

- There are at the present not sufficient scientific data to allow for determination of dose-response relationships and thresholds for these allergens.

Further, SCCNFP is of the opinion that information should be provided to consumers about the known presence in cosmetic products of fragrance ingredients with a well-recognised potential to cause contact allergy.

Information regarding these fragrance chemicals should be given to consumers if deliberately added to a fragrance formulation either in the form of a chemical or as an identified constituent of an ingredient.

In the future other fragrance chemicals may be included (or excluded) depending on the epidemiological and safety data available.

This is required to improve the protection of the consumer by ensuring that the correct diagnosis of contact allergy to well-recognised fragrance allergens can be made without undue

delay and by providing information that will help the consumer to avoid specific substances that they may not tolerate.

Natural materials, such as oak moss, will according to the interim position statement given by the SCCNFP be considered in a separate document (SCCNFP/0202/99).

In the DG Enterprise's request the term "labelling" of cosmetic products with respect to fragrance ingredient is used. This term belongs to the sector of risk management which is beyond the remit of the SCCNFP. It is up to the legislator to consider the best way to provide the information to the consumers.

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FRAGRANCE ALLERGY IN CONSUMERS

Appendix

Background information on fragrance chemicals, which have been identified as contact allergens in consumers

Fragrance chemicals most frequently reported as contact allergens

Ref n° *	Common name	cas no	comment
1	Amyl cinnamal	122-40-7	Ingredient of the Fragrance mix
2	Amylcinnamyl alcohol	101-85-9	May cross react with amyl cinnamal (Ref no 1)
3	Benzyl alcohol	100-51-6	
4	Benzyl salicylate	118-58-1	
5	Cinnamyl alcohol	104-54-1	Ingredient of the Fragrance mix
6	Cinnamal	104-55-2	Ingredient of the Fragrance mix
7	Citral	5392-40-5	
8	Coumarin	91-64-5	
9	Eugenol	97-53-0	Ingredient of the Fragrance mix
10	Geraniol	106-24-1	Ingredient of the Fragrance mix
11	Hydroxycitronellal	107-75-5	Ingredient of the Fragrance mix
12	Hydroxymethyl-pentylcyclo-hexenecarboxaldehyde	31906-04-4	
13	Isoeugenol	97-54-1	Ingredient of the Fragrance mix

* Ref n° refers to the number of the short review presented on the following pages.

Fragrance chemicals less frequently reported as contact allergens

Ref no*	Common name	Cas. Number	Comments
14	Anisyl alcohol	105-13-5	
15	Benzyl benzoate	120-51-4	
16	Benzyl cinnamate	103-41-3	
17	Citronellol	106-22-9	
18	Farnesol	4602-84-0	
19	Hexyl cinnamaldehyde	101-86-0	positive control substance in animal sensitisation tests.
20	Lilial	80-54-6	2-(4-tert-butylbenzyl)propionaldehyde
21	d-Limonene	5989-27-5	Oxidization products are strong sensitisers. Mostly reported as an occupational allergen.
22	Linalool	78-70-6	
23	Methyl heptine carbonate	111-12-6	
24	3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	127-51-5	= γ -Methylionone

*: Ref no refers to the number of the short review presented on the following pages

Background documentation

Common name Chemical name	Amyl cinnamal 2-benzylideneheptanal Ref n° 1
Synonyms	α -amyl cinnamic aldehyde
Cas. no	122-40-7
Einecs	204-541-5
Data	Amyl cinnamal is one of 8 constituent of the fragrance mix that is used for diagnosing contact allergy to fragrances. General eczema patient population: •In an European multicentre study a total of 1072 patients were patch tested in 9 different centres. 5/1072 (0.47%) had a positive reaction to amyl cinnamal 1% in pet (1). Subgroups of patients: •176 patients suspect of fragrance sensitivity were patch tested with selected fragrance substances. 167 of these were tested with amyl cinnamal 5% and 5 patients (3 %) gave an allergic reaction (2). •713 patients with cutaneous reactions to cosmetic products were identified. In 578 cases sensitization were observed. In 2 subjects amyl cinnamal was one of the causative ingredients as judged by patch testing (3). •Among 156 patients with pure contact allergy to cosmetic products, amyl cinnamal was one of the causative ingredient in 2 (1.3%) of the cases (4). • The causative ingredients were identified in 119 patients with contact allergy to cosmetic products. 1/119 (0.8%) were allergic to amyl cinnamal, patch tested in 5% in pet. (5). •13 patients sensitive to α-amyl cinnamal were identified over 3 years, 10 of these were also sensitive to α-amyl cinnamic alcohol (6). •179 patients suspected of cosmetic allergy were patch tested with 16 fragrance materials among these amyl cinnamal 10% in pet. 7 cases (3.9%) were found positive to α-amyl cinnamal (7). As part of the fragrance mix: •In an European multicentre study involving 6 countries, 78 patients who were positive to one or the other of two different fragrance mixes, one contained amyl cinnamal. The 78 patients were tested with the individual constituents of the mixes. 2/78 (2.6%) were positive to amylcinnamal 2% (8). •The frequency of contact allergy to amyl cinnamal in patients positive to the fragrance mix, is reported in a range of studies from different countries: 1.9 % of the fragrance mix reactions were due to amyl cinnamal in Italy (9), 2.3% in Denmark (10) and 2,5% in France (11). Test concentration: •Test concentration: 1% cinnamyl alcohol is the standard concentration used in routine testing, however 2% in pet may be used according to De Groot et al (12)and 5% in pet gave no irritant reactions when tested in 100 control individuals (24).
Summary	Amyl cinnamal is a wellknown allergen as part of the diagnostic test, the fragrance mix. It accounts for 2%-3% of the reactions to the fragrance mix and has been identified as a cause of allergic reactions in persons with eczema from cosmetic products.

Common name Chemical name	Amylcinnamyl alcohol 2-pentyl-3-phenylprop-2-en-1-ol Ref n° 2
Synonyms	α -amyl cinnamic alcohol
Cas. no	101-85-9
Einecs	202-982-8
Data	Patient subgroups • 8 patients with contact allergy to ethylenediamine and the perfume in mycolog cream were patch tested with the ingredients of the perfume (14). α-amyl cinnamyl alcohol 5% in pet were found to be strongly positive in 5/8 cases. α-amyl cinnamyl alcohol was present in the cream in 0.001%. Reactions to other fragrance ingredients, but less frequently, were also found (14). • 20 perfume allergic patients were tested with several screening series of fragrances. 15 were allergic due to perfumes in cosmetics. 2/20 reacted with a positive reaction to α-amyl cinnamyl alcohol 5% in petrolatum. (15). • 11 patients sensitive to α-amyl cinnamic alcohol were identified over 3 years, 10 of these were also sensitive to α-amyl cinnamic aldehyde. Some of the patients primarily sensitized by the perfume in a medicament. Test concentration according to authors way below an irritant level (6). • 179 patients suspected of cosmetic allergy were patch tested with 16 fragrance materials among these α-amyl cinnamyl alcohol 20% in pet. 7 cases (3.9%) were found positive to α-amyl cinnamyl alcohol. Test concentrations were chosen based on existing recommendations or a pilot study involving 60 eczema patients. Test concentrations were chosen deliberately high, but non irritant, to avoid false negative reaction (7) • 460 patients were considered to have contact allergy related to cosmetics. 80 patients were positive to the fragrance mix and by testing with ingredients of the mix and other fragrances 3 cases of allergic reactions to amyl cinnamyl alcohol were identified. Test concentrations unknown (16). Test concentrations: • Amyl cinnamyl alcohol 2% in pet is recommended for patch testing according to De Groot (12). Higher concentrations have been used in a number of studies (7, 15).
Summary	Five single cases reported of contact allergy to amyl cinnamyl alcohol and allergic reactions by patch testing <u>were</u> found in 7/179-2/20 (4-10%) of patients with contact eczema from cosmetics. Amyl cinnamyl alcohol probably cross reacts with amyl cinnamal.

Common name Chemical name	Benzyl alcohol (INCI) Benzyl alcohol	Ref n° 3
Synonyms		
Cas. No	100-51-6	
Einecs	202-859-9	
Data	General eczema patient population: •0.5% of tested patients in Japan gave a positive reaction to benzyl alcohol 5%. Number of included patients unknown (17). Subgroups of patients: •20 perfume allergic patients were tested with several screening series of fragrances. 15 were allergic due to perfumes in cosmetics. Benzyl alcohol 5% gave a positive reaction in 3/20 patients (15). •176 patients suspect of fragrance sensitivity were patch tested with selected fragrance substances. 167 of these were tested with benzyl alcohol 5% and 2 patients (1.2 %) gave an allergic reaction (2). •182 patients suspected of contact allergy to cosmetics were patch tested with a series of 22 fragrance substances. 3 (1.6%) of the patients had a positive patch test reaction to benzyl alcohol 10% which was also found in 10/79 cosmetic products sent for analysis by the patients or their physicians. Benzyl alcohol 10% was negative in a pilot study for irritant reactions in 81eczema patients (18). •1.3% (2/156) of patients with contact allergy to cosmetic products were sensitized to benzyl alcohol (4). •713 patients with cutaneous reactions to cosmetic products were identified. In 578 cases sensitization were observed. In 3 subjects benzyl alcohol was one of the causative ingredients as judged by patch testing (3). •242 randomly selected patients with proven contact allergy from different origin were tested with 7 perfume components. 4(1.6%) had a positive patch test to benzyl alcohol (19). Case reports: •A 46 year old man with atopic eczema were allergic to the perfume of a preparation used. Benzyl alcohol was found to be the causative ingredient by patch testing with 5% in pet and found in the perfume in a high concentration (13). •2 cases of contact allergy to a perfume and an after shave lotion were reported together with contact allergy to benzyl alcohol, patch tested in 1 % in pet (20) Other studies: •Occurs in minor amounts in balsam of Peru, which is used as a screening agent for fragrance contact allergy (21). Benzyl alcohol is also used as a preservative. Test concentrations: •Benzyl alcohol 5% is recommended (12). Benzyl alcohol 10% was negative in 20 eczema patients and 61 patients with cosmetic eczema, when tested for irritancy (18).	
Summary	Benzyl alcohol is found in several studies as a cause of allergic reactions in 1.2-15% (2-4 cases in each study)of patients with eczema from cosmetic products.	

Common name Chemical name	Benzyl salicylate (INCI) Benzyl-o-hydroxybenzoate	Ref n° 4
Synonyms		
Cas. No	118-58-1	
Einecs	204-262-9	
Data	General eczema patient population: •2.3% of tested patients in Japan gave a positive reaction to benzyl salicylate 2%. Number of included patients unknown (17). •1943 consecutive eczema patients were examined with regard to sensitivity to perfumes from toilet soap and detergents. 78 patients (4%) showed positive reactions to perfumes and in 75% of the cases the reaction was found to be associated with sensitivity to benzyl salicylate patch tested in 2% pet. Benzyl salicylate was present in the positive perfumes (22). •241 patients were tested with a perfume screening series. 6 (2.5%) had a positive reaction to benzyl salicylate 2% in pet (23). Subgroups of patients: •20 perfume allergic patients were tested with several screening series of fragrances. 15 were allergic due to perfumes in cosmetics. Benzyl salicylate 2% gave a positive reaction in 2/20 patients (15). •176 patients suspect of fragrance sensitivity were patch tested with selected fragrance substances. 167 of these were tested with benzyl salicylate 5 and 2%. 8 and 5 patients (4.8%-3%) reacted with an allergic reaction, respectively. Benzyl salicylate was a more common cause of positive patch test reactions in Japan than in Europe and US (2). •713 patients with cutaneous reactions to cosmetic products were identified. In 578 cases sensitization were observed. In 1 subject benzyl salicylate was one of the causative ingredients as judged by patch testing (3). Test concentrations: •Benzyl salicylate 1% is recommended for patch testing (24).	
Summary	Benzyl salicylate is as a cause of allergic reactions in 0.2-10% of patients with eczema from cosmetic products and in one study accounted for 75% of reactions to commercial perfumes.	

Common name Chemical name	Cinnamyl alcohol [INCI] Cinnamic alcohol	Ref n° 5
Synonyms		
Cas. no	104-54-1	
Einecs	203-212-3	
Data	Cinnamyl alcohol is one of 8 constituent of the fragrance mix, that is used for diagnosing contact allergy to fragrances. General eczema patient population: •In an European multicentre study a total of 1072 patients were patch tested in 9 different centres. 6/1072 (0.56%) had a positive reaction to cinnamyl alcohol 1% (1). Subgroups of patients: •20 perfume allergic patients were tested with several screening series of fragrances. Cinnamyl alcohol 5% gave a positive reaction in 15/20 (75%) patients. 50 control patients were tested with the fragrance allergens and were negative (15). •713 patients with cutaneous reactions to cosmetic products were identified. In 578 cases sensitization were observed. In 17 subjects cinnamyl alcohol was one of the causative ingredients as judged by patch testing. This constituted 10% of fragrance ingredients causing cutaneous reactions (3). •Among 156 patients with pure contact allergy to cosmetic products, cinnamyl alcohol was one of the causative ingredient in 6 (3.8%) of the cases (4). •The causative ingredients were identified in 119 patients with contact allergy to cosmetic products. 2/119 (1.7%) were allergic to cinnamyl alcohol, patch tested in 5% in pet. (5). •176 patients suspect of fragrance sensitivity were patch tested with selected fragrance substances. 167 of these were tested with cinnamyl alcohol 5% in lanolin and 11(6.6%) had an allergic reaction (2). As part of the fragrance mix: •In an European multicentre study involving 6 countries, 78 patients were positive to one or the other of two different fragrance mixes, both containing cinnamyl alcohol. The patients were tested with the individual constituents of the mixes. 5/78 (6.4%) were positive to cinnamyl alcohol 1% (8). •The frequency of contact allergy to cinnamyl alcohol in patients positive to the fragrance mix, is reported in a range of studies from different countries: 9.3% of the fragrance mix reactions were due to cinnamyl alcohol in Italy (9), 10.8% in Denmark (10), 8% in Hungary (25), 5.5% in Germany (26) and 14% in France (11). Other: •Cinnamyl alcohol is restricted in the IFRA guideline to a maximum use concentration of 0.8%. In a children's toy perfume cinnamyl alcohol has been found in a concentration of 3.7% (27). •Cinnamyl alcohol has induced sensitization in 2.7% (4/150) healthy volunteers at exposure to a 4% concentration (28). Test concentration: •Test concentration: 1% cinnamyl alcohol is the standard concentration used in routine testing, however 2% in pet may be used according to De Groot et al (12) and 5% in pet gave no irritant reactions when tested in 100 control individuals (24).	

Summary	Cinnamyl alcohol is a well-known allergen as part of the diagnostic test, the fragrance mix. It accounts for 5-14% of the reactions to the fragrance mix. In addition it has been shown to be a cause of allergic reactions by patch test in 1.7%-75% of patients with eczema from cosmetic products.

Common name Chemical name	Cinnamal [INCI] Cinnamaldehyde; 3-phenyl-2-propenal	Ref n° 6
Synonyms	Cinnamic aldehyde	
Cas. no	104-55-2	
Einecs	203-213-9	
Data	Cinnamal is one of 8 constituent of the fragrance mix, that is used for diagnosing contact allergy to fragrances. General eczema patient populations: •In an European multicentre study a total of 1072 patients were patch tested in 9 different centres. 10/1072 (0.9%) had a positive reaction to cinnamal 1% (1). •Cinnamal is a part of the North American Standard patch test series, that is used for testing all eczema patients. In 1985-89 3.1% of 3964 patients and in 1994/96 2.4% of 3112 patients were positive to cinnamal 1% (10). 85% of these cases were of current definite, probable or possible relevance (46). Subgroups of patients: •20 perfume allergic patients were tested with several screening series of fragrances. Cinnamal 1% gave a positive reaction in 6/20 (30%) patients (15). •182 patients suspected of contact allergy to cosmetics were patch tested with a series of 22 fragrance substances. 3.7% of the patients had a positive patch test reaction to cinnamal 0.5%. Cinnamal was found in 8/79 cosmetic products sent in for analysis by the patients or their physicians (18). •713 patients with cutaneous reactions to cosmetic products were identified. In 578 cases sensitization were observed. In 6 subjects cinnamal was one of the causative ingredients as judged by patch testing (3). •176 patients suspect of fragrance sensitivity were patch tested with selected fragrance substances. 167 of these were tested with cinnamal 1% in pet and 24 (14.4%) gave an allergic reaction (2). As part of the fragrance mix: •In an European multicentre study involving 6 countries, 78 patients positive to one or the other of two different fragrance mixes, both containing cinnamal, were tested with the individual constituents of the mixes. 10/78 (12.8%) were positive to cinnamal 1% (8). •The frequency of contact allergy to cinnamal in patients positive to the fragrance mix, is reported in a range of studies from different countries: 5.5% of the fragrance mix reactions were due to cinnamal in Italy (9), 16.9% in Denmark (10), 24% in Hungary (25) , 21% in Germany (26) and 36% in France (11). Others: •Cinnamal has actively sensitized 8%-44% of healthy volunteers using different concentrations and experimental methods (29). According to the IFRA guideline, cinnamal must only be used in conjunction with substances preventing sensitization, but no restrictions is made on the concentrations. Test concentrations: 1% cinnamal is the standard concentration used in routine testing (12). Higher concentrations may give irritant reactions (23).	
Summary	Cinnamal is a well proven allergen as part of the diagnostic test, the fragrance mix. It accounts for 5-36% of the reactions to the fragrance mix. It gives reactions in 2-3% of consecutively patch tested patients, most of which are of relevance. In addition it has been shown to be a cause of allergic reactions by patch test in 1%-30%patients with eczema from cosmetic products in several studies.	

Common name	Citral Ref n° 7
Chemical name	3,7-dimethyl-2,6-octadien-1-al, mix of cis and trans isomers
Cas. no	5392-40-5
Einecs	226-394-6
Data	General eczema patient population: •228 patients were tested by the North American Contact Dermatitis Research Group in 1973/74 with citral 1% in pet. 1.7% had a positive patch test reaction to citral (30). •2455 eczema patients were patch tested with two separate mixes: one standard fragrance mix and a new mix containing dihydrocoumarine and citral instead of oak moss and amyl cinnamic aldehyde. 6.7% of the patients reacted to the new mix. 78 patients positive to either of the mixes were patch tested with the individual ingredients. Isoeugenol gave most reactions and next citral 2% in pet that gave reactions in 13 individuals, 16.7% of those positive to the mix (8). •19/1855, (1.0%) consecutively patch tested eczema patients in a European multicentre study gave a positive reaction to Citral tested in 2% (31-personal communication). Subgroups of patients: •182 patients suspected of contact allergy to cosmetics were patch tested with a series of 22 fragrance substances. 2.6% of the patients had a positive patch test reaction to citral 2% in pet. Citral was found in 4/79 cosmetic products sent in for analysis by the patients or their physicians (18). Occupational setting: •4 bakers with hand eczema were patch tested with fragrance/flavours. One reacted with a positive reaction to citral 0.5% in pet. Relevance unknown (32). Other data: •Citral from different sources and in different concentrations have been studied by the Human Maximization Test. In all tests citral induced sensitization in 12%-64% of human volunteers, 3/25-16/25 (33). Citral was also studied in the repeated insult patch procedure at 4-8% and sensitized 48% of a panel of 40 human volunteers (33). According to IFRA guideline citral must only be used in conjunction with substances preventing sensitization, but no restrictions is made on the use concentrations. Test concentrations: •2% in petrolatum is the recommended concentration (12).
Summary	Citral is a cause of allergic reactions in about 1% of consecutive patch tested patients. It was the most frequent cause of reactions to a new diagnostic test for fragrance contact allergy and proved to cause contact allergic reactions in 2.6% of patients with eczema from cosmetic products.

Common name Chemical name	Coumarin (INCI) 1-benzopyran-2-one; cis-o-coumarinic acid lactone	Ref n° 8
Synonyms	occurs naturally in tonka beans and other plants.	
Cas. no	91-64-5	
Einecs	202-086-7	
Data	General eczema patient population: •241 consecutive patients were patch tested with coumarin 5%. 2 (0.8%) had a positive reaction (23). •14.000 consecutive eczema patients were patch tested with coumarin 5 % in pet or for a short period 8% in pet. 58 (0.4%) showed a positive reaction. 20/58 cases were not identified by other markers of perfume allergy and would have been missed if coumarin had not been patch tested (34). Subgroups of patients: •20 perfume allergic patients were tested with several screening series of fragrances. 15 had dermatitis related to the use of a cosmetic product. Coumarin 5% gave a positive reaction in 2/20 (10%) patients (15). •176 patients suspect of fragrance sensitivity were patch tested with selected fragrance substances. 167 of these were tested with coumarin 5% and 2 patients (1.2%) reacted with an allergic reaction (2). •182 patients suspected of contact allergy to cosmetics were patch tested with a series of 22 fragrance substances. 6.8% of the patients had a positive patch test reaction to coumarin 8% in pet. Coumarin was found in 16/79 (20.3%) cosmetic products sent in for analysis by the patients or their physicians. The concentration of 8% coumarin was tested in a total of 54 controls and no reactions were found (18). •713 patients with cutaneous reactions to cosmetic products were identified. In 578 cases sensitization were observed. In 4 subjects coumarin was one of the causative ingredients as judged by patch testing (3). •The causative ingredients were identified in 119 patients with contact allergy to cosmetic products. 1/119 (0.8%) were allergic to coumarin patch tested in 5% in pet. (5). •A group of 242 randomly selected eczema patients were patch tested with perfume components. 9 (3.7%) were positive to coumarin 5.8% at patch testing (19). Case reports: •A women developed severe eczema from using a perfumed lotion. Patch testing showed a strong reaction to coumarin 0.5% in pet and the diluent, which was constituents of the perfume formulation of the lotion (35). •A women developed severe eczema from a perfume. She was positive at patch testing to the perfume. By chemical fractionation it was established that coumarin was the causative ingredient. It was contained in a concentration of 0.23% in the perfume (36). Test concentrations: •Coumarin 5% is the recommended patch test concentration (12).	
Summary	Coumarin is a cause of allergic reactions in about 0.4-0.8% of consecutive patch tested patients. 1/3 of these cases is not detected by the fragrance mix. Coumarin has caused contact allergic reactions in 0.8-10% of patients with eczema from cosmetic products	

Common name Chemical name	Eugenol (INCI) Eugenol	Ref n° 9
Synonyms		
Cas. no	97-53-0	
Einecs	202-589-1	
Data	Eugenol is one of 8 constituent of the fragrance mix, that is used for diagnosing contact allergy to fragrances. Consecutive patients: •In an European multicentre study a total of 1072 patients were patch tested in 9 different centres. 13/1072 (1.2%) had a positive reaction to eugenol 1% (1). Subgroups of patients: •20 perfume allergic patients were tested with several screening series of fragrances. Eugenol 2% gave a positive reaction in 4/20 (20%) patients (15). •713 patients with cutaneous reactions to cosmetic products were identified. In 578 cases sensitization were observed. In 4 subjects eugenol was one of the causative ingredients as judged by patch testing (3). •Among 156 patients with pure contact allergy to cosmetic products, eugenol was one of the causative ingredient in 11 (7.1%) of the cases (4). •176 patients suspect of fragrance sensitivity were patch tested with selected fragrance substances. 167 of these were tested with eugenol 5% and 13 patients (7.8 %) gave an allergic reaction (2). As part of the fragrance mix: •In an European multicentre study involving 6 countries, 78 patients positive to one or the other of two different fragrance mixes, both containing eugenol, were tested with the individual constituents of the mixes. 8/78 (10.3%) were positive to eugenol 2% (8). •The frequency of contact allergy to eugenol in patients positive to the fragrance mix, is reported in a range of studies from different countries: 16.7% of the fragrance mix reactions were due to eugenol in Italy (9), 12.2% in Denmark (10), 4% in Hungary (25), 6.8% in Germany (26) and 22% in France (11). Test concentrations: •1% eugenol is the standard concentration used in routine testing, however 2% have been used for a number of years and is recommended by De Groot (12). In a study 5% eugenol in pet was tested in 100 healthy volunteers and gave no reactions (24)	
Summary	Eugenol is a well known contact allergen. Many investigations have been performed. It is the cause of sensitization in 1.2% of consecutive eczema patients and accounts for 4%-16% of reactions to the fragrance mix. Eugenol has caused contact allergic reactions in 0.7-20% of patients with eczema from cosmetic products	

Common name Chemical name	Geraniol (INCI) Ref n° 10
Synonyms	
Cas. no	106-24-1
Einecs	203-377-1
Data	Geraniol is one of 8 constituent of the fragrance mix, that is used for diagnosing contact allergy to fragrances. Consecutive patients: •In an European multicentre study a total of 1072 patients were patch tested in 9 different centres. 4/1072 (0.4%) had a positive reaction to geraniol 1% (1). Subgroups of patients: •20 perfume allergic patients were tested with several screening series of fragrances. Geraniol 5% gave a positive reaction in 6/20 (30%) patients (15). •182 patients suspected of contact allergy to cosmetics were patch tested with a series of 22 fragrance substances. 1.6% of the patients had a positive patch test reaction to geraniol 1%. Geraniol was found in 4/79 cosmetic products sent for analysis by the patients or their physicians (18). •713 patients with cutaneous reactions to cosmetic products were identified. In 578 cases sensitization were observed. In 8 subjects geraniol was one of the causative ingredients as judged by patch testing (3). •Geraniol caused contact allergy in 2/156 (1.2%) patients suffering from contact allergy to cosmetic products (4). •176 patients suspect of fragrance sensitivity were patch tested with selected fragrance substances. 167 of these were tested with geraniol 5% and 5 patients (3.0%) gave an allergic reaction (2). As part of the fragrance mix: •In an European multicentre study involving 6 countries, 78 patients positive to one or the other of two different fragrance mixes, both containing geraniol, were tested with the individual constituents of the mixes. 4/78 (5.1%) were positive to geraniol 1% (8). •The frequency of contact allergy to geraniol in patients positive to the fragrance mix, is reported in a range of studies from different countries: 7.4% of the fragrance mix reactions were due to geraniol in Italy (9), 3.3 % in Denmark (10), 4% in Hungary (25), 6.8% in Germany (26) and 22% in France (11). Test concentration: •1% geraniol is the standard concentration used in routine testing (12), however 2% have been used for a number of years and is recommended by De Groot (12). In a study 5% geraniol in pet was tested in 100 healthy volunteers and gave no irritant reactions (24)
Summary	Geraniol is a well-known contact allergen as an ingredient in the diagnostic test, the fragrance mix. It is a cause of sensitization in 0.4% of consecutive eczema patients and accounts 3%-7% of reactions to the fragrance mix. Geraniol has caused contact allergic reactions in 1.2-30% of patients with eczema from cosmetic products

Common name Chemical name	Hydroxycitronellal 7-Hydroxycitronellal	Ref n° 11
Synonyms	Laurine	
Cas. No	107-75-5	
Einecs	203-518-7	
D Data	Hydroxycitronellal is one of 8 constituent of the fragrance mix, which is used for diagnosing contact allergy to fragrances. General eczema patients population: •In an European multicentre study a total of 1072 patients were patch tested in 9 different centres. 8/1072 (0.75%) had a positive reaction to Hydroxycitronellal 1% (1). Subgroups of patients: •20 perfume allergic patients were tested with several screening series of fragrances. Hydroxycitronellal 4% gave a positive reaction in 9/20 (45%) of the patients (15). •182 patients suspected of contact allergy to cosmetics were patch tested with a series of 22 fragrance substances. 19/182 (10.5%) of the patients had a positive patch test reaction to hydroxycitronellal 10%. Hydroxycitronellal was found in 47/79 (59%) of cosmetic products sent in for analysis by the patients or their physicians (18). •713 patients with cutaneous reactions to cosmetic products were identified. In 578 cases sensitization were observed. In 11 subjects hydroxycitronellal was one of the causative ingredients as judged by patch testing (3). • 156 patients with contact allergy to cosmetic products were identified. Hydroxycitronellal was one of the causative ingredients in 6 cases (3.8%), as determined by patch testing (4). •23 cosmetic products, which had caused contact allergic reactions in 11 patients with perfume allergy, were subjected to chemical analysis. The products of all patients sensitive to hydroxycitronellal, n=6, was found to contain the substance. The content of hydroxycitronellal was at average 5 times higher in cosmetics from hydroxycitronellal sensitive patients compared with hydroxycitronellal negative patients (37) •176 patients suspect of fragrance sensitivity were patch tested with selected fragrance substances. 167 of these were tested with hydroxycitronellal 4% and 23 patients (13.8%) reacted with an allergic reaction (2). As part of the Fragrance mix: •In an European multicentre study involving 6 countries, 78 patients positive to one or the other of two different fragrance mixes, both containing hydroxycitronellal, were tested with the individual constituents of the mixes. 7/78 (9%) were positive to hydroxycitronellal 5% (8). •The frequency of contact allergy to hydroxycitronellal in patients positive to the fragrance mix, is reported in a range of studies from different countries: 16% of the fragrance mix reactions were due to hydroxycitronellal in Italy (9), 7.4 % in Denmark (10), 10% in Hungary (25) 6.2% in Germany (26) and 5% in France (11). Others: •Hydroxycitronellal is restricted in the IFRA guideline to 1% in consumer products due to its sensitizing properties. 5% hydroxycitronellal has been found to cause sensitization in 36% of healthy volunteers (38). Test concentration: •1% hydroxycitronellal is the standard concentration used in routine testing, however 2% have been used for a number of years and is recommended by De Groot (12). In a study 5% hydroxycitronellal in pet was tested in 100 healthy volunteers and gave no irritant reactions (24)	
Summary	Hydroxycitronellal is a well known contact allergen as ingredient in the diagnostic test, the fragrance mix. It is a cause of sensitization in 0.75% of consecutive eczema patients and accounts 6%-16% of reactions to the fragrance mix. Hydroxycitronellal has caused contact allergic reactions in 10%-45% of patients with eczema from cosmetic products	

Common name Chemical name	Hydroxymethyl-pentylcyclo-hexenecarboxaldehyde 4-(4-Hydroxy-4-methylpentyl)cyclohex-3-enecarbaldehyde	Ref n° 12
Synonyms	Lyrall	
Cas. No	31906-04-4	
Einecs	250-863-4	
Data	General eczema patient population: •106 patients were tested with Lyrall 5% and 1% in petrolatum as part of a screening study for fragrance contact allergy. 3 (2.8%) had a positive patch test reaction to Lyrall 5% and 1 (0.9%) to Lyrall 1%. Clinical relevance was not firmly established, but may have been present in 2 patients (1). •1855 eczema patients were tested with a screenings series of 11 fragrance allergens among these Lyrall 5% in pet. 50/1855 (2.7%) were positive at patch testing to Lyrall. Judged by history 2/3 Lyrall positive cases were either definitively or probably relevant. In 4 cases of Lyrall allergy an extended exposure evaluation was performed. Lyrall was identified in one or more cosmetic products, that had caused contact dermatitis (41). Subgroups of patients: • The causative ingredients were identified in 75 patients with contact allergy to cosmetic products. One patient was allergic to Lyrall which was present in a deodorant cream, patch test concentration unknown (42). Case-reports: • A 28 year old man developed allergic contact dermatitis to two separate underarm deodorants. By patch testing with fractions of the perfumes from the products it was established that Lyrall was responsible for the reaction to both products. An additional fragrance allergen, acetyl cedrene, was found in one of the two deodorants (43). • A 20 year-old woman presented with a 5-months history of severe dermatitis in both axillae, related to the use of her underarm deodorant of a particular brand. Testing with Standard series was negative, including the Fragrance mix. Subsequent patch testing with the ingredients of her deodorant showed that she was allergic to Lyrall, tested in 10% in pet, contained in the fragrance compound in the deodorant (59). Test concentration: •Lyrall 10% in pet has been reported to be non-irritant under patch test conditions (12)	
Summary	Lyrall was a cause of allergic reactions in about 2.8% of consecutive patch tested patients. 2/3 of the cases <u>were</u> relevant. In addition three relevant cases of contact allergy to Lyrall from cosmetic products are established.	

Common name Chemical name	Isoeugenol Isoeugenol	Ref n° 13
Synonyms		
Cas. No	97-54-1	
Einecs	202-590-7	
Data	Isoeugenol is one of 8 constituent of the fragrance mix, that is used for diagnosing contact allergy to fragrances. General eczema patients population: •In an European multicentre study a total of 1072 patients were patch tested in 9 different centres. 20/1072 (1.86%) had a positive reaction to Isoeugenol 1% (1). Subgroups of patients: •20 perfume allergic patients were tested with several screening series of fragrances. Isoeugenol 2% gave a positive reaction in 5/20 (25%) of the patients (15). •713 patients with cutaneous reactions to cosmetic products were identified. In 578 cases sensitisation were observed. In 10 subjects isoeugenol was one of the causative ingredients as judged by patch testing (3). • 156 patients with contact allergy to cosmetic products were identified. Isoeugenol was one of the causative ingredients in 16 cases (10.3%), as determined by patch testing (4). As part of the fragrance mix: •In an European multicentre study involving 6 countries, 78 patients positive to one or the other of two different fragrance mixes, both containing isoeugenol, were tested with the individual constituents of the mixes. 16/78 (20,5%) were positive to isoeugenol 2%, which was the most frequent allergen (8). •The frequency of contact allergy to isoeugenol in patients positive to the fragrance mix, is reported in a range of studies from different countries: 22% of the fragrance mix reactions were due to Isoeugenol in Italy (9), 18.5 % in Denmark (10), 6% in Hungary (25), 16.6% in Germany (26) and 17% in France (11). Others: •Isoeugenol was restricted in the IFRA guideline to 0.2% until May 1998, where the concentration was lowered to 0.02%. Isoeugenol has been found to cause sensitisation in 12-36% of healthy volunteers (39,40). Test concentrations: •1% isoeugenol is the standard concentration used in routine testing , however 2% have been used for a number of years (12). In a study 5% isoeugenol in pet was tested in 100 healthy volunteers and gave no irritant reactions (24)	
Conclusion	Isoeugenol is a well-known contact allergen as ingredient in the diagnostic test, the fragrance mix. It is a cause of sensitisation in 1.9% of consecutive eczema patients and accounts for 6%-22% of reactions to the fragrance mix. Isoeugenol has caused contact allergic reactions in 2-25% of patients with eczema from cosmetic products	

Common name Chemical name	Anisyl alcohol 4-methoxybenzyl alcohol	Ref n° 14
Synonyms		
Cas. No	105-13-5	
Einecs	203-273-6	
Data	Subgroups of patients •176 patients suspect of fragrance sensitivity were patch tested with selected fragrance substances. 167 of these were tested with anisyl alcohol 5% in pet and 3 (1.6%) reacted with an allergic reaction (2). •20 perfume allergic patients were tested with several screening series of fragrances. 15 were allergic due to perfumes in cosmetics. Anisyl alcohol 5% in pet. gave a positive reaction in 4/20 (20%)(15). Test concentration: • 5% anisyl alcohol has been reported to be a non-irritant patch test concentration (12)	
Conclusion	Two studies reported with contact allergy to anisyl alcohol among patients with cosmetic eczema. 3 and 4 cases were documented (1.6-20%).	

Common name Chemical name	Benzyl benzoate (INCI) Benzyl benzoate	Ref n° 15
Synonyms		
Cas. no	120-51-4	
Einecs	204-402-9	
Data	Consecutive patients: •335 and 284 patients were tested by the North American Contact Dermatitis Research Group in 1979/80 with benzyl benzoate 2% in pet. 1% of the 284 had a positive patch test reaction to benzyl benzoate and none of the 335 patients (30). •241 patients were tested with a perfume screening series. 1 (0.4%) had a positive reaction to benzyl benzoate 2% in pet (23). Subgroups of patients: •20 perfume allergic patients were tested with several screening series of fragrances. 15 were allergic due to perfumes in cosmetics. Benzyl benzoate 5% gave a positive reaction in 1/20 patients (15). •713 patients with cutaneous reactions to cosmetic products were identified. In 578 cases sensitization were observed. In 1 subject benzyl benzoate was one of the causative ingredients as judged by patch testing (3). •Benzoyl benzoate is one of the main components of Peru Balsam, which is used as a screening agent for fragrance contact allergy (24). 103 cases with contact allergy to Peru balsam was tested with known ingredients. 12 (12%) were positive to Benzoyl benzoate tested 5% in pet. (44). Other studies: •Occurs in fairly large amounts in a number of blossom concretes and absolutes as tuberose and hyacinth (45). Test concentrations: •Benzyl benzoate 5% in pet is the recommended patch test concentration according to De Groot (12).	
Summary	Benzyl benzoate is positive in several studies, but only a single case are reported in each except for patients sensitive to Peru balsam	

Common name Chemical name	Benzyl cinnamate (INCI) Benzyl 3-phenyl-2-propenoate	Ref n° 16
Synonyms	Cinnamein	
Cas. No	103-41-3	
Einecs	203-109-3	
Data	Subgroups of patients: •182 patients suspected of contact allergy to cosmetics were patch tested with a series of 22 fragrance substances. 6 (3.2%) of the patients had a positive patch test reaction to benzylcinnamate 8%. No Benzylcinnamate was found in 79 cosmetic products sent in for analysis by the patients or their physicians (18). •Benzyl cinnamate is a component of balsam of Peru (21), which is used as a screening agent for fragrance contact allergy.103 cases with contact allergy to Peru balsam was tested with known ingredients. 19 (18%) were positive to Benzyl cinnamate tested 5% in pet. (44). Test concentrations: •Benzyl cinnamate 5% is recommended for patch testing according to De Groot (12)	
Summary	Benzyl cinnamate positive in one study of patients with contact allergy to cosmetic products and in a high proportion of patients with contact allergy to Peru balsam.	

Common name Chemical name	Citronellol (INCI) 3,7-Dimethyl-6-octenol	Ref n° 17
Synonyms		
Cas. no	106-22-9	
Einecs	203-375-0	
Data	General eczema patient population: • 100 patients were tested with citronellol 5% and 1% in petrolatum as part of a screening study for fragrance contact allergy. 1 (1%) had a positive patch test reaction to citronellol 1%, but none to 5%. The patient was without a history of fragrance sensitivity (1) Subgroups of patients: • 20 perfume allergic patients were tested with several screening series of fragrances. 15 had dermatitis related to cosmetic products. Citronellol 5% gave a positive reaction in 7/20 (35%) patients (15). • The causative ingredients were identified in 75 patients with contact allergy to cosmetic products. 1 patient was allergic to citronellol, concentration unknown, present in a lotion (42). • The causative ingredients were identified in 119 patients with contact allergy to cosmetic products. 2/119 (1.7%) were allergic to citronellol, patch tested in 2% in pet. (5). Test concentrations: • Citronellol 1-2% in petrolatum is the recommended patch test concentration (12).	
Summary	One case of contact allergy to citronellol of uncertain relevance found by testing consecutive patients. Two studies of eczema patients with cosmetic eczema show more than one case each of patch test reactions to citronellol.	

Common name Chemical name	Farnesol (INCI) 3,7,11 trimethyldodeca-2,6,10 trienol	Ref n° 18
Synonyms		
Cas. no	4602-84-0	
Einecs	225-004-1	
Data	General eczema patients population: • 466 patients were patch tested by the Japanese society of contact dermatitis. Farnesol 2%, 5% and 10% was used. 1.1% of the patients reacted positively to farnesol 10% or 5% and 0.2% to farnesol 2% (17). Subgroups of patients: • 182 patients suspected of contact allergy to cosmetics were patch tested with a series of 22 fragrance substances. 2 (1.1%) of the patients had a positive patch test reaction to farnesol 4% in pet. Farnesol was not found in any of 79 cosmetic products sent in for analysis by the patients or their physicians. The patch test concentration of Farnesol 4% was based on a negative result in testing 20 control eczema patients (18) • 111 patients were tested with farnesol 1% in lanolin. 8 cases of sensitization to farnesol <u>were</u> found. 6 of the 8 also reacted to the balsam of Peru (47). Case Reports: • A women with an axillary dermatitis due to a deodorant tested positive to farnesol 5% in pet, probably used as preservative in the deodorant (47). Other: • Farnesol has been mentioned as a constituent of Peru Balsam (18,21). • 1/230 Patients with contact allergy to Peru balsam reacted also to Farnesol (44). • Restricted in IFRA –guideline: Farnesol should be at least 96% pure. The recommendation is based on a private communication that farnesol containing impurities caused sensitization while preparations of at least 96% purity did not. Test concentrations: • Farnesol 4% is the recommended patch test concentration (12).	
Summary	One study of eczema patients with cosmetic eczema shows two cases of contact allergy to farnesol. Additional cases in Peru balsam positive patients are found.	

Common name Chemical name	Hexyl cinnamaldehyde α -hexyl cinnamaldehyde	Ref n° 19
Synonyms		
Cas. No	101-86-0	
Einecs	202-983-3	
Data	Subgroups of patients: •20 perfume allergic patients were tested with several screening series of fragrances. Hexylcinnamal 2% was positive in one patient (15). •Ingredients responsible for allergy to cosmetics were determined in 119 patients suffering from cosmetic-related contact dermatitis. One was allergic to hexylcinnamic aldehyde 5% (5). •179 patients suspected of cosmetic allergy were patch tested with a series of 16 fragrance substances. 7/179 (3.9%) had a positive patch test to hexyl cinnamic aldehyde 10% (7). Other information: • Hexyl cinnamic aldehyde is a positive control substance in the OECD guideline for animal sensitization tests (48) Test concentration: •Hexylcinnamic aldehyde 2% is the recommended patch test concentration according to De Groot (12)	
Summary	Two studies with one case and one study with 7 cases of contact allergy to hexyl cinnamic aldehyde <u>were</u> found among patients with eczema from cosmetic products.	

Common name Chemical name	Ref n° 20 2-(4-tert-Butylbenzyl)propionaldehyde (Lilial) 2-(4-tert-Butylbenzyl)propionaldehyde;4-(1,1-Dimethylethyl)- α -methylbenzenepropanal; p-tert-Butyl- α -methylhydrocinnamaldehyde
Synonyms	Lilial , Lilestral
Cas. No	80-54-6
Einecs	201-289-8
Data	General eczema patient population: •3/685 (0.44) consecutive, Japanese patients with eczema had an allergic reaction to lilial 10% (17). Subgroups of patients: •176 patients suspect of fragrance sensitivity were patch tested with selected fragrance substances. 167 of these were tested with lilial 5% and 2 patients (1.2%) reacted with an allergic reaction (2). •179 patients suspected of cosmetic allergy were patch tested with a series of 16 fragrance substances. 5/179 (2.8%) had a positive patch test to lilial 20%. However some of these may have been false positive reactions due to the excited skin syndrome (7). Case reports: • A young man developed axillary dermatitis after using a new roll-on antiperspirant. He was patch test positive to the deodorant and the perfume from the deodorant. Fractionation and subsequent patch testing of the perfume showed that the offending allergen was lilial (49). Test concentrations: •Patch tests were performed with 0%, 2%,5% and 10% lilial in 685 patients with contact dermatitis. Only one irritant reaction was found to each concentration and 10% lilial was recommended for patch testing (17). De Groot recommends 1% lilial for patch testing (12)
Summary	Two cases of contact allergy to Lilial <u>were</u> found in a study of 176 eczema patients with cosmetic eczema and a case with contact allergy to lilial from a deodorant. More cases found but these may have been false positive.

Common name Chemical name	d-Limonene (R)- <i>p</i> -Mentha-1,8-diene	Ref n° 21
Synonyms		
Cas. no	5989-27-5	
Einecs	227-813-5	
Data	General eczema patient population: • Oxidised d-limonen were tested on consecutive patients. Two batches were used one oxidised for 10 weeks and one for 20 weeks. Patients were tested in both Stockholm and Leuven. 4/153 (2.6%) and 2/216 (0.9%) were positive to the first batch of d-limonen, test concentration 3% in the two participating clinic. 8/413 (1.9%) and 14/953 (1.6%) reacted to the second batch at a 3% concentration. Many of the limonene positive cases reacted to markers of fragrance contact allergy, such as the fragrance mix, Peru balsam and colophony (50). • 2/88 (2.3%) of patch tested patients reacted to oxidised limonene in 2% concentration. Probable relevance was documented in one case of a mechanic using a d-limonen hand cleanser (51) Subgroups of patients: • 179 patients suspected of cosmetic allergy were patch tested with a series of 16 fragrance substances. 2/179 (1.1%) had a positive patch test to d-limonene 10% (7). Occupational setting: • 2/105 (1.9%) car mechanics were allergic to d-limonene 5% in pet at patch testing (52) Other information: • d-limonene is apart from being a fragrance substance also used to degrease metal and for hand cleansing in industry. • Air-oxidation of d-limonene is essential for its sensitizing effects. Some of the oxidation products of d-limonene has been demonstrated to be potent sensitizers in animal assays (53). • IFRA-guideline recommends that d-limonene and natural products containing substantial amount of it, should only be used when the level of peroxides is kept to the lowest practical level, for instance by adding antioxidants at the time of production (IFRA guideline, dec 95). • The addition of the antioxidant butylated hydroxytoluene (BTH) prevent autooxidation of d-limonene for periods depending on the purity of d-limonene and room temperature (54). • d-Limonene is classified, labelled and warned as a sensitizer under the EU Dangerous Substances and Preparations Directive due to its ability to form allergenic oxidation products.	
Summary	Oxidisation products of d-limonene are strong allergens. A number of cases of contact allergy from occupational exposures to d-limonene is reported. The frequency of contact allergy to oxidised limonene is 1-2% in consecutive eczema patients. The relationship between contact allergy to oxidised d-limonene and fragrances in cosmetic products need to be further examined.	

Common name Chemical name	Linalool (INCI) Linalool	Ref n° 22
Synonyms		
Cas. no	78-70-6	
Einecs	201-134-4	
Data	Subgroups of patients: • 75 patients with contact allergy to cosmetic products were identified among 1781 patients tested . 3 patients gave an allergic reaction to Linalool, which was present in a shampoo, hair lotion and a shaving foam (42). • The causative ingredients were identified in 119 patients with contact allergy to cosmetic products. One patient was allergic to linalool, patch tested in 10% in pet. (5). Case-reports: • A 52 year old man developed contact allergy to his after-shave. Linalool and hydroxycitronallal present in the after-shave was determined as the causative ingredient by patch testing (55). Test concentrations: Linalool 30% may be used for patch testing according to De Groot (12)	
Summary	One study with one case and one study with 3 cases of contact allergy to linalool <u>were</u> found among patients with eczema from cosmetic products.	

Common name Chemical name	Methyl heptine carbonate Methyl oct-2-ynoate Ref n° 23
Synonyms	
Cas. no	111-12-6
Einecs	203-836-6
Data	Subgroups of patients: •182 patients suspected of contact allergy to cosmetics were patch tested with a series of 22 fragrance substances. 2 patients (1.1%) had a positive patch test to methylheptine carbonate 0.5%. The test concentration was based on a pilot study were 1/34 patients with contact dermatitis to cosmetics had a positive reaction to 0.5% methyl heptine carbonate (18). Methylheptine carbonate was detected in 3/79 cosmetic products brought in by the patients (18). •278 patients were tested by the North American Contact Dermatitis Research Group with methylheptine carbonate 1%, as part of a screening series for fragrance contact sensitivity. 1/278 (0.4%) reacted (30). Occupational setting: • 4 bakers with hand eczema were patch tested with fragrances/flavours. One reacted with a one plus reaction to methyl heptine carbonate 0.5% in pet. Relevance unknown (32). Case-reports: •A 19 year old women developed contact dermatitis after having worked for 3 years with mixing fragrances in a fragrance laboratory. She regularly worked with methyl octine carbonate and occasionally with methyl heptine carbonate. She was positive at patch testing to each of these tested separately at 1% in pet (56). •A 32 year old barber developed hand eczema. Patch testing showed an allergic reaction to an after shave. Further patch testing with a fragrance series showed positive reactions to: Methyl heptine carbonate 0.5% pet, hydroxycitronellal 10% in pet and cinnamic alcohol 5% in pet. All three fragrance materials were shown to be present in the after shave by chemical analysis (57). Other data: Methyl heptine carbonate is restricted to 0.01% in consumer products (IFRA guideline), due to a strong sensitizing potential. This is based on a private communication to IFRA. Test concentration: •Methyl heptine carbonate 0.5% is recommended for patch testing according to De Groot (12)
Summary	Single cases <u>were</u> reported. Is a strong sensitizer according to IFRA.

Common name Chemical name	Ref n° 24 3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one 3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one
Synonyms	γ -methylionone
Cas. No	127-51-5
Einecs	204-846-3
Data	Subgroups of patients: • 179 patients suspected of cosmetic allergy were patch tested with a series of 16 fragrance substances. 2/179 (1.1%) had a positive patch test to γ-methylionone 10% (7). • The causative ingredients were identified in 75 patients with contact allergy to cosmetic products. 1 patient was allergic to γ-methylionone , concentration unknown. Its presence was detected in a rouge (42). • The causative ingredients were identified in 119 patients with contact allergy to cosmetic products. 1/119 (0.8%) was allergic to γ-methylionone, patch tested in 5% in pet. (5). Case-reports: • 86 year old women developed a rash from using a cologne. Patch testing with the cologne gave a strong positive reaction. 18 components from the cologne were tested and reactions were found to ionones, one of these was γ-methylionone (58). Test concentration: • γ-Methylionone 10% in pet is recommended for patch testing according to De Groot (12).
Summary	One study with two cases and two studies of 1 case of contact allergy to γ -methylionone <u>were</u> found among patients with eczema from cosmetic products.

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Benzyl alcohol and benzoic acid group used as excipients

Report published in support of the 'Questions and answers on benzyl alcohol used as an excipient in medicinal products for human use' (EMA/CHMP/508188/2013) and the 'Questions and answers on benzoic acid and benzoates used as excipients in medicinal products for human use' (EMA/CHMP/508189/2013)



Benzyl alcohol and benzoic acid group used as excipients

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Executive summary

This document and the related questions and answers documents [23, 24] have been written in the context of the revision of the Annex of the European Commission Guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' [4, 9].

The use of benzoic acid and benzyl alcohol as excipients and mainly employed as solubilising agent and/or preservative in medicinal products. However, in pre-term and full-term neonates, concerns have been raised with the use of benzyl alcohol and benzoic acid. Benzyl alcohol administered intravenously has led to "gasping syndrome" in several pre-term neonates with metabolic acidosis involving deterioration of the neurological state, cardio-vascular failure and haematological anomalies. The majority of poisonings were fatal. This syndrome was associated with the accumulation of benzyl alcohol and its metabolite, benzoic acid. Benzyl alcohol must not be used in pre-term and full-term neonates. It is recommended to revise and implement information on these excipients in the package leaflet of medicinal products.

Introduction

Benzoic acid and benzyl alcohol are aromatic chemical compounds used for the improvement of active substance solubility and added as antibacteriostatic compound. Benzyl alcohol is mainly used in medicinal products wherein the route of administration is parenteral and to a lesser extend topical; whereas the two main routes of administration for benzoic acid are topical and per os.

Scientific discussion

1. Characteristics

1.1. Category (function)

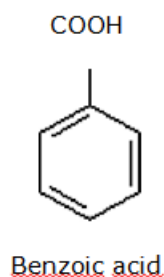
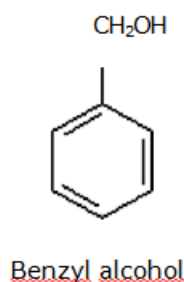
Benzyl alcohol is mainly used as a preservative. The minimal inhibitory concentrations (MIC) are as follows:

Microorganism	MIC* (µg / ml)
Aspergillus niger	5000
Candida albicans	2500
Escherichia coli	2000
Pseudomonas aeruginosa	2000
Staphylococcus aureus	25

* Minimal inhibitory concentration

Benzoic acid is a bacteriostatic antiseptic that is only active in an acidic environment (pH 2.5 to 4.5). It is mainly used as a preservative.

1.2. Physico-chemical Properties



Natural sources of benzy alcohol are Tolu or Peru Balsam as well as plant extracts such as that of jasmine. Industrial preparation of benzy alcohol is done by the hydrolysis of benzy chloride, using the Cannizaro or the Tischenko reaction starting with benzaldehyde.

It has to be noted that benzy benzoate, a molecule used as a plasticiser, solubilising agent, solvent or therapeutic agent, generates benzoic acid and benzy alcohol after hydrolysis.

1.3. Use in medicinal products

Benzy alcohol is used as both an excipient (preservative, solubilising agent) and as an active principal (antiseptic, local anaesthetic).

Benzy alcohol is used for different purposes:

- preservative (2% in oral or parenteral pharmaceutical preparations, 3% in cosmetics)
- solubilising agent (concentration greater than or equal to 5%)
- disinfectant (10% solutions)
- local anaesthetic (in certain injections, cough remedies, ophthalmic solutions, ointments, dermatological aerosols)

The most common routes of administration are parenteral and to a lesser extend topical.

Benzy alcohol is notably used via intramuscular administration in antibiotics, anti-inflammatory products or neuroleptics for its anaesthetic properties, in order to reduce pain at the injection site. The median concentration is 150 mg per injection (30 mg/ml). The range of injected dose varies from 20 to 600 mg/day.

Benzy alcohol is also used as a preservative and may possibly be substituted by other preservative substances. However, benzy alcohol also acts as a local anaesthetic, a property that is highly valued for reducing pain associated with intra-muscular injection.

2. Pharmacokinetics

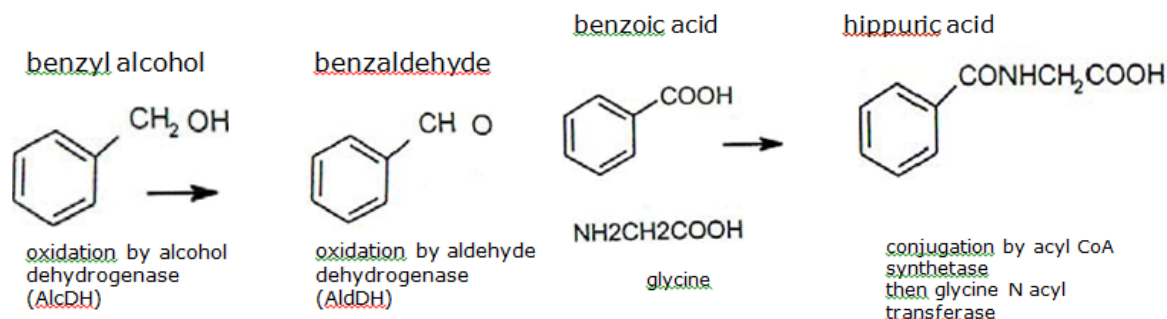
2.1. Absorption

Route of Administration	Absorption	
	Benzy alcohol	Benzoic acid
cutaneous	Up to 60%	43%

oral	100 %	100%

The oral absorption is complete. The cutaneous absorption of benzyl alcohol is significant.

2.2. Metabolism



Benzyl alcohol is oxidised by alcohol dehydrogenase (AldDH), a cytoplasmic enzyme present mainly in the liver, but also in the intestine and kidney. This reaction is saturable. The benzaldehyde formed is oxidised by aldehyde dehydrogenases (AldDH), cytoplasmic and mitochondrial enzymes mainly present in the liver, but also in the intestine and numerous organs.

Secondary metabolism:

Benzaldehyde can react with biogenic amines according to the Pictet and Spengler reaction and lead to the formation of corresponding tetrahydroisoquinolines and beta-carbolines, which are pharmacologically active derivatives.

As benzyl alcohol is quickly metabolized in benzoic acid after administration, it is not present at measurable level in the blood but its oxidation product, benzoic acid, is present and may be used for kinetic.

A perturbation of metabolism (anomaly, ADH2*3 allelic variant [30] or immaturity) or elimination of benzyl alcohol may lead to toxicity. Indeed, there is a greater accumulation of benzyl alcohol in serum of pre-term neonates expressed in peak level and in area under curve than in full-term neonates [16].

2.3. Elimination

Benzoic acid undergoes conjugation by acyl CoA synthetase then by glycine N acyl transferase to form hippuric acid. The newly synthesised hippuric acid is then eliminated in the urine.

For an adult, after intravenous administration of a dose of 15 mg/kg, the elimination half-life from plasma is 16 minutes for benzoic acid and 31 minutes for hippuric acid. Eighty per cent of the dose is eliminated in the urine in the form of hippuric acid. Clearance is approximately 600 ml/min.

The benzoic acid detoxification process is immature in those pre-term neonates [16] and conjugation of benzoic acid is saturated more quickly. This reaction is furthermore limited by the glycine available. This results in an accumulation of benzoic acid.

3. Toxicology

3.1. Toxicity after single administration of benzyl alcohol

Species (adult animals)	Route	LD50	Observations
rat	orally	1230-3200 mg/kg	
mouse	orally	1580 mg/kg	
rabbit	orally	1040 mg/kg	
rat	IV	50 mg/kg	convulsions, dyspnea, change in behaviour, haemolysis
mouse	IV	324 mg/kg	
rat	SC	1700 mg/kg	
rat	IP	400 mg/kg	
rabbit	Dermal	2000 mg/kg	
Guinea pig	Dermal	<5 mg/kg	
rat	Inhalation	>4.178 mg/l	

3.2. Toxicity after single administration of benzoic acid

Species (adult animals)	Route	LD50 *
cat	orally	2 g/kg
dog	orally	2 g/kg
mouse	IP	1.46 g/kg
mouse	orally	1.94 g/kg
rat	orally	1.7 g/kg

* Lethal dose 50%

3.3. Toxicity after repeated administration of benzyl alcohol

Species	Route	Duration	Doses	Observations	NOAEL
Rat adult	orally	16 days	125–2000 mg/kg	Diffuse haemorrhages	M: 250 mg/kg F : 125 mg/kg
Mouse adult	orally	16 days	125–2000 mg/kg	Lethargy, respiratory anomalies	M: 250 mg/kg F: 500 mg/kg

Rat adult	orally	13 weeks	50–800 mg/kg	Neurotoxicity, respiratory difficulties, diffuse bleeding, necrosis of the brain, muscles, thymus congestion, renal haemorrhages and damage	100 mg/kg
Rat adult	orally	103 weeks	200–400 mg/kg	Diffuse haemorrhages and neurological lesions	< 200 mg/kg
Rat juvenile (d22)	orally	6 weeks	100–600 mg/kg/d	Gasping syndrome at the higher dose levels	300 mg/kg/d (Foulon, 2005) [7]

No data have been reported for parenteral use, or topical use. Furthermore, there are no chronic studies related to toxicity on newborn or infant animals. There is only one study performed on juvenile rats establishing a NOAEL close to the adult one after oral administration [7].

3.4. Hypersensitivity

Some allergic reactions have been reported in humans. Benzyl alcohol may lead to local reactions (contact dermatitis) and general reactions, probably linked to a direct histamine release [19].

3.5. Local tolerance

Both Benzyl alcohol and Benzoic acid are mucous membrane irritant [28]; it causes serious irritation following application to the skin of mice.

3.6. Genotoxicity

A clastogenic effect has been shown in vitro [20]. However, the results obtained in vivo are negative.

3.7. Carcinogenicity

JECFA (joint FAO/WHO expert committee on food additives) considered that, although long term carcinogenicity and toxicity studies carried out with sodium benzoate in rats and mice were not very good quality, all of the available data, in particular new studies with benzyl acetate, lead to the conclusion that compounds from the benzoic acid family are not carcinogenic [14].

3.8. Reproductive function toxicity

In rats, no action on the growth, fertility, lactation or survival has been observed during a multigeneration reproductive study (because of the consumption of glycine occurring during elimination of benzoic acid and the possibility that glycine is essential for growth, a detrimental effect on growth could be envisaged).

Toxicity on foetal development has only been observed at doses that are toxic to the mother.

Three reproductive studies and 3 developmental toxicity studies exist, which lead to the conclusion that the no observable adverse effect level (NOAEL) after ingestion is in the order of 500 mg/kg/day.

4. Clinical safety data

4.1. Pharmacovigilance

Sixteen neonatal deaths thought to be caused by the benzyl alcohol preservative used in some intravascular solutions have been reported to the Food and Drug Administration (FDA)¹ by 2 medical centres [5, 8]. The deaths occurred in pre-term neonates weighing 2500 g who had central intravascular catheters flushed periodically each day with bacteriostatic normal saline containing 9 mg/ml benzyl alcohol. Ten deaths occurred in 1 institution over a 6-month period and 6 deaths occurred in the other institution over a 16-month period. Investigators in the 2 hospitals have reported that similar deaths have not occurred since flush solutions without preservatives have been substituted for those with the benzyl alcohol.

Onset of toxic illness in the infants occurred between several days and a few weeks of age with a characteristic clinical picture that included metabolic acidosis progressing to respiratory distress and gasping respirations. Many infants also had central-nervous-system dysfunction, including convulsions and intracranial haemorrhage; hypotension leading to cardiovascular collapse was a late finding usually presaging death.

Gas chromatographic analysis demonstrated benzyl alcohol or its metabolites in blood and urine samples from infants in 1 hospital. Retrospective analysis of urine samples from 5 infants in the other hospital for organic acid profile by gas-liquid chromatography showed urine benzoate levels of 4.4–16.1 mg/mg creatinine and hippurate levels of 7.4–33.3 mg/mg creatinine (normal values = 0-trace); serum benzoic acid levels were 8.4–28.7 mEq/L (normal = 0). Review of the medical records of the affected infants resulted in estimates of daily intake of benzyl alcohol ranging from 99 to 405 mg/kg/day.

Data type	Population	Exposure vector	Route	Benzyl alcohol dose	Observations	Reference
Retrospective study	10 premature newborns	catheters* rinsed with heparin and 0.9% benzyl alcohol solutions and Bacteriostatic water used for dilution and reconstitution	IV	99–234 mg/kg/day	gasping syndrome, neurological deterioration, metabolic acidosis, haematological anomalies, hepatocellular insufficiency, renal failure, cardiovascular collapse, 6 out of 10 deaths**	Gershanik, 1981 [8]

¹ FDA WARNING IN 1982: Neonatal Deaths Associated With Use of Benzyl Alcohol - United States

Retrospective study	11 premature newborns	catheters* rinsed with heparin and 0.9% benzyl alcohol solutions and Bacteriostatic water used for dilution and reconstitution	IV	130-405 mg/kg	progressive encephalopathy, convulsions, hypotension, cardiovascular collapse, 10 out of 11 deaths	Brown, 1982 [5]

* Umbilical catheterisation for monitoring following respiratory distress requiring ventilation

** No new cases have been reported by the authors after having prohibited the procedure of rinsing catheters with benzyl alcohol. The authors conclude that pre-term neonates who receive preparations containing benzyl alcohol intravenously accumulate the alcohol and its metabolites. This accumulation may be explained by the very high doses of benzyl alcohol relative to the size and weight of the neonates, and the reduced metabolic capability in pre-term neonates.

Data type	Population	Exposure vector	Route	Benzyl alcohol dose	Observations	Reference
Retrospective study	Neonates	Amiodarone Atracurium Dexamethasone Glycopyrrolate Phenobarbital Midazolam	continuous or intermittent infusions	0.6–319.5 mg/kg/d	No outcome data described	Shehab, 2009 [29]
Reported case	Pre-term neonate (24w gestation)	Clindamycin	IV	unknown	Desaturation, chest splinting requiring resuscitation	Hall, 2004 [10]
Reported case	Neonate (day 4)	Amiodarone	IV instead of orally	Approximately 20 mg/kg*	Amiodarone contains BA and polysorbate 80, exerting hemodynamic effects. Hypotension requiring cardiopulmonary resuscitation, systemic and peripheral hypoperfusion, lactic acidosis, multiple organ failure (hepatic	Masi, 2008 [18]

					and renal), myocardial ischaemia, encephalopathy.	
Reported case	Girl, 5 year-old	Valium	IV	180 mg/kg/day over 36 hours	Valium contains BA, benzoic acid and propylene glycol, exerting hemodynamic effects, hypotension, metabolic acidosis, death.	Lopez, 1995 [17]
Reported case	Male, 53 years old	Vepesid (etoposide)	IV	90 mg/kg in one perfusion	Haemolysis, metabolic acidosis, death	Smith, 2001 [30]
Reported case	Female, 65 years old	(mupirocin, pimecrolimus cream), prednisone IM and triamcinolone	Cutaneous, IM	Unknown	Chronic conjunctival inflammation and concomitant eyelid dermatitis with ectropion	Jacob, 2008 [11]

* The loading dose (47 mg/kg of amiodarone) was administrated intravenously. Amiodarone (150 mg of amiodarone/ml) contains 20 mg/ml of Benzyl alcohol.

4.2. Conclusion regarding the toxicity of benzyl alcohol

In adults, a single case of toxicity linked to the administration of benzyl alcohol has been reported in the literature even if the role of benzyl alcohol is uncertain. The dose administered was estimated as 90 mg/kg intravenously [30].

The main problem linked to the use of benzyl alcohol concerns neonates (pre-term and full-term) due to the immaturity of the metabolising enzymes and the risk of subsequent accumulation. Benzyl alcohol administered intravenously has led to "gasping syndrome" in several pre-term neonates with metabolic acidosis involving deterioration of the neurological state, cardio-vascular failure and haematological anomalies. The majority of poisonings were fatal. This syndrome is associated with the accumulation of benzyl alcohol. In one case in a five-year-old girl, adverse events occur at 20 mg/kg of benzyl alcohol intravenously although benzyl alcohol was not the only excipient allowing this effect in amiodarone (there was also polysorbate 80).

4.3. Conclusions regarding the toxicity of benzoic acid

All of the data examined have enabled the Acceptable Daily Intake (ADI) to be set at 5 mg/kg orally (in food) for benzoic acid and its salts on the basis of a NOAEL of 500 mg/kg determined during the reprotoxicity studies. The ADI is valid for adults and children over 4 weeks [13].

Benzoic acid may be used for managing hyperammonaemia in urea cycle disorders in the neonates. However, the main problem linked to the use of benzoic acid concerns neonates (pre-term and full-term) who have immature metabolising enzymes leading to accumulation. The risk associated with the

accumulation of benzoic acid is an increase in bilirubinaemia following displacement of bilirubin from albumin and which may cause newborn jaundice to develop into kernicterus [12] (non-conjugated bilirubin deposits in the brain tissue) [2].

5. Updated information for the package leaflet

Benzyl alcohol

Benzyl alcohol is classified in the list of excipients with known effects.

It appears that the table of excipients with known effects is incomplete and erroneous:

- The risk inherent to benzyl alcohol is due to its accumulation in the organism. Elimination of benzyl alcohol is highly variable as both the age (immaturity) and ethnic polymorphism of alcohol deshydrogenase may lead to accumulation of benzyl alcohol and thus toxicity. It is thus difficult to establish an exemption threshold.
- The actual recommendation does not recommend benzyl alcohol for children up to 3 years.

Benzoic acid

Benzoic acid is classified in the list of excipients with known effects.

It appears that the table of excipients with known effects is incomplete and erroneous: there is a risk of jaundice also by oral administration, for which absorption is 100%.

Neither benzyl alcohol nor benzoic acid should be used in neonates (pre-term and full-term).

Benzyl alcohol and benzoic acid should be used with caution for children older than four weeks.

Clinician should be aware of the following:

- Accumulation of benzyl alcohol may lead to toxicity
- Avoid a chronic use
- Use with caution

Therefore the following updated information is proposed:

Benzyl alcohol

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Benzyl alcohol	All routes of administration	Zero	This medicine contains x mg benzyl alcohol in each <dosage unit> <unit volume> <which is equivalent to x mg/<weight><volume>>. Benzyl alcohol may cause allergic reactions.	
	Oral, parenteral	Zero	Benzyl alcohol has been linked with the risk of severe side effects including breathing problems (called "gasping syndrome") in young children. Do not give to your newborn baby (up to 4 weeks old), unless recommended by your doctor.	Intravenous administration of benzyl alcohol has been associated with serious adverse events and death in neonates ("gasping syndrome"). The minimum amount of benzyl alcohol at which toxicity may occur is not known. Warning in section 4.4 in the SmPC should be given if used in neonates.
			Do not use for more than a week in young children (less than 3 years old), unless advised by your doctor or pharmacist.	Increased risk due to accumulation in young children.
			Ask your doctor or pharmacist for advice if you are pregnant or breast feeding. This is because large amounts of benzyl alcohol can build-up in your body and may cause side effects (called "metabolic acidosis").	

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
			Ask your doctor or pharmacist for advice if you have a liver or kidney disease. This is because large amounts of benzyl alcohol can build-up in your body and may cause side effects (called "metabolic acidosis").	High volumes should be used with caution and only if necessary, especially in subjects with liver or kidney impairment because of the risk of accumulation and toxicity (metabolic acidosis).
	Topical	Zero	Benzyl alcohol may cause mild local irritation.	

Benzoic acid

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Benzoic acid (E 210) and benzoates e.g.:	All routes of administration	Zero	This medicine contains x mg <benzoic acid/benzoate salt> in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>.	
Sodium benzoate (E 211) Potassium benzoate (E212)	Oral, parenteral	Zero	<Benzoic acid/benzoate salt> may increase jaundice (yellowing of the skin and eyes) in newborn babies (up to 4 weeks old).	Increase in bilirubinaemia following its displacement from albumin may increase neonatal jaundice which may develop into kernicterus (non-conjugated bilirubin deposits in the brain tissue).

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
	Topical	Zero	<Benzoic acid/benzoate salt> may cause local irritation.	May cause non-immunologic immediate contact reactions by a possible cholinergic mechanism.
			<Benzoic acid/benzoate salt> may increase jaundice (yellowing of the skin and eyes) in newborn babies (up to 4 weeks old).	Absorption through the immature skin of neonates is significant.

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Provisional Peer-Reviewed Toxicity Values for

Benzyl alcohol
(CASRN 100-51-6)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Commonly Used Abbreviations

BMD	Benchmark Dose
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
LOAEL _{ADJ}	LOAEL adjusted to continuous exposure duration
LOAEL _{HEC}	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
NOAEL _{ADJ}	NOAEL adjusted to continuous exposure duration
NOAEL _{HEC}	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration
p-RfD	provisional oral reference dose
RfC	inhalation reference concentration
RfD	oral reference dose
UF	uncertainty factor
UF _A	animal to human uncertainty factor
UF _C	composite uncertainty factor
UF _D	incomplete to complete database uncertainty factor
UF _H	interhuman uncertainty factor
UF _L	LOAEL to NOAEL uncertainty factor
UF _S	subchronic to chronic uncertainty factor

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR BENZYL ALCOHOL (CASRN 100-51-6)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (U.S. EPA) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

- 1) U.S. EPA's Integrated Risk Information System (IRIS).
- 2) Provisional Peer-Reviewed Toxicity Values (PPRTVs) used in U.S. EPA's Superfund Program.
- 3) Other (peer-reviewed) toxicity values, including
 - ▶ Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
 - ▶ California Environmental Protection Agency (CalEPA) values, and
 - ▶ EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in U.S. EPA's IRIS. PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the U.S. EPA IRIS Program. All provisional toxicity values receive internal review by two U.S. EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multiprogram consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all U.S. EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a 5-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV documents conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and Resource Conservation and Recovery Act (RCRA) program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the

adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV document and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other U.S. EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

There is no assessment of benzyl alcohol on IRIS (U.S. EPA, 2008), and there is no RfD for benzyl alcohol on the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006). The HEAST (U.S. EPA, 1997) lists subchronic and chronic RfD values for benzyl alcohol of 1×10^0 and 3×10^{-1} mg/kg-day, respectively. The source document for these assessments is a Health and Environmental Effects Document (HEED) (U.S. EPA, 1989). The subchronic RfD is based on a NOAEL of 143 mg/kg-day in a 13-week study in rats (NTP, 1989); the reported critical effect is decreased body weight, and an uncertainty factor (UF) of 100 is used. The chronic RfD is based on a LOAEL of 286 mg/kg-day in a 2-year study in rats (NTP, 1989a); the reported critical effect is epithelial hyperplasia in the forestomach, and the UF was 1000. Other than the HEED discussed above, the CARA list (U.S. EPA, 1991, 1994) does not include any relevant documents. Benzyl alcohol was included in two reviews of benzyl derivatives prepared by the WHO Expert Committee on Food Additives (WHO, 2005; FAO/WHO, 1996). The Committee derived an ADI of 0–5 mg/kg for benzyl alcohol (WHO, 2005). The National Toxicology Program (NTP, 1989a) has conducted 16-day and 13-week oral studies and a 2-year oral exposure cancer bioassay of benzyl alcohol. Neither CalEPA (2008a,b) nor ATSDR (2008) has assessed the toxicity of benzyl alcohol.

An RfC for benzyl alcohol is not available on IRIS (U.S. EPA, 2008) or in the HEAST (U.S. EPA, 1997). The HEED (U.S. EPA, 1989) reported that there were no inhalation toxicity data in humans or animals at the time of publication. Occupational exposure limits for benzyl alcohol have not been derived by ACGIH (2008), NIOSH (2008), or OSHA (2008).

A cancer assessment for benzyl alcohol is not available on IRIS (U.S. EPA, 2008) or in the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006). A Weight of Evidence classification for benzyl alcohol is not listed in the HEAST. Using the U.S. EPA (1986) guidelines, the HEED (U.S. EPA, 1989) assigned benzyl alcohol to Weight of Evidence

Group D (not classifiable as to human carcinogenicity) in the executive summary and Group E (evidence of noncarcinogenicity) in the text. NTP (1989a) concluded that there was no evidence of carcinogenic activity in male or female rats or mice administered benzyl alcohol via oral gavage for 2 years. Benzyl alcohol is not included in the 11th Report on Carcinogens (NTP, 2005). The carcinogenicity of benzyl alcohol has not been assessed by IARC (2008).

Benzyl alcohol is used as a topical anesthetic and was shown to induce a spectrum of behavioral responses identical to ethyl alcohol intoxication. Benzyl alcohol is also used as a preservative in medicinal products and was identified as a component associated with brain hemorrhage in infants and neonates (NTP, 1989a).

Literature searches were conducted from 1960s through May 28, 2009 for studies relevant to the derivation of provisional toxicity values for benzyl alcohol. Databases searched include MEDLINE, TOXLINE (with NTIS), BIOSIS, TSCATS/TSCATS2, CCRIS, DART, GENETOX, HSDB, RTECS, Chemical Abstracts, and Current Contents (last 6 months). An OECD SIDS Initial Assessment Report (OECD, 2001) and a Cosmetic Ingredient Review Expert Panel Report (Nair, 2001) were also consulted for relevant information. An updated literature search was performed in MEDLINE, TOXLINE (with NTIS), BIOSIS, PUBMED, and Current Contents from October 2008 to May 2009.

REVIEW OF PERTINENT DATA

Human Studies

Available data in humans are limited to case reports where exposure to benzyl alcohol occurred with other solvents or when administered as a bacteriostatic agent with other medications. These reports provided no dose-response in humans for quantitative assessments. Workers occupationally exposed for an undetermined duration to a high vapor concentration of a mixture containing benzyl alcohol, benzene, and ester solvents reported temporary headaches, vertigo, nausea, diarrhea, and weight loss (Treon and Stasik, 1983). Also, several reports describe toxicity in preterm neonates exposed intravascularly to solutions that contained benzyl alcohol as a preservative (Brown et al., 1982; Gershanik et al., 1982). Brown et al. (1982) gave details of 10 of 16 low birth weight infants (<1250 g) who died following exposure to bacteriostatic normal saline containing benzyl alcohol at a dose between 130 and 405 mg/kg-day. Symptoms of toxicity were observed between Days 2 and 4 of administration and included slowly progressive bradycardia, often with gasping respiration (resulting in the name “gasping syndrome”), seizures, unresponsiveness, and extremely depressed EEGs characteristic of progressive metabolic acidosis. Gershanik et al. (1982) reported that 10 low birth weight or premature infants developed gasping syndrome following hospitalization and exposure to benzyl alcohol intravenously in medications. Effects were similar to those reported by Brown et al. (1982). Infants that developed gasping syndrome received benzyl alcohol doses ranging from 99–234 mg/kg-day. A matched control group of infants who did not develop gasping syndrome received 27–99 mg/kg-day of benzyl alcohol intravenously. Menon et al. (1984) and Benda et al. (1986) reported a possible association between benzyl alcohol and brain hemorrhages in low birth weight infants given medicine with benzyl alcohol added as a preservative. Benda et al.

(1986) also reported significant ($p < 0.001$) decreases in the incidences of cerebral palsy and developmental delay in surviving low birth weight infants after benzyl alcohol use was discontinued in a neonatal intensive care unit. Hiller et al. (1986) reported a strong association with benzyl alcohol exposure and gasping syndrome and death in a large cohort of 61 low birth weight infants born prematurely. Significant decreases in neurotoxicity and mortality were reported in infants who did not receive benzyl alcohol compared to those who received. Neurotoxicological findings, such as kernicterus, intraventricular hemorrhage, and mortality in preterm infants have also been reported in other case studies (Jardine and Rogers, 1989).

Animal Studies

McClogskey et al. (1986) reported minimal toxic effects in CD mice to benzyl alcohol at doses below 800 mg/kg in a 4-hour observation period whereas mortality (LD50) was observed at 650 mg benzyl alcohol dose administered intraperitoneally to adult and neonatal mice during a seven day observation period. In the metabolism part of this study, the authors observed that the toxicity was related to the parent compound not the metabolite of benzyl alcohol. The subchronic and chronic toxicity and carcinogenicity of benzyl alcohol following oral exposure has been studied by NTP (1989a). Several screening assays that investigated the developmental toxicity of orally administered benzyl alcohol were also conducted (York et al., 1988; York et al., 1986; Hazelden, 1983; Hardin et al., 1987). Subchronic/chronic studies evaluating the toxicity of benzyl alcohol following inhalation exposure were unavailable.

In 13-week repeated-dose studies, groups of rats and mice (10 per sex and dose) were administered 0, 50, 100, 200, 400, or 800 mg/kg-day of benzyl alcohol in corn oil 5 days/week via oral gavage (NTP, 1989a). Dose selection was based on results from 16-day studies in rats and mice that observed treatment-related effects (including increased mortality, lethargy, decreased mean final body weight, and presence of blood around the nose, mouth, GI tract, and urinary tract) at ≥ 1000 mg/kg-day. In the 13-week studies, animals were observed twice daily for signs of toxicity and mortality. Animals were weighed individually at the beginning and end of the studies and were weighed as groups weekly during the study. All animals were necropsied after 13 weeks of treatment (or killed *in extremis* during the study), and unspecified tissues were microscopically examined from all control and high-dose animals. The brain was microscopically examined at 0, 400, and 800 mg/kg-day in rats and mice and in all mice that died prior to the scheduled sacrifice. No clinical pathology examinations (e.g., clinical chemistry, hematology, urinalysis) were conducted.

In rats, 8/10 males and 2/10 females in the 800 mg/kg-day group died; 4/10 of the deaths in males and 1/10 deaths in females were attributed to gavage error (NTP, 1989a). Signs of neurotoxicity (including staggering, labored breathing, and lethargy) were observed in male and female rats at 800 mg/kg-day, and 5/10 males at this dose were observed with blood around the nose and mouth after 8 weeks of treatment. No toxicologically significant decrease in final mean body weight was observed at any dose. No treatment-related gross lesions were reported. Histopathological effects attributable to treatment at 800 mg/kg-day included necrosis of the dentate gyrus of the hippocampus (9/9 males; 7/7 females); skeletal muscle necrosis (5/10 males); thymic congestion, hemorrhage, and atrophy (8/10 males); and renal nephrosis (6/9 males) similar to spontaneous age-related renal disease. The NTP report states that these lesions were not observed at lower doses. Therefore, treatment-related effects (mortality and

neurotoxicity) were only observed at the high dose (800 mg/kg-day) in rats administered benzyl alcohol for 13 weeks. The NOAEL and FEL values in rats are 400 mg/kg-day and 800 mg/kg-day, respectively.

In mice, there was no clear dose-related trend in mortality incidence (NTP, 1989a). Final mean body weights of mice in all treatment groups were within 10% of controls. In the first 2 weeks of the study, male and female mice staggered after receiving 800 mg/kg-day of benzyl alcohol. No other treatment-related clinical signs were observed. No gross or microscopic lesions attributable to treatment were seen in mice at any dose. Interstitial pneumonia observed in all groups was consistent with Sendai infection. Treatment-related effects in this study included clinical observation of staggering in high-dose males and females; no effects were observed at 400 mg/kg-day. Therefore, the NOAEL and LOAEL values in mice are 400 mg/kg-day and 800 mg/kg-day, respectively.

In the 2-year studies conducted by NTP (1989a), technical-grade benzyl alcohol (99% pure) dissolved in corn oil was administered by gavage 5 days/week, for 103 weeks, to 50 male and female F344/N rats at concentrations of 0, 200, or 400 mg/kg and B6C3F₁ mice at concentrations of 0, 100, or 200 mg/kg. Mice were unintentionally given α -methylbenzyl alcohol for 4 days during Week 80 with no observed toxicological syndromes. Animals were observed twice daily for signs of toxicity. Body weight was recorded at study initiation, weekly for 12 weeks, then monthly thereafter. After 2 years of treatment, all animals were necropsied, and a comprehensive set of tissues was microscopically examined from all female rats, control and high-dose male rats and mice, male rats and mice that died prior to 22 months of treatment, and male rats and mice with gross lesions. In addition, the pituitary gland and testes were microscopically examined in low-dose male rats; the adrenal gland, brain, kidney, liver and lungs were examined in low-dose male mice; and the brain, liver, spleen, and uterus were examined in low-dose female mice. When mortality at the high dose was $\geq 15\%$ above the control group for a particular sex and species, then a complete histopathological examination was performed on all animals of that sex and species in the low- and high-dose groups. Clinical chemistry, hematology, urinalysis, or ophthalmology parameters were not evaluated.

Survival of the benzyl alcohol treated female rats was significantly lower than controls after Week 50 in high-dose animals and, after Week 71, in low-dose animals (NTP, 1989a). Many of the deaths were attributed to gavage error (1, 17, and 13 in the control, low-, and high-dose groups, respectively); however, there was an apparent dose-response trend in nonaccidental deaths in females (13/50, 16/50, and 20/50, respectively, at 0, 200, and 400 mg/kg-day). Because of the high incidence of gavage-related deaths, it is not clear if the apparent increase in nonaccidental mortality in treated female rats was related to benzyl alcohol toxicity. In males, an apparent dose-related increase in gavage-related deaths was observed (4/50, 8/50, and 14/50 at 0, 200, and 400 mg/kg-day, respectively); however, survival curves in males were similar in both treated groups compared with controls. No effects on body weight or incidences of clinical signs of toxicity were observed.

High-dose male rats were observed with a higher incidence of epithelial hyperplasia in the forestomach than controls (0/48, 0/19, and 4/50 at 0, 200, and 400 mg/kg-day, respectively) (NTP, 1989a). It is possible that this observation was related to benzyl alcohol treatment because

benzyl acetate, which is metabolized to benzyl alcohol (NTP, 1989a), induced forestomach hyperplasia and squamous cell neoplasms in male and female mice dosed via oral gavage at 1000 mg/kg-day (molar equivalent to 720 mg/kg-day of benzyl alcohol) (NTP, 1986), but not in male or female rats or mice exposed at up to 350 mg/kg-day (molar equivalent to 252 mg/kg-day benzyl alcohol) in the diet for 2 years (NTP, 1993). Because benzyl acetate is metabolized to benzyl alcohol (NTP, 1989a), these data provide suggestive evidence that the forestomach is a potential target organ for benzyl alcohol in gavaged animals. However, the incidence of forestomach hyperplasia in high-dose benzyl alcohol treated male F344/N rats (4/50) was low. Increased incidences of microscopic lesions of the respiratory tract, larynx, and lungs were also observed in treated rats; however, these effects appear to have been caused by gavage error or by reflux of the gavage material and aspiration into the lungs due to the anesthetic properties of the test substance. High-dose rats were also observed with an increased incidence of cataracts and retinal atrophy, but this was attributed to those animals being housed in cages on the top racks, permitting greater exposure to fluorescent lighting. The data from this study indicate that there were no toxic effects that were associated with benzyl alcohol administration to male or female rats at any dose; therefore, the NOAEL in rats observed in this study is 400 mg/kg-day, and a clear LOAEL is not established. There is some uncertainty, however, as meaningful interpretation of the mortality data is not possible due to the high rate of gavage errors—particularly in females. Thus, the data cannot be used for dose-response assessments.

In mice, no effects on survival, clinical signs of toxicity, or body weight were observed (NTP, 1989a). High-dose male and female mice had an increased incidence of corpora amylacea (incidence of 15/49, 21/48, and 22/50 in males and 14/50, 15/48, and 25/50 in females in the control, low-, and high-dose groups, respectively). The lesion was described as consisting of “one or several small foci of mineralization in the thalamus” and was noted to be a common, spontaneously occurring lesion. Inspection of 2-year studies published in 1989 by the National Toxicology Program indicates that the incidences of corpora amylacea in the 2-year benzyl alcohol study in mice were within the vehicle control incidence of brain mineralization in mice in these studies (NTP, 1989e,f). Therefore, it does not appear that this lesion was likely related to benzyl alcohol treatment. There was also an increased incidence ($p = 0.044$ by the life table and incidental tumor tests) in adrenal cortex adenomas in high-dose male mice (adjusted terminal rates of 0/33, 0/30, and 3/35 in the control, low-, and high-dose groups, respectively). The incidence at the high dose, however, was within the historical control incidence and was, therefore, not attributed to benzyl alcohol treatment. Overall, no treatment-related effects were observed in this study at any dose. Therefore, the NOAEL in mice is 200 mg/kg-day (143 mg/kg-day when adjusted for a 5 day/week dosing schedule), the highest dose tested, and a LOAEL is not achieved.

Interpretation of the 2-year study in mice is unclear because it appears that this species, like rats, could have been given doses higher than 200 mg/kg-day—the highest dose tested. The study authors indicated that mice were given lower doses than rats based on reduced relative body weight gain observed at ≥ 400 mg/kg-day and on the anesthetic properties of benzyl alcohol, which may have contributed to the gavage related deaths in the 13-week studies (NTP, 1989a). However, the 13-week studies appeared to indicate that rats were more sensitive to benzyl alcohol toxicity than mice, and rats tolerated doses up to 400 mg/kg-day for two years; therefore,

it appears that mice could have tolerated doses substantially higher than 200 mg/kg-day for 2 years.

The developmental toxicity of benzyl alcohol has been evaluated in several screening assays (Hazelden, 1983; York et al., 1986; Hardin et al., 1987; York et al., 1988). In one study, groups of 50 SPF, pregnant female CD-1 mice, 6–8 weeks old, were gavaged with benzyl alcohol in distilled water at 0 or 750 mg/kg-day on gestation days (GDs) 6–13 (presence of vaginal sperm plug = Day 0) (Hardin et al., 1987; Hazelden, 1983). Mice were observed twice daily for clinical signs of toxicity and mortality during treatment, once daily during GDs 14–17, and twice daily after GD 18 for signs of parturition. Mice that died early were necropsied for the purpose of excluding gavage error as a cause of death. Body weights of the dams were recorded at study initiation, on GDs 6 and 17, and on Day 3 postpartum. Number of pups/litter and total litter weights were recorded within 12 hours of parturition (Day 1 postpartum) and again 48 hours later. The following parameters were evaluated: maternal mortality and weight change, number of viable fetuses, number of live births per litter, and pup birth weight, percent survival and weight gain. Nineteen of the 50 treated mice died. Reproductive and gestation indices and mean gestation lengths were not significantly different in treated and control mice. Maternal body weight was significantly less than controls throughout the exposure period and on Day 3 postpartum. Other signs of maternal toxicity included increased mortality, cyanosis, dyspnea, hypothermia, behavioral pathologies, and piloerection, which could lead to mortality. Mean litter weight was also significantly less than controls on Days 1 and 3 postpartum. A NOAEL is not achieved.

In a similar study, York et al. (1986) administered benzyl alcohol by oral gavage to 50 mice/dose at 0 or 550 mg/kg-day on GDs 6–15. All dams were allowed to deliver naturally. Body weight was recorded daily and all animals were observed for clinical signs of toxicity and mortality daily throughout treatment until Day 3 postpartum. No clear effects on mortality, clinical signs of toxicity, maternal body weight, or body weight gain, gestation index, average number of live pups per litter, postnatal survival, or pup body weight on Days 0 and 3 postpartum were observed. The NOAEL in this study is 550 mg/kg-day, the only dose tested.

In another preliminary, short-term developmental toxicity test in mice, benzyl alcohol was given a score of intermediate priority classification or “no decision” when rated relative to five indices of potential developmental toxicity (i.e., the proportion of pregnant survivors that produced a litter of at least one live born pup, average litter sex ratio and pup weight at birth, and average pup survival and weight gain) to 3 days of age (York, et al., 1988). No other information was given.

Taken together, the available data are not adequate to fully evaluate the ability of benzyl alcohol to induce developmental toxicity because the presence of visceral or skeletal malformations are not systematically evaluated in any of the studies. Reproductive organs in the NTP (1993) studies were unaffected by benzyl alcohol in both mice and rats. The data generated at NIOSH laboratory (York et al., 1986; Hardin et al., 1987) and the extensive evaluation reported by the Flavor and Extract Manufacturers Association (FEMA) expert panel (Adams et al., 2005) do not provide adequate information to evaluate developmental toxicity of benzyl alcohol.

Other Studies

Benzyl alcohol did not induce reverse mutations in seven *Salmonella typhimurium* tester strains or in two strains of *E. coli* with or without addition of an exogenous metabolic activation system (S9) (Fluck et al., 1976; Florin et al., 1980; Ishidate et al., 1984; Mortelmans et al., 1986; Rogan et al., 1986; NTP, 1989a). Benzyl alcohol at cytotoxic concentrations produced forward mutations (trifluorothymidine resistance) in the mouse L5178Y/TK+/- lymphoma assay without S9 addition but not with S9 (NTP, 1989a). The chemical also caused DNA damage in *Bacillus subtilis* (Yoo, 1986; Kuroda et al., 1984). Benzyl alcohol did not induce DNA damage in human alveolar cells (Waters et al., 1982). Benzyl alcohol was not positive in an in vitro chromosomal aberrations test in Chinese Hamster Lung cells without metabolic activation (Ishidate et al., 1988); in Chinese Hamster ovary (CHO) cells, benzyl alcohol induced chromosomal aberrations in the presence of S9 mix—but not without S9 (NTP, 1989a). An equivocal increase in sister chromatid exchanges was observed in CHO cells in the presence or absence of S9 activation (NTP, 1989a). The substance did not induce micronucleus formation in mice in vivo (Hayashi et al., 1988). Overall, benzyl alcohol has produced mixed results in genotoxicity assays; however, the data suggest that benzyl alcohol is not likely a genotoxic agent.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR BENZYL ALCOHOL

Available human studies have several limitations, such as exposure as mixtures and exposure as bacteriostatic agents with other medications. Benzyl alcohol was the subject of subchronic and chronic animal studies conducted by the National Toxicology Program (1989a), but no other relevant repeated-dose toxicity studies were located. In the subchronic studies (NTP, 1989a), treatment-related effects were only observed at the high dose, 800 mg/kg-day, and included mortality in male and female rats, increased incidences of clinical signs of neurotoxicity (including staggering, labored breathing, and/or lethargy) in male and female rats and mice, presence of blood around the nose and mouth in male rats, necrotic lesions of the brain in male and female rats, thymic congestion, hemorrhage, and atrophy in male rats, and nephrosis in male rats. Although these effects in rats may lead to FEL, there were several uncertainties, possibly due to significant gavage errors. No effects were observed at lower doses (≤ 400 mg/kg-day). Therefore, the NOAEL in this study in both rats and mice was 400 mg/kg-day.

In the chronic studies (NTP, 1989a), benzyl alcohol was administered by gavage 5 days/week, for 103 weeks to groups of 50 male and 50 female F344/N rats at 0, 200, or 400 mg/kg and B6C3F₁ mice at 0, 100, or 200 mg/kg for 2 years. The study featured comprehensive histopathological examination of the test animals. No effects, other than increased incidence of corpora amylacea, were clearly related to benzyl alcohol administration in either species. The NOAEL in rats and mice was 400 and 200 mg/kg-day, respectively, the highest dose tested in both species. The rat study, however, experienced a high incidence of accidental deaths, precluding a meaningful interpretation of the mortality data in this study.

Benzyl alcohol has been evaluated in several reproductive and developmental screening assays. Data on developmental and reproductive toxicity are limited to single dose-level studies in mice (York et al., 1988; Hardin et al., 1987). Benzyl alcohol administered at 550 mg/kg-day by gavage during 6–15 days of gestation produced no adverse effects (York et al., 1988). Hardin et al. (1987) reported both maternal toxicity and fetotoxicity in mice when exposed to 750 mg benzyl alcohol/kg-day during 6–13 days of gestation. This LOAEL is similar to the dose at which neurotoxicity was observed in both male and female rats exposed to 800 mg benzyl alcohol by gavage reported in the NTP (1989a) subchronic study. These studies are not adequate to derive p-RfD values because they do not evaluate a comprehensive set of developmental or reproductive toxicity parameters; however, the results of these assays suggest that maternal toxicity, rather than developmental and reproductive endpoints, may be a sensitive indicator of benzyl alcohol toxicity.

A subchronic p-RfD for benzyl alcohol can be derived based on the subchronic NTP (1989a) study in mice. This study identifies a LOAEL of 800 mg/kg-day and a NOAEL of 400 mg/kg-day in mice based on clinical signs of neurotoxicity, such as staggering, labored breathing, and lethargy in both males and females. The 400-mg/kg-day NOAEL is supported by the results of the chronic rat study, which found no treatment-related lesions in the brain or other tissues. The NOAEL/LOAEL approach was used to derive the p-RfD. The data provide no indication of the shape of the dose-response curve in this dose range and are not suitable for benchmark dose modeling (U.S. EPA, 1996, 2000). Although the high dose (800 mg/kg-day) reported in rats was associated with neurotoxicity and mortality, the NTP (1989a) reported uncertainties in dose-response caused by gavage error; thus, the rat study is precluded for development of toxicity values.

The NOAEL in mice of 400 mg/kg-day was adjusted to a continuous exposure basis ($400 \text{ mg/kg-day} \times 5 \text{ day/7 days} = 286 \text{ mg/kg-day}$) and divided by a composite uncertainty factor of 1000 ($UF_A = 10$ [extrapolation from animal studies], $UF_H = 10$ [human variability], and $UF_D = 10$ [database deficiencies, including lack of adequately conducted reproductive and neurological toxicity tests]) to derive a subchronic p-RfD as follows:

$$\begin{aligned}\text{Subchronic p-RfD} &= \text{NOAEL} \div \text{UF} \\ &= 286 \text{ mg/kg-day} \div 1000 \\ &= \mathbf{0.3 \text{ or } 3 \times 10^{-1} \text{ mg/kg-day}}\end{aligned}$$

Confidence in the key study is low. The study evaluates a sufficient number of doses and animals per dose in two species (rats and mice), and a sufficient number of tissues appears to be evaluated. However, key studies have several other limitations, such as high mortality due to gavage error, poor documentation of microscopic examinations, and lack of clinical pathology evaluations. Confidence in the database is low. No supporting studies other than the ensuing chronic study were located, and adequate reproductive and neurological toxicity studies are not available. Because the brain was shown to be a target, it has not been established that the most sensitive toxicological endpoint has been adequately characterized. Therefore, confidence in the subchronic p-RfD is low.

The chronic NTP (1989a) study in mice provides a suitable basis for derivation of a chronic p-RfD for benzyl alcohol. This study provides a NOAEL of 200 mg/kg-day for effects on survival, growth, and tissue histopathology; a LOAEL is not identified. The mouse chronic study reported mineralization of thalamus in female mice and adenomas in adrenal cortex in high-dose males; these were within historical control incidence, and were therefore, not attributed to benzyl alcohol treatment. The mouse study has been chosen over the similar rat study, with a NOAEL of 400 mg/kg-day, as the critical study because the rat study is compromised by a high number of accidental deaths, precluding a meaningful interpretation of the mortality data from this study. The NOAEL in mice of 200 mg/kg-day has been adjusted to a continuous exposure basis ($200 \text{ mg/kg-day} \times 5 \text{ days/7days} = 143 \text{ mg/kg-day}$) and divided by a composite UF of 1000 (10 for interspecies extrapolation; 10 for human variability; and 10 for database deficiencies, which includes the lack of adequately conducted reproductive, developmental, and neurological toxicity tests). A chronic p-RfD is derived as follows:

$$\begin{aligned}\text{Chronic p-RfD} &= \text{NOAEL} / \text{UF} \\ &= 143 \text{ mg/kg-day} / 1000 \\ &= \mathbf{0.1 \text{ mg/kg-day or } 1 \times 10^{-1} \text{ mg/kg-day}}\end{aligned}$$

Confidence in the principal study is medium. Although the study authors performed a comprehensive histopathology examination, no effects clearly associated with benzyl alcohol administration are identified. Confidence in the database is low; supporting data for the critical study are available only from the subchronic study, and only limited information has been located on the potential of ingested benzyl alcohol to induce developmental or reproductive effects. Benzyl alcohol has been shown to affect the nervous system in rats and mice in the 13-week studies; however, a comprehensive neurological toxicity study has not been located. Therefore, it has not been established that the most sensitive toxicological endpoint has been adequately characterized. Confidence in the chronic p-RfD is low.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR BENZYL ALCOHOL

No adequate human or animal data regarding the toxicity of benzyl alcohol following subchronic or chronic inhalation exposure are available, precluding derivation of p-RfC values.

DERIVATION OF A PROVISIONAL CARCINOGENICITY ASSESSMENT FOR BENZYL ALCOHOL

There are no human carcinogenicity data for benzyl alcohol. Benzyl alcohol did not induce tumors in a 2-year study in male or female rats and mice (NTP, 1989a). The sensitivity of the studies to detect carcinogenicity may have been compromised by the lack of a maximum tolerated dose achieved in mice of both sexes. No toxic effects occurred in male or female mice at up to 200 mg/kg-day, and only mild toxic effects (staggering during the first two weeks of the

study) occurred at 800 mg/kg-day in mice during the 13-week range-finding study. Also, mice appeared to be less sensitive than rats to benzyl alcohol toxicity in the 13-week study, and rats tolerated up to 400 mg/kg-day for 2 years. Therefore, it appears that mice could have been given substantially higher doses than 200 mg/kg-day for 2 years. Although a clear NOAEL is not observed in rats either, severe effects are noted in the 13-week range-finding study (NTP, 1989a) at a dose only 2-fold higher than the highest dose tested in the 2-year bioassay. Therefore, it appears that the maximum tolerated dose was approached in the rat study. Although not clearly related to treatment, survival is significantly reduced at both doses in female rats due to a large number of accidental deaths. However, the increased mortality in the treatment groups does not appear to substantially affect the sensitivity of the bioassay because survival rates at 12, 18, and 24 months were within U.S. EPA (1986) guidelines. NTP (1989a) considered survival to be adequate for cancer assessment. Benzyl alcohol has produced mixed results in genotoxicity assays; however, the data suggest that benzyl alcohol is not likely a potent genotoxic agent. Under the U.S. EPA (2005) cancer guidelines, there is “*Inadequate Information to Assess Carcinogenic Potential*” based on the available data.

Derivation of quantitative estimates (p-OSF or p-IUR) of cancer risk for benzyl alcohol is precluded by the absence of data.

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**Toxicological evaluation of some food
additives including anticaking agents,
antimicrobials, antioxidants, emulsifiers
and thickening agents**

WHO FOOD ADDITIVES SERIES NO. 5

The evaluations contained in this publication
were prepared by the Joint FAO/WHO Expert
Committee on Food Additives which met in Geneva,
25 June - 4 July 1973¹

World Health Organization
Geneva
1974

¹ Seventeenth Report of the Joint FAO/WHO Expert Committee on
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FAO Nutrition Meetings Report Series, 1974, No. 53.

BENZOIC ACID AND ITS POTASSIUM AND SODIUM SALTS

Explanation

These substances have been evaluated for acceptable daily intake
by the Joint FAO/WHO Expert Committee on Food Additives (see Annex 1,
Refs No. 6 and No. 13) in 1961 and 1965.

Since the previous evaluation, additional data have become
available and are summarized and discussed in the following monograph.
The previously published monographs have been expanded and are

reproduced in their entirety below.

BIOLOGICAL DATA

BIOCHEMICAL ASPECTS

The liver is the main site of conjugation with glycine in both man and most experimental animals (rabbits, rats) with the exception of the dog. Where the kidney is the main site of biosyntheses (Snapper et al., 1924; Friedmann & Tachau, 1911), sheep appear to have reduced capabilities of conjugating benzoic acid with glycine. Infusion of increasing amounts of benzoic acid into the rumen at levels up to 1.8 g/kg led to progressive fall in conjugation and increasing excretion of free benzoic acid in the urine. Doses of 1.1 and 1.8 g/kg were toxic leading to death. Potassium deficiency also occurred as shown by the usual symptoms of severe muscular weakness and tremors (Martin, 1966). For many years a liver function test was used in man based on the urinary excretion of hippuric acid after a test dose of benzoic acid (6 g orally or 1.5-2.0 g intravenously). Hence there exists a large amount of experience on the excretion of benzoic acid and hippuric acid in man. In the blood benzoates exist in the free state and not bound to proteins (Knoefel & Huang, 1956). In the dog the kidney clearance was estimated to be 0.90-1.89 (Knoefel & Huang, 1956).

Normal urinary excretion of hippuric acid in man was estimated to be 1.0-1.25 g/day, equivalent to 0.7-1.7 g of benzoic acid (Stein et al., 1954). Other determinations of the normal excretion in man and rat yield values lying between 1-3 mg/kg bw (Armstrong et al., 1955). The maximum rate of the hippuric acid excretion after ingestion of benzoic acid was observed to be 17 mg/min and for benzoyl glucuronic acid 0.57 mg/min equivalent to 24 g/day calculated as benzoic acid (Schachter, 1957). Up to 10 g of benzoate is quantitatively excreted by man (Barnes, 1959). At high intakes of sodium benzoate up to 3% is conjugated with glucuronic acid and all metabolites eliminated completely within 14 hours (Schachter, 1957). 75 to 80% of administered benzoic acid is eliminated by man in six hours (Quick, 1931). Sodium benzoate also decreases uric acid excretion (Quick, 1931), urea and ammonia excretion in man (Lewis, 1914).

Precursors of endogenous benzoate are phenylalanine and tyrosine. Experiments with labelled phenylalanine showed that about 1 to 2% is metabolized by this pathway. Rabbits given 50-400 mg/kg bw per day of deuterio-phenylalanine for six to 12 days, humans given 14-28 mg/kg bw per day for four to six days and guinea-pigs given 300 mg/kg bw per day for 12 days were examined (Bernard et al., 1955). $1\text{-}^{14}\text{C}$ acetate, however, did not produce labelled benzoic acid in rabbits and guinea-

pigs (Bernard et al., 1955). 3-¹⁴C-dl phenylalanine given intraperitoneally to rats produced 0.6 to 1% of activity as urinary hippuric acid (Altman et al., 1954).

1,3,4,5-tetrahydroxycyclohexanoic acid (quinic acid) may also serve as precursor of benzoic acid in intermediary metabolism (Dickens & Pearson, 1951). Several human subjects were given 6 g quinic acid orally or 250 g prunes and excreted hippuric acid in increased amounts during the following 24 hours (Quick, 1931). When deuterio-benzoic acid was administered to man and rats it was excreted with its deuterium content unchanged. Feeding guinea-pigs, with body fluids enriched in D₂O, with hydro-aromatic compounds led to urinary excretion of deuterio-benzoic acid with high D content. A similarly prepared rat, when fed 750 mg hydroxy benzoic acid over five days, excreted urinary benzoic acid enriched in D. When human subjects and guinea-pigs were given quinic acid over several days, 47 to 72% was converted to benzoic acid and excreted in the urine (Bernard et al., 1955). Four rats irradiated with 700 roentgens and four controls were given intraperitoneally carboxyl-¹⁴C-labelled sodiumbenzoate and fasted. Irradiation had no effect on the conjugating ability but the irradiated rats excreted less labelled hippuric acid due to dilution by endogenously produced benzoic acid (Schreier et al., 1954).

Benzoic acid inhibits pepsin digestion and sodium benzoate inhibits trypsin digestion of fibrin but they have no effect on amylase or lipase. Trypsin digestion of casein is only initially depressed by sodium benzoate (Kluge, 1933). Benzoic acid is a specific powerful inhibitor of the D-amino acid oxidase (50% inhibition by 10⁻⁴M) (Klein & Kamin, 1941). Concentrations in the range of 10⁻³M exert some unspecific inhibitory effects on the metabolism of fatty acids, e.g. on acetoacetate formation (Avigan et al., 1955).

Benzoic acid is rapidly absorbed (Schanker et al., 1958) and rapidly and completely excreted in the urine (Schachter, 1957; Barnes, 1959). One healthy man given 6, 9, 13.9, 34.7 and 69.3 millimoles of sodium benzoate showed a complete elimination of the drug within 10 to 14 hours (Schachter, 1957). Cumulation does not occur as shown by experiments on the distribution and elimination of sodium benzoate-l-C¹⁴ administered i.p., orally or s.c. to the rat. Practically quantitative excretion occurs in the urine within one to two days, less than 1% of radioactivity appears in the faeces, a few

ppm appear in organs. All radioactivity was identified as labelled benzoic acid (Lang & Lang, 1956). Orally or s.c. administered labelled benzoic acid appeared to 90% in the urine as hippuric acid, 0.1% of radioactivity occurred in the expired CO₂ and 2% remained in the carcass (Bernard et al., 1955).

Two urinary metabolites of benzoic acid are known, namely hippuric acid and benzoyl-glucuronic acid. Conjugation with glycine and glucuronic acid occurs in preference to oxidation because benzoic acid strongly inhibits fatty oxidation in the liver. In man, rabbit and rat, benzoic acid is almost entirely excreted as hippuric acid, whereas dogs excrete more conjugated glucuronic acid than hippuric acid (Williams, 1959). Sheep are less able to excrete hippuric acid and excrete much free benzoic acid in their urine (Martin, 1966). The urine of man, pig, rabbit and sheep contains up to 10% of benzoyl-glucuronic acid.

The maximum urinary excretory rate achieved depends on the dose of benzoate given. Limiting values of hippuric acid excretion were approached in man at a dose of 13.9 millimoles (Schachter, 1957). Limitations in availability of glycine account for this (Quick, 1933). In the rat the tolerance of large doses of benzoic acid depends on the addition of adequate amounts of glycine to the diet leaving sufficient glycine for protein synthesis. Normally preformed glycine is used though some is synthesized as well by the rat (Quick, 1931; Barnes, 1959). When rats were fed 1.5% benzoic acid (as the sodium salt) in the diet, they excreted 95% and more of the drug as hippuric acid in the urine. As the benzoate in the diet was increased to 3.75% so the ratio of hippuric acid to total benzoic acid in the urine decreased. Additional glycine raised elimination to 86 to 99%. The only other derivative, found in significant amounts in the urine, was benzoyl glucuronide (Griffith, 1929). Dogs and rabbits excrete hippuric acid independent of the route of administration of benzoic acid (Quick, 1931).

TOXICOLOGICAL STUDIES

Special studies on carcinogenicity

See under long-term studies (Hosino, 1951).

Special studies on reproduction

Mouse

From the animals subjected to a 17-month study some were mated and reproduction studied over five generations. Only body weights are given in the results (Shtenberg & Ignat'ev, 1970).

Acute toxicity

Animal	Route	LD ₅₀ (mg/kg bw)	Reference
Rat	Oral (Na salt)	2 700	Deuel et al., 1954
	i.v. (Na salt)	1 714 ± 124	Spector, 1956
Rabbit	Oral (Na salt)	2 000	Spector, 1956
	s.c. (Na salt)	2 000	Spector, 1956
Dog	Oral (Na salt)	2 000	Spector, 1956

Data on the LD₅₀ of potassium benzoate are not available. Benzoic acid is not acutely toxic to man (Lehman, 1908) or to test animals in moderate doses (Rost et al., 1913; Smyth & Carpenter, 1948).

Outbreaks of poisoning affecting 28 cats have followed ingestion of meat containing 2.39% benzoic acid. The effects were nervousness, excitability and loss of balance and vision. Convulsions occurred and 17 cats died or were killed. Autopsies showed damage to intestinal mucosa and liver. The sensitivity of the cat may be due to its failure to form benzoyl glucuronide and toxicity may develop with quantities greater than 0.45 g/kg single doses or 0.2 g/kg repeated doses (Bedford & Clarke, 1971).

Short-term studies

Mouse

Mice fed 3 g sodium benzoate daily for 10 days showed a 10% reduction of their creatine output, probably due to depletion of the glycine pool (Polonowski, 1941). Groups of 50 male and 50 female mice were given benzoic acid at the rate of 80 mg/kg/day, sodium bisulfite at 160 mg/kg/day and a mixture of the two at the same levels by gavage. The highest mortality was observed in mice given the combination (60% of 32% benzoic acid alone) and weight gain was reduced. A five-day period of food restriction at 2.5 months produced an 85% mortality in both groups (Shtenberg & Ignat'ev, 1970).

Rat

Groups of 10 rats (five males and five females) were fed sodium benzoate for 30 days at levels ranging from 16-1090 mg/kg bw. There were no observable effects on body weight, appetite or mortality nor any histological changes in the organs (Smyth & Carpenter, 1948).

Groups of three male and three female rats were fed on 0%, 2% and 5% sodium benzoate for 28 days. All animals on the 5% level died during the first two weeks showing hyperexcitability, urinary incontinence and convulsions. At the 2% level male rats showed a significant decrease in body weight. The food intake of male and female animals was decreased at the 2% level compared with controls (Fanelli & Halliday, 1963). Four groups of 15 rats were given 0%, 5% sodium benzoate and 5% benzoate + 1% glycine in their diet for three weeks. Body weight was reduced at the 5% level but less so when 1% glycine was added. Total cholesterol content of the liver was unaffected but phospholipids significantly reduced in the liver at the 5% level. Potassium concentration of skeletal muscle at the 5% level was also low. Glycine corrected the potassium and phospho-lipid deficiencies (Kowalewski, 1960). Twenty-eight young rats were given a diet containing 5% sodium benzoate for three weeks. Nineteen animals died within two weeks. Food consumption was significantly reduced and most animals developed severe diarrhoea. Autopsy changes were gut haemorrhage, nasal blood crust but normal urine. Five adult rats died within five weeks with severe weight loss on a similar diet (Kieckebusch & Lang, 1960). Groups of four to 19 male rats were fed diets containing 0%, 1.5%, 2.0%, 2.5%, 3%, 3.25% and 3.75% sodium benzoate for 40 days. Average growth was less than in controls at all levels but above 3% mortality was high, food efficiency poor and growth severely depressed. Addition of glycine reduced the toxic effects. Animals died with incoordination, tremor or convulsions and had severe eye inflammation. Feeding other groups of 10 to 15 young male rats on restricted amounts of diet containing 0%, 1.5%, 2.0%, 2.5% and 3% sodium benzoate revealed no difference in weight gain at the 3% level. Glycine addition again improved this weight loss (Griffith, 1929).

Ninety-day feeding tests were carried out on groups of eight to 10 rats on diets containing 1%, 2%, 4% and 8% of sodium benzoate. In the group on the 8% diet there were four deaths (average number of days to death 13). The weight gain of the four survivors was two-thirds of that of the controls on an identical food intake. Kidney and liver weights were significantly higher than those of the control group. At the lower levels no demonstrable effect was observed (Deuel et al., 1954).

Guinea-pig

Experiments on groups of four animals showed that doses of benzoate + benzoic acid of 150 mg/kg bw given daily up to 65 days had no adverse effects. When the same dose was fed to scorbutic animals a shortening of the life-span was observed (Kluge, 1933).

Dog

Feeding tests on 17 dogs over 250 days with sodium benzoate or benzoic acid at the rate of 1000 mg/kg bw had no effect on growth, appetite and wellbeing. Above this level ataxia, epileptic convulsions and death occurred (Rost et al., 1913).

Long-term studies

Mouse

Parenteral administration of benzoic acid has been shown not to cause tumour development (Hosino, 1951). Groups of 25 male and 25 female mice were given benzoic acid in doses of 40 mg/kg/day, sodium bisulfite in doses of 80 mg/kg/day and a mixture of the two at the same levels for 17 months. Mortality was increased in the groups receiving the mixture (62%) compared with the individual substance groups (32%) at eight months. Mortality at 17 months is not given and pathology is not reported (Shtenberg & Ignat'ev, 1970).

Rat

Three groups of 20 male and 20 female rats were pair fed for eight weeks on diets containing 0%, 0.5% and 1% benzoic acid and thereafter fed ad libitum over four generations. Two generations were fed for their whole life-span, the third and fourth generations were autopsied after 16 weeks. No harmful effects were observed on growth, fertility, lactation and life-span. The post-mortem examination showed no abnormalities (Kieckebusch & Lang, 1960). In another experiment 20 male and 30 female rats were fed on a diet containing 1.5% benzoic acid with 13 male and 12 female rats as controls for 18 months. Fifteen animals died in the test group, in the control three only. The test animals showed reduced body weight and food intake. Repeat experiments on groups of 20 test animals and 10 controls taken from another strain showed similar findings (Marquardt, 1960).

Groups of 10 rats, males and females, received benzoic acid at 40 mg/kg/day or sodium bisulfite at 80 mg/kg/day or a mixture of the two in the diet for 18 months. The growth was slightly reduced and ESR was increased. Rats fed benzoic acid developed some tolerance to a lethal dose of the compound given terminally. No pathology is reported (Shtenberg & Ignat'ev, 1970).

OBSERVATIONS IN MAN

In man tolerance appears to vary, 5.7 g sodium benzoate causing marked gastrointestinal disturbances in some (Meissner & Shepard,

1866) others tolerating 25 g to 40 g (Bignami, 1924). Up to 12 g daily have been given therapeutically without ill effects (Senator, 1879) yet over five days have produced gastric burning and anorexia in 30% of other subjects (Waldo et al., 1949). Toxic symptoms are local gastrointestinal mucosal irritation or CNS effects with convulsions in animals. Acute toxicity in man is readily reversible and probably due to the disturbance in acid-base equilibrium rather than associated with any tissue damage (Barnes, 1959).

Six men were given 0.3 g to 0.4 g of benzoic acid in their diet for periods up to 62 days. No abnormalities were seen in blood picture, urine composition, nitrogen balance and wellbeing (Chittenden et al., 1909). Nine patients receiving penicillin treatment were given 1200 mg of benzoic acid daily divided into eight doses over a period of five days in eight of the subjects and 14 days in one case. No effect was observed, particularly, in no case did the endogenous creatinine clearance show significant changes nor did routine urine analysis show any abnormality (Waldo, 1949).

Comments:

Considering the metabolic and toxicological data the margin of safety seems to be very large in animals and man. Benzoic acid is effectively and rapidly metabolized and eliminated by the body without apparent tissue injury. The rat seems closest to man as far as the metabolism of benzoate is concerned. There is no reason to believe that potassium benzoate differs toxicologically from benzoic acid and sodium benzoate when these are used as food additives. The cat seems to be much more sensitive than other species. Allergic responses to benzoic acid have been reported.

EVALUATION

Level causing no toxicological effect

Rat: 10 000 ppm (1%) in the diet equivalent to 500 mg/kg bw.

Estimate of acceptable daily intake for man

0-5 mg/kg bw.*

* As sum of benzoic acid and Na and K benzoate (expressed as benzoic acid).

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See Also:

Toxicological Abbreviations



Danish Ministry of the Environment
Environmental Protection Agency

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Chemical substances in car safety seats and other textile products for children

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However, publication does indicate that, in the opinion of the Danish Environmental Protection Agency, the content represents an important contribution to the debate surrounding Danish environmental policy.

Sources must be acknowledged.

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Foreword

This study is part of the Environmental Agency's programme for surveying chemicals in consumer products, which in 2014 had a special focus on chemical substances in products for children.

The objectives of the study are:

- To determine which chemicals that can be problematic to human health may be present in car safety seats and other textile products for children.
- To examine parts of the car safety seats and other selected product groups for substances that may potentially pose a risk to children.
- To provide health and environmental assessments of the substances identified in the survey.

The project was conducted from March to December 2014, in a collaboration between COWI (project management, mapping, health and environmental assessment) and the Danish Teknologisk Institute, TI (chemical analyses and migration tests). Jesper Kjølholt, COWI was the overall project manager while Inge Bondgaard Nielsen was project responsible at TI.

The project supervised by a steering committee consisting of the following:

- Shima Dobel, Danish Environmental Protection Agency (Chair)
- Lene Gede, Danish Environmental Protection Agency
- Jesper Kjølholt, COWI

Please note, that this English version of the original report (in Danish) contains seven appendices of which five are in Danish. However, with the exception of appendix 3, the appendices consist almost entirely of tables with chemical names and data and, hence, we believe that the majority of the contents should be possible to interpret.

Conclusion and Summary

Background and objectives

This study was undertaken as part of the Danish Environmental Protection Agency's programme for the identification of problematic chemicals in consumer products, which in 2014 had a special focus on chemical substances in products for children. The overall objective has been to examine and assess whether there are chemical substances in car safety seats and other textile products for children that may pose a risk, and to evaluate whether the regulations on the area are met.

Survey

Through a survey based on literature and interviews/questionnaire an overview is provided of which product types would be relevant to include in the practical investigations of this project, and a variety of chemical substances that can be expected to occur in the relevant product types were identified. The results of the survey have formed the basis for the subsequent project activities, where specific products were selected and purchased for chemical analysis.

The project deals with car safety seats and other textile products for children. The focus of the survey is on articles for babies and infants, i.e. children of 0-3 years of age, because this age group is considered particularly vulnerable to exposure to chemicals. The study includes product types with textile used specifically and solely for children, and which children will typically sit on or lie in. The main focus has been on car safety seats, but other relevant product types such as cradle seats, prams and strollers, baby slings, bunting bags, travel-/weekend beds and baby mattresses have also been included in the survey. Children's clothing, furnishing fabrics and furniture for children as well as toys are not covered by the study.

In the survey, a literature search for publications with information about the chemical substances in textiles and childcare articles was carried out. The main sources have been studies undertaken by environmental authorities and consumer organizations in the Nordic countries and Germany. Additionally, the market for these products has been identified through personal communication and questionnaires to a number of market players, including consumer organizations as well as retailers and producers of children's articles.

The product types studied are typically composed of different components, e.g. a car seat consists of both textiles (upholstery, belts/straps), foam (fill) and plastic parts (shell, buckles). The occurrence and possible release of chemicals depends to a large extent on the material in which the substances are used. Chemicals in the plastic parts are considered to be better immobilised in the material than chemical substances in textiles and foam for which reason the focus has been on the latter two materials.

Five out of the 11 contacted distributors of car safety seats and other children's articles have returned the questionnaire; two distributors have additionally attached analysis reports for their own products, and some companies have provided other additional information on chemical substances in car safety seats.

No production of car safety seats takes place in Denmark. It is estimated that 75 - 80% of car safety seats on the Danish market originate from countries outside the EU, and of these, the majority from China. The remaining 20-25% are produced in the EU, mostly in Germany but also in Italy. Car safety seats produced in the EU are typically more expensive than the Asian products. Regarding

the other children's articles, there is a major production of prams in Europe (roughly covering half of the market), while most other children's articles such as cradle seats, baby slings and buntings mostly originate from countries outside the EU (about 80%).

In general, it seems that distributors do not know whether hazardous chemicals are present in the products or if chemicals are added to meet certain technical or functional requirements such as water repellancy or fire retardant properties. One producer has, however, indicated that some functional requirements are made with respect to fire resistance and UV protection for car safety seats and certain other products. The occurrence of flame retardants can according to the producer be explained by strict requirements to fire safety in the UK, making the addition of flame retardants necessary in products that are (also) intended for the British market. Additionally, PAH compounds may be present as impurities in foam parts.

Previous surveys and studies show that a large number of chemicals are used in textiles. The Swedish Chemicals Inspectorate, for example, has compiled a list of 1,900 chemicals known to be used in textile production. The substances are not necessarily present in the final products.

Danish and German consumer organizations in collaboration have made a larger study of car safety seats, in which a number of new car safety seats on the market are analysed every year. The results from 2011 to 2013 are included in this survey together with the results of a survey of car safety seats made by the Swedish Chemicals Inspectorate. In these studies, phosphorus-based flame retardants were detected in a large number of samples in several studies, and in concentrations that indicate an intentional use of the substances in the products. Two other studies also showed a significant occurrence of phthalates and brominated flame retardants in a few seats. The concentrations of most of the other substances detected suggest that the substances primarily occur as impurities in the materials and that they do not have a technical function in the final products. This applies to e.g. PAH compounds, organotin compounds, perfluorinated substances and azo dyes.

In two studies, which analysed 15 baby slings for a number of substances, only phosphorus-based flame retardants and nonylphenol ethoxylates were found in significant concentrations.

The presence of chemicals in prams was previously investigated in two studies. The phthalate DEHP was found in a single pram in a very high concentration but apart from that only the phosphorus-based flame retardants were detected in measurable concentrations. Tin was found in some of the products but the content could not be related to the occurrence of selected organic tin compounds. Some prams contained bromine in relatively high concentrations in the fabric (two out of four samples), which could indicate the presence of brominated flame retardants.

In one of the consumer product surveys published by the Danish EPA with focus on products for babies, two nursing pillows and two baby mattresses were analysed. In one of the nursing pillows high concentrations of phthalates were found while tin was found in both nursing pillows and mattresses. The content of tin could, however, not be related to content of organotin compounds.

Selection of products and substances

Based on the results of the survey, a preliminary hazard assessment was carried out of the key substances/-groups that were identified in previous studies of relevant textile products as well as an initial screening level exposure assessment concerning textile products for children, with special emphasis on products for small children.

In the exposure assessment, parameters such as distribution of the product, frequency and duration of use, overall usage duration and exposure pathways were evaluated qualitatively. On the basis of this assessment car safety seats, baby slings and baby mattresses were identified as the three product groups which, overall, resulted in the highest exposure, especially of small children, and

they were therefore selected for further testing in the chemical analysis programme and a total of 30 products were purchased for the purpose.

Chemical analyses and migration test

Previous studies have shown a very large variation in the occurrence of chemical substances in textile products and it was therefore considered that there was a need for an initial screening analysis as a supplement to the results from the survey to support the decision on, which specific quantitative analyses should be performed on each product. A screening analysis by GC/MS of all 30 products was conducted. Sub-samples of several different materials were taken from many of the products and in total 59 sub-samples were screened. All samples were additionally analysed specifically for formaldehyde, which has previously been found in related textile products and which cannot be detected by GC/MS screening. Furthermore, the samples of car safety seats were screened by X-ray for content of the elements tin and bromine as indicators of organotin compounds and brominated flame retardants.

The results from the screening analyses showed several unidentifiable compounds in low concentrations, i.e. in concentrations indicating that the substances probably did not have an intended function in the textile product. Only a few substances were detected in concentrations, that were considered important in relation to health, and formaldehyde was not detected in any of the samples. The X-ray screening analysis did, however, show a significant content of bromine, which could indicate the presence of brominated flame retardants.

The specific substances selected for quantitative chemical analysis based on the results of the screening analyses and initial hazard/exposure assessment were phosphorus-based flame retardants, the phthalate DIDP, azo dyes (through certain aromatic amines as indicators), isocyanates and brominated flame retardants. The substances found in most samples in significant concentrations were the phosphorus-based flame retardants TCEP, TCPP and TDCP and two isomers of diisodecyl phthalate (DIDP), all of which were found in concentrations of 10,000 mg/kg material in one or more samples.

Previous studies of textile products have shown the presence of several substances with well-known health or environmentally hazardous properties in some products, but these substances (e.g. various phenols, formaldehyde, phthalates other than DIDP, PAHs, organic tin compounds) were found only in very low concentrations in the 30 products analysed as a part of this project.

Azo dyes, which can release one or more of the 22 aromatic amines listed in Appendix 8 to Annex XVII of the REACH Regulation, are not permitted to be used in textile or leather articles, which may come into prolonged direct dermal or oral contact with humans. For the aromatic amines a general limit value of 30 mg/kg (each) has been set. The compound 4-methyl-m-amino-phenylenediamine was detected at or above this threshold in two products, while 3 of the other 11 tested products contained low levels of the relevant amines. The violations have been reported to the Danish Chemicals Inspectorate.

Isocyanates were, despite indications of their presence in the samples at the preliminary screening, not found in concentrations above 1 mg/kg in any of the samples examined by the specific quantitative analysis. It is therefore concluded that the conventional GC/MS screening method is not suitable to indicate a possible content of isocyanates.

The X-ray screening analysis indicated that brominated flame-retardants could be present in two products. This could, however, not be verified by the quantitative analyses of selected brominated flame retardants where only a content up to about 1 mg/kg was found. It is assessed that the products may have been treated with polymeric brominated flame retardants, which are not

possible to detect by the analysis performed but are considered to be less harmful to human health than the traditional brominated flame retardants.

In another consumer product project for the Danish EPA, conducted concurrently with this project, textiles from eight car safety seats were analysed for 39 polyfluoroalkyl substances (PFAS). The concentrations of PFAS were below the detection limit in all of the eight samples.

From the results of the quantitative chemical analyses, it was concluded that testing of migration was only relevant to perform for the phosphorus-based flame retardants. The seven products having the highest content of one or more of these substances – 4 car safety seats, 2 baby slings and 1 baby mattress – were tested for migration to artificial sweat over a period of 3 hours. Migration was observed in all tests, varying from <1 % up to 30% of the material's content (by weight).

Health assessment

On the basis of the results of the quantitative chemical analysis an assessment of health risks was performed for the substances showing a significant migration out of the products, since this migration may result in exposure of children via the individual product types. The hazard assessment therefore focuses on the three phosphorus-based flame retardants (TCEP, TCPP and TDCP), while no risk assessment has been conducted for either diisodecyl phthalate (DIDP) or the aromatic amines from azo dyes.

Phosphorus-based flame retardants

TCEP is associated with a low to moderate acute toxicity. In rat studies, it appears that up to 90% of the substance is absorbed after oral intake. Lesions in the kidney after repeated exposure is assessed to be the most sensitive endpoint with a LOAEL of 12 mg/kg bw/d. The substance has not been shown to be mutagenic, but is classified as a carcinogen in category 2 (Carc 2), with a carcinogenic effect related to the effects on the kidneys. TCEP has also been shown to be toxic to reproduction in mice and rats (Repr. 1B), and a NOAEL of 175 mg/kg bw/d is established because of impairment of fertility in both sexes.

TCPP is considered to have a low acute toxicity. Up to 80% of the substance is rapidly absorbed after oral intake, and dermal absorption studies have shown that a dermal absorption of 40% can be used for TCPP. Based on analogy with TCEP, TCPP is also classified as Carc. 2, H451. In a 90 day study in rats, a LOAEL value of 52 mg/kg bw/day was determined based on effects on the liver and thyroid gland. *In vitro* studies also indicate some endocrine disruption potential.

TDCP is also assessed to have low acute toxicity. Animal studies indicate absorption of 100%, 100% and 30% for oral intake, inhalation and dermal absorption, respectively. Neurotoxic effects were observed in studies with repeated exposure but a dose-response factor has not been established. TDCP is mutagenic *in vitro* but not *in vivo*, and it is classified Carc 2. on the basis of effects on the kidneys with a LOAEL of 5 mg/kg bw/d.

Health risk assessment

Based on the results of the chemical analyses and the worst-case exposure scenarios for the use of car safety seats, baby slings and baby mattresses, the health risk associated with the above substances has been estimated. This was done based on the procedures in the ECHA guidance document and on DNELs identified in the literature or calculated. The results, which were based on very conservative assumptions, indicated that there may be a risk associated with the exposure of children to particularly TCEP in car safety seats and baby slings, and TDCP in car safety seats, baby slings and baby mattresses, if the children are exposed as described in the worst-case scenarios. The scenarios were therefore adjusted in order to make the assumptions more realistic. The assumptions were adjusted with regard to the skin surface area in contact with the products, the migration from the products and the absorption of the substances through the skin.

The results of the more realistic calculations also indicated an undesirable risk associated with a single car seat, a baby sling and a baby mattress.

With regard to baby mattresses it should be stressed that they are usually expected to be protected with a cover and in addition with a sheet during use and these two layers of textile are expected to act as a barrier to migration. This is supported by the results from the initial quantitative analyses, which showed that the content of TDCP in the mattress cover was approximately 1000 times lower than the content in the foam. It is therefore assumed that the textile cover is an effective barrier which can provide considerable protection of the children using the mattress. However, based on the results, it cannot be excluded that there may be a risk associated with the use, if the children, are in direct contact with the mattress without cover.

The test samples from the car seat and the baby sling have been taken from areas expected to be in direct contact with the face. Samples include both textile / felt and foam and as the results are obtained from the samples immersed in the sweat simulant, it will not be reflected if the textile part may be a barrier to the migration of the substances in the foam.

The risk assessment carried out does not account for exposure from other sources of TCEP, TCP and TDCP. The assessment by the Scientific Committee, SCHER, of TCEP in toys indicates that there is exposure to TCEP from dermal contact with furniture and dust, inhalation and drinking water, which may also be possible for the other substances. In addition, it is also possible that children are exposed to substances from car safety seats, baby slings and mattresses within the same period of time.

Environmental assessment

Following the initial exposure assessment, which indicated a limited environmental load with chemicals from the selected product groups, it was decided to limit the environmental risk assessment to a conservative screening assessment of environmental impact. This was based on an arbitrary substance, "Substance X", in an arbitrary product at a concentration of 10,000 mg/kg and 100% release to wastewater within a period of one year. Assuming that all children in the age group 0-3 years use such textiles, the resulting concentrations (PEC) in sewage effluent and sludge, respectively, were estimated under worst case conditions and compared to the lowest Predicted No-Effect Level (PNEC) reported for any of the relevant, quantified substances – i.e. TCEP, TCP, TDCP, DIDP and azo dyes (aromatic amines).

It was from this conservative screening assessment concluded that the risk of environmental effects in aquatic or soil environments due to release of the assessed substances from washing of children's textiles was insignificant and therefore it was not considered necessary to perform a more detailed assessment. Similarly, an environmental impact from emission of the substances to air was estimated to be insignificant.

1. Introduction

1.1 Background

The use of chemicals in the production of textile fibres, finished fabrics and textile products is intensive. This applies to all phases of the life cycle. Many of the chemicals used are potentially hazardous to human health and/or the environment. Most of the fabrics that can be found in articles are manufactured in countries outside the EU (KemI, 2013). While some of the substances used react chemically or are removed from the textiles during the production, and therefore not found in significant amounts in the finished products, other chemicals continue to exist in their original form and may be released later from the finished product thus resulting in exposure of consumers and the environment. Exposure of consumers can take place directly during use or, for certain substances, indirectly via the environment (release during washing and waste management).

In this project, the focus has been on products with textiles aimed for use by children. Products with textiles are found anywhere in the children's immediate environment, both as clothing and home textiles or as components of products developed specifically for children. The main focus of this project is on car safety seats as it is a product type that is used on a daily basis by a large fraction of Danish children from birth until approximately 12 years of age. The regulatory rules state that children under 135 cm in height must sit in an approved car safety seat when being transported in a car. Moreover, it is a product group, which in previous studies has been found to contain problematic chemicals.

1.2 Objectives

The objectives of the study are:

- To determine which chemicals that can be problematic to human health may be present in car safety seats and other textile products for children.
- To examine parts of the car safety seats and other selected product groups for substances that may potentially pose a risk to children.
- To provide health and environmental assessments of the substances identified in the survey.

2. Survey of products and chemicals

2.1 Surveying method

2.1.1 Literature search

A literature search was performed partly by a search on the internet with relevant search terms and partly by searching on the web sites of specific agencies and organizations, including:

- Danish Environmental Protection Agency
- Chemicals Inspectorate (KemI, Sweden)
- Environment Directorate (formerly SFT and Klif, Norway)
- Umweltbundesamt (Germany)
- Federal Institute for Risikobewertung (BfR, Germany)

For the internet search, search words such as "textiles", "car safety seats", "chemicals", "surveys", combined with "children" in Danish, English and German.

2.1.2 Information from market players

The following organizations and companies were contacted by telephone and/or by mail and requested to provide information on chemicals in car safety seats and other textile products, among others by completing a questionnaire, which can be found in Appendix I:

Organizations:

- Danish Consumer Council
- FDM (Federation of Danish Motorists), the interest organization of Danish car owners
- Stiftung Warentest, the German testing organization
- ADAC, the German association for car owners
- Ecology Center, USA/American environmental and consumer NGO

Importers, distributors and vendors:

- BabySam
- T. Hansen
- Ønskeborn
- COOP Denmark
- Britax Roemer
- IKEA A / S
- Dansk Supermarked
- Børnebiksen
- Jysk
- Fætter BR
- Silvan

2.2 Identification of relevant products

2.2.1 Delimitation of the product groups covered in this project

This project deals with car safety seats and other products with textiles for children. The survey focused on product types with textile used specifically by children, and that they typically sit or lie in, while children's clothing, home textiles, furniture and toys are not included. The main focus has been on car safety seats but other relevant product types such as cradle seats, prams and strollers, baby slings, buntings and baby mattresses have also been included in the survey.

Although car safety seats and/or booster seats can be used by children up to 12 years of age (for children under 135 cm), the investigation focused on articles for young children from 0-3 years of age. This age group represents the primary users of the mentioned product types, and children in this group of age are considered particularly vulnerable because they often lick/suck on or put objects in the mouth.

The product types mentioned are typically composed of a number of components. E.g. a car seat typically consists of textiles (upholstery, belts), foam (buffer) and plastic parts (shell, buckles). Chemical substances in the plastic parts are considered less prone to be released from the material than chemical substances in textiles, i.e. they are less prone to be released during use and, hence, significant skin contact with these product parts is not expected. Therefore, within the framework of the investigation, it has not been a priority to carry out a comprehensive survey of the plastic parts and no chemical analyses of these product components were performed. However, to the extent that previous studies have included analyses of chemical substances in parts other than textiles in relevant products, these results are reported.

2.2.2 Child car safety seats and booster seats

Product description

In Denmark, it is a legal requirement that children under 135 cm must be secured in safety equipment that is suitable considering both height and weight. Babies (up to approx. 1 year) are typically fixed in rear-facing infant seats, toddlers (1-4 years) in forward as well as rear-facing seats, while children from 4-5 years of age should use booster seats with or without backrest (Road Safety Council , 2014).



FIGURE 1
EXAMPLES OF HIGHCHAIRS / BELT PADS TO: A - BABIES (CA. 0- 1 YEAR); B - YOUNG CHILDREN (1-4 YEARS), AND C - LARGER CHILDREN (APPROX 4-12 YEARS) (PICTURES FROM WWW.BABYSAM.DK).

A car seat is a complex product composed of several different parts and materials (Tønning et al., 2008 and own observations):

- Frame made of metal or hard plastic.
- Shell, buckles and possible armrests made of hard plastic.
- Foam materials for fillings and upholstery of the chair itself and possible support cushions, for example polyurethane foam, polystyrene foam or polyester fiber. Cotton wool may occur.
- Textile cover for chair and support pads. The cover is typically made of cotton, polyester, or a mixture of these.
- Potentially, some buckles or labels consisting of soft plastic or rubber.
- Straps or belts consisting of textile.

The market for car safety seats

In the trade statistics from Statistics Denmark there is no specific product code for car safety seats. There is no production of car safety seats in Denmark. According to FDM (personal communication, 2014) around 75-80% of car safety seats on the Danish market originate from non-EU countries, and of these the majority are imported from China. The remaining 20-25% are produced in the EU, mostly in Germany but also in Italy. Typically, car safety seats produced in the EU are more expensive than the Asian products, and therefore the vast majority of car safety seats found in discount stores, DIY centres and supermarket chains typically come from countries outside the EU. Many brands are marketed as being European, although the actual production takes place outside the EU.

Several businesses are reported to be contract manufacturers, i.e. they first commence a production when a retail company has commissioned a certain number of seats. By this the products are sent directly sent from the factory to the customer, which reduces the need for preservatives and biocides for storage (FDM, 2014).

FDM (2014) estimates that about 100,000 to 500,000 seats per year are sold in Denmark, but the actual number is probably in the low end of the range. Over the past seven years, on average 61,000 children per year were born in Denmark (Statistics Denmark, 2013). Assuming that each child needs approximately 3 car safety seats through its childhood, that the lifetime of a car seat is 5 - 10 years and that a chair is re-used 1-2 times (the models often become obsolete before they are worn out), the number of sold seats roughly equals the birth rate and therefore believed rather to be in the order of 60,000-100,000 seats per year than the above estimate by FDM.

A very large number of different car safety seats are on the market. Danish and German consumer organisations (Danish Consumer Council, FDM, Stiftung Warentest and ADAC) have teamed up to finance a larger study of child car safety seats. In total, 131 different car safety seats from a large number of producers have been tested as part of the study (Danish Consumer Council, 2014c). There is no information indicating that the market is dominated by only a few actors, and in many Internet shops seats from many different producers are sold. Child car safety seats can be purchased e.g. in a variety of Internet shops, baby and children's equipment shops and department stores, at FDM and in certain DIY centres, car accessories stores and supermarkets.

There is a widespread re-use of car safety seats, e.g. they are used by younger siblings or given to family and friends, and there is also widespread private sale through Internet sites such as the Den Blå Avis.

Reply from market players on chemicals in car safety seats

Five out of the 11 contacted distributors/vendors of car safety seats and other children's articles have responded to the questionnaire. Two respondents have additionally attached analysis reports for their own products, and some have provided additional information by e-mail or telephone.

In general, it seems that the distributors/vendors have no information about possible presence of harmful chemicals in car safety seats or information about chemicals are added to meet certain technical or functional requirements such as water-resistance or flame-retardant properties.

One company with a significant market share in Denmark has indicated that there are functional requirements with respect to fire resistance and UV protection for car safety seats. However, flame retardants are added only to the foam parts and not to the textiles, while it is not known whether chemicals are added to achieve UV protection. Additionally, it is indicated that PAH (polycyclic aromatic hydrocarbons) may be present in the foam, although this chemical is not intentionally added to achieve a specific function. The occurrence of flame retardants can be explained by the stricter fire safety requirements to car safety seats in the UK, making the addition of flame retardants necessary in products intended for the British market.

2.2.3 Other textile articles for children

In addition to car safety seats the distributors/vendors were also asked for information regarding import, production and possible use of chemical additives used in the following products:

- Nursing pads, -cushions and -pillows
- Prams and strollers
- Buntings, carrier- and sleeping bags
- Baby slings
- Cradle seats and the like
- Travel- and weekend beds

Baby mattresses were only later selected as an independent product type (representing the, from an exposure point of view, most relevant parts of prams, carry cots, travel-/weekend beds etc.) for possible purchase and subsequent chemical analysis, and therefore information on this product type has only indirectly been answered by the respondents to the questionnaire.

Product descriptions

The above products are typically composed of a variety of materials which will result in different extent of exposure of the child. Prams and strollers, cradle seats, travel-/weekend beds are expected to be made of the same materials as car safety seats, while nursing pillows, mattresses and buntings only contain the textile parts and foam/fillers.

Some of these products were previously tested in a consumer product project by the Danish EPA on products for babies (Tønning et al., 2008). The following information on materials in such products (except travel-/weekend beds) is extracted from this study. The breakdown into product groups in the investigation by Tønning et al. (2008) is not completely identical with the breakdown of products here. Significant changes in the composition of the materials are not expected for the products since the compilation of data in the previous survey.

Nursing pads / cushions / pillows - Cover of plastic film or textile (cotton or cotton/polyester) with a coating of plastic or similar, and also covers of nylon fabric occur. The plastic may be PVC, polyurethane (PU), acrylic plastic or other. Covers are often decorated with prints. Fill material of foam (polyurethane foam, PU) or polyester.

Carrycots / pram upholsteries – Cover/exterior fabrics made of textile often with a plastic coating either on the outside or inside, or with a water-repellent impregnation. The textile material is often polyester but can also be cotton or cotton/polyester. The coating may be polyurethane (PU), which is of type FR (= containing a flame retardant product). Upholstery/fill of polyurethane foam is (PU) and/or polyester.

Cot bumpers and head protection in prams - Outer fabric of cotton or cotton/polyester. Filling of polyester fibre, or possibly PU foam.

Bunting bags / sleeping bags – Cover/exterior of polyester, cotton or polyester/cotton. Inside textile of cotton, polyester/cotton or polyester fleece (furry fabric). Fill/interior of polyester, wooly fibres or down/feathers.

Baby slings - Outer fabric of cotton, cotton/polyester or polyester. Fill/upholstery of polyester fiber or polyurethane (PU).

Baby chairs and loose chair linings / -cushions, cradle seats, sedan chairs, bouncy chairs - Exterior of cotton or cotton/polyester. Filling of polyester fiber.

Baby mattresses and mattress pads - Cover of cotton and cotton/polyester. Fill of polyurethane (PU), polyester, latex foam, PU foam with flame retardant, felt of flax.

Travel- and weekend beds - These products are not described by Tønning et al. (2008). The materials for the mattresses in these types of beds are supposed to be identical to the materials described for baby mattresses and mattress pads.

The market for the products

While there also is a major production of prams in Europe (approximately 50%), it is stated that other children's articles such as cradle seats, baby slings and buntings mostly come from countries outside the EU (approximately 80%). As with car safety seats, many of the manufacturers have their headquarters in Europe while the production takes place outside the EU. For most of the products it is estimated that one unit of the product is bought for each child. Since there is a certain re-use of the products, the market for each product is estimated to be less than the number of newborn children, which in Denmark is about 60,000 a year. The number of new products sold are on this background estimated to be in the range of 25,000-50,000 pcs./year.

The consumer product project on baby products by Tønning et al. (2008) estimated that the number of prams sold in Denmark were 25,000 to 40,000 pcs./year while the number of each of the other products was estimated at 40,000 to 60,000 pcs./year. In the current study it is assessed to be unlikely that the number of products sold corresponds to the number of babies (60,000 newborns per year), since all products will be recycled within the families to a certain degree of.

Products are sold in many of the same places as car safety seats, however, probably a larger share of the market covered by baby and children equipment stores and internet stores specialised in baby and children's equipment.

Response from distributors/vendors on chemical substances in other products

As with car safety seats it does not seem that distributors/vendors have any knowledge on the presence of harmful chemicals in these children's products, or if chemicals are added to achieve specific technical or functional properties such as water-repellancy or flame-retarding properties. A single producer stated that UV filters are used in hoods for prams, that flame retardants are used in the foam materials, and that PAHs can be present as impurities in the foam parts.

2.3 Chemical substances in textiles

2.3.1 Problematic chemical substances occurring in textiles

Table 2-1 gives an overview of substances or groups of substances that can potentially be found in textiles. The list is based on previous consumer product projects prepared for the Environmental Protection Agency (Poulsen et al., 2011; Rasmussen et al. 2013, Andersen et al., 2014) and a publication on chemicals in textiles from the Swedish Chemicals Agency (KemI, 2013a).

Some of the listed substance groups comprise many individual substances, of which only a few examples of the most wellknown individual substances are mentioned. It is emphasized that the list is not complete, but it gives a good overview of substances that potentially can be found in textile products. The substances are added either to achieve specific functional properties or are present as residues from the use of various process chemicals in the production of textiles.

TABLE 2-1

OVERVIEW OF CHEMICAL SUBSTANCES THAT POTENTIALLY CAN BE FOUND IN TEXTILES (SEE CLARIFICATION IN TEXT).

Substance function	Substance groups or substances	Example of a substance from the substance group	CAS no.	Restrictions on the use of the substance in relation to textiles according to REACH Annex XVII
Biocides, including preservatives, disinfectants and insecticides	Phenols, such as: Pentachlorophenol (PCP) Tetrachlorophenol (TeCP), trichlorophenol (TrCP), o-phenylphenol (OPP)	Pentachlorophenol	87-86-5	Shall not be placed on the market, or used, —as a substance, —as a constituent in other substances, or in mixtures, in a concentration equal to or greater than 0,1 % by weight
		Trichlorophenol	88-06-2	-
		<i>Ortho</i> -phenylphenol	90-43-7	-
	Organic tin compounds	Tributyltin	688-73-3	Tri-substituted organostannic compounds such as tributyltin (TBT) compounds and triphenyltin (TPT) compounds shall not be used after 1 July 2010 in articles where the concentration in the article, or part thereof, is greater than the equivalent of 0,1 % by weight of tin. Dibutyltin (DBT) compounds shall not be used after 1 January 2012 in mixtures and articles for supply to the general public where the concentration in the mixture or the article, or part thereof, is greater than the equivalent of 0,1 % by weight of tin. Diocetyl tin (DOT) compounds shall not be used after 1 January 2012 in the following articles for supply to, or use by, the general public, where the concentration in the article, or part thereof, is greater than the equivalent of 0,1 % by weight of tin: – textile articles intended to come into contact with the skin, – gloves, – footwear or part of footwear intended to come into contact with the skin,
		Triphenyltin		
		Diocetyl tin		

Substance function	Substance groups or substances	Example of a substance from the substance group	CAS no.	Restrictions on the use of the substance in relation to textiles according to REACH Annex XVII
				– wall and floor coverings, – childcare articles, – female hygiene products, – nappies, – two-component room temperature vulcanisation moulding kits (RTV-2 moulding kits)
	Dimethylfumarate (DMFu)		624-49-7	Shall not be used in articles or any parts thereof in concentrations greater than 0,1 mg/kg. Articles or any parts thereof containing DMF in concentrations greater than 0,1 mg/kg shall not be placed on the market.
	Formaldehyde		50-00-0	-
	Triclosan			-
	Silver compounds	Silver	7440-22-4	-
		Silver nitrate	7761-88-8	-
Flame retardants	Polybromo biphenyls (PBB)		59536-65-1	1. Shall not be used in textile articles, such as garments, undergarments and linen, intended to come into contact with the skin 2. Articles not complying with paragraph 1 shall not be placed on the market.
	Polybrominated	Octabromodiphenyl	32536-52-0	1. Shall not be placed on the market, or used:

Substance function	Substance groups or substances	Example of a substance from the substance group	CAS no.	Restrictions on the use of the substance in relation to textiles according to REACH Annex XVII
	diphenyl ethers (PBDE)	ether		– as a substance, – as a constituent of other substances, or in mixtures, in concentrations greater than 0,1 % by weight. 2. Articles shall not be placed on the market if they, or flame-retardant parts thereof, contain this substance in concentrations greater than 0,1 % by weight.
	Phosphorus-based flame retardants	Tris (1,3-dichloro-2-propyl) phosphate (TDCP)	13674-87-8	-
		Tris (1-chloro-2-propyl) phosphate (TCPP)	13674-84-5	-
		Tris (2-chloroethyl) phosphate (TCEP)	115-96-8	-
UV absorbers and stabilizers.	Benzophenones	2-Hydroxybenzophenone	117-99-7	-
		2,4-Dihydroxybenzophenone	131-56-6	-
		2,2'-Dihydroxy-4,4'-dimethoxybenzophenone	131-54-4	-
	Triazoles	2-Hydroxyphenyl-benzotriazoles		-
		Bumetrizole	3896-11-5	-

Substance function	Substance groups or substances	Example of a substance from the substance group	CAS no.	Restrictions on the use of the substance in relation to textiles according to REACH Annex XVII
	Triazines	2-Hydroxyphenyl-s-triazines		-
Plasticizers	Phthalates	Bis (2-ethylhexyl) phthalate (DEHP)	117-81-7	1. Shall not be used as substances or in mixtures, in concentrations greater than 0,1 % by weight of the plasticised material, in toys and childcare articles.
		Dibutyl phthalate (DBP)	84-74-2	2. Toys and childcare articles containing these phthalates in a concentration greater than 0,1 % by weight of the plasticised material shall not be placed on the market.
		Benzyl butyl phthalate (BBP)	85-68-7	3. The Commission shall re-evaluate, by 16 January 2010, the measures provided for in relation to this entry in the light of new scientific information on such substances and their substitutes, and if justified, these measures shall be modified accordingly. 4. For the purpose of this entry 'childcare article' shall mean any product intended to facilitate sleep, relaxation, hygiene, the feeding of children or sucking on the part of children.
		Di-isononyl-phthalate (DINP)	28553-12-0	1. Shall not be used as substances or in mixtures, in concentrations greater than 0,1 % by weight of the plasticised material, in toys and childcare articles which can be placed in the mouth by children.
		Di-isodecyl- phthalate (DIDP)	26761-40-0	2. Such toys and childcare articles containing these phthalates in a concentration greater than 0,1 % by weight of the plasticised material shall not be placed on the market.
		Di-n-octyl phthalate (DNOP)	117-84-0	3. The Commission shall re-evaluate, by 16 January 2010, the measures provided for in relation to this entry in the light of new scientific information on such substances and their substitutes,

Substance function	Substance groups or substances	Example of a substance from the substance group	CAS no.	Restrictions on the use of the substance in relation to textiles according to REACH Annex XVII
				and if justified, these measures shall be modified accordingly. 4. For the purpose of this entry ‘childcare article’ shall mean any product intended to facilitate sleep, relaxation, hygiene, the feeding of children or sucking on the part of children.
Dyes and dyeing ancillary materials	Azo dyes			1. Azodyes which, by reductive cleavage of one or more azo groups, may release one or more of the aromatic amines listed in Appendix 8, in detectable concentrations, i.e. above 30 mg/kg (0,003 % by weight) in the articles or in the dyed parts thereof, according to the testing methods listed in Appendix 10, shall not be used, in textile and leather articles which may come into direct and prolonged contact with the human skin or oral cavity, such as: – clothing, bedding, towels, hairpieces, wigs, hats, nappies and other sanitary items, sleeping bags, – footwear, gloves, wristwatch straps, handbags, purses/wallets, briefcases, chair covers, purses worn round the neck, – textile or leather toys and toys which include textile or leather garments, – yarn and fabrics intended for use by the final consumer. • 2. Furthermore, the textile and leather articles referred to in paragraph 1 shall not be placed on the market unless they conform to the requirements set out in that paragraph. 3. Azodyes, which are contained in Appendix 9, ‘List of azodyes’ shall not be placed on the market, or used, as substances, or in mixtures in concentrations greater than 0,1 % by weight, where the
	Aniline		62-53-3	
	Benzidine and/or its derivatives		92-87-5	

Substance function	Substance groups or substances	Example of a substance from the substance group	CAS no.	Restrictions on the use of the substance in relation to textiles according to REACH Annex XVII
				substance or the mixture is intended for colouring textile and leather articles.
Surfactants and wetting agents	Alkylphenols and alkylphenol ethoxylates	Nonylphenol	25154-52-3	Shall not be placed on the market, or used, as substances or in mixtures in concentrations equal to or greater than 0,1 % by weight for the following purposes: ... 3) textiles and leather processing except – processing with no release into waste water, – systems with special treatment where the process water is pre-treated to remove the organic fraction completely prior to biological waste water treatment (degreasing of sheepskin);
		Nonylphenol ethoxylater	-	
		Octylphenol		-
		Octylphenol Ethoxylates		-
Solvents	N-Methyl-2-pyrrolidone (NMP)		872-50-4	(Listed in Appendix 6 – Toxic to reproduction) Shall not be placed on the market as: – substances – as constituents of other substances or – in mixtures • for supply to the general public when the individual concentration in the substance or mixture is equal to or greater than: – either the relevant specific concentration limit specified in Part 3 of Annex VI to Regulation (EC) No 1272/2008, or, the relevant concentration specified in Directive 1999/45/EC

Substance function	Substance groups or substances	Example of a substance from the substance group	CAS no.	Restrictions on the use of the substance in relation to textiles according to REACH Annex XVII
				where no specific concentration limit is set out in Part 3 of Annex VI to Regulation (EC) No 1272/2008.
	N,N-Dimethylacetamide (DMAc)		127-19-5	(Listed in Appendix 6 – Toxic to reproduction) Shall not be placed on the market as: – substances – as constituents of other substances or – in mixtures • for supply to the general public when the individual concentration in the substance or mixture is equal to or greater than: – either the relevant specific concentration limit specified in Part 3 of Annex VI to Regulation (EC) No 1272/2008, or, the relevant concentration specified in Directive 1999/45/EC where no specific concentration limit is set out in Part 3 of Annex VI to Regulation (EC) No 1272/2008.
	N,N-dimethylformamide; dimethyl formamide (DMF)		68-12-2	Shall not be used in articles or any parts thereof in concentrations greater than 0,1 mg/kg. Articles or any parts thereof containing DMF in concentrations greater than 0,1 mg/kg shall not be placed on the market.
Impregnation agents	Fluorotelomer alcohols	6:2 FTOH		-
		8:2 FTOH		-
	Fluorotelomer	6:2 FTA		-

Substance function	Substance groups or substances	Example of a substance from the substance group	CAS no.	Restrictions on the use of the substance in relation to textiles according to REACH Annex XVII
	acrylates	8:2 FTA		-
	Perfluorinated alkyl sulfonates	Perfluorooctan sulfonic acid (PFOS)		(Listed in Appendix 6 – Toxic to reproduction) Shall not be placed on the marked as: – substances – as constituents of other substances or – in mixtures • for supply to the general public when the individual concentration in the substance or mixture is equal to or greater than: – either the relevant specific concentration limit specified in Part 3 of Annex VI to Regulation (EC) No 1272/2008, or, – the relevant concentration specified in Directive 1999/45/EC where no specific concentration limit is set out in Part 3 of Annex VI to Regulation (EC) No 1272/2008.
		Perfluorohexane sulfonic acid		-
	Perfluoroalkyl carboxylic acids	Perfluorohexane acid		-
		Perfluorooctanoic acid (PFOA)		-
	Perfluoroalkyl sulfonamides	Perfluorooctanesulfonamide (PFOSA)		-
		Perfluorooctanesulfonamidoethanol (N-Me-		-

Substance function	Substance groups or substances	Example of a substance from the substance group	CAS no.	Restrictions on the use of the substance in relation to textiles according to REACH Annex XVII
		FOSE)		
Pigments for printing and dyeing	Heavy metals	Chromium		A number of chromium compounds and salts (both Chromium (III), - (IV) and (VI) are listed in Appendix 1 and 2, entry 28 – Carcinogens and in Appendix 4, Entry 29 – Mutagens: 1. Shall not be placed on the marked as: – substances – as constituents of other substances or – in mixtures • for supply to the general public when the individual concentration in the substance or mixture is equal to or greater than: – either the relevant specific concentration limit specified in Part 3 of Annex VI to Regulation (EC) No 1272/2008, or, – the relevant concentration specified in Directive 1999/45/EC where no specific concentration limit is set out in Part 3 of Annex VI to Regulation (EC) No 1272/2008. Without prejudice to the implementation of other Community provisions relating to the classification, packaging and labelling of substances and mixtures, suppliers shall ensure before the placing on the market that the packaging of such substances and mixtures is marked visibly, legibly and indelibly as follows: ‘Restricted to professional users’.
		Nickel		Shall not be used: ...

Substance function	Substance groups or substances	Example of a substance from the substance group	CAS no.	Restrictions on the use of the substance in relation to textiles according to REACH Annex XVII
				b) in articles intended to come into direct and prolonged contact with the skin such as: ... – rivet buttons, tighteners, rivets, zippers and metal marks, when these are used in garments if the rate of nickel release from the parts of these articles coming into direct and prolonged contact with the skin is greater than 0,5 µg/cm²/week.
		Copper		-
		Cobalt		A number of cobalt compounds and salts are listed in Appendix 1 and 2, entry 28 – Carcinogens and in Appendix 6, Entry 30 – Toxic to reproduction: 1. Shall not be placed on the marked as: – substances – as constituents of other substances or – in mixtures • for supply to the general public when the individual concentration in the substance or mixture is equal to or greater than: – either the relevant specific concentration limit specified in Part 3 of Annex VI to Regulation (EC) No 1272/2008, or, – the relevant concentration specified in Directive 1999/45/EC where no specific concentration limit is set out in Part 3 of Annex VI to Regulation (EC) No 1272/2008. Without prejudice to the implementation of other Community

Substance function	Substance groups or substances	Example of a substance from the substance group	CAS no.	Restrictions on the use of the substance in relation to textiles according to REACH Annex XVII
				provisions relating to the classification, packaging and labelling of substances and mixtures, suppliers shall ensure before the placing on the market that the packaging of such substances and mixtures is marked visibly, legibly and indelibly as follows: 'Restricted to professional users'.
Other substances with no particular function, but may be present as impurities or of unknown origin	Polycyclic aromatic hydrocarbons (PAHs)	Benzo[a]pyrene (BaP)	50-32-8	Cf. Regulation 1272/2013 the five named PAHs should each occur only at concentrations lower than 0.5 mg / kg (weight percent) in toys and childcare articles that are marketed for the first time on and after 27 December 2015 and which contains rubber parts or plastic components that come into direct as well as prolonged or short-term repetitive contact with the human skin or the oral cavity under normal or reasonably foreseeable conditions of use.
		Benzo[e]pyrene	192-97-2	
		Benzo[a]anthracene	56-55-3	
		Chrysene	218-01-9	
		Benzo[b]fluoranthene	205-99-2	

The Swedish Chemicals Agency (KemI, 2013) has compiled a list of 1,900 chemicals that are known to be used in textile production. From this list 165 substances were identified as harmful based on their classification as either carcinogenic, endocrine-disrupting, mutagenic, toxic to reproduction, skin sensitizing and/or harmful to the environment. A few of the substances or substance groups in the above table, such as PAHs or triclosan, are not listed in Chemical Agency's list but have been included here.

2.3.2 Previous studies of chemicals in car safety seats

A number of studies on chemicals in car safety seats have been conducted in Denmark, Sweden, Germany and the United States. The survey results are summarized in the following paragraphs and in Table 2-2.

Danish and German Consumer Organisations (Danish Consumer Council, FDM, Stiftung Warentest and ADAC) have teamed up to be able to carry out a larger study of child car safety seats. The Danish Consumer Council has kindly made last year's results available for this survey (Danish Consumer Council, 2011; 2012; 2013).

The following parts of car safety seats were analysed:

- Cover (textile)
- Straps/belts
- The textile from the pads placed on the straps/belts (to improve comfort).

The plastic parts and the fill material was not included in the analysis programme, which comprised the following substances: 18 PAHs, 11 phthalates, 7 phosphorus-based flame retardants, 3 phenols (phenol, nonylphenol, bisphenol A), 6 organotin compounds, 57 azo dye indicators (aromatic amines), 8 metals, 6 brominated flame retardants and formaldehyde. A full list of the analysed substances can be found in Appendix 2. However, some of the materials were not analysed for all substances.

Table 2-2 only shows results for substances that actually were present in the respective materials in 2013. Phosphorus-based flame retardants were the only substances which were found in a large number of samples (eg. TCPP was found in the cover of the 14 seats) and in concentrations that suggests an intended functionality. A single car seat had a high concentration of the phthalate DEHP in the cover. The concentrations of most other substances, which were detected in only a few seats, were so low that they were considered residues or impurities in the materials. This applies to e.g. PAHs, organotin compounds and formaldehyde.

Overall in the 2013 analysis programme, 34 chemicals were found in the various materials (from 40 seats), while in 2012, 22 chemicals were identified (from 31 seats), and in 2011, there were 50 findings (from 38 seats). In the studies from 2011 and 2012, it was the phosphorus-based flame retardants that occurred in the greatest number of samples and in the highest concentrations (up to 143,600 mg TDCP/kg and up to 24,060 mg TCPP/kg). In 2011, also phthalates were found in significant concentrations in 6 out of 38 seats (up to 14,025 mg/kg) and brominated flame retardants in 4 out of 38 seats (up to 7,750 mg/kg). In 2012, no phthalates were detected, while brominated flame retardants were found in the cover of 3 out of 31 seats in concentrations up to 6,300 mg/kg.

In 2011, the US consumer organization Ecology Center (HealthyStuff, n.n.) studied 150 seats including many brands, which are also sold in Europe and Denmark. According to the summary of the study there were harmful chemicals in 60% of car safety seats. However, it has not been possible to identify which substances were included or how the car safety seats were tested.

The Swedish Chemicals Inspectorate (KemI) has in 2013 analysed 12 car safety seats and additionally two other childrens' chair covers for brominated flame retardants, dyes, perfluorinated compounds (PFOS/PFOA), phthalates, lead, formaldehyde and organic tin compounds (KemI, 2013b; unpublished).

The samples were taken from different parts of car safety seats:

- Textiles
- Fill and foam
- Hard and soft plastics.

The substances were found in a few samples, while the vast majority of the samples did not contain any of the mentioned substances. Formaldehyde was an exception, which was detectable in approximately one third of the fill and foam samples, but not the textile parts (KemI, 2013b).

TABLE 2-2

OVERVIEW OF SUBSTANCES FOUND IN PREVIOUS STUDIES ON CAR SAFETY SEATS. ONLY SUBSTANCES ACTUALLY DETECTED IN ONE OR MORE SAMPLES ARE INCLUDED IN THE TABLE. ADDITIONAL SUBSTANCES, WHICH WERE EXAMINED, ARE MENTIONED IN THE BODY TEXT. IN IF THE INVESTIGATION NOT COVERED ANALYSES OF EACH SUBSTANCE IN CERTAIN MATERIALS IS THIS SET.

Substance or substance group	Material	Detected substances, concentration range, comments (if any)	No. detect./ No. of samples	Source
Brominated flame retardants	Textile (cover)	Decabromodiphenyl ether 19 mg/kg	1/50	KemI, 2013b
	Plastic, fill and foam	These materials were not analysed for brominated flame retardants	-	KemI, 2013b
	Textile (pads)	Decabromodiphenyl ether 500 mg/kg	1/40	Danish Consumer Council, 2013
Azo dye indicators	Textile (cover)	Diaminodiphenylmethane 6 mg/kg, 1,4-Phenylenediamine 96 mg/kg, Aniline 157 mg/kg	2/50	KemI, 2013b
	Textile (pads)	Disperse orange 97 mg/kg	1/40	Danish Consumer Council, 2013
Phthalates	Plastic	DEHP 130 mg/kg – 113,000 mg/kg	2/18	KemI, 2013b
	Textile (cover)	DEHP 33,000 mg/kg	1/40	Danish Consumer Council, 2013

Substance or substance group	Material	Detected substances, concentration range, comments (if any)	No. detect./ No. of samples	Source
	Textile (pads)	DINP 175 mg/kg DIDP 3,175 mg/kg	1/40	Danish Consumer Council, 2013
Lead	Textile	7.5 mg/kg	1/50	KemI, 2013b
Formaldehyde	Fill and foam	< LoD - 28 mg/kg	9/24	KemI, 2013b
	Textile (cover)	15 -45 mg/kg	3/40	Danish Consumer Council, 2013
	Textile (pads)	27 mg/kg	1/40	Danish Consumer Council, 2013
Organic tin compounds	Fill and foam	DBT 0.012 mg/kg	1/24	KemI, 2013b
	Textile (cover)	MOT 0.31 – 2.15 mg/kg DOT 0.41 – 35 mg/kg DBT 0.08 – 0.9 mg/kg MBT 0.13 mg/kg	3/40 4/40 2/40 1/40	Danish Consumer Council, 2013
	Textile (pads)	MOT 0.15 – 1.7 mg/kg DOT 0.41 – 35 mg/kg DBT 0.068 mg/kg	4/40 4/40 1/40	Danish Consumer Council, 2013
PAH	Textile (cover)	Naphthalene 3 mg/kg Phenanthrene 0.54 mg/kg Pyrene 0.38 (all detected in the same seat)	1/40	Danish Consumer Council, 2013

Substance or substance group	Material	Detected substances, concentration range, comments (if any)	No. detect./ No. of samples	Source
	Textile (pads)	Phenanthrene 0,2 – 0,47 mg/kg Fluoranthene 0,68 mg/kg Pyrene 0,39 – 1,5 mg/kg BaP 0,34 mg/kg Chrysene 3,7 mg/kg Benzo[a]anthracene 0,45 mg/kg	3/40	Danish Consumer Council, 2013
Phosphorus-based flame retardants	Textile (cover)	TCEP 173 mg/kg	1/40	Danish Consumer Council, 2013
		TCP 5.6 – 19,000 mg/kg	14/40	
		TDMP 20 – 148,000 mg/kg	6/40	
		Triphenylphosphate 8.3 – 250 mg/kg	6/40	
	Textile (pads)	TCEP 255 mg/kg	1/40	Danish Consumer Council, 2013
		TCP 5.7 – 12,000 mg/kg	11/40	
		TDMP 26 – 56,900 mg/kg	6/40	
		Triphenylphosphat 5.1 - 750 mg/kg	3/40	
Phenols	Textile (cover)	Phenol 27 – 255 mg/kg Bisphenol A < LoD	6/40	Danish Consumer Council, 2013
	Textile (pads)	Phenol 23 – 460 mg/kg Nonylphenol 160 mg/kg	1/40 1/40	Danish Consumer Council, 2013

2.3.3 Previous studies of chemical substances in other textile products for children

Baby slings

The Danish Consumers' Council published in early 2014 a study on baby carriers, in which 13 products were analysed for a variety of substances. In the analysis programme the following substances were included: PAHs, phthalates, phosphorus-based flame retardants, formaldehyde and nonylphenol (Danish Consumer Council, 2014a). In contrast to other studies, the various components of the products (belts, plastic parts, textiles and possible fill materials) were mixed to produce composite samples. Excluded from this approach were the analyses for formaldehyde and nonylphenol, which were only applied to the textile parts. Only phosphorus-based flame retardants and nonylphenol ethoxylates were found to a significant degree in the baby slings as can be seen from Table 2-3.

In a previous consumer product project on baby products (Tønning et al., 2008) the textile parts of two baby slings were analysed. The following substances (-groups) were included in the specific

analysis program: azo dyes, chlorinated benzenes and toluene, chlorinated phenols, phthalates, formaldehyde, bromine, chlorine, tin, antimony, organic tin compounds and selected phosphorus-based flame retardants. Not all substances were analysed in all products or in all parts of the individual products. For example the baby slings were not analysed for phosphorus-based flame retardants. In the baby slings, chlorine, tin and phthalates were detected in all samples tested, as shown in Table 2-3. The occurrence of tin could, however, not be related to the presence of selected organic tin compounds.

TABLE 2-3
OVERVIEW OF SUBSTANCES FOUND IN PREVIOUS STUDIES OF BABY SLINGS.

Substance or substance group	Material	Detected substances, concentration range, comments (if any)	No. detect./ No. of samples	Source
PAH	Composite	Phenanthrene 0.36 mg/kg No ther PAH's were found	1/13	Danish Consumer Council, 2014a
Phosphorus-based flame retardants	Composite	TCEP 18 mg/kg TCPP 14 – 5,500 mg/kg TDCP 4,400 mg/kg	1/13 3/13 1/13	Danish Consumer Council, 2014a
Nonylphenol-ethoxylates	Textile	> 300 mg/kg	1/13	Danish Consumer Council, 2014a
Chlorine	Fill and foam	49 – 110 mg/kg	2/2	Tønning al., 2008
Tin	Fill and foam	381 – 468 mg/kg	2/2	Tønning al., 2008
Phthalates	Plastic parts	DEP 60-350 mg/kg DIBP 25 – 760 mg/kg	1/1	Tønning al., 2008

Prams

In another study by the Danish Consumer Council the presence of chemicals in 7 prams was investigated (results available for 6 products, Danish Consumer Council, 2014b). Composite samples consisting of parts from the liner/lining of, apron and hood were analysed for phthalates and phosphorus-based flame retardants. Samples from the apron alone was additionally analysed for polyfluorinated alkyl compounds (PFAS). The phthalate DEHP was found in a single pram at a very high concentration but apart from that only phosphorus-based flame retardants occurred in measurable concentrations (Table 2-4).

The aforementioned consumer product project on baby products (Tønning et al., 2008) also included analyses of upholstery, foam and plastic parts from two pram aprons. Tin was found in both foam and fabric, but the presence of tin could not be related to the presence of selected organic tin compounds. The prams also contained bromine in relatively high concentrations in the textile (2 out of 4 samples), which could indicate the presence of brominated flame retardants. Some samples also contained phthalates, phosphorus and polyfluorinated compound 6:2 FTOH, however only in very low concentrations.

The study of pram aprons included a number of volatile organic compounds, which were analysed by a semi-quantitative screening. Some substances were found in relatively low concentrations but could not be detected in the majority of samples. The results from the screening are not presented here.

TABLE 2-4
OVERVIEW OF SUBSTANCES FOUND IN PREVIOUS SURVEYS OF PRAMS

Substance or substance group	Material	Concentration range, comments (if any)	No. detect./ No. of samples	Source
Bromine	Textile	1220 – 1320 mg/kg	2/4	Tønning al., 2008
	Foam	< LoD	0/4	Tønning al., 2008
Chlorine	Textile	513 -1.600 mg/kg	2/2	Tønning al., 2008
	Foam	22 – 106 mg/kg	3/3	Tønning al., 2008
Fosfor	Textile	64 mg/kg	1/2	Tønning et al., 2008
	Foam	< LoD	0/4	Tønning et al., 2008
Tin	Textile	41 mg/kg	1/2	Tønning et al., 2008
	Foam	258 – 658 mg/kg	3/4	Tønning et al., 2008
Phthalates	Textile	DEHP 40 mg/kg	1/4	Tønning et al., 2008
	Composite	DEHP 74.000 mg/kg	1/6	Danish Consumer Council, 2014b
	Hood	DEHP 211,000 mg/kg The analysis was repeated on the hood due to the high conc. found in the composite sample	1/6	Danish Consumer Council, 2014b
Phosphorus-based flame retardants	Textile	TCEP < LoD Tributyl phosphate 2 mg/kg Triphenyl phosphate 0.4 mg/kg	1/3	Tønning et al., 2008
	Foam	TCEP < LoD Triphenyl phosphate 5 mg/kg	1/4	Tønning et al., 2008

Substance or substance group	Material	Concentration range, comments (if any)	No. detect./ No. of samples	Source
	Composite	TCEP 320 mg/kg TCPP 6.9 – 23 mg/kg TDCP 3300 – 44 mg/kg Triphenyl phosphate 5.1 – 30 mg/kg	1/6 4/6 2/6 3/6	Danish Consumer Council, 2014b
Polyfluorinated alkyl substances (PFAS)	Textile*	6:2 FTOH 6 mg/kg	1/1	Tønning et al., 2008
	Textile	PFOA 0.023 – 0.026 mg/kg	2/6	Danish Consumer Council, 2014b

* From the screening analysis (Tønning et al., 2008), not the specific analysis.

Nursing cushions

In the consumer product project on baby products by Tønning et al. (2008) also two nursing cushions were analysed. In one of them, high concentrations of phthalates (144,000 to 220,000 mg/kg) were detected. Tin was found in both cushions but could not be related to the presence of selected organic tin compounds.

Baby mattresses

The same project on baby products (Tønning et al., 2008) also included chemical analyses of two baby mattresses, which were analysed for a few substances (formaldehyde, bromine, phosphorus, chlorine, tin, antimony, and selected organic tin and phosphorus compounds). Only tin and chlorine was detected in foam samples from both mattresses, but the content of tin could not be related to the presence of selected organic tin compounds.

2.4 Ecolabel and criteria

There are a number of eco-labels that have established requirements to the content of certain chemical substances in textiles. The requirements are briefly reviewed below as they can give an indication of the problematic substances that may occur in textiles, which are not eco-labeled.

Oeko-Tex

The concept of the Oeko-Tex ® Standard 100 is to achieve uniform standards for the use of chemicals in textile production and to set up environmental criteria for potentially hazardous substances in textiles.

The Oeko-Tex tests and criteria are divided into different classes of products depending on the intensity of skin contact:

- Product Class I: Textiles and toys for babies and small children up to 3 years of age, e.g. underwear, rompers, bedding, soft toys etc.
- Product Class II: Textiles with a large part of their surface in direct contact with the skin, such as underwear and linen.

- Product Class III: Textiles with no or only a small part of their surface in direct contact with the skin, e.g. jackets.
- Product Class IV: Home textiles such as table cloths, curtains and carpets.

In many cases the limit values for products in Product Class 1 are lower than in the other product classes, which is because the products in this class are intended for children under 3 years of age. Table 2-5 shows the substances that are banned or restricted in relation Oeko-Tex 100 standard for Product Class 1.

TABLE 2-5
LIST OF SUBSTANCES WITH OEKO-TEX CRITERIA

Substance or substance group	Examples	Product Class I criteria (mg/kg) ¹
Formaldehyde	-	<LoD
Extractable metals	Sb (Antimony)	30.0
	As (Arsenic)	0.2
	Pb (Lead)	0.2
	Cd (Cadmium)	0.1
	Cr (Chromium) / Cr(VI)	1.0 / <LoD
	Co (Cobalt)	1.0
	Cu (Copper)	25.0
	Ni (Nickel)	1.0
	Hg (Mercury)	0.02
Dissolved heavy metals	Pb (Lead)	90.0
	Cd (Cadmium)	50.0
Pesticides	Sum (up to 60 pesticides) ²	0.5
Chlorophenols	Pentachlorophenol (PCP)	0.05
	Tetrachlorophenols (TeCP), Sum	0.05
	Trichlorophenols (TrCP), Sum	0.2
Phthalates	DINP, DNOP, DEHP, DIDP, BBP, DBP, DIBP, DIHP, DHNUP, DHP, DMEP, DPP, Sum	0.1 w-%
Organic tin compounds [mg/kg]	TBT	0.5

Substance or substance group	Examples	Product Class I criteria (mg/kg) ¹
	TPhT	0.5
	DBT	1,0
	DOT	1,0
Other chemical residues	OPP (o-Phenylphenol)	50
	Aryl amines ²	No limit value
	SCCP [w-%]	0.1 w-%
	TCEP [w-%]	0.1 w-%
	DMFu	0.1
Dyes	Cleavable aryl amines ²	Not used
	Carcinogens ²	Not used
	Allergens ²	Not used
	Others ²	Not used
Chlorobenzenes and Toluenes	Sum	1.0
Polycyclic aromatic hydrocarbons	Benzo[a]pyrene	0.5
	Benzo[e]pyrene	0.5
	Benzo[a]anthracene	0.5
	Chrysene	0.5
	Benzo[b]fluoranthene	0.5
	Benzo[j]fluoranthene	0.5
	Benzo[k]fluoranthene	0.5
	Dibenzo[a,h]anthracene	0.5
	Sum PAH ²	5.0
Biologically active products	-	None
Flame retardants	General	None
	PBB, TRIS, TEPA, pentaBDE, octaBDE, DecaBDE, HBCDD , SCCP, TCEP ²	Not used

Substance or substance group	Examples	Product Class I criteria (mg/kg) ¹
Solvents	NMP ³	0.1
	DMAc	0.1
	DMF	0.1
Detergents, moisturizers and residues hereof	OP, NP, Sum	10.0
	OP, NP, OP(EO)1-20, NP(EO)1-20 Sum	250.0
Polyfluorinated substances	PFOS	1.0 µg/m ²
	PFOA	0.05
	PFUdA	0.05
	PFDaA	0.05
	PFTTrDA	0.05
	PFTeDA	0.05
Emissions of volatile substances	Formaldehyde	0.1 mg/m ³
	Toluene	0.1 mg/m ³
	Styrene	0.005 mg/m ³
	Vinyl cyclohexene	0.002 mg/m ³
	4-Phenylcyclohexene	0.03 mg/m ³
	Butadiene	0.002 mg/m ³
	Vinyl chloride	0.002 mg/m ³
	Aromatic hydrocarbons	0.3 mg/m ³
	Volatile organic substances	0.5 mg/m ³

¹ In some cases other concentration units are used.

² The full list of substances is available at https://www.oeko-tex.com/en/manufacturers/test_criteria/limit_values/limit_values.html.

³ With the exception of spun dyed fibres used for PPE (polyphenyl ethers): 3,0%

The EU Flower and the Swan

An updated criteria document establishing the ecological criteria for the award of the EU Ecolabel to textile products was published in July 2014 (European Commission, 2014). The Criteria Document sets limit values for a wide range of chemicals, divided into categories according to the production stages of the supply chain for textile products.

The criteria for the EU Flower are largely the same as the Oeko-Tex criteria. E.g. the threshold values for heavy metals and phosphorus-based flame retardants are the same. A detailed list of the restrictions on chemicals can be found in Appendix 1 of the criteria document (European Commission, 2014).

The Nordic Ecolabel (the Swan) has also developed a criteria document for eco-labeling of textiles, skin and leather (Nordic Ecolabelling, 2013). The Swan generally follows the EU Flower criteria but it should be noted that the Nordic criteria have not yet been updated.

2.5 Mapping of waste streams

No information on the specific product groups in relation to waste disposal and recycling etc. has been identified. Thus, there is no identified data on volumes of the various product types in the waste streams.

As for car safety seats, it is known that some reception stations/recycling centres prescribe delivery of car safety seats in the fraction "hard plastic", presumably for reuse/recycling of this part of the seat. The other components of car safety seats, mainly the textile and foam parts, are assumed just to be dropped off in the "small combustibles" fraction and thus go to waste incineration and subsequent end disposal (including use of slag for different (construction/fill) purposes).

Mattresses (other than spring mattresses, which are not relevant in this context) are generally considered to be "small combustibles" and should thus also prevailingly be disposed of by incineration. There were no specific regulations for baby slings, however these are also likely to be part of the fraction "small combustibles" just like the textile-based parts of the other textile product types in the survey.

Metal frames of prams, strollers and weekend beds etc. are disposed of as "metal", but this is not a relevant waste fraction in relation to this project.

3. Preliminary hazard and exposure assessment

A preliminary hazard screening was carried out with emphasis on the health hazard aspects related to the substances/substance groups that, according to the studies identified in the literature- and questionnaire based survey, previously have been found in safety car safety seats and other textile products for children. The purpose of this screening was primarily to identify the substances that from a purely hazard point of view (based primarily on the substances' classification) would be most relevant to include in the chemical analysis programme making up the next phase of the project. As the survey included a significant number of product types, the screening hazard assessment was supplemented by an initial exposure assessment to be able to set priorities and focus the analysis phase as deemed necessary in light of the limitations in the overall framework of the project.

The initial assessments have provided the basis for the more detailed descriptions and assessments in phase 3 of the project i.e. the actual human health and environmental assessment based on the quantitative results obtained in phase 2 (chemical analysis phase). To avoid unnecessary repetition, the majority of the descriptions and –assessments made in the screening have been moved and are elaborated further in Chapter 6 (health assessment) and Chapter 7 (environmental assessment), respectively. Please see these two chapters for details.

The initial review of the health and environmental classifications for a number of substances or substance groups identified in the survey, which may occur in textiles for children (but not necessarily in the specific products purchased) can be found in Annex 3.

3.1 Preliminary health hazard screening

3.1.1 Health effects

The substances/groups of substances that on basis of the performed survey were included in the preliminary hazard assessment are the following:

- Chlorophenols and alkylphenols (and their ethoxylates)
- Organic tin compounds
- Dimethylfumarate (DMFu)
- Formaldehyde
- Triclosan
- Silver and silver compounds
- Brominated flame retardants
- Phosphorus-based flame retardants
- UV absorbers and stabilizers.
- Phthalates
- Azo dyes
- N-methyl-2-pyrrolidone (NMP)
- N, N-dimethylacetamide (DMAc)
- N, N-dimethylformamide (DMF)
- Fluoroalkylated substances (PFAS)
- Heavy metals
- Polycyclic aromatic hydrocarbons (PAH)

Below is a brief description of the health properties of the above, potentially occurring substances, primarily from their classifications, but also a few other data sources (see Annex 3):

Some of the **chlorinated phenols** are suspected of being carcinogenic, while nearly all of them are known irritants when in contact with skin. 2,4-dichlorophenol is on the EU list of substances suspected of being endocrine disruptors (Cat. 2).

Alkylphenol ethoxylates are degraded to **alkylphenols** among which nonylphenol is the best studied. Nonylphenol may impair fertility and the unborn child during pregnancy. Furthermore, the substance is suspected of causing allergy and is considered to be an endocrine disruptor. Five alkylphenols are on the EU list of suspected endocrine disruptors. Of these, nonylphenol and 4-tert-octylphenol are classified in Cat. 1 while 4-tert-butylphenol is classified in Cat. 2.

For the group of **tributyltin compounds (TBT)** and for **triphenyltin hydroxide** a harmonised classification has been adopted, while for other substances from this substance group no such classification exist. TBT can cause moderate to severe skin and eye irritation, and there are indications of irritation of the respiratory tract after inhalation. The biggest concern with respect to organotin compounds is however the environmental effects, since some substances from the group have been shown to be endocrine disrupting in the environment and affect the immune system in the long term.

Dimethyl fumarate (DMFu) has no harmonized classification. Harmful when in contact with skin, skin irritant, may cause allergic skin reaction, and eye irritant are the most frequent effects mentioned in the self-classifications of the substance. In the EU thousands of cases of allergy to DMFu have been reported, and the substance is therefore now regulated under REACH and is not permitted to occur in articles in concentrations higher than 0.1 mg/kg.

Formaldehyde can cause cancer in humans. Furthermore, the substance is classified as toxic by ingestion, inhalation or skin contact and can cause allergic skin reaction and irritate the skin and eyes.

Triclosan is suspected of being an endocrine disruptor and, in addition, suspected to increase the risk of developing allergies in children to other substances because of triclosan's effect on bacteria and thereby the immune system (forbruger kemi.dk). The substance has recently been banned as a biocide in textiles and is also regulated in cosmetic products.

Silver or silver compounds can, like triclosan, be added to textiles as an antibacterial agent. Nano-sized silver has been found in clothes, especially sportswear, where its function is to prevent bad odours. The health effects of nano-silver are not well documented, while the high toxicity in the aquatic environment is assessed primarily to be due to dissolved silver (silver ions).

Some of the **brominated flame retardants** are suspected of being harmful to fetuses, to induce cancer, disrupt the hormone balance and reduce fertility. The group of polybrominated biphenyls and four of the brominated diphenyl ethers are on the EU list of endocrine disrupting substances (Cat. 1 and 2, respectively).

The most common **phosphorus-based flame retardants** in textile products are TCPP, TDCP and TCEP. The substances are used only in the foam and fill material, not in the fabric itself. Two of the substances are classified as carcinogenic (TDCP and TCEP), while TCEP is also classified as toxic to reproduction.

There are three main groups of **UV protective substances** in textiles: Benzophenones, triazoles and triazines. Some benzophenones are suspected of being carcinogenic, endocrine disrupting

and/or harmful to the unborn child, while others are not considered to be problematic to the same degree. None of the identified substances have a harmonized classification.

The **phthalates** include several substances of which six (DEHP, DBP, BBP, DINP, DIDP, DNOP) are regulated under REACH. Several of the phthalates affect fertility and/or are harmful to the unborn child. Additionally, many are suspected of being endocrine disruptors, while some phthalates are considered to be largely unproblematic.

Azo dyes may give rise to the formation of a series of aromatic amines in textiles such as biphenyl-4-amine, or benzidine. Many of the aromatic amines can cause cancer and a number of the substances are classified as skin allergens or toxic to reproduction.

The health effects of **N-methyl-2-pyrrolidone (NMP)** include irritation of the mucous membranes of the eyes and respiratory tract in humans, drowsiness and irregular breathing, and effects on fetus development. Additionally, NMP penetrates easily through the skin and may thus increase skin penetration of other substances.

N, N-Dimethyl acetamide is classified as toxic to reproduction (may harm the unborn child) and as hazardous by inhalation and skin contact. The chronic effects include possible "chronic liver and kidney damage." The substance may produce systemic injuries when inhaled or absorbed through skin over an extended period.

Dimethyl formamide is classified as hazardous by skin contact and inhalation, as well as eye irritating and toxic to reproduction (may harm the unborn child). Although animal studies failed to demonstrate that the substance is mutagenic or carcinogenic, several tests of persons who have been exposed to dimethyl formamide indicate that the substance may contribute to the development of testicular cancer.

Polyfluorinated alkyl substances, PFAS, can be divided into a large number of sub-groups in which the substances' health properties seem to be more closely linked to the length of the perfluoroalkyl (C_nF_{2n+1} or $F(CF_2)_n$) than to the functional group of the molecule. Substances with a perfluoroalkyl chain of 6-8 carbon atoms appear to be the most harmful. Only the C_8 compounds PFOS and its derivatives have a harmonized classification under CLP. According to the classification the substances are carcinogenic as well as toxic to reproduction.

Among the **heavy metals**, which occur mainly in the textile part, either due to their use for dyeing or printing, or simply as an impurity, are chromium, nickel, copper and cobalt.

Chromium (VI) compounds are highly toxic and can cause allergy by skin contact. Following long-term exposure to chromium (VI) various forms of gastrointestinal discomfort and increased incidence of lung cancer have been observed.

Nickel can cause skin allergy and sensitization to nickel can lead to contact dermatitis. In addition, exposure via inhalation in the work environment has led to increased risk of lung cancer.

Long-term oral exposure to copper can lead to liver damage. Copper and copper salts can produce allergic reactions by skin contact in sensitive individuals.

Cobalt is sensitizing and has the classification "may cause an allergic skin reaction" and "may cause allergy or asthmatic symptoms or breathing difficulties if inhaled."

PAH (polycyclic aromatic hydrocarbons) is a group of substances comprising more than 100 individual substances, which are composed of two or more fused benzene rings. Benzo[a]pyrene

(BaP) is often used as an indicator of the occurrence and health effects of PAHs and is also the most studied substance from the group. BaP and certain other PAHs have been shown to be toxic by inhalation, ingestion, or skin absorption. Some are carcinogenic, mutagenic and toxic to reproduction.

3.1.2 Health exposure assessment

Exposure to chemicals in children's articles

The project focuses on product types with textile parts specially developed for (small) children that the child will lie or sit in and thereby give rise to significant skin contact or that children can be expected to lick/suck on or put in the mouth. Clothes and toys are not included in this survey.

Children's possible exposure via the different product types have been evaluated qualitatively by the project team by considering the different routes of exposure (dermal, oral and inhalation exposure), the length of the use period and the distribution of the different product types in Denmark. The exposure scenarios are summarized in Table 3-1.

It can be seen from the table that car safety seats generally are considered to have a high exposure score, owing both to a long period of use, the frequent use, possibility of relatively high skin contact and oral exposure and the ubiquity of such seats among Danish families. Baby mattresses (as a separate product or as a component part of carrycots, pushchairs, weekend beds etc.) were also assessed to result in high exposure.

TABLE 3-1
OVERALL INITIAL EXPOSURE ASSESSMENT OF TEXTILE PRODUCTS FOR CHILDREN.

Product	Exposure pathway	Typical age group and period of use	Typical duration of exposure	Distribution in the population	Overall exposure assessment
Car safety seats (incl. booster seats)	Both dermal, oral and inhalation. Dermal exposure will occur especially in the summer when kids are sitting in the car seat in short-sleeved clothes/short pants and also sweats. Oral exposure will occur to a lesser extent with certain parts of the seat, for example belts and buckles or the side of the headrest. Inhalation exposure will especially occur in the first period of the useful life and in the summer where high temperatures cause greater evaporation of possible volatile substances. Car safety seats are furthermore used inside a car where the air exchange typically is limited.	0 – up to 12 years of age, thus used for almost the entire childhood.	Daily / several times a week with a useful life of 0.5 - 1 hours/event.	Very common	High
Baby mattresses	Found in beds, carrycots and prams. Both dermal, oral and inhalation. There will be limited skin contact as beddings or similar is typically used in the pram and kids will typically also be wearing clothes. Dermal exposure will therefore primarily be the hands and feet. Oral exposure can occur, especially from the textile cover of the mattress, but not from the foam part. Inhalation exposure will occur especially in the first period of their useful life. Bed mattresses are used indoors, where there is generally poor air exchange, while the carrycot and lift mattresses are used both indoors and outdoors.	Pram/carrycot mattresses: 0 - 2 years, daily Bed mattresses: from 0 years, daily	Pram/carrycot mattresses: 3 hours/event Bed mattresses: 12 hours/daily	Very common	High
Baby sling	Both dermal, oral and inhalation. Dermal exposure will occur especially in the summer when kids are wearing short-sleeved clothes/shorts and also sweats.	0-1 year, daily – several times per week	1 hour/event	Common	Medium

Product	Exposure pathway	Typical age group and period of use	Typical duration of exposure	Distribution in the population	Overall exposure assessment
	Oral exposure will occur to a lesser extent with certain parts of the seat, for example belts and buckles or the side of the headrest. Inhalation exposure will especially occur in the first period of the useful life.				
Cradle seat	Both dermal, oral and inhalation. Dermal exposure will occur especially in summer when the kids are sitting in the cradle seat in short-sleeved clothes/short pants and also sweats. Oral exposure will occur to a lesser extent with certain parts of the seat, for example belts and buckles or the side of the headrest. Inhalation exposure will especially occur in the first period of the useful life.	Babies of less than 6 months for approx. 3 months	1 – 2 times daily, about 15 min/event	Very common	Medium
Prams	Both dermal, oral and inhalation. There will be limited skin contact as beddings or similar is typically used in the pram and kids will typically also be wearing clothes. Dermal exposure will therefore primarily be the hands and feet exposed to the sides of the pram. Oral exposure can occur, especially from the textile cover of the mattress, but not from the foam part. Inhalation exposure will especially occur in the first period of the useful life and in the summer where high temperatures cause greater evaporation of possible volatile substances. Prams are however typically used outside where the air exchange is good.	0 - 2 years, daily	3 hours/event	Very common	Medium
Strollers	Both dermal, oral and inhalation. Dermal exposure will occur especially in summer when the kids are	0.5 - 3 years, daily	3 hours/event	Very common	Medium

Product	Exposure pathway	Typical age group and period of use	Typical duration of exposure	Distribution in the population	Overall exposure assessment
	sitting in the stroller in short-sleeved clothes/short pants and also sweats. Oral exposure will occur to a lesser extent with certain parts of the seat, for example belts and buckles or the side of the headrest. Inhalation exposure will especially occur in the first period of the useful life and in the summer where high temperatures cause greater evaporation of possible volatile substances. Strollers are however typically used outside where the air exchange is good.				
Bunting bags	Both dermal, oral and inhalation. Dermal exposure will be limited as the children typically will be wearing long-sleeved shirts and pants in those seasons where the bunting bags are used the most. Children will primarily touch the bunting bag with their hands/arms, but will otherwise be dressed. Loose parts such as straps and the sides of the head piece can be placed in the mouth and may result in an oral exposure. Inhalation exposure will especially occur in the first period of the useful life, but will be limited as the product is typically used outdoors in the colder seasons.	Up to 2 years of age, approx. half of the year	Daily/several times per week, about 2 hours/event on the average	Very common or common	Medium / low
Travel and weekend beds	Both dermal, oral and inhalation. There will be limited skin contact as bed linen and night clothes are typically used. Dermal exposure will therefore primarily be the hands and feet exposed to the sides of the pram. Oral exposure will occur to a lesser extent with certain parts of the bed, for example the sides. Inhalation exposure may occur especially in the first part of the period of	2 years, approx. every 2nd week	10 hours/event	Very common	Low

Product	Exposure pathway	Typical age group and period of use	Typical duration of exposure	Distribution in the population	Overall exposure assessment
	use				
Baby joggers	Similar to the pathways of the stroller	0.5 - 3 years, several times per week	1 hour/event	Less common	Low
Carriers	Both dermal, oral and inhalation. Dermal exposure will occur especially in the summer when the kids are wearing short-sleeved clothes/shorts and are also sweating. Oral exposure will occur to a lesser extent with certain parts of the carrier, for example belts and buckles or the side of the headrest. Inhalation exposure will especially occur in the first period of the useful life. Carriers are primarily used outdoor.	From 1 - 2 years, approx. every 2nd week	1 hour/event	Common	Low

Selection of product groups for further investigation (chemical analysis)

Based on the initial exposure assessment, car safety seats, baby slings, prams/strollers/carrycots and cradle seats appear to be the product types that overall give rise to the largest exposures. Prams, strollers and carrycots, however mainly because of the baby mattresses being part of the products. The Environmental Protection Agency has presently a special focus on phosphorus-based (chlorinated) flame retardants, which leads to focusing also on products with foam materials, where these substances are considered likely to occur.

Moreover, in consultation with the Danish EPA it was decided to limit the number of product types to be included in the specific studies (chemical analysis), to three in order to be able to obtain a satisfactory coverage of the individual product types within the project's economic framework. Due to these considerations in combination with previous findings of chemical substances in products as well as a desire to focus particularly on products for babies/infants, it was decided that the analysis and test programme should include the following product types:

- Car safety seats
- Baby slings
- Baby mattresses (incl. mattresses in prams, baby lifts, etc.)

The calculation of the exposure, which children may be exposed to, is made on the basis of realistic worst-case scenarios to illustrate the potential dermal, oral and inhalatory exposure of children primarily aged 0-3 years. The exposure scenarios follow the REACH guidance, "Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.15 - Consumer exposure estimation", Version 2.1 from April 2012 (ECHA, 2012b).

The concrete method for calculation and assessment of oral, dermal and inhalatory exposure of children to chemicals in the mentioned products is described in detail in Chapter 6.

3.2 Initial environmental hazard screening

An environmental hazard screening has been performed for the same substances/groups of substances that have been identified for the health screening in section 3.1. The environmental screening is also based mainly on the the official CLP-classification of the substances.

3.2.1 Environmental Effects

Table 3-2 below gives an overview of the substances/groups of substances listed under the health screening (and discussed in detail in Annex 3), which also have a harmonized classification that includes environmental characteristics.

It appears from the table that a significant number of substances are classified for chronic toxicity to aquatic organisms, which is considered to be the appropriate ecotoxicity classification to focus on since, in the current context, it seems unlikely that exposure should occur at levels where acute effects are relevant to consider. Many of the substances are classified as Aquatic Chronic 1, which implies that the chronic No-Observed-Effect Concentration (NOEC) in fish, crustaceans and/or algae is ≤ 0.1 mg/L.

TABLE 3-2
HARMONISED ENVIRONMENTAL CLASSIFICATION OF SUBSTANCES (CLP REGULATION 1272/2008).

Index No.	Substance	CAS No.	Environmental Classification	
			Hazard category	Hazard statement codes
604-002-00-8	Pentachlorophenol	87-86-5	Aquatic Acute 1 Aquatic Chronic 1	H400 H410
604-013-00-8	2,3,4,6-tetrachlorophenol	58-90-2	Aquatic Acute 1 Aquatic Chronic 1	H400 H410
604-018-00-5	2,4,6-trichlorophenol	88-06-2	Aquatic Acute 1 Aquatic Chronic 1	H400 H410
604-020-00-6	2-Phenylphenol	90-43-7	Aquatic Acute 1	H400
-	Tributyltin compounds	-	Aquatic Acute 1 Aquatic Chronic 1	H400 H410
050-004-00-1	Triphenyltin hydroxide	76-87-9	Aquatic Acute 1 Aquatic Chronic 1	H400 H410
604-070-00-9	Triclosan	3380-34-5	Aquatic Acute 1 Aquatic Chronic 1	H400 H410
047-001-00-2	Silver nitrate	7761-88-8	Aquatic Acute 1 Aquatic Chronic 1	H400 H410
015-102-00-0	Tris(2-chloroethyl)-phosphate (TCEP)	115-96-8	Aquatic Chronic 2	H411
015-188-00-X	(1-methylethylidene)di-4,1-phenylenetetraphenyl diphosphate	5945-33-5	Aquatic Chronic 4	H413
607-318-00-4	Dibutylphthalate (DBP)	84-74-2	Aquatic Acute 1	H400
612-042-00-2	Benzidine	92-87-5	Aquatic Acute 1 Aquatic Chronic 1	H400 H410
612-008-00-7	Aniline	62-53-3	Aquatic Acute 1	H400
601-053-00-8	Nonylphenol	25154-52-3	Aquatic Acute 1 Aquatic Chronic 1	H400 H410
607-624-00-8	Perfluorooctan sulfonic acid (PFOS); and its potassium-, diethanol-amine-, ammonium- and lithium salts	1763-23-1 2795-39-3 70225-14-8 29081-56-9 29457-72-5	Aquatic Chronic 2	H411
024-017-00-8	Chromium (VI) compounds	-	Aquatic Acute 1 Aquatic Chronic 1	H400 H410
028-002-01-4	Nickel (powder)	7440-02-0	Aquatic Chronic 3	H412
601-032-00-3	Benzo[a]pyrene	50-32-8	Aquatic Acute 1 Aquatic Chronic 1	H400 H410
601-049-00-6	Benzo[e]pyrene	192-97-2	Aquatic Acute 1 Aquatic Chronic 1	H400 H410

Index No.	Substance	CAS No.	Environmental Classification	
			Hazard category	Hazard statement codes
601-033-00-9	Benz[a]anthracene	56-55-3	Aquatic Acute 1 Aquatic Chronic 1	H400 H410
601-048-00-0	Chrysene	218-01-9	Aquatic Acute 1 Aquatic Chronic 1	H400 H410
601-034-00-4	Benz[e]acephenanthrylene	205-99-2	Aquatic Acute 1 Aquatic Chronic 1	H400 H410

The table includes only those substances for which there is a harmonized classification. It is considered likely that an additional number of substances would be classified as chronically toxic in the aquatic environment, if a classification had been available.

3.2.2 Environmental exposure

The primary way in which a substance in one of the textile products in this project could be released to the aquatic environment is by discharge to the sewage system following washing of the product. Many of the mentioned substances will mainly end up in the sludge phase at the sewage treatment plants but some of the more water-soluble substances will probably remain in the water phase and finally enter a surface water body with the treated effluent. This exposure pathway to the water environment, and to the environment as such, is possibly the most important, even though the textile products in this project undoubtedly are washed significantly less frequent than clothing.

As regards the release of the chemical substances to air it will typically be very slow and be mediated by migration to and evaporation from the textile surface. For substances with very low vapor pressure the release will be minimal.

Environmental exposure in the waste phase of the relevant products is assessed to be low as the textile part of the products are expected to be disposed of ultimately in a waste incineration plant where an efficient high temperature combustion in combination with flue gas cleaning will ensure that only negligible amounts are emitted to the atmosphere. It cannot be ruled out that very low amounts could occur in the slag or in residuals from the flue gas cleaning.

The exposure of the aquatic environment via sewage

It is proposed to calculate the environmental exposure resulting from the release of substances into urban wastewater from the washing of the textile products as follows:

By textile washing among consumers the spent washwater will predominantly be released to the public sewage system, through which it is transported to a sewage treatment plant, in which partly a transformation and partly a distribution of the substance between the water phase and the solid phase (sewage sludge) will take place. The treated effluent is discharged to a receiving surface water body, typically a river or the sea near the coast, while the sludge is either incinerated, converted into other products or applied to soil (mostly farmland).

The daily release of a chemical substance from a standard household/family to the sewage system can be calculated by the following formula:

Daily discharge of Substance-X from textile washing per household = Number of machine wash/day * Amount (w) of textile/wash * Proportion (%) of textile containing Substance-X * Proportion (%) of Substance-X in the textile * Proportion (%) of Substance-X released/wash.

Exposure of the soil environment via sewage

As a realistic worst-case exposure of the soil by application of sewage sludge it is assumed that the entire amount of substance-X from the washing of textiles ending up in a sewage treatment plant will be distributed to the sludge phase, after which the produced sludge is applied to farmland in the maximum allowable amount. In need of a more refined assessment, estimates of degradation during the treatment process and a more realistic distribution between sludge and water phase will be applied.

Exposure via the air

Emissions to the air are assessed in the health part of the project and the estimates from that assessment, if deemed relevant, will be used as worst case for environmental loads via air.

4. Selection of products and substances for analysis

4.1 Products and product parts

In consultation with the EPA, chemical analyses were to be performed only on three selected product groups to ensure a sufficient coverage for a given product type within the project's economic framework. The criteria for selection/prioritization were given partly by the initial exposure assessment for the identified product types, partly from the expected presence of substances in specific products or product parts. Finally, the focus has particularly been on products intended for babies/infants as these groups are considered the most sensitive to the effects of chemical substances.

In light of the considerations in the previous chapter, the following product groups were selected for procurement of products for subsequent chemical analysis:

- Car safety seats
- Baby slings
- Baby mattresses (incl. mattresses for carrycots and prams).

With regard to **Car safety seats**, 10 products in the price range from about 600 to about 1500 kr. each were purchased in both physical stores and Internet stores. Preferably, brands and models were selected that have not been analysed before and brands in the low end of the price scale as these are usually produced outside the EU where less control of the use of chemicals in such products can be expected. In addition, the focus was on car safety seats intended for the youngest age group, i.e. 0-13 kg. An overview of the purchased car safety seats is given below in Table 4-1.

TABLE 4-1
OVERVIEW OF PURCHASED CAR SAFETY SEATS.

Product no.	Size	Origin	Place of purchase (type)
A1	0 - 13 kg	China	Specialty store for car accessories
A2	9 - 36 kg	China	Specialty store for car accessories
A3	9 - 36 kg	Italy	Specialty store for car accessories
A4	0 - 13 kg	France	Supermarket
A5	0 - 18 kg	France	Supermarket
A6	0 - 4 years	China	Specialty store for car accessories
A7	0 - 13 kg	Probably China	Specialty store for baby/infant articles
A8	0 - 13 kg	Probably Asia	Specialty store for baby/infant articles
A29	0 - 13 kg	No information	Internetshop (baby/infant articles)
A30	0 - 13 kg	Probably Asia	Internetshop (baby/infant articles)

Baby slings (10 items) were purchased partly in physical stores and partly via Danish websites, which have a greater variety of brands (Table 4-2). Particularly, brands and models were purchased that were not analysed in previous studies and/or models where the babies can sit in different positions. The latter type is particularly interesting because the largest oral exposure is expected when the baby sits on the wearer's stomach, and hence can suck on the edge of the carrier belt, or sits on the wearer's back with the possibility of oral contact with the shoulder straps. The price of the baby slings were in the range from about 300 to about DKK 1,300.

TABLE 4-2
OVERVIEW OF PURCHASED BABY SLINGS

Product no.	Size	Origin	Place of purchase (type)
B9	0 - 3 år	China	Specialty store for baby/infant articles
B10	3 – 24 months	Latvia	Specialty store for baby/infant articles
B11	3,5 -15 kg	Romania	Internetshop (baby/infant articles)
B12	3,5 - 6 kg	China	Internetshop (baby/infant articles)
B13	4 – 48 months	India	Internetshop (baby/infant articles)
B14	3.5 – 9 kg	No information	Internetshop (baby/infant articles)
B15	3,5-15 kg	Probably China	Internetshop (baby/infant articles)
B16	3,5 – 18 kg	No information	Internetshop (baby/infant articles)
B17	6 months – 12 kg	Probably Asia	Internetshop (baby/infant articles)
B18	3,6 - 18,2 kg	China	Internetshop (baby/infant articles)

The final product group was **baby mattresses**, including both separate mattresses intended for carrycots/prams, and mattresses already being part of such products (Table 4-3). The 10 products purchased lie in the price range from about 80 to about 80 DKK 1,300. and they represent a wide range of the baby mattresses available on the Danish market. Most brands offer mattresses in different sizes, so that there are models suitable for carrycots, prams and baby beds, respectively. The materials appear to be identical in the different size groups. Although linen is typically used, which reduces the dermal exposure, exposure by inhalation could still be relevant due to the total time the babies lie on mattresses (in either carrycot, pram or bed).

TABLE 4-3
OVERVIEW OF PURCHASED MATERESSES AND CACRYCOTS.

Product no.	Product type	Origin	Place of purchase (type)
M19	Carrycot mattress	Netherlands	Specialty store for baby/infant articles
M20	Carrycot mattress	Poland	Specialty store for baby/infant articles
M21	Carrycot	Sweden/Poland	Specialty store for baby/infant articles
M22	Carrycot	Poland	Specialty store for baby/infant articles
M23	Soft carrycot	China	Specialty store for baby/infant articles
M24	Carrycot	No information	Specialty store for baby/infant articles
M25	Carrycot mattress	No information	Specialty store for baby/infant articles
M26	Carrycot mattress	No information	Specialty store for baby/infant articles

Product no.	Product type	Origin	Place of purchase (type)
M27	Carrycot mattress	No information	Specialty store for baby/infant articles
M28	Carrycot mattress	No information	Specialty store for baby/infant articles

4.2 Chemical substances

Previous studies of articles for babies/infants suggest that primarily the phosphorus-based flame retardants (in particular TCPP and TDCP), brominated flame retardants, formaldehyde and phthalates can occur in such high concentrations that a health risk can not be excluded.

Additionally, some substances classified due to health effects have either not been studied or have only been studied in very few products or materials, e.g. chlorophenols and PFAS.

However, the available studies are not considered to provide a basis that with sufficient certainty is able to identify the most relevant substances for quantitative analysis in the analysis part of the project. Therefore, an initial semi-quantitative screening analysis by GC-MS on the selected products was conducted to provide the necessary data basis for the selection of substances for quantitative analysis and possibly subsequent migration testing.

The GC-MS screening analysis was supplemented with a specific analysis of formaldehyde as this substance has been previously demonstrated to occur in several textile products, while it is undetectable by GC-MS screening.

5. Chemical analysis and test programme

5.1 Introduction

Based on the results of the literature survey and the preliminary assessment of the potentially occurring substances and the possible exposure of children in the studied product types (see Chapter 4), the following three product types were selected for the chemical analysis programme: Car safety seats, baby slings and baby mattresses. A total of 30 products were purchased; 10 products within each of the three selected groups of products.

As the results of the survey could not clarify which substances would be the most relevant to analyse quantitatively (as discussed in the previous chapter), screening analyses by gas chromatography-mass spectrometry (GC/MS) and by X-ray diffraction (XRD) were performed initially. The screening analysis by GC/MS was performed to identify the possible content of volatile and semi-volatile organic substances in the products while the screening analyses by XRD were performed to elucidate the possible content of elements in the products that could indicate a content of inorganic compounds and metal-containing organic compounds, e.g. organotin compounds.

From most of the products several sub-samples have been for analysis and they have as far as possible (and relevant) been taken from the parts of the product where children will have the most direct contact to reflect a worst-case exposure scenario. Most often this will be the zones near of the baby's face, where the child can suck on the textiles. The products selected for analysis typically consists of a number of different types of materials, for example textiles and foam, which is another reason to take more subsamples from many of the products. Sub-samples consisting of several different materials have been taken as well as sub-samples consisting of only one material.

An overview of the sub-samples within each product type is given in the tables below (by product type). In the sample numbering, the first letter refers to the type of product, i.e. "A" for "autostol" (car seat), "B" for "bæresele" (baby sling) and "M" for "madras" (mattress). The subsequent figure is simply the sample serial number, while a possible final letter (A, B and, if necessary, C) denotes different sub-samples of the same main sample.

TABLE 5-1
CAR SAFETY SEATS – OVERVIEW OF SUB-SAMPLES FOR GC/MS SCREENING ANALYSIS.

Product number	Sub-sample for GC/MS screening
A1	Black textile and foam
A2A	Grey textile and foam
A2B	Black plastic
A3A	Black textile and foam + backside
A3B	Grey textile and foam + backside
A4	Blue textile and foam + backside

Product number	Sub-sample for GC/MS screening
A5A	Red tekstile and foam + ribbon
A5B	Black backside made of rubber
A6A	Grey textile with foam
A6B	Backside
A7A	Black textile and foam
A7B	Textile with print
A8A	Black textile and foam
A8B	Red textile and foam
A29	Black textile and foam + "a heart"
A30	Grey textile and foam

TABLE 5-2
BABY SLINGS – OVERVIEW OF SUB-SAMPLES FOR GC/MS SCREENING ANALYSIS.

Product number	Description
B9A	Bright textile with foam
B9B	Foam "mat" between inner and outer textile
B9C	Small strap with grey plastic and bright textile
B10A	Foam from red and grey bib
B10B	Red textile with felt, grey textile and bib
B10C	White foam mat in the bib
B11	Grey textile and foam
B12A	Foam from belt strap, foam and felt
B12B	Grey, black and white textile from bib
B12C	Plastic "panel" on bib
B13A	Foam from belt strap with plastic and felt in the bib
B13B	Perforated textile from belt strap with fibres, black textile from belt strap and black textile from inner side of bib
B14A	Foam in the bib
B14B	White textile from backside of the bib + grey and black textile on the strap
B15A	Foam with felt on the bib
B15B	Black strap and dark blue textile
B16A	Foam in the strap
B16B	Black and grey textile from the inside of the sling
B17A	Foam in the bib and in the sling itself

Product number	Description
B17B	White and black textile from the bib
B18A	Foam from strap and felt from bib
B18B	Grey textile from strap and black textile from ribbon
B18C	White "net" textile in the sling and white textile on the bib

TABLE 5-3
BABY MATTRESSES – OVERVIEW OF SUB-SAMPLES FOR GC/MS SCREENING ANALYSIS.

Product number	Description
M19A	Textile from separate mattress for carrycots
M19B	Foam from separate mattress for carrycots
M20A	Textile from separate mattress for carrycots
M20B	Foam from separate mattress for carrycots
M21A	Textile from carrycot
M21B	Foam from carrycot
M22A	Textile from carrycot
M22B	Textile from carrycot
M23A	Textile from soft carrycot
M23B	Textile from soft carrycot
M24A	Textile from carrycot
M24B	Textile from carrycot
M25A	Textile from separate mattress for carrycots
M25B	Foam from separate mattress for carrycots
M26A	Textile from separate mattress for carrycots
M26B	Foam from separate mattress for carrycots
M27A	Textile from separate mattress for carrycots
M27B	Foam from separate mattress for carrycots
M28A	Textile from separate mattress for carrycots
M28B	Foam from separate mattress for carrycots

Based on the results from the screening analyses the decision was made on quantitative analyses of relevant substances by specific methods. The results of the quantitatively determined content were subsequently compared with the exposure scenarios as a basis for decision on the possible conduct of a migration test. This means that migration was only tested for the substances detected in significant concentrations, and where migration was considered to be a relevant route of exposure.

5.2 Screening analyses

Screening analyses for organic compounds by GC/MS have been carried out on all subsamples mentioned in the above summary tables.

The screening analyses by X-ray analysis (XRD) were performed with the primary objective to identify the possible presence of organotin compounds, and these analyses were limited to car safety seats as such substances were considered primarily to occur within this product group, if at all. Only the textile part of the seats were analysed as it was assumed that it would primarily be these sub-samples that could contain organotin compounds (or brominated flame retardants).

5.2.1 Screening analysis by GC/MS

The screening analyses cover a large number of volatile and semi-volatile organic compounds, but the method is not equally suitable for all substances. E.g. volatile aldehydes (including formaldehyde, which in previous studies has been found in some textiles) are not detected by the method. Isocyanates, which may occur as a residual monomer in foam, also require a specific method of analysis, and the same applies to aromatic amines derived from azo dyes.

As, by the GC/MS screening, the contents of all substances are quantified based on the same internal standard, the results can only be regarded as semi-quantitative. The response factor for some of the substances in the analysis is close to the response factor of the internal standard, while the response factor for others may deviate significantly and therefore will result in a more uncertain determination of the concentration in the sample.

Analytical method - volatile and semi-volatile organic compounds by GC/MS

A known sub-amount of the product of between 0.5 and 2 grams is weighed, extracted with solvent, and then analysed by gas chromatography combined with mass-selective detection (GC/MS). Internal standards of deuterium-labeled naphthalene and phenanthrene were used. The individual compounds detected were identified by comparing the actual mass spectra with mass spectra from the NIST MS library¹. The NIST library is a database of spectra for more than 500,000 chemical substances.

All volatile and semi-volatile organic substances detected by the GC/MS screening are semi-quantified using the response factor for naphthalene.

The limits of detection vary between 1 and 50 mg/kg, depending on the matrix and the specific response of the substances. The contents of all substances found above the detection limit are reported in mg/kg of the sample.

Results of the screening analysis by GC/MS

The detailed results of the screening analyses can be found in Appendix 4. Many different volatile and semi-volatile components have been found in the samples, and the results vary considerably between samples and, hence, it has been difficult to find any clear pattern in the results.

In a few samples no volatile or semi-volatile substances were detected and in other samples only very few, while in other samples more than 15 different components were detected. If one should point at some substances occurring in many of the samples, particularly the phosphorus-based flame retardants are noteworthy. In addition, it various hydrocarbons (primarily aliphatic hydrocarbons), (aromatic) amines and individual phthalates and certain isocyanates are present in a number of samples.

¹ National Institute of Standards and Technology (NIST), USA.

A significant number of substances were detected by the screening that could not be identified. The substances are not included in the NIST library, and therefore considered not to be commonly known substances.

The results of the screening analysis by GC/MS has formed the basis for the selection of substances to be quantified by specific analytical methods, see section 5.3.

5.2.2 Screening analysis by X-ray

By X-ray screening analysis all elements can in principle be detected, however not elements with low molecular weights such as hydrogen, oxygen, carbon and nitrogen. In this project, however, the focus of the elemental screening has been on the metal tin, as the use of organotin compounds in certain textile products previously has been reported, and as these substances only have a poor recovery by the GC/MS screening method. In addition, samples can be simultaneously analysed for content of bromine (as an indicator of content of brominated flame retardants).

Analytical method - screening analysis by X-ray

The samples were analysed for their content of tin and bromine by wavelength-dispersive (WD) X-ray analysis; Philips PW2400 / UNIQUANT ver. 5.49.

The detection limit of the method is 20 mg/kg and the variability is estimated to be 5-15 % RSD.

Results of the screening analysis by X-ray

In Table 5-4 the results of the screening analyses performed by X-ray are shown. Only car seat samples were screened by X-ray analysis.

TABLE 5-4
RESULTS OF X-RAY SCREENING ANALYSES OF CAR SAFETY SEATS.

Sample no.	Sample type	Tin mg/kg	Bromine mg/kg
A1	Textile	< 20	5,700
A2A	Textile	< 20	220
A3A	Textile	< 20	170
A3B	Textile	65	410
A4	Textile	< 20	450
A5A	Textile	< 20	< 20
A6A	Textile	< 20	430
A6B	Textile	< 20	< 20
A7A	Textile	< 20	< 20
A8A	Textile	< 20	< 20
A8B	Textile	< 20	290
A29	Textile	< 20	27,000
A30	Textile	< 20	90

< Less than the stated limit of detection.

As the table shows, tin was not found in significant quantities in any of the samples. Therefore, it was considered unnecessary to carry out specific analyses for organotin compounds.

However, bromine was detected in 9 out of the 13 samples of textiles from car safety seats. A high content of bromine may indicate the use of brominated flame retardants and therefore it was decided to carry out quantitative analyses for those brominated flame retardants that are most often used in textiles in samples with the highest content found by the screening: 1, 3B, 4, 6A and 29.

5.3 Quantitative analyses

Based on the results from the screening analyses by GC/MS and X-ray, quantitative analyses for selected, specific organic substances were performed. No quantitative determinations of inorganic substances were performed as it was considered that the relevant element identified (bromine) would most likely occur in the form of organic compounds.

Some of the substances listed in the survey (chapter 2) were deselected for the chemical analysis programme due to the following considerations:

- The substance or indications of the presence of the substance was not detected in any of the screening analyses conducted;
- the substance was only detected at low (i.e. not relevant) concentrations;
- the substance was not found to be of toxicological concern in the initial risk screening;
- the substance was considered to be already sufficiently documented in other studies.

This applies, for example to substances such as chlorophenols, aliphatic hydrocarbons, carboxylic acids, aldehydes, ketones, alcohols and amines, triazoles/triazines, certain solvents, perfluorinated alkyl compounds (covered by a separate project under consumer product program in 2014²), a series of biocides, PAHs, and various phthalates.

Based on screening results the following substances were selected for specific analysis:

- Phosphorus-based flame retardants, based on detection of significant content in many of the products, i.e. content in amounts estimated to be due to intentional use and not just residues from previous process stages or contamination by migration from other materials.
- Azo dyes, due to detection of aromatic amines, and because the specific aromatic amines derived from azo dyes cannot be detected by the GC/MS screening method. Additionally, in other studies these substances have been shown to be present in some textile products. Azo dyes are measured analytically by the derived aromatic amines.
- Phthalates, based on the detection of significant (i.e. intentional) contents in a single sample.
- Isocyanates, only in foam sub-samples, as these substances have been found in other studies and because indications of content of TDI were found by the screening analysis.
- Formaldehyde, as the substance cannot be detected by the screening method and the substance has previously been found in other studies of textile, including car safety seats.
- Brominated flame retardants as the X-ray screening indicated high bromine content in several of the screened samples.

² Lassen *et. al.* (in press). "Perfluoralkyl substances (PFAS) in textiles for children". In this project, 8 car safety seats, among many other textile products, were analysed for their content of PFAS but no such substances were detected in any of the seats.

The specific analyses of the above substances/-groups were carried out on sub-samples of products within the three selected product types as presented in Table 5-5, Table 5-6 and Table 5-7 below.

TABLE 5-5
OVERVIEW OF QUANTITATIVE ANALYSES OF CAR SAFETY SEATS BY SPECIFIC METHODS.

Product	P-based flame retardants	Formaldehyde	Isocyanates	Phthalates	Azo dyes	Brominated flame retardants
A1	Black textile and foam	Black textile	-	-	Black textile	Black textile
A2A	Grey textile and foam	Grey textile	-	-	Grey textile	-
A2B	Black plastic	-	-	-	-	-
A3A	Sort betræk inklusiv skum	Black textile	Foam from chair	-	-	-
A3B	Black textile and foam	Black textile	-	-	-	Black textile
A4	Blue textile, foam and backside	Blue textile	-	-	-	Blue textile
A5A	Red textile, foam and ribbon	Red textile	Foam from chair	-	-	-
A5B	Black rubber backside	Black rubber backside	-	-	-	-
A6A	-	Grey textile	Foam from chair	-	-	Grey textile
A6B	-	Black textile from back of belt	-	Black textile from back of belt	Black textile from back of belt	-
A7A	-	Black textile	Foam from chair	-	Black textile	-
A7B	-	Printed textile	-	-	-	-
A8A	Black textile and foam	Black textile	Foam from chair	-	Black textile fom belt	-
A8B	Red textile and foam	Red textile	-	-	Red textile	-
A29	Black textile, foam and "heart"	Black textile	-	-	Black textile (two types)	Black textile
A30	Grey textile and foam	Grey textile	-	-	-	-

- : The analysis was not carried out on this sample.

TABLE 5-6
OVERVIEW OF QUANTITATIVE ANALYSES OF BABY SLINGS BY SPECIFIC METHODS.

Product	P-based flame retardants	Formaldehyde	Isocyanates	Phthalates	Azo dyes	Brominated flame retardants
B9A	-	Bright textile		-	-	-
B9B	Foam "mat" between textile layers	-	Foam "mat" between textile layers	-	-	-
B9C	Small plastic "panel"	Bright textile	-	-	-	-
B10A	-	-	-	-	-	-
B10B	Red textile with felt, gery textile and bib	Red textile with felt, grey textile and bib	-	-	-	-
B10C	-	-	-	-	-	-
B11	-	Grey textile	-	-	-	-
B12A	Foam from strap, foam and felt	White textile	-	-	-	-
B12B	Grey, black and white textile from bib	Grey and black textile	-	-	Black textile	-
B12C	Plastic "panel" in bib	-	-	-	-	-
B13A	-	Perforated textile from strap	-	-	-	-
B13B	-	Black textile from strap and inner side of bib	-	-	Perforated textile and black textile	-
B14A	-	-	-	-	-	-
B14B	-	White textile from bib + grey and black textile from strap	-	-	-	-
B15A	-	-	-	-	-	-
B15B	-	Black strap and dark blue textile	-	-	Black strap and dark blue textile	-
B16A	Foam from belt	-	-	-	-	-
B16B	-	Black and grey textile from inside of strap	-	-	Black and grey textile from inside of strap	-

Product	P-based flame retardants	Formaldehyde	Isocyanates	Phthalates	Azo dyes	Brominated flame retardants
B17A	-	-	-	-	-	-
B17B	White and black textile from strap	White and black textile from strap	-	-	Black textile from bib	-
B18A	Foam from belt and felt from bib	-	-	-	-	-
B18B	Grey textile from strap and black textile from ribbon	Grey textile from strap and black textile from ribbon	-	-	Grey textile from strap and black textile from ribbon	-
B18C	White "net" textile in strap and white textile from bib	White "net" textile in strap and white textile from bib	-	-	-	-

- : The analysis was not carried out on this sample.

TABLE 5-7
OVERVIEW OF QUANTITATIVE ANALYSES OF BABY MATTRESSES BY SPECIFIC METHODS.

Product	P-based flame retardants	Formaldehyde	Isocyanates	Phthalates	Azo dyes	Brominated flame retardants
M19A	-	Textile cover	-	-	-	-
M19B	-	-	Foam	-	-	-
M20A	-	Textile cover	-	-	-	-
M20B	-	-	Foam	-	-	-
M21A	-	Textile cover	-	-	-	-
M21B	-	-	-	-	-	-
M22A	-	Textile cover	-	-	-	-
M22B	-	-	Foam	-	-	-
M23A	-	Textile cover	-	-	-	-
M23B	-	-	-	-	-	-
M24A	Textile cover	Textile cover	-	-	-	-
M24B	Foam	-	Foam	-	-	-
M25A	-	Textile cover	-	-	-	-
M25B	-	-	Foam	-	-	-
M26A	-	Textile cover	-	-	-	-
M26B	-	-	Foam	-	-	-

Product	P-based flame retardants	Formaldehyde	Isocyanates	Phthalates	Azo dyes	Brominated flame retardants
M27A	-	Textile cover	-	-	-	-
M27B	-	-	Foam	-	-	-
M28A	-	Textile cover	-	-	-	-
M28B	-	-	Foam	-	-	-

- : The analysis was not carried out on this sample.

5.3.1 Phosphorus-based flame retardants

Based on the GC/MS screening results, 25 samples or subsamples of the parts of the products that may come into direct contact with the child were selected for phosphorus-based flame retardant analysis (primarily the chlorinated ones). The subsamples were completely similar to the corresponding sub-samples analysed by GC/MS.

All samples and subsamples were analysed for the content of the following phosphorus-based flame retardants:

TCEP: Tris (2-chloroethyl) phosphate, CAS no. 115-96-8

TCPP: Tris (1-chloro-2-propyl) phosphate, CAS no. 13674-84-5

TDCP: Tris (1,3-dichloro-2-propyl) phosphate, CAS no. 13674-87-8

TPP: Triphenyl phosphate, CAS no. 115-86-6

Analytical method for phosphorus-based flame retardants

Known aliquots were weighed and extracted with solvent, and the extracts subsequently analysed by gas chromatography combined with mass selective detection (GC/MS). The internal standards used were deuterium-labeled naphthalene and phenanthrene. All substances were determined quantitatively against an external calibration standard for each flame retardant. True duplicate determinations were made. The results are expressed as the mean of the duplicate determinations.

The detection limits ranged from 1 to 50 mg/kg depending on the matrix and the specific response of the substance. The relative standard deviations ranged from 5-20% (RSD), however, with a single outlier sample (5B) having a RSD of 73%.

Results of analyses for phosphorus-based flame retardants

The following Table 5-8 present the results of the analyses for phosphorus-based flame retardants in sub-samples from the three product groups: car safety seats, baby slings and baby mattresses.

TABLE 5-8
RESULTS OF ANALYSES FOR P-BASED FLAME RETARDANTS IN CAR SAFETY SEATS, BABY SLINGS AND BABY MATTRESSES (MEAN OF DUPLICATE DETERMINATIONS).

P-based flame retardants, result in mg/kg				
	TCEP	TCPP	TDCP	TPP
Car safety seats				
A1, textile + foam	-	720	2,700	44
A2A, textile + foam	- / -	810	3,700	-
A2B, black plastic	-	30	14	-

P-based flame retardants, result in mg/kg				
	TCEP	TCPP	TDCP	TPP
A3A, textile + foam	-	2,200	20,300	34
A3B, textile + foam	-	4,800	21,100	65
A4, textile + foam	-	56	42,600	84
A5A, textile + foam	-	46	31,500	54
A5B, black rubber	-	-	23	-
A8A, textile + foam	840	18,100	5,100	28
A8B, textile + foam	41	2,490	6,700	30
A29, textile + foam	-	-	-	330
A30, textile + foam	-	-	-	28
Baby slings				
B9B, foam mat	-	21	-	18
B9C, textile and plastic	-	17	-	-
B10B, textile	-	-	-	14
B12A, foam	75	11,200	160	43
B12B, textile	20	720	23	14
B12C, plastic	-	340	-	-
B16A, foam	-	-	-	86
B17B, textile	-	-	-	14
B18A, foam and felt	4,700	16,300	13,000	-
B18B, textile	57	140	45	-
B18C, textile	44	41	48	-
Baby mattresses				
M24A, textile	-	-	92	-
M24B, foam	-	-	89,700	74

- :Below the stated limit of detection: 5-20 mg/kg

TCEP: Tris(2-chloroethyl)phosphate, CAS nr. 115-96-8

TCPP: Tris(1-chloro-2-propyl)phosphate, CAS nr. 13674-84-5

TDCP: Tris(1,3-dichloro-2-propyl)phosphate, CAS nr. 13674-87-8

TPP: Triphenyl phosphate, CAS nr. 115-86-6

High levels of flame retardants were detected in seven of the samples, i.e. a content of more than 1% (> 10,000 mg/kg) indicating an intended addition for a functional purpose.

Seven samples with concentrations greater than 10,000 mg/kg were selected for additional testing for migration (migration to sweat) for all four flame retardants: TCEP, TCPP, TDCP and TPP. However, only one subsample was selected per product (the sub-sample with the highest content). Samples of all three product types were selected, however most car seat samples.

See the results of the migration tests in section 5.4.

5.3.2 Formaldehyde

Formaldehyde cannot be determined by the screening method by GC/MS. Therefore, sub-samples from all products were selected for the quantitative analysis of formaldehyde as previous studies have shown that formaldehyde can be found in textile products, including car safety seats. Only subsamples made of textile were examined for the content of formaldehyde.

Analytical method for formaldehyde

Sub-samples of the textiles were taken in the areas of the samples, which may come into direct contact with the child. The sub-samples were cut into smaller pieces and a known aliquot was weighed. The sample was extracted with purified water for one hour in a water bath at 40 °C and shaking at regular intervals. Acetylacetone reagent was added to an aliquot of the samples solution and subsequently spectrophotometric analysis was performed at a wavelength in the range 412-415 nm. The analysis was performed according to accreditation under Japanese law no. 112: 1972 without any modifications.

Limit of detection: 2 - 5 mg/kg. True duplicate determinations were made.

Results of analyses for formaldehyde

No content of formaldehyde was found in any of the 37 textile samples analysed with a detection limit of 2-5 mg/kg. See detailed table in Appendix 5.

5.3.3 Isocyanates

Quantitative determination of isocyanates were made on 14 samples selected based on the results of the screening analyses. The screening analyses had indicated content of isocyanates in the pure foam samples as well as in sub-samples containing both textile and foam. Since it is assessed that isocyanates do not occur in textiles, and the content in the composite samples therefore must be due to the foam part, only sub-samples of pure foam were analysed specifically for isocyanates.

The samples were analysed for content of the isocyanates 2,4- and 2,6-toluene diisocyanate (2,4- and 2,6-TDI, CAS no. 584-84-9 and 91-08-7) as these are the only ones of the identified isocyanates that are (semi)volatile and therefore can be emitted from the foam. In addition, the analysis included 4,4'-diphenylmethane diisocyanate (MDI, CAS no. 101-68-8).

Analytical method for isocyanates

Sub-samples with known weight were extracted with dichloromethane with an internal standard added and subsequent derivatization. The substances 2,4- and 2,6-TDI and MDI were analysed for. The analysis was performed by HPLC with fluorescence detector.

True triplicate determinations were made since isocyanates can be randomly distributed within the foam system. The results are reported as the mean of the triplicate determinations.

Results of analyses for isocyanates

As described previously, the screening analyses indicated that in several samples there could be a content of isocyanates, probably caused by the release of unreacted monomer from the foam materials. Since TDI and MDI has problematic health properties such as sensitization, the 14 most relevant samples were selected for specific analysis for isocyanates. The results of this analysis are shown in Table 5-9.

TABLE 5-9
RESULTS OF SPECIFIC ANALYSES FOR ISOCYANATES (MEAN OF TRIPPLICATE DETERMINATIONS).

		Concentration (mg/kg)		
Sample no.	Sub-sample	2,4-TDI	2,6-TDI	MDI
Car safety seats				
A3A	Foam from chair	0,17	0,43	< 0,1
A5A	Foam from chair	< 0,1	< 0,1	< 0,1
A6A	Foam from chair	< 0,1	< 0,1	< 0,1
A7A	Foam from chair	< 0,1	< 0,1	< 0,1
A8A	Foam from strap	< 0,1	< 0,1	< 0,1
Baby slings				
B9B	Foam mat between textiles	< 0,1	< 0,1	< 0,1
Baby mattresses				
M19B	Foam	0,14	< 0,1	< 0,1
M20B	Foam	< 0,1	< 0,1	< 0,1
M22B	Foam	< 0,1	< 0,1	< 0,1
M24B	Foam	0,91	0,64	< 0,1
M25B	Foam	< 0,1	< 0,1	< 0,1
M26B	Foam	< 0,1	< 0,1	< 0,1
M27B	Foam	0,23	0,17	< 0,1
M28B	Foam	< 0,1	< 0,1	< 0,1

< Content below the stated limit of detection.

While the screening analysis by GC/MS had indicated contents of the isocyanates 2,4- and 2,6-TDI in the 14 samples at concentrations between 10 and 940 mg/kg, the specific quantitative analyses could not confirm these high concentrations. Thus, isocyanates (2,4- and 2,6-TDI) were only detected in the samples 3A, 19B, 24B and 27B with 0.64 mg/kg as the highest concentration.

The results of the specific analysis thus clearly show that the screening method by GC/MS cannot be used for the determination of isocyanates. It was expected that the method would be able to give an indication of the isocyanate content, but this is apparently not the case.

5.3.4 Phthalates

In the screening by GC/MS only one single sample of a car seat (sample A6B) was found, which had a high content of phthalates, namely isomeric di-decylphthalates (DIDP). The concentration of the isomeric di-decylphthalate was considered to be outside the screening range, and therefore the concentration was probably underestimated by the screening analysis. In the specific analysis, the sample was diluted to an appropriate level in order to determine the exact concentration.

Analytical method for isomeric di-isodecyl phthalate (DIDP)

The sub-sample (about 0.5 g, accurately weighed) was extracted with dichloromethane by ultrasound for 1 h and mechanical shaking for 1 hour. Deuterium-labelled internal standards of dibutyl phthalate-d4 and di- (2-ethylhexyl)-phthalate-d4 had been added to the solvent prior to the extraction. The extract was analysed by capillary gas chromatography with mass spectrometric detection (GC/MS).

The analysis was carried out by an accredited method for phthalates; however, the isomeric di-isodecyl phthalates, CAS. 26761-40-0 and 68515-49-1, are not covered by the accreditation. True duplicate determinations were performed. The results are reported as the average of the duplicate determinations.

Results of analyses for isomeric di-isodecyl phthalate

By the GC/MS screening analysis only very low concentrations of phthalates were found, if at all, and only in one sample the content was high enough (functional level) to justify a specific analysis. The result of this is shown in Table 5-10.

TABLE 5-10
RESULTS OF SPECIFIC ANALYSES FOR PHTHALATES IN CAR SEAT SAMPLE A6B (MEAN OF DUPLICATE DETERMINATIONS).

Sample No.	Material	Phthalate concentration (mg/kg)	
		Di-isodecyl-phthalate isomer 1	Di-isodecyl-phthalate isomer 2
A6B	Textile	41,000	350,000

The screening analysis by GC/MS indicated a content of di-isodecyl phthalates reported as >12,100 mg/kg and >3320 mg/kg, respectively, for the two isomers. These concentrations were considered, as mentioned above, not to be correct as the MS detector was probably overloaded with the isomeric di-decylphthalates at the screening, and the true concentrations therefore way out of the linear calibration range. In the specific quantitative measurements the extracts were diluted so that the concentration was quantifiable and the total content of DIDP was then found to be about 390,000 mg/kg (39% w/w), which is a exceedance of the provisions regarding certain phthalates in childcare articles in REACH. The Environmental Protection Agency has therefore reported this finding of DIDP to the Danish Chemicals Inspectorate.

5.3.5 Azo dyes

Determination of azo dyes (determined as the derived aromatic amines) requires a specific method of analysis for all the relevant amines, and, hence, the GC/MS screening method cannot be used to select the samples for quantitative determination of azo dyes. Therefore, 13 samples of textiles from car safety seats and baby sling were selected, which had the darkest colors as it was assessed most likely that azo dyes, if at all, would occur in dark colored textile samples. No samples of textiles from the baby mattresses were selected, as they all were made of relatively bright fabrics.

Analytical method for aromatic amines derived from azo dyes

The analyses were performed according to DS/EN 14362-1 by gas chromatography combined with mass spectrometric detection (GC/MS) as indicated in REACH Annex XVII, Section 43, which comprise 23 substances (all aromatic amines) and sets a limit for the content of each of the substances of 30 mg/kg. In addition, 4 additional amines were determined by the analysis, including aniline and p-phenylenediamine, which, when they both are present in significant concentrations, indicate that the samples contain 4-aminoazobenzene. Duplicate determinations were made.

The aromatic amines derived from azo dyes and aniline and p-phenylenediamine, were quantified by comparison with calibration standards of all named substances. 2,6-Dichloro-p-phenylene-diamine and 4,5-dichloro-o-phenylenediamine was identified by comparing the current mass spectra with mass spectra in the NIST library. The amounts stated for these two substances were calculated from the response of the internal deuterium-labeled standard naphthalene-d8 (semi-quantitative determination).

The standard method EN 14362-3: 2012 deals with specific analysis of 4-aminoazobenzene, which is only performed if the presence of aniline and 4-phenylenediamine in significant quantities, i.e. more than 5 mg/kg for both substances. Therefore, this analysis was not performed as the indicator substances were not detected in significant quantities.

Results of analyses for amines derived from azo dyes and other amines

The results of the analyses for azo dyes as represented by the derived aromatic amines are shown in Table 5-11 below. The results are reported as the mean of the performed duplicate determinations. Only substances found above the limit of detection are reported. See the list of all examined specific substances in Annex 6.

TABLE 5-11
RESULTS OF QUANTITATIVE ANALYSES FOR AZO DYE-DERIVED AMINES AND OTHER AROMATIC AMINES IN CAR SAFETY SEATS AND BABY SLINGS (MEAN OF DUPLICATE DETERMINATIONS).

Component	CAS-no.	Concentration, mg/kg												
		Car safety seats							Baby slings					
		A1	A2A	A6B	A7a	A8A	A8B	A29	B12B	B13B	B15B	B16B	B17B	B18B
4-Methyl-m-phenylene diamine (2,4-TDA)	95-80-7	50	-	-	-	30	6,7	-	11	-	-	-	-	-
Benzidine	92-87-5	-	-	-	-	-	-	-	-	-	-	7,5	-	-
3,3'- Dimethoxy-benzidine	119-90-4	-	-	-	-	-	-	-	-	-	-	6,4	-	-
Other aromatic amines		-	-	-	-	-	-	-	-	-	-	-	-	-
Aniline	62-53-3	0,57	-	-	-	-	-	-	-	0,95	-	8,2	-	0,96
p-Phenylene diamine		20	-	-	-	-	-	-	-	-	-	-	-	-
2,6-Dichloro-p-phenylene diamine 4,5-Dichloro-o-phenylene diamine	609-20-1/ 5348-42-5	280	90	-	-	130	-	24	200	-	38	98	230	-

"-" Concentration below the LoD: 2 – 5 mg/kg.

Generally, only very low amounts of amines as defined in REACH Annex XVII, paragraph 43 were detected, and all samples except one thus comply with the limit of 30 mg/kg for the aromatic amines set out in REACH Annex XVII. The sample exceeding the limit of 30 mg/kg is sample made

of black textile from car seat A1, in which was detected 50 mg of 4-methyl-m-phenylene diamine/kg textile. This result has been reported to the Danish Chemicals Inspectorate.

5.3.6 Brominated flame retardants

The screening analysis by X-ray showed high levels of bromine in five of the textile samples, and these textiles were therefore selected for quantitative analysis for brominated flame retardants. The analysis include the following brominated flame retardants, which historically have been used in textiles, i.e.:

- Polybrominated diphenyl ethers (PBDEs)
- Hexabromocyclododecane-dodecane (HBCD)

and

- polybrominated biphenyls (PBB) and
- tetrabromobisphenol-A (TBBPA).

The polybrominated biphenyls (PBB) and polybrominated diphenyl ethers (PBDEs) include a variety of individual components, all of which were determined by the analysis. Of these, decaBDE is the substance most commonly used in textiles.

Analytical method for brominated flame retardants

Sub-samples in known quantities were extracted by Soxhlet, ASE or liquid/liquid extraction with addition of deuterium-labeled standards. The extracts were purified on columns, and analysis was performed by capillary gas chromatography with mass spectrometric detection (GC/MS) or HPLC/MS-MS, depending on the substance. Tetrabromobisphenol-A (TBBPA) was derivatized with BSTFA prior to analysis.

The detection limits ranged between 0.00002 and 0.01 mg/kg depending on the matrix and the response of the substances. The relative standard deviations (RSD) ranged from 5 to 25%. True duplicate determinations were performed.

Results of analyses for brominated flame retardants

The results of the specific analyses for brominated flame retardants in Table 5-12 are reported as the average of the performed duplicate determinations. See the complete list of individual substances in Annex 7.

TABLE 5-12
RESULTS OF THE QUANTITATIVE ANALYSES FOR BROMINATED FLAME RETARDANTS IN TEXTILES
FROM CAR SAFETY SEATS (AVERAGE OF DUPLICATE DETERMINATIONS).

Substance	Concentration in mg/kg				
	A1	A3B	A4	A6A	A29
Polybrominated biphenyls (PBB), individual substances	-	-	-	-	-
Sum of polybrominated biphenyls (PBB)	< 0,03	< 0,04	< 0,02	< 0,02	< 0,02
2,4,4'-TriBDE (BDE-28)	0,00088	0,00037	0,00094	0,14	0,047
Sum of other polybrominated diphenyl ethers (PBDE)	< 0,07	< 0,08	< 0,05	< 0,05	< 0,05

Substance	Concentration in mg/kg				
	A1	A3B	A4	A6A	A29
Tetrabromobisphenol-A (TBBPA)	< 0,03	< 0,04	< 0,02	< 0,02	< 0,01
Hexabromocyclo dodecane (HBCD) (alpha, beta, gamma)	< 0,002	1,2*	0,031	< 0,001	0,042

- Means content below the stated limit of detection.

* The content was only found in one of the duplicate determinations.

A few brominated flame retardants were detected in low concentrations:

- 2,4,4-TriBDE (BDE-28) was found in 3 samples with 0.047 mg/kg as the highest concentration. BDE-28 is present in low concentrations in pentaBDE, but if this was the source, the sum of PBDE should be much higher.
- Hexabromocyclododecane (HBCD) was detected in 3 samples with 1.2 mg/kg as the highest concentration.

Thus, the findings of relatively high concentrations of bromine by X-ray analysis cannot be explained by presence of the investigated brominated flame retardants.

In recent years, however, a number of alternative brominated flame retardants have been introduced on the market including some polymeric brominated flame retardants, which are considered to be less harmful than traditional brominated flame retardants. Since it is not possible to point at other sources to the bromine found in the textile samples, it is considered the most likely explanation that polymeric brominated flame retardants have been used, which could not be determined by the analytical method applied.

5.4 Migration testing

The results of the quantitative analyses in combination with the exposure scenarios led to the selection of phosphorus-based flame retardants for migration testing. Seven sub-samples were tested for migration to sweat (as this migration type was assessed to be the main pathway of exposure). In practice, the seven sub-samples with the highest content of P-based flame retardants were selected, however max. one sub-sample per product and in a way so that all three product types were represented. Migration tests were not performed with any of the other substances because the concentration levels were assessed to be too low for migration of health concern to take place. The aromatic amine 4-methyl-*m*-phenylene diamine is automatically considered to pose a risk when the limit value is exceeded. Hence, in this context it was found unnecessary to test it for migration.

5.4.1 Migration test method

A migration test fluid of artificial sweat was prepared according to ISO 105-E04: 2008 in accordance with Oeko-Tex®, wherein the simulator comprises 1-histidine monohydrochloride 1-hydrate, sodium chloride, sodium dihydrogen phosphate and sodium hydroxide, and a pH adjusted to 5.5. The artificial sweat was heated to 37 °C to mimic body temperature. The amount of test sample relative to migration fluid was approximately 2.5 grams textile to 50 ml simulant.

A sub-sample of known weight was taken of each of the 7 samples. The sub-samples were placed in a known volume of the sweat simulant in a heating cabinet at 37 °C, and static contact with the simulant for 3 hours. After 3 hours of migration to the artificial sweat, the sub-samples were removed from the migration liquid, and the liquid was immediately extracted with dichloromethane and stored refrigerated until assayed for the specific substances. The migration test was performed in duplicate.

5.4.2 Analytical method - phosphorus-based flame retardants in migration liquid

A known aliquot of the migration fluid sample was weighed and extracted with dichloromethane after which the extract was analysed by gas chromatography combined with mass selective detection (GC/MS). Internal standards of deuterium-labeled naphthalene and phenanthrene were used. The substances found were quantified by comparison to calibration standards. All analyses were performed as true duplicate determinations.

Quantitative determinations were made against calibration standards for each of the four P-based flame retardants:

TCEP: Tris (2-chloroethyl) phosphate, CAS no. 115-96-8

TCPP: tris (1-chloro-2-propyl) phosphate, CAS no. 13674-84-5

TDCP: Tris (1,3-dichloro-2-propyl) phosphate, CAS no. 13674-87-8

TPP: Triphenyl phosphate, CAS no. 115-86-6

The detection limits ranged between 1 -5 mg/kg, while the relative standard deviations (RSD) varied from 10 to 15 % for the determination in mg/kg. The RSD for the determination in mg/m² was higher due to uncertainty in the measurements of the surface areas of the subsamples.

5.4.3 Results of the migration tests with phosphorus-based flame retardants

The results of the quantitative analyses of the content of phosphorus-based flame retardants in the product samples are reported in Table 5-8.

Table 5-13 presents the results of tests for migration of the four flame retardants to artificial sweat performed on selected samples (four car safety seats, two baby slings and one baby mattress). It should be noted that TPP was not detected in significant concentrations in any of the textile samples, yet it was included in the migration test for completeness. The results are reported as the mean of duplicate determinations on the basis of weight and surface area, respectively (mg/kg and mg/m², respectively).

TABLE 5-13
RESULTS OF THE TESTS/ANALYSES FOR PHOSPHORUS-BASED FLAME RETARDANTS IN ARTIFICIAL SWEAT MIGRATION LIQUID FOR CAR SAFETY SEATS, BABY SLINGS AND BABY MATTRESSES, RESPECTIVELY.

	Extracted amount after 3 hours, weight basis (mg/kg)			
	TCEP	TCPP	TDCP	TPP
Car safety seats				
A3B, textile with foam	< 1	140	95	< 1
A4, textile with foam	< 1	11	460	< 1
A5A, textile with foam	< 1	12	560	< 1
A8A, textile with foam	840	1700	130	< 1
Baby slings				

	Extracted amount after 3 hours, weight basis (mg/kg)			
	TCEP	TCPP	TDCP	TPP
B12A, foam	19	1200	< 1	< 1
B18A, foam and felt	1400	2400	150	< 1
Baby mattresses				
M24B, foam	< 1	< 5	500	< 1

	Amount migrated after 3 hours, surface area basis (mg/m ²)			
	TCEP	TCPP	TDCP	TPP
Car safety seats				
A3B, textile with foam	< 0.5	63	42	< 0.5
A4, textile with foam	< 0.1	1.1	47	< 0.1
A5A, textile with foam	< 0.3	3.2	150	< 0.3
A8A, textile with foam	130	260	20	< 0.2
Baby slings				
B12A, foam	11	710	< 0.6	< 0.6
B18A, foam and felt	620	1100	66	< 0.5
Baby mattresses				
M24B, foam	< 0.5	< 3	210	< 0.5

< : Means content below the stated limit of detection.

TCEP: Tris(2-chloroethyl) phosphate, CAS nr. 115-96-8

TCPP: Tris(1-chloro-2-propyl) phosphate, CAS nr. 13674-84-5

TDCP: Tris(1,3-dichloro-2-propyl) phosphate, CAS nr. 13674-87-8

TPP: Triphenyl phosphate, CAS nr. 115-86-6

Three of the four tested phosphorus-based flame retardants (TCEP, TCPP and TDCP) were found in artificial sweat following migration testing for 3 hours. TPP, which was present only in low concentrations in the textile samples, was not detected. The relationship between the content in the migration fluid and the content determined in the products vary both between the individual flame retardants and between individual samples. The former difference may be explained by differences in the polarity and boiling point of the substances, while the latter difference is believed to be due to differences in the physical and chemical availability of the flame retardants among the different products.

6. Health assessment

6.1 Introduction

The health assessment in this chapter includes a hazard assessment of three phosphorus-based flame retardants that have been shown to migrate out of the analysed products to a significant extent. Based on the results of the migration tests and on the established exposure scenarios a risk assessment has been performed.

The chemical analyses also showed presence of the phthalate DIDP in a single sample and an aromatic amine derived from azo dyes in two samples, in levels, which are at or above the applicable respective threshold limit. No health assessment has been conducted for these substances.

The quantitative chemical analysis also included substances such as formaldehyde, certain isocyanates and brominated flame retardants, but none of these substances/substance groups occurred in concentrations, which are believed to give rise to health concerns. These substances/substance groups are therefore also omitted from the health assessment.

The hazard assessment was conducted only for those substances that were found in relevant concentrations in the quantitative analyses, since this occurrence can indicate exposure via the individual product types. The hazard assessment focuses on the above mentioned substances.

The exposure assessment was performed for relevant routes of exposure based on the REACH guidance, "Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.15 - Consumer exposure estimation" (ECHA, 2012b). It was assumed in the exposure assessment that the children were 1-12 months of age. For the phosphorus-based flame retardants the exposure calculation was carried out on the basis of the migration found in the tests performed.

The risk assessment was carried out by comparing the estimated exposure with DNEL/DMEL values calculated for the substances.

6.2 Hazard assessment

In the following sections, the phosphorus-based flame retardants TCEP, TCPP and TDCP are described in terms of their harmonized classification according to the CLP Regulation, their health effects and the DNEL (Derived No Effect Level) values identified in the literature or calculated from the NOAEL/LOAEL values. TPP is not included in the assessment since no migration was detected in concentrations above the quantification limit in any of the samples analysed.

6.2.1 Method for calculation of DNEL

DNEL indicates the exposure level at which, according to available knowledge, health effects due to exposure to a given substance are not expected to occur. If no DNELs were identified in the literature, DNEL values have been calculated using the ECHA guideline "Guidance on information requirements and chemical safety assessment. Chapter R.8: Characterisation of dose [concentration] - response for human health" (ECHA, 2012a).

DNEL values for effects with a threshold value were calculated based on an adjusted NOAEL (no observed adverse effect level) or LOAEL (lowest observed effect level) and the appropriate

uncertainty factors (AF: Assessment Factors), selected based on the study, which forms the basis of the NOAEL or LOAEL value. DNEL values can be set for all relevant exposure pathways and situations, or the most sensitive effect can be used as basis for setting the DNEL, taking into account whether the DNEL reflects external or internal exposure. For substances without a threshold, a DMEL (Derived minimal-effect-level) may in some cases be derived, e.g. if a carcinogenicity study is available.

Table 6-1 shows the uncertainty factors used to calculate DNELs.

TABLE 6-1
OVERVIEW OF THE UNCERTAINTY FACTORS USED FOR THE DERIVATION OF DNEL.

Uncertainty	Uncertainty factor
Extrapolation between species (inter-species): allometric scaling (correction for differences in body weight and metabolism between animals and humans)	4 for rats 7 for mice 1.4 for dogs
Extrapolation between species (inter-species): other differences between animals and humans	2.5
Difference between individuals of the same species (intra-species variation)	10
Extrapolation from LOAEL to NOAEL	3 – 10 (depending on the effect)
Duration of exposure in the study	2 – sub-chronic to chronic

6.2.2 Phosphorus-based flame retardants

The following phosphorus-based flame retardants have been included in the quantitative analyses:

- TCEP: Tris (2-chloroethyl) phosphate, CAS no. 115-96-8
- TCPP: Tris (1-chloro-2-propyl) phosphate, CAS no. 13674-84-5
- TDCP: Tris (1,3-dichloro-2-propyl) phosphate, CAS no. 13674-87-8
- TPP: Triphenylphosphate, CAS no. 115-86-6

All four compounds were found in the textile and foam parts, in some of the samples in high concentrations. However, as TPP was not identified above the quantification limit in the migration analyses, no hazard evaluation is performed for TPP.

Harmonised classification

Two of the substances are classified in the category carcinogenic (TDCP and TCEP), and TCEP is also classified as toxic to reproduction (

Table 6-2). There is no harmonised classification of TCPP. The industry proposal for the classification of TCPP, which is available from the ECHA website, does not include the classification of carcinogenicity or reproductive toxicity effects. However, the EU risk assessment report (RAR), and the EU Scientific Committee on Health and Environmental Risks (SCHER) consider it possible to make assessments by analogy in relation to cancer data for the substances TCEP and TDCP.

TABLE 6-2
HARMONISED CLASSIFICATION OF PHOSPHORUS-BASED FLAME RETARDANTS (CLP REGULATION
1272/2008)

Index No.	Substance	CAS No.	Classification	
			Hazard Class and Category Code(s)	Hazard statement Code(s)
015-199-00-X	tris[2-chloro-1-chloromethyl)ethyl] phosphate (TDCP)	13674-87-8	Carc. 2	H351
015-102-00-0	tris(2-chloroethyl)-phosphate (TCEP)	115-96-8	Acute Tox. 4 * Carc. 2 Repr. 1B Aquatic Chronic 2	H302 H351 H360F H411

* There are specific cut-off values for the substances with respect to the selected hazard class.

Health Effects

The health effects of TCEP, TCPP and TDCP are described in EU risk assessment reports (EU RAR, 2009; 2008a and 2008b). Effects of TCPP and TDCP are also summarised in a recent project on consumer products project from the Danish Environmental Protection Agency (Andersen et al., 2014) based on the EU risk assessment reports (EU RAR). The following sections are based on those sources.

TCEP

A study in rats has shown that TCEP to a large extent is absorbed following oral ingestion (about 90% of the dose) and is distributed in the body organs. In the EU risk assessment, absorption rates (oral, dermal and inhalation) of 100% are used (EU RAR, 2009).

Three studies in animals indicate low to moderate toxicity of TCEP. A rat study showed an LD50 in the range of 430-1230 mg/kg bw following oral exposure. The inhalation toxicity appears to be low based on results from tests with rats that survived the 8-hour exposure to aerosols saturated with TCEP. The acute dermal toxicity has also been shown to be low, with a dermal LD50 of > 2150 mg/kg bw in a rabbit study.

TCEP is not considered to be a skin and eye irritant, and is not corrosive.

With regard to repeated dose toxicity, 12 studies are identified in the EU RAR. Most NOAEL values were derived for non-neoplastic effects and ranged between 44-350 mg/kg bw/d. Based on one study in mice, a LOAEL of 12 mg/kg bw/d was established for renal lesions following oral exposure for 18 months. Effects on the kidneys were assessed to be the most sensitive endpoint why this LOAEL is used in the EU risk assessment.

The available mutagenicity data show that TCEP is not mutagenic.

A number of carcinogenicity studies in rats and mice have been conducted with TCEP in accordance with established guidelines. These studies show that TCEP is carcinogenic in rats and mice and form the basis for the classification of TCEP as Carc. 2. TCEP causes benign and malignant tumours in multiple organs in rats and mice. The carcinogenicity of TCEP is considered to be related to non-genotoxic mechanisms and effects on the kidneys (increased cell growth). A LOAEL of 12 mg/kg bw/d was established based a study showing tumour formation at higher concentrations.

The available studies in mice and rats show that TCEP is toxic to reproduction. Exposure of mice resulted in significant impairment of reproduction in both sexes and in a NOAEL of 175 mg/kg

bw/d. A NOAEL of 200 mg/kg bw/d was established in a study on developmental toxicity in rats and a NOAEL of 100 mg/kg bw/d was determined for maternal toxicity.

In light of the foregoing, injuries to the kidneys are assessed to be the critical effect with a LOAEL of 12 mg/kg bw/d, and this value is therefore used in the risk assessment in this report. Since the absorption of TCEP is high (about 90% of the dose by oral intake), the assessments in this report are based on 100% absorption at both oral and dermal exposure, and inhalation.

TCPP

Toxicokinetic properties and health effects of **TCPP** are described in Andersen et al. (2014):

"TCPP is absorbed rapidly and extensively (approximately 80% of the dose) following oral administration and is widely distributed in the organs of the body. TCPP is extensively metabolised in the body, and the metabolites are excreted via urine and faeces. Dermal absorption studies have shown that a dermal absorption of 40% can be used for TCPP (EU RAR, 2008a). The acute toxicity is low because most oral LD50 values are below³ 2000 mg/kg body weight according to a classification as Acute Tox. 4, H302.

Based on an oral 28-day study in rats, a NOAEL of 100 mg/kg body weight/day (in terms of liver effects) was established, and in an oral 90-day study in rats, a LOAEL value of 52 mg/kg body weight/day was found, regarding effects on liver and thyroid.

There are no available cancer studies for TCPP. But the EU Risk Assessment Report and the EU Scientific Committee on Health and Environmental Risks consider it possible to make analogies in relation to cancer data on substances TCEP and TDCPP. On this background, TCPP should be classified as Carc. 2, H451, as this is the EU harmonised classification for the two analogy substances TCDP and TCEP.

In terms of effects on the foetus and fertility, a LOAEL value of 99 mg/kg body weight has been derived from a 2-generation reproduction toxicity study in rats, based on the effects on uterine weight seen in all dosed females in the Fo generation. A LOAEL value of 99 mg/kg body weight is derived for developmental toxicity in the offspring based on the increased number of dwarfism observed in all dose groups in the Fo generation (EU RAR, 2008a). Toxic effects in dams may play a role in these results, but the substance may need to be classified as Repr 2; H361.

TCPP's endocrine disrupting potential has also been studied in an in vitro study using a H295R cell line where the testosterone concentration was increased by TCPP concentrations of 1, 10 and 100 mg/L. Furthermore, data from the 2-generation reproduction study indicate endocrine disruption of TCPP due to findings of uterine weight and extension of the estrogenic cycle. These results indicate that TCPP may change the sex hormonal balance, which may support the classification as presented above. However, it remains to be determined whether increased testosterone levels will also occur in in vivo studies, and this may be associated with the decrease in uterine weight" (Andersen et al., 2014).

TCPP is not considered to be allergenic by skin contact, but in irritation tests a slight degree of skin and eye irritation was demonstrated. TCPP was not genotoxic or mutagenic neither in *in vitro* nor in *in vivo* animal studies (Andersen et al., 2014).

In view of the above, the effect on the liver is assessed to be the critical effect with a LOAEL value of 52 mg/kg bw/day, and this value is therefore used in the risk assessment in this report. The highest

³ In the english version of Andersen et al. (2014) available from the Danish EPA it is stated that "The acute toxicity is low because most oral LD50 values are below 2000 mg/kg body". The authors of this report believe that the correct wording would be "... above 2000 mg/kg".

absorption occurs for oral exposure, where 80% of TCPP is absorbed why this value is used in the calculation of oral exposure. The dermal absorption is 40 % and this value is therefore used in the calculation of dermal exposure in this report.

TDCP

The absorption of **TDCP** based on animal studies is described in the EU RAR (EU RAR, 2008b) to be 100%, 100% and 30% for oral intake, inhalation and dermal absorption, respectively (the latter for handling of foam containing TDCP). These values are used in the exposure calculations. TDCP is extensively metabolised in the body and the metabolites are excreted primarily via the urine, but also via faeces and exhaled air.

A number of animal studies to elucidate the acute toxicity, skin, eye and respiratory tract irritation and sensitization are described in the EU RAR and it is concluded that TDCP has a low acute toxicity (LD50 values > 2000 mg/kg) and no significant potential for either irritation or sensitization.

With regard to repeated dose toxicity, a 2-year cancer study in rats has been conducted, in which groups of 60 male and female rats received diets containing TDCP at doses of 0, 5, 20 and 80 mg/kg bw/day. Significantly higher mortality was observed in the high dose group of males. This study resulted in a LOAEL of 5 mg/kg bw/day for effects on kidneys and testicles. The effects on the kidney (hyperplasia) can be interpreted as a pre-neoplastic lesion, which may lead to tumour formation. Therefore, the established LOAEL of 5 mg/kg bw/day is also considered to be the effect concentration for carcinogenic effects. Several studies of mutagenicity suggest that TDCP is mutagenic *in vitro* but not *in vivo*. The classification of TDCP as Cat. 2 Carc. is based on the rat study and the mutagenicity studies.

In another animal study with repeated dosing, neurotoxic effects were studied but dose-related effects could not be demonstrated. One epidemiological study from a company producing TDCP showed no adverse health effects associated with exposure to TDCP.

A NOAEL value derived on the basis of a reproduction toxicity study in rats exposed to 0, 25, 100 and 400 mg/kg/d on days 6-15 of gestation was used for risk assessment. A NOAEL of 100 mg/kg bw/d was determined based on a statistically significant increased bone resorption, reduced fetal viability and maternal toxicity.

Based on the foregoing, the effect on kidneys and potentially subsequent tumour formation is assessed to be the critical effect with a LOAEL of 5 mg/kg bw/d and this value is therefore used in the risk assessment. The highest absorption occurs by oral exposure, which results in 100% absorption (in animal studies), whereas dermal exposure results in 30% absorption. These values are used in the exposure calculations in this report.

Determination of Derived-no-effect-levels (DNEL)

The determination of DNEL values for the phosphorus-based flame-retardants is described below and the values are listed in **Fejl! Henvisningskilde ikke fundet..**

The DNEL value for **TCEP** is calculated based on a LOAEL for kidney damage of 12 mg/kg bw/d from a 18-month oral carcinogenicity study in mice (EU RAR, 2009). As the scientific committee SCHER has already evaluated TCEP in toys (SCHER, 2012), the same uncertainty factors as used by SCHER are used in the present assessment, i.e. 10 for interspecies variation, 10 for intraspecies variability and 3 for extrapolation from LOAEL to NOAEL. An additional factor of 3 to account for the uncertainty about the importance of TCEP with regard to formation of tumours in humans was also introduced. The latter takes into account that there is a lack of knowledge about the mode-of-action of the substance as well as indications of a threshold for tumour formation. This gives a total

uncertainty factor of 900. As the mice are exposed orally and 100% absorption of TCEP is assumed, the DNEL value for systemic effects becomes 0.013 mg/kg bw/d. For comparison, use of default uncertainty factors recommended by ECHA and shown in Table 6-1 would result in a factor of 17.5 for interspecies variation contrary to the factor of 10 used by SCHER.

The DNEL value of 0.007 mg/kg bw/d for **TCPP** is based on a LOAEL of 52 mg/kg bw/day determined in an oral 90-day rat study corresponds to an internal dose of 41.6 mg/kg bw/day when taking 80% uptake of TCPP by oral exposure into account. The following uncertainty factors are used: interspecies variation (10 (allometric scaling (4), other differences (2.5)), intraspecies variation (10), extrapolation from LOAEL to NOAEL (3) and for the duration of the study (2), i.e. a total uncertainty factor of 600 (Andersen et al., 2014). The DNEL value of 0.07 mg/kg bw/d is within the interval indicated by SCHER for the TDI of 0.057-0.17 mg/kg bw/d.

The DNEL value of 0.005 mg/kg bw/d for **TDCP** is based on an oral LOAEL of 5 mg/kg bw/day determined in the 2-year cancer study. The calculations are based on 100% oral uptake of TDCP and therefore the internal dose is equal to the values found. The total uncertainty factor of 1000 is composed of the following: interspecies variation (10 (allometric scaling (4), other differences (2.5)), intraspecies variation (10) and extrapolation from LOAEL to NOAEL (10). The uncertainty factor for extrapolation from LOAEL to NOAEL is in this case set to 10, since hyperplasia in the kidney is considered a serious effect (Andersen et al., 2014). The DNEL value of 0.005 mg/kg bw/d is within the interval indicated by SCHER for the TDI of 0.005-0.016 mg/kg bw/d.

For the above-mentioned substances, the Commission Directive 2014/79/EC of 20 June 2014 on toy safety sets a limit content of 5 mg/kg for TCEP, TCPP and TDCP in toys intended for children under 36 months and toys intended to be put in the mouth, applicable to each of the three substances.

TABLE 6-3
DNEL VALUES FOR PHOSPHORUS-BASED FLAME RETARDANTS.

Substance	Cas no.	Systemic DNEL	Source
TCEP	115-96-8	0.013 mg/kg bw/d	Determined from a LOAEL of 12 mg/kg bw/d and a total AF of 900 as suggested by SCHER.
TCPP	13674-84-5	0.07 mg/kg bw/dag	Andersen et al., 2014
TDCP	13674-87-8	0.005 mg/kg bw/dag	Andersen et al., 2014

6.3 Assessment of human exposure

Based on the results of the survey and the assessment of the potential for exposure, three product types (car safety seats, baby slings and baby mattresses) are selected for further exposure and risk assessment. The exposure assessment is based on the results of the migration tests from samples taken from car safety seats, baby slings, and mattresses, supplemented with data from the literature.

6.3.1 Method for calculation of human exposure

Calculations of the potential exposure of children using the products from the survey will be based on worst-case scenarios to illustrate the potential dermal and oral exposure of children, mainly aged 1-12 months. If the assumptions used in the worst-case scenarios indicate a risk, the scenarios will be refined using more realistic assumptions. If the worst-case scenarios do not indicate a risk, no further calculations will be carried out.

Dermal exposure

Dermal exposure is assumed to be the most common route of exposure regarding car safety seats, baby slings and baby mattresses. Exposure calculation will as a starting point assume that children only wear diapers/pants, but otherwise are undressed so that direct exposure to chemical substances migrating from the analysed materials is possible on the remaining part of the body.

Development of scenarios follow the principles in the REACH guidance, "Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.15 - Consumer exposure estimation", version 2.1 of October 2012 (ECHA, 2012b). Parameters used in the exposure calculation are shown in Table 6-4.

TABLE 6-4
PARAMETERS USED IN THE EXPOSURE SCENARIOS FOR DERMAL ABSORPTION (FROM ECHA, 2012A).

Parameter	Description	Unit
Migr.	Amount of substance migrating to artificial sweat per surface area of the product per unit time	mg/cm ² /hour
T _{contact}	Contact duration between article and skin per event	hour/event
n	Mean number of events per day	/d
A _{skin}	Area of contact between product and skin	cm ²
bw	Body weight	kg
F _{abs} *	Dermal absorption	%
Resulting parameter	Description	Unit
L _{der}	Dermal load on the skin that is expected due to migration	mg/cm ²
D _{der, external}	Dermal dose per day and body weight	mg/kg bw/d
D _{der, internal}	Dermal absorbed dose per day and body weight	mg/kg bw/d

* For a Tier 1 estimate a dermal absorption of 100 % is usually applied.

The dermal load can, based on measurements of migration to artificial sweat, be calculated in accordance with the following formula:

$$L_{der} = Migr \cdot T_{contact}$$

The external dermal dose is calculated according to the following formula:

$$D_{der,ekstern} = \frac{L_{der} \cdot A_{skin} \cdot n}{BW}$$

If the dermal absorption is included in the formula, the internal dermal dose (the quantity that can be absorbed and transferred to the bloodstream) can be calculated as follows:

$$D_{der,intern} = \frac{L_{der} \cdot A_{skin} \cdot F_{abs} \cdot n}{BW}$$

Oral exposure

Oral exposure related to the tested products is possible in varying degrees. The exposure time will typically be shorter than the useful life, as the children are not expected to suck on products all the time. In relation to the exposure calculations, the time at which the child will be able to suck on the products per day will be estimated

The development of the exposure scenarios will likewise be in line with the REACH guidelines (ECHA, 2012b). No migration tests with saliva have been conducted. As migration to sweat simulant is expected to provide results in the same order of magnitude as saliva simulant, the calculation of the oral exposure will be based in the results from the artificial sweat. Children will only be exposed to a part of the product surface. In relation to the exposure calculations, the proportion of the total surface of the product that the child's mouth can be in contact with will be determined.

Parameters used in the exposure calculation are shown in Table 6-5.

TABLE 6-5

PARAMETERS USED IN THE EXPOSURE SCENARIOS FOR ORAL EXPOSURE (FROM ECHA, 2012B).

Parameters	Description	Unit
Migr.	Amount of substance migrating to artificial sweat per surface area of the product per unit time	mg/cm ² /hour
T _{contact}	Contact duration between article and saliva per hour per day	hour/day
A _{product}	Area of total surface of contact between product and saliva	cm ²
bw	Body weight	kg
F _{abs} *	Oral absorption	%
Resulting parameters	Description	Unit
D _{oral}	Oral dose per day and body weight	mg/kg bw/d

* For a Tier 1 estimate an oral absorption of 100 % is usually applied.

In connection with the chemical analyses only migration test to artificial sweat and not specifically to the artificial saliva have been conducted. The calculation of the daily oral dose is therefore carried out based on measurements of the migration to artificial sweat in accordance with the following formula:

$$D_{oral} = \frac{Migr \cdot T_{contact} \cdot A_{product}}{BW}$$

If the absorption by oral intake is inserted in the formula, the internal daily dose can be calculated as follows:

$$D_{oral,int} = \frac{Migr \cdot T_{contact} \cdot A_{produkt} \cdot n \cdot F_{abs}}{BW}$$

6.3.2 Estimation of dermal exposure

The parameters used for the scenarios for dermal exposure via car safety seats, baby slings and mattresses are shown for each of the substances TCEP, TCPP and TDCP in Table 6-6 , Table 6-7 and **TABLE 6-8** , respectively. The values are based on children aged 6-12 months and with a weight of 9.2 kg for the investigated car safety seats, children aged 1-3 months and weighing 5.6 kg for the investigated baby slings and children aged 3-6 months and with a weight of 7.4 kg for the investigated baby mattresses (US EPA, 2011). The exposed skin surfaces reflect the age of the children, as well as the different body parts that are expected to be in contact with the textile surfaces from the different products.

Different contact surface areas (A_{skin}) between the product and skin are assumed in the worst-case scenarios. For car safety seats a total skin area of 1835 cm² is applied, based on an assumption that half of the 6-12 months child's torso, legs and head, and a quarter of the child's arms are in contact with the surface of the textile product. For baby slings a skin area of 740 cm² is used, based on an assumption that half of the 1-3 month baby's torso and a quarter of the baby's head are in contact with the product's textile surface. For baby mattresses it is assumed that half of the 3-6 month baby's skin surface in contact with the textile surface of the product, which means a contact surface of 1900 cm² (US EPA, 2011). For all products, it is assumed that children sweat when using the products. It is generally assumed that the results of the individual samples are representative of the entire contact area, although this will not be the case in all situations.

Dermal exposure based on the results of the migration test from artificial sweat. The substance specific parameters used for the exposure calculations are shown in the tables below.

TABLE 6-6

PARAMETERS FOR THE CALCULATION OF WORST-CASE SCENARIOS FOR DERMAL ABSORPTION OF TCEP FROM CAR SAFETY SEATS, BABY SLINGS AND BABY MATTRESSES. AN ABSORPTION OF 100% IS APPLIED ($F_{\text{ABS}} = 1$).

TCEP	T _{contact}	n	A _{skin}	bw	Migration	L _{der}	D _{der, external}	D _{der, internal}
Product	h	d ⁻¹	cm²	Kg	(mg/cm²/h)	(mg/cm²)	mg/kg bw/d	
Car safety seats								
A3B ¹	1	1	1835	9.2	< 1.67 × 10 ⁻⁵	< 1.67 × 10 ⁻⁵	< 3.32 × 10 ⁻³	< 3.32 × 10 ⁻³
A4 ²	1	1	1835	9.2	< 3.3 × 10 ⁻⁶	< 3.3 × 10 ⁻⁶	< 6.65 × 10 ⁻⁴	< 6.65 × 10 ⁻⁴
A5A ²	1	1	1835	9.2	< 1 × 10 ⁻⁵	< 1 × 10 ⁻⁵	< 1.99 × 10 ⁻³	< 1.99 × 10 ⁻³
A8A ³	1	1	1835	9.2	4.3 × 10 ⁻³	4.3 × 10 ⁻³	0.864	0.864
Baby slings								
B12A ⁴	1	1	740	5.6	3.67 × 10 ⁻⁴	3.67 × 10 ⁻⁴	3.67 × 10 ⁻²	3.67 × 10 ⁻²
B18A ⁵	1	1	740	5.6	2.07 × 10 ⁻²	2.07 × 10 ⁻²	2.07	2.07
Baby mattresses								
M24B ⁴	3	1	1900	7.4	< 1.67 × 10 ⁻⁵	< 5 × 10 ⁻⁵	< 1.70 × 10 ⁻²	< 1.70 × 10 ⁻²

1 Grey textile and foam + backside; 2 textile with foam; 3 textile and foam; 4 foam; 5 foam and felt

TABLE 6-7

PARAMETERS FOR THE CALCULATION OF WORST-CASE SCENARIOS FOR DERMAL ABSORPTION OF TCP P FROM CAR SAFETY SEATS, BABY SLINGS AND BABY MATTRESSES. AN ABSORPTION OF 40 % IS APPLIED ($F_{\text{ABS}} = 0.4$)

TCP P	T _{contact}	n	A _{skin}	bw	Migration	L _{der}	D _{der, external}	D _{der, internal}
Product	h	d ⁻¹	cm ²	Kg	(mg/cm ² /h)	(mg/cm ²)	mg/kg bw/d	
Car safety seats								
A3B ¹	1	1	1835	9.2	2.1 × 10 ⁻³	2.1 × 10 ⁻³	0.42	0.17
A4 ²	1	1	1835	9.2	3.67 × 10 ⁻⁵	3.67 × 10 ⁻⁵	7.31 × 10 ⁻³	2.93 × 10 ⁻³
A5A ²	1	1	1835	9.2	1.07 × 10 ⁻⁴	1.07 × 10 ⁻⁴	2.13 × 10 ⁻²	8.51 × 10 ⁻³
A8A ³	1	1	1835	9.2	8.67 × 10 ⁻³	8.67 × 10 ⁻³	1.73	0.69
Baby slings								
B12A ⁴	1	1	740	5.6	2.37 × 10 ⁻²	2.37 × 10 ⁻²	3.13	1.25
B18A ⁵	1	1	740	5.6	3.67 × 10 ⁻²	3.67 × 10 ⁻²	4.85	1.94
Baby mattresses								
M24B ⁴	3	1	1900	7.4	< 1 × 10 ⁻⁴	< 3 × 10 ⁻⁴	7.70 × 10 ⁻²	3.08 × 10 ⁻²

1 Grey textile and foam + backside; 2 textile with foam; 3 textile and foam; 4 foam; 5 foam and felt

Table 6-8

PARAMETERS FOR THE CALCULATION OF WORST-CASE SCENARIOS FOR DERMAL ABSORPTION OF TDCP FROM CAR SAFETY SEATS, BABY SLINGS AND BABY MATTRESSES. AN ABSORPTION OF 30% IS APPLIED ($F_{ABS} = 0.3$)

TDCP	T _{contact}	n	A _{skin}	bw	Migration	L _{der}	D _{der, external}	D _{der, internal}
Product	h	d ⁻¹	cm ²	Kg	(mg/cm ² /h)	(mg/cm ²)	mg/kg bw/d	
Car safety seats								
A3B ¹	1	1	1835	9.2	1.40 × 10 ⁻³	1.40 × 10 ⁻³	0.28	8.38 × 10 ⁻²
A4 ²	1	1	1835	9.2	1.57 × 10 ⁻³	1.57 × 10 ⁻³	0.31	9.37 × 10 ⁻²
A5A ²	1	1	1835	9.2	5 × 10 ⁻³	5 × 10 ⁻³	1.00	0.30
A8A ³	1	1	1835	9.2	6.67 × 10 ⁻⁴	6.67 × 10 ⁻⁴	0.13	3.99 × 10 ⁻²
Baby slings								
B12A ⁴	1	1	740	5.6	< 2 × 10 ⁻⁵	< 2 × 10 ⁻⁵	< 2.64 × 10 ⁻³	< 7.93 × 10 ⁻⁴
B18A ⁵	1	1	740	5.6	2.20 × 10 ⁻³	2.20 × 10 ⁻³	0.29	8.72 × 10 ⁻²
Baby mattresses								
M24B ⁴	3	1	1900	7.4	7 × 10 ⁻³	2.10 × 10 ⁻²	5.39	1.62

1 Grey textile and foam + backside; 2 textile with foam; 3 textile and foam; 4 foam; 5 foam and felt

6.3.3 Oral exposure

The parameters used for the scenarios for oral exposure via car safety seats, baby slings and mattresses are shown for each of the substances TCEP, TCPP and TDCP in Table 6-9, Table 6-10 and Table 6-11, respectively. The values are based on children aged 6-12 months and with a weight of 9.2 kg for the investigated car safety seats, children aged 1-3 months and weighing 5.6 kg for the investigated baby slings and children aged 3-6 months and with a weight of 7.4 kg for the investigated baby mattresses (US EPA, 2011).

It is assumed in the worst-case scenario that the child sucks on the textile from the car seat and baby sling, respectively, for half of the total use period, equivalent to 30 min. and that the child sucks on an area of 10 cm² textile, which corresponds to the area normally considered in relation to toys. For baby mattresses, it is assumed that the child sucks on the textile in contact with the mattress for 30 min. of the total usage time of 3 hours per occurrence and likewise an area of 10 cm². In this case, the worst-case assumption is that the substances will migrate from the mattress foam to the textile cover in an area corresponding to the analysed samples. Migration analyses have been performed on samples with a thickness of 2.8 cm and the estimated exposure is based on the contents herein. An oral absorption of 100% is applied for TDCP and TCEP and an absorption of 80% is used for TCPP.

TABLE 6-9

PARAMETERS FOR THE CALCULATION OF WORST-CASE SCENARIOS FOR ORAL ABSORPTION OF TCEP FROM CAR SAFETY SEATS, BABY SLINGS AND BABY MATTRESSES. AN ABSORPTION OF 100% IS APPLIED ($F_{ABS} = 1$).

TCEP	T _{contact}	n	A _{product}	bw	Migration	Doral, external	Doral, internal
Product	h	d ⁻¹	cm ²	Kg	(mg/cm ² /h)	mg/kg bw/d	
Car safety seats							
A3B ¹	0.5	1	10	9.2	< 1.67 x 10 ⁻⁵	< 9.06 x 10 ⁻⁶	<9.06 x 10 ⁻⁶
A4 ²	0.5	1	10	9.2	< 3.3 x 10 ⁻⁶	< 1.81 x 10 ⁻⁶	< 1.81 x 10 ⁻⁶
A5A ²	0.5	1	10	9.2	< 1 x 10 ⁻⁵	< 5.43 x 10 ⁻⁶	< 5.43 x 10 ⁻⁶
A8A ³	0.5	1	10	9.2	4.3 x 10 ⁻³	2.36 x 10 ⁻³	2.36 x 10 ⁻³

TCEP	T _{contact}	n	A _{product}	bw	Migration	D _{oral, external}	D _{oral, internal}
Product	h	d ⁻¹	cm ²	Kg	(mg/cm ² /h)	mg/kg bw/d	
Baby slings							
B12A ⁴	0.5	1	10	5.6	3.67 x 10 ⁻⁴	3.27 x 10 ⁻⁴	3.27 x 10 ⁻⁴
B18A ⁵	0.5	1	10	5.6	2.07 x 10 ⁻²	1.85 x 10 ⁻²	1.85 x 10 ⁻²
Baby mattresses							
M24B ⁴	0.5	1	10	7.4	< 1.67 x 10 ⁻⁵	< 1.13 x 10 ⁻⁵	< 1.13 x 10 ⁻⁵

1 Grey textile and foam + backside; 2 textile with foam; 3 textile and foam; 4 foam; 5 foam and felt

TABLE 6-10

PARAMETERS FOR THE CALCULATION OF WORST-CASE SCENARIOS FOR ORAL ABSORPTION OF TCP P FROM CAR SAFETY SEATS, BABY SLINGS AND BABY MATTRESSES. AN ABSORPTION OF 80% IS APPLIED (F_{ABS} = 0.8).

TCP _P	T _{contact}	n	A _{product}	bw	Migration	D _{oral, external}	D _{oral, internal}
Product	h	d ⁻¹	cm²	Kg	(mg/cm²/h)	mg/kg bw/d	
Car safety seats							
A3B1	0.5	1	10	9.2	2.1 × 10 ⁻³	1.14 × 10 ⁻³	9.13 × 10 ⁻⁴
A42	0.5	1	10	9.2	3.67 × 10 ⁻⁵	1.99 × 10 ⁻⁵	1.59 × 10 ⁻⁵
A5A2	0.5	1	10	9.2	1.07 × 10 ⁻⁴	5.80 × 10 ⁻⁵	4.64 × 10 ⁻⁵
A8A3	0.5	1	10	9.2	8.67 × 10 ⁻³	4.71 × 10 ⁻³	3.77 × 10 ⁻³
Baby slings							
B12A4	0.5	1	10	5.6	2.37 × 10 ⁻²	2.11 × 10 ⁻²	1.69 × 10 ⁻²
B18A5	0.5	1	10	5.6	3.67 × 10 ⁻²	3.27 × 10 ⁻²	2.62 × 10 ⁻²
Baby mattresses							
M24B4	0.5	1	10	7.4	< 1 × 10 ⁻⁴	< 6.76 × 10 ⁻⁵	< 5.41 × 10 ⁻⁵

1 Grey textile and foam + backside; 2 textile with foam; 3 textile and foam; 4 foam; 5 foam and felt

TABLE 6-11

PARAMETERS FOR THE CALCULATION OF WORST-CASE SCENARIOS FOR ORAL ABSORPTION OF TDCP FROM CAR SAFETY SEATS, BABY SLINGS AND BABY MATTRESSES. AN ABSORPTION OF 100% IS APPLIED (F_{ABS} = 1).

TDCP	T _{contact}	n	A _{product}	bw	Migration	D _{oral, external}	D _{oral, internal}
Product	h	d ⁻¹	cm²	Kg	(mg/cm²/h)	mg/kg bw/d	
Car safety seats							
A3B ¹	0.5	1	10	9.2	1.40 × 10 ⁻³	7.61 × 10 ⁻⁴	7.61 × 10 ⁻⁴
A4 ²	0.5	1	10	9.2	1.57 × 10 ⁻³	8.51 × 10 ⁻⁴	8.51 × 10 ⁻⁴
A5A ²	0.5	1	10	9.2	5 × 10 ⁻³	2.72 × 10 ⁻³	2.72 × 10 ⁻³
A8A ³	0.5	1	10	9.2	6.67 × 10 ⁻⁴	3.62 × 10 ⁻⁴	3.62 × 10 ⁻⁴
Baby slings							
B12A ⁴	0.5	1	10	5.6	< 2 × 10 ⁻⁵	< 1.79 × 10 ⁻⁵	< 1.79 × 10 ⁻⁵
B18A ⁵	0.5	1	10	5.6	2.20 × 10 ⁻³	1.96 × 10 ⁻⁴	1.96 × 10 ⁻⁴
Baby mattresses							
M24B ⁴	0.5	1	10	7.4	7 × 10 ⁻³	4.73 × 10 ⁻³	4.73 × 10 ⁻³

1 Grey textile and foam + backside; 2 textile with foam; 3 textile and foam; 4 foam; 5 foam and felt

6.4 Health risk assessment

Based on the results of the exposure assessment and the identified or predicted DNELs, the risk characterization ratio is calculated as:

$$RCR = \frac{Eksponering}{DNEL}$$

The calculation of the risk characterization ratio is based on internal exposure and the determined DNEL values for systemic effects. If $RCR > 1$ there is a risk.

The dermal exposure is based on the results of the migration test and the maximum values observed and an assumption of 100, 40 and 30 % absorption for TCEP, TCPP and TDCP, respectively. The oral exposure is also based migration to sweat (from an area of 10 cm²) since no migration test with artificial saliva have been conducted, as well as an assumption of 100, 80 and 100 % absorption for TCEP, TCPP and TDCP, respectively (see section 6.2.2).

It is assumed that the child sits for 1 hour in the car seat and in the baby sling and lies for 3 hours on the mattress (see Table 3-1)

TABLE 6-12

CALCULATION OF RISK CHARACTERISATION RATIO FOR ORAL AND DERMAL EXPOSURE TO TCEP.

TCEP	DNEL: 0.013 mg/kg bw/d			
Product	D _{der, internal}	D _{oral, internal}	RCR _{dermal}	RCR _{oral}
	mg/kg bw/d	mg/kg bw/d		
Car safety seats				
A3B1	< 3.32 x 10 ⁻³	< 4.53 x 10 ⁻⁶	0.26	6.97 x 10 ⁻⁴
A42	< 6.65 x 10 ⁻⁴	< 9.06 x 10 ⁻⁷	0.05	1.39 x 10 ⁻⁴
A5A2	< 1.99 x 10 ⁻³	< 2.72 x 10 ⁻⁶	0.15	4.18 x 10 ⁻⁴
A8A3	0.864	1.18 x 10 ⁻³	66.49	0.18
Baby slings				
B12A4	4.85 x 10 ⁻²	1.64 x 10 ⁻⁴	3.73	2.52
B18A5	2.73	9.23 x 10 ⁻³	210.1	1.42
Baby mattresses				
M24B4	< 1.28 x 10 ⁻²	< 5.63 x 10 ⁻⁶	0.99	8.66 x 10 ⁻⁴

TABLE 6-13

CALCULATION OF RISK CHARACTERISATION RATIO FOR ORAL AND DERMAL EXPOSURE TO TCPP.

TCPP	DNEL: 0.07 mg/kg bw/d			
Product	D _{der, internal}	D _{oral, internal}	RCR _{dermal}	RCR _{oral}
	mg/kg bw/d	mg/kg bw/d		
Car safety seats				
A3B1	0.17	4.57 x 10 ⁻⁴	2.39	1.3 10 ⁻²
A42	2.93 x 10 ⁻³	7.97 x 10 ⁻⁶	0.04	2.28 10 ⁻⁴
A5A2	8.51 x 10 ⁻³	2.32 x 10 ⁻⁵	0.12	6.63 10 ⁻⁴
A8A3	0.69	1.88 x 10 ⁻³	9.88	5.38 10 ⁻²
Baby slings				
B12A4	1.25	8.45 x 10 ⁻³	17.87	0.24
B18A5	1.94	1.31 x 10 ⁻²	27.69	0.37

TCPP	DNEL: 0.07 mg/kg bw/d			
Product	D _{der} , internal	D _{oral} , internal	RCR _{dermal}	RCR _{oral}
	mg/kg bw/d	mg/kg bw/d		
Baby mattresses				
M24B4	3.08 x 10 ⁻²	< 2.70 x 10 ⁻⁵	0.44	7.72 x 10 ⁻⁴

TABLE 6-14

CALCULATION OF RISK CHARACTERISATION RATIO FOR ORAL AND DERMAL EXPOSURE TO TDCP.

TDCP	DNEL: 0.005 mg/kg bw/d			
Product	D _{der} , internal	D _{oral} , internal	RCR _{dermal}	RCR _{oral}
	mg/kg bw/d	mg/kg bw/d		
Car safety seats				
A3B1	8.38 x 10 ⁻²	3.80 x 10 ⁻³	16.75	0.15
A42	9.37 x 10 ⁻²	4.26 x 10 ⁻⁴	18.75	0.17
A5A2	0.30	1.36 x 10 ⁻³	59.84	0.54
A8A3	3.99 x 10 ⁻²	1.81 x 10 ⁻⁴	7.98	7.25 x 10 ⁻³
Baby slings				
B12A4	< 7.93 x 10 ⁻⁴	< 8.93 x 10 ⁻⁶	0.16	3.57 10 ⁻³
B18A5	8.72 x 10 ⁻²	9.82 x 10 ⁻⁴	17.44	0.39
Baby mattresses				
M24B4	1.62	2.36 x 10 ⁻³	323.51	0.95

First step in the risk assessment is to set up very conservative assumptions in order to stop the assessment here if possible, provided not even these conservative assumptions will lead to RCR values above 1.

As the calculations in Table 6-12 to Table 6-14 show, the RCR values indicate, based on the applied worst-case assumptions, that there may be a unacceptable risk associated with dermal exposure to TCEP and TCPP in some of the car safety seats and in baby slings and oral exposure to TCEP in one of the baby slings. For TDCP the RCR values indicate a risk associated with car safety seats, baby slings and baby mattresses.

Since the results as mentioned are based on very conservative assumptions, these assumptions are reviewed again for the affected products.

6.4.1 Revised health risk assessment

In the following section, the preconditions for risk calculations are discussed and adjusted in order to make them more realistic. It is estimated that it is not immediately relevant to adjust the uncertainty factors used in the DNEL calculations.

Car safety seats

A_{skin}: In the worst case calculation it is assumed that the textile of the car seat comes in contact with an area equivalent to 40% of the child's skin surface. This is extremely conservative as children of 6-12 months only in rare cases and only limited to the summer months can be expected to sit in the car seat wearing only a diaper. A more realistic estimate would be that 25% of the surface area of the childrens arms, hands and legs come into contact with the textile, corresponding to approximately 10% of the total area and 445 cm².

Migration: It is assumed in the worst case scenario that the same amount, which was measured in the migration test to the artificial sweat, will also migrate out of the material under normal use and come into contact with the child. This assumption may not be realistic, as the amount of sweat is likely to be limited in most of cases where children are sitting in the car seat. Many cars are equipped with air conditioning, and if not, it is likely that users would otherwise provide ventilation when the temperature is so high that children will sweat. Furthermore the migration analyses have been performed in a way where the sample (i.e. both the textile and the underlying foam) has been immersed in the sweat simulant and thus the substances contained in both textile and foam can be released directly to the artificial sweat. Under normal conditions, the textile in itself will act as a barrier to migration from the foam part. The amount that migrates out of the material is therefore assumed to represent a maximum of 10% of the quantity found in the migration analysis.

F_{abs}: In the worst case calculation it is assumed that 100% of the amount of TCEP that could potentially migrate out of the material in contact with sweat is absorbed through skin. Although the substance is problematic when it comes in contact with skin, 100% absorption, during the period of time the child is sitting in chairs is a very conservative assumption, as dermal absorption is expected to take place very slowly. A more realistic estimate of F_{abs} for TCEP is 50%. The dermal absorption for TCP and TDCP is not adjusted, thus still assumed to be 40% and 30%

Baby slings

A_{skin}: It is assumed in the worst-case calculation that the textile from the baby sling comes into contact with an area equivalent to 22% of the child's skin surface. This is also considered conservative, and a more realistic assumption is that a maximum of 25% of the torso, corresponding to 295 cm² comes into contact with the baby sling, since the children are assumed to be wearing at least a diaper, and only one side of the body is exposed.

Migration: Also for baby slings, it is assumed in the worst-case scenario that the same amount, which was measured in the migration test to the artificial sweat, also will migrate out of the material under normal use and come into contact with the child. Analyses were performed on foam and on foam and felt together, respectively, since the initial screening analyses indicated that the substances were only available in the foam. The sample has been immersed in the sweat simulant and have thus the substances contained in all materials can be released directly to the liquid. For the baby sling, the felt will act as a barrier to migration from the foam part. The amount that migrates out of the material and through the felt is therefore assumed to represent a maximum of 10% of the quantity found in the migration analysis.

F_{abs}: As with car safety seats, it is initially assumed that 100% of the amount of TCEP that could potentially migrate out of the material in contact with sweat is absorbed through skin. A more realistic estimate of F_{abs} for TCEP is 50%, as the absorption is expected to take place very slowly. The dermal absorption for TCP and TDCP is not adjusted, thus still assumed to be 40 and 30%, respectively. For TCEP the oral absorption is still assumed to be 100%.

Baby Mattresses

A_{skin}: In the worst-case calculation, it is assumed that the mattress is in contact with about 50% of the child's skin surface. This estimate is also be considered conservative, as it will not be possible during the total exposure period, when the child is laying on a flat surface, and a more realistic estimate of the contact surface is considered to be 25% of the surface skin, i.e. 950 cm². In addition children are expected to wear night clothes a major part of the year.

Migration: Also for baby mattresses, it is assumed in the worst-case scenario that the same amount, which was measured in the migration test to the artificial sweat, will migrate out of the material under normal use and come into contact with the child. Baby mattresses are expected to have a textile cover to protect the mattress and in addition to be covered by a sheet during use. The

analysis was performed on the foam itself, which was immersed in the sweat simulant was able to release the substances directly to the liquid; however, during use covers and bed sheets will act as barriers to migration to the child's skin. The amount that migrates out of the material and through the textile is therefore assumed to be maximum 10% of the quantity found in the migration analysis. This is supported by results from the initial quantitative analysis of the foam from a mattress, where TDCP were found in a level of 89,700 mg/kg, whereas the content of TDCP in textile cover on the mattress 92 mg/kg, thus almost 1000 times lower.

F_{abs}: F_{abs} of TDCP assumed to continue to be 30%, since the child is expected to be in contact with mattress for a longer time than in the case of car safety seats and baby slings.

TABLE 6-15
CALCULATION OF REVISED RISK CHARACTERISATION RATIO FOR DERMAL EXPOSURE

Product	A _{skin}	Migration	F _{abs}	L _{der}	D _{der, external}	D _{der, internal}	RCR _{Dermal}
	cm ²	(mg/cm2/h)		(mg/cm ²)	mg/kg bw/d		
TCEP (DNEL: 0.013 mg/kg bw/d)							
Car safety seats							
A8A ³	445	4.33 x 10 ⁻⁴	0.5	4.33 x 10 ⁻⁴	2.01 x 10 ⁻²	1.05 x 10 ⁻²	0.81
Baby slings							
B12A ⁴	295	3.67 x 10 ⁻⁵	0.5	3.67 x 10 ⁻⁵	1.93 x 10 ⁻³	9.66 x 10 ⁻⁴	0.07
B18A ⁵	295	2.07 x 10 ⁻³	0.5	2.07 x 10 ⁻³	0.11	5.44 x 10 ⁻²	4.19
TCPP (DNEL: 0.07 mg/kg bw/d)							
Car safety seats							
A3B ¹	445	2.1 x 10 ⁻⁴	0.4	2.1 x 10 ⁻⁴	1.02 x 10 ⁻²	4.06 x 10 ⁻³	0.06
A8A ³	445	8.67 x 10 ⁻⁴	0.4	8.67 x 10 ⁻⁴	4.19 x 10 ⁻²	1.68 x 10 ⁻²	0.24
Baby slings							
B12A ⁴	295	2.37 x 10 ⁻³	0.4	2.37 x 10 ⁻³	0.13	4.99 x 10 ⁻²	0.71
B18A ⁵	295	3.67 x 10 ⁻³	0.4	3.67 x 10 ⁻³	0.19	7.73 x 10 ⁻²	1.1
TDCP (DNEL: 0.005 mg/kg bw/d)							
Car safety seats							
A3B ¹	445	1.40 x 10 ⁻⁴	0.3	1.40 x 10 ⁻⁴	6.77 x 10 ⁻³	2.03 x 10 ⁻³	0.41
A4 ²	445	1.57 x 10 ⁻⁴	0.3	1.57 x 10 ⁻⁴	7.58 x 10 ⁻³	2.27 x 10 ⁻³	0.45
A5A ²	445	5 x 10 ⁻⁴	0.3	5 x 10 ⁻⁴	2.42 x 10 ⁻³	7.26 x 10 ⁻³	1.45
A8A ³	445	6.67 x 10 ⁻⁵	0.3	6.67 x 10 ⁻⁵	3.22 x 10 ⁻³	9.67 x 10 ⁻⁴	0.19
Baby slings							
B18A ⁵	295	2.2 x 10 ⁻⁴	0.3	2.2 x 10 ⁻⁴	1.16 x 10 ⁻²	3.48 x 10 ⁻³	0.70
Baby mattresses							
M24B ⁴	950	7 x 10 ⁻⁴	0.3	2.1 x 10 ⁻³	0.27	8.09 x 10 ⁻²	16.18

With regard to oral exposure, the assumption of 10% migration of TCEP from the baby sling, B18A, will result in a risk characterisation ratio of 0.14 and thus an indication that the exposure is not considered unacceptable.

With regard to dermal exposure, it appears from Table 6-15 that the risk characterization ratios calculated after the revision using more realistic assumptions still indicate an unacceptable risk associated with the dermal exposure from the following products:

- **Car safety seats:**
 - Migration of TDCP from the car seat A5A (RCR = 1.45)
- **Baby slings:**
 - Migration of TCEP from the baby sling B18A (RCR = 4.19)
 - Migration of TCPP from the baby sling B18A (RCR = 1.1)
- **Baby mattresses:**
 - Migration of TDCP from the mattress M24B (RCR = 16.18)

It should be emphasized that in the estimation of migration from baby mattress is does not take into account the fact that mattresses are normally expected to be protected with a cover and an additional sheet. These two layers of textiles are expected to act as an effective barrier towards the migrating substances based on the initial quantitative analysis, and to provide substantial protection of the children.

However, if the child is lying directly on the mattress without clothes, then the calculation indicates that there may be an undesirable risk associated with the skin contact.

The test samples from the car seat and the baby sling have been taken from areas expected to be in direct contact with the face. Samples include both felt and foam and as the results are obtained from the samples immersed in the sweat simulant, it will not be reflected if the textile part may be a barrier against the migration of the substances in the foam.

The risk assessment carried out does not account for exposure from other sources of TCEP, TCPP and TDCP. The assessment by the Scientific Committee, SCHER, of TCEP in toys indicates that there is exposure to TCEP from dermal contact with furniture and dust, inhalation and drinking water, which may also be possible for the other substances. In addition, it is also possible that children are exposed to substances from car safety seats, baby slings and mattresses within the same period of time.

7. Environmental assessment

7.1 Introduction

The preliminary hazard assessment also included a screening for harmonized environmental classification of substances/groups of substances that, according to the literature survey (see Chapter 2), had been identified in various previous studies of car safety seats or other textile products intended for children.

The results of this screening are presented in Appendix 3, but virtually none of the substances identified in this study have a harmonized environmental classification (with the phosphorus-based flame retardant TCEP classified "Aquatic Chronic 2" with hazard statement H411 as an exception). This does of course not exclude substances without harmonized classification from having inherent hazardous properties.

Environmental exposure to chemicals from the selected product groups - car safety seats, baby slings and baby mattresses - is estimated to be very limited. This is partly because the chemicals in those products are released only to a very limited degree in the products' lifetime. For example, they are usually not washed regularly, and that the consumption of the chemical substances used in car safety seats, baby slings and/or baby mattresses represent only a small part of the total consumption of those chemicals.

The specific chemical analyses in this project has included phosphorus-based flame retardants, brominated flame retardants, some phthalates, azo dyes, isocyanates and formaldehyde. However, as shown in Chapter 5, only a modest number of substances have been detected in quantities that are relevant for the further (primary health) assessment. The substances are a number of phosphorus-based, chlorinated flame-retardants in a number of samples, one phthalate (diisodecylphthalate, DIDP) and aromatic amines as indicators of azo dyes in a few samples.

7.2 Environmental effects

In the following, the main ecotoxicological data of relevance for environmental risk assessment are presented for the substances detected in the samples at concentrations that cannot automatically be considered negligible.

7.2.1 Phosphorus-based flame retardants

At the initial GC/MS screening the presence of phosphorus-based flame retardants was demonstrated in 25 sub-samples representing 15 products (8 car safety seats, 6 baby slings and 1 baby mattress), which were then analysed quantitatively for four P-based flame retardants:

- TCEP: Tris (2-chloroethyl) phosphate, CAS no. 115-96-8
- TCPP: Tris (1-chloro-2-propyl) phosphate, CAS no. 13674-84-5
- TDCP: Tris (1,3-dichloro-2-propyl) phosphate, CAS no. 13674-87-8
- TPP: Triphenyl phosphate, CAS no. 115-86-6

As shown in Section 5.3.1, the substances TCPP and TDCP were identified most frequently and in the highest concentrations, while content above 0.1% of TCEP only occurred in two products. TPP was not detected in relevant concentrations and is not discussed further here.

For TCPP and TDCP the published REACH registration data found on the EU Chemicals Agency's (ECHA) website suggested the following "Predicted No Effect Concentration (PNEC)" values, i.e. the concentrations below which effects on aquatic organisms are not expected to occur:

- TCPP: PNEC (freshwater): 0.64 mg/liter
PNEC (soil): 1.7 mg/kg soil dw
- TDCP: PNEC (freshwater): 0.01 mg/liter
PNEC (soil): 0.33 mg/kg soil dw

Thus, TDCP is the most toxic of the two substances, and since this was also the flame retardant that was detected in high concentrations in most samples it seems reasonable to base a realistic worst-case assessment for the aquatic environment on the data for this substance, i.e. a PNEC = 0.01 mg/L.

7.2.2 Phthalates (DIDP)

An EU environmental risk assessment of di-isodecyl phthalate (DIDP) is available (EU RAR, 2003), in which the available ecotoxicological data for the substance are reviewed and assessed. The report presents the following most sensitive endpoints for the main groups of test organisms, i.e. fish, daphnia and algae:

- LC50 (fish): $\geq 0,47$ mg/L
- EC50 (Daphnia): $\geq 0,18$ mg/L
- EC50 (algae): $\geq 1,3$ mg/L

There was no evidence of impact at the stated values, and since no reliable chronic test results were reported, it was found in the EU assessment that it was not possible to derive a PNEC.

For the assessment in this report we propose instead to apply the EU environmental quality standards (EQS) for the related phthalate DEHP (di (2-ethylhexyl)phthalate), which is 1.3 $\mu\text{g/L}$ (= 0.0013 mg/L) (implemented in Denmark by Statutory Order No. 1022 of 25.08.2010) and is the lowest of the EQS's set for phthalates in this Order.

In the EU risk assessment of DEHP, a PNEC for soil of >13 mg/kg soil dw is derived.

7.2.3 Azo dyes

Azo dyes are determined not directly, but through some characteristic aromatic amines (see Chapter 5) that they may degrade to and for which limits are set under the REACH Regulation. In this study, the substance 4-methyl-m-phenylenediamine (= toluene-2,4-diamine; TDA) was found in two samples at levels around or above the permitted limit of 30 mg/kg. Other amines listed in Appendix 8 of the REACH Regulation Annex XVII were not detected or only found at trace level.

TDA is described and environmentally assessed in an EU risk assessment (EU RAR, 2008). Based on the available data the following PNECs for fresh water and soil were derived for TDA:

PNEC (freshwater): 1.6 mg/L
PNEC (soil): 0.32 mg/kg soil dw

This value is proposed to be used for risk assessment of azo dyes.

7.3 Environmental exposure

Chapter 6 on health assessment describes how the identified chemicals could be released from a textile product during use, including via air, and lead to exposure of humans. Release to **air** will typically occur very slowly through migration to and evaporation from the textile surface.

With regard to the **aquatic** environment, chemical substances in textiles for children will primarily be released indirectly via the public wastewater system in connection with washing of the products. Many of the substances end up primarily in the sludge phase at the sewage treatment plants, but some of the more water-soluble will to some extent remain (unchanged) in the aqueous phase and be discharged to the aquatic environment with the treated effluent. For the products in question it is, however, assessed that the frequency of washing is low (baby sling most frequently) or absent (foam part of baby mattresses).

With regard to **soil** the only relevant exposure route is assessed to be via application of sewage sludge on agricultural land.

Environmental exposure in the **waste** phase of the products is considered to low as the textile and foam parts of the products are expected to be disposed of/destroyed in a waste incineration plant.

7.3.1 Exposure of the aquatic environment via sewage

At textile washing by consumers the spent wash water is assumed to be discharged into the public sewage system and on to a sewage treatment plant, where a substance will undergo transformation to varying degrees and there will be a distribution between the water phase and solid phase (sewage sludge), depending on the specific properties of the substance.

According to the ECHA guidance document (ECHA, 2012c) a standard EU sewage treatment plant is to be used for the assessment of the environmental exposure resulting from domestic washing. Such a plant has a capacity of 10,000 PE (person equivalents) and each PE contributes with 200 liters of water/day and 0,011 kg sludge dw/day. This corresponds for a sewage treatment plant of this size 730,000 m³ of wastewater/year and 40 tons of sludge dw/year. In the current context it is assumed that the 10,000 PE are distributed over 2,500 households of four persons each.

In principle, the daily discharge of the studied compounds with sewage from a standard household /family can be calculated using the following formula:

Daily discharge of Substance-X from textile washing per household = Number of machine wash/day * Amount (w) of textile/wash * Proportion (%) of textile containing Substance-X * Proportion (%) of Substance-X in the textile * Proportion (%) of Substance-X emitted/wash.

Here, however, initially a **simplistic worst-case exposure calculation** will be made for an arbitrary substance, "Substance X", in an arbitrary product since the exposure beforehand is assessed to be very limited:

It is assumed that the content of Substance X in the product is 10,000 mg/kg, and that the product is washed one or more times in the course of a year, whereby the entire content of Substance-X (i.e. 100%) is released and discharged with the wastewater. The weight of the textile part of the product is set to 0.5 kg.

According to data retrieved from the Danish Statistics Bank (www.statistikbanken.dk), the proportion of 0-3 year olds (inclusive) in the Danish population per 1 January 2014 was approximately 4%. For the calculation here the fraction is, a bit more conservatively, set to 5%, corresponding to 500 children aged 0-3 years in the model catchment area (10,000 PE).

From this the total annual release of Substance-X to wastewater from washing of the childrens' textile products is calculated to be:

$$0.5 \text{ kg} \times 10,000 \text{ mg/kg} \times 100\% \times 500 = 2,500,000 \text{ mg} = 2.5 \text{ kg}.$$

The concentration in the wastewater becomes:

$$2,500,000 \text{ mg} / 730,000 \text{ m}^3 = 0.0034 \text{ mg/L} = 3.4 \text{ } \mu\text{g/L}.$$

In relation to the aquatic environment is assumed as worst case, that there will be no degradation of Substance-X place in the sewage treatment plant and that the entire amount is released to the water environment (i.e. 100 % allocation to the aqueous phase in the STP). Thereby, the Predicted Environmental Concentration, PEC, at the discharge point becomes 3.4 µg/L.

7.3.2 Exposure of soil environment via sewage sludge

As a worst-case exposure of soil during application of sewage sludge is assumed that Substance-X undergoes no degradation in the STP and is distributed 100 % to the sludge phase, after which the produced sludge is applied to farmland.

According to the REACH scenario the annual production of sludge at the standard STP is 0.011 kg sludge dw/day/PE x 10,000 PE x 365 days = 40.150 kg sludge dw/year ~ around 40 tonnes of sludge dw/year. According to the Danish Statutory Order on the use of sludge (Order no. 1650 of 13.12.2006), the maximum amount of sludge that can be applied on arable land is 7 tonnes dw/hectare/year (as an average over 10 years), which is equivalent to 7/40 (17.5%) of the sludge produced at the plant. This means that 17.5% of the released amount of Substance-X (i.e. 437,500 mg) is applied to 1 hectare (ha) of land.

If it is assumed that the sludge is mixed homogeneously in the upper 20 cm of the soil, and that the soil density is 1.5 t/m³, the amount of directly exposed soil per hectare will be about 3000 tonnes. The concentration of Substance-X in the soil will then be 437,500 mg/3,000,000 kg soil dw = 0.15 mg/kg soil dw.

7.3.3 Environmental exposure through air

Emissions to air are evaluated under the health part of the project. The environmental impact of this emission from childrens textiles is estimated to be marginal and will not be quantified here.

7.4 Environmental risk assessment

A worst-case environmental risk assessment is carried out based on the lowest PNEC (or EQS value) that is identified for any substance in section 7.2, which is 1.3 µg/L for the substance di-isodecyl phthalate, DIDP (actually, the value applies to DEHP, but is used here to represent DIDP). The second lowest value is 10 µg/L for TDCP.

If the PNEC (EQS) for DIDP (i.e. 1.3 µg/L) is compared to the estimated worst-case concentration (PEC) of Substance-X in the effluent from the sewage treatment plant of 3.4 µg/L the following risk quotient (= PEC/PNEC) can be derived: **PEC/PNEC (freshwater) = 3.4/1.3 = 2.6.**

In other words, a dilution of 2.6 times in the receiving water body is required to meet the environmental (water) quality standard for Substance-X in the worst case. This is normally considered to be a low dilution factor (commonly a standard initial dilution factor of 10 is used). Given the conservative assumptions that were made for derivation of the PEC and that the lowest PNEC/EQS for any of the substances in question was used, it is not considered necessary to refine the calculation further as the risk to the aquatic environment from washing of childrens textiles obviously will be very small.

Regarding soil, the lowest PNEC value is for toluene-2,4-diamine (TDA), namely 0.32 mg/kg soil dw and since the PEC for Substance-X was calculated to be 0.15 mg/kg soil this gives the following risk quotient: **PEC/PNEC (soil) = 0.15/0.32 = 0.47.**

As the PEC/PNEC is <1, the risk from exposure of the soil as a result of the release of Substance-X from washing of childrens textiles is assessed to be negligible.

8. Conclusion

8.1 Survey

The results of the literature (and questionnaire) survey can be summarized as follows:

It is estimated that 75 - 80% of the car safety seats on the Danish market originate from countries outside the EU, and of these, the majority from China. The remaining 20-25% are produced in the EU, mostly in Germany but also in Italy. Car safety seats produced in the EU are typically more expensive than the Asian products. Regarding the other children's articles, there is a major producer of prams in Europe (covering roughly half of the market), while other children's articles as cradle seats, baby slings and bunting bags mostly originate from countries outside the EU (approximately 80%).

In general, it seems that dealers do not know whether harmful chemicals are present in the products or if chemicals are added to meet certain technical or functional requirements such as water repellancy or fire retardant properties.

Previous studies have shown that the following substances/substance groups can be present in significant concentrations:

- Phosphorus-based flame retardants (many car safety seats and other product groups).
- Phthalates (some car safety seats and other product groups).
- Brominated flame retardants (in a few car safety seats, and detection of bromine as an indicator in other product groups)
- Nonylphenol (baby slings)

A preliminary hazard assessment was performed for the key substances/groups that were previously found in relevant textile products, including car safety seats. Further, a preliminary exposure assessment was made related to products with textiles for children, especially small children. Based on these assessments, car safety seats, baby slings and baby mattresses were selected as product groups for the chemical analysis programme and a total of 30 products were purchased for the purpose.

8.2 Chemical analysis and testing

The first phase in the chemical analysis programme was a screening analysis of all 30 product samples by GC/MS. This was because the survey not had clearly identified specific substances or groups of substances which occurred frequently enough in the products to justify their selection for the further programme solely on basis of the survey. In the screening analyses several unidentifiable compounds occurred in low concentrations, i.e. concentrations that did not indicate that the substance had deliberately been added to provide the textile product with a specific functionality. Only few identifiable substances occurred in concentrations that were assessed to be significant in relation to possible health impacts. A screening analysis by X-ray (only comprising car safety seats) showed a high content of bromine in some samples, which could be due to content of brominated flame retardants, while a specific analysis of formaldehyde (not detectable by GC/MS) did not detect occurrence of this substance in any sample.

The specific substances that based on the results of the GC/MS screening and initial hazard and exposure assessment were selected for quantitative chemical analysis were: phosphorus-based flame retardants, the phthalate DIDP, azo dyes (through certain aromatic amines as indicators), isocyanates and brominated flame retardants. Of these only the substances TCEP, TCPP and TDCP (all phosphorus-based flame retardants) and the phthalate DIDP occurred in significant concentrations, i.e. above the general lower limit for classification of hazardous substances in articles = 0.1 % = 1000 mg/kg. Further, a content of the aromatic amine, 4-methyl-m-amino-phenylenediamine (= 2,4-TDA) was detected at or above the limit value of 30 mg/kg in two products. The substance can be released from certain azo dyes that are regulated in textile and leather articles.

In contrast to this, the specific analyses revealed no presence of isocyanates or brominated flame retardants in concentrations above about 1 mg/kg, despite indications of some content by the screening analyses. It is therefore concluded for isocyanates that GC/MS screening is not a useful method to give indication of presence of these substances in various matrices, while for the brominated flame retardants it is assessed that the content of bromine in the samples probably is due to polymeric brominated flame retardants, which can not be detected by the analysis performed.

Testing of migration (to sweat) was performed for phosphorus-based flame retardants. The seven products having the highest content of one or more of the flame retardants were tested and migration was observed in all tests, albeit in greatly varying extent.

Overall, it is concluded that in previous studies of textile products several chemical substances considered potentially hazardous to health or the environment have been found in a number of products, but these substances were only to a very limited extent found to be present in the 30 products analysed in this project.

8.3 Health assessment

On the basis of the results of the quantitative chemical analysis an assessment of health risks was performed for the substances that migrate out of the products to a significant extent, since this occurrence may result in exposure via the individual product types. The hazard assessment therefore focuses on the three phosphorus-based flame retardants (TCEP, TCPP and TDCP), while no risk assessment has been conducted for either diisodecyl phthalate (DIDP) or the aromatic amines from azo dyes.

Phosphorus-based flame retardants

TCEP is associated with a low to moderate acute toxicity. In rat studies, it appears that up to 90% of the substance is absorbed after oral intake. Lesions in the kidney after repeated exposure is assessed to be the most sensitive endpoint with a LOAEL of 12 mg/kg bw/d. The substance has not been shown to be mutagenic, but is classified as a carcinogen in category 2 (C2), with a carcinogenic effect related to the effects on the kidneys. TCEP has also been shown to be toxic to reproduction in mice and rats (Repr. 1B), and a NOAEL of 175 mg/kg bw/d is set because of impairment of fertility in both sexes.

TCPP is considered to have a low acute toxicity. Up to 80% of the substance is rapidly absorbed after oral intake, and dermal absorption studies have shown that a dermal absorption of 40% can be used for TCPP. Based on analogy with TCEP, TCPP is also classified as Carc. 2, H451. In a 90 day study in rats, a LOAEL value of 52 mg/kg bw/day was determined based on effects on liver and the thyroid gland. *In vitro* studies also indicate some endocrine disruption potential.

TDCP is also assessed to have low acute toxicity. Animal studies indicate an absorption of 100%, 100% and 30% for oral intake, inhalation and dermal absorption, respectively. Neurotoxic effects

were observed in studies with repeated exposure but a dose-response factor has not been established. TDCP is mutagenic *in vitro* but not *in vivo*, and it is classified Carc 2. on the basis of effects on the kidneys with a LOAEL of 5 mg/kg bw/d.

Health risk assessment

Based on the results of the chemical analyses and the worst-case exposure scenarios for the use of car safety seats, baby slings and baby mattresses, the health risk associated with the above substances has been estimated. This was done based on the procedures in the ECHA guidance document and on DNELs identified in the literature or calculated. The results, which were based on very conservative assumptions, indicated that there may be a risk associated with the exposure of children to particularly TCEP in car safety seats and baby slings, and TDCP in car safety seats, baby slings and baby mattresses, if the children are exposed in the way described in the worst-case scenarios. The scenarios were therefore adjusted in order to make the assumptions more realistic. The assumptions in the Tier 1 calculations were adjusted with regard to the skin surface area in contact with the products, the migration from the products and the absorption of the substances through the skin.

The results of the Tier 1 calculation indicated an undesirable risk associated with a single car seat, a baby sling and a baby mattress.

With regard to baby mattresses it should be stressed that they are usually expected to be protected with a cover and in addition with a sheet during use and these two layers of textile are expected to act as a barrier to migration. This is supported by the results from the initial quantitative analyses, which showed that the content of TDCP in the mattress cover was approximately 1000 times lower than the content in the foam. It is therefore assumed that the textile cover is an effective barrier which can provide considerable protection of children using the mattress. However, based on the results, it cannot be excluded that there may be a risk associated with the use, if the children, are in direct contact with the mattress without cover.

The test samples from the car seat and the baby sling have been taken from areas expected to be in direct contact with the face. Samples include both felt and foam and as the results are obtained from the samples immersed in the sweat simulant, it will not be reflected if the felt part may be a barrier against the migration of the substances from the foam.

The risk assessment carried out does not account for exposure from other sources of TCEP, TCPP and TDCP. The assessment by the Scientific Committee, SCHER, of TCEP in toys indicates that there is exposure to TCEP from dermal contact with furniture and dust, inhalation, and drinking water, which may also be possible for the other substances. In addition, it is also possible that children are exposed to substances from car safety seats, baby slings and mattresses within the same period of time.

8.4 Environmental assessment

Following the initial exposure assessment, which indicated a limited environmental load with chemicals from the selected product groups, it was decided to limit the environmental risk assessment to a conservative screening assessment of environmental impact based on an arbitrary substance, "Substance X", in an arbitrary product at a concentration of 10,000 mg/kg and 100% release to wastewater within a period of one year. Assuming that all children in the age group 0-3 years use such textiles, the resulting concentrations (PEC) in sewage effluent and sludge, respectively, were estimated under worst case conditions and compared to the lowest Predicted No-Effect Level (PNEC) reported for any of the relevant, quantified substances – i.e. TCEP, TCPP, TDCP, DIDP and azo dyes (aromatic amines).

It was from this conservative screening assessment concluded that the risk of environmental effects in aquatic or soil environments due to release of the assessed substances from washing of childrens' textiles was insignificant and therefore it was not considered necessary to perform a more detailed assessment. Similarly, an environmental impact from emission of the substances to air was estimated to be insignificant.

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Appendix 1: Questionnaire for suppliers and producers of car safety seats and other textile products for children

Questions concerning chemical substances in car safety seats and textile products for children on the Danish market

COWI is in collaboration with the Danish Technological Institute preparing a survey concerning chemical substances in car safety seats and other textile products for children on the Danish market for the Danish EPA. As a part of the project, companies are asked to support the survey by providing information on the topic. Please consider the following questions, which will presumably take 10 – 30 min to fill in depending on the amount of information and knowledge available.

Thank you very much in advance!

Your company:

Contact person:

Email:

Phone:

1. The following product groups are included in the survey. Which of the following **textile products** for children does your company sell?

Please also indicate the region of manufacture for each product category. In the case that products originate from different regions, please give an estimate on the relative distribution (e.g. EU 20%, non-EU 80%).

	<i>Region of manufacture</i>			<i>Distribution</i>			Don't know
	DK	EU	non-EU	% from DK	% from EU	% from non-EU	
☐ Car safety seats (Danish: Autostole)	☐	☐	☐				☐
☐ Buggies (Danish: Barnevogn)	☐	☐	☐				☐
☐ Cradle seats (Danish: Skråstole)	☐	☐	☐				☐
☐ Carriers (Danish: Bærestole)	☐	☐	☐				☐
☐ Changing mats (Danish: Skifteunderlag)	☐	☐	☐				☐
☐ Buggy sleeping bags (Danish: Køreposer)	☐	☐	☐				☐
☐ Travel- or camping beds	☐	☐	☐				☐
☐ Children's beanbag chair (Danish: Sækkestole)	☐	☐	☐				☐

2. With respect to car safety seats, would it be possible for you to indicate the *approximate* number of car safety seats (or alternatively the value of articles) that your company is selling yearly and/or the number of articles that are sold in Denmark yearly?

Number of car safety seats sold by your company: _____

Number of car safety seats sold in Denmark: _____

3. Do any of the product types have the listed functional requirements (either because suppliers label their products with those properties or because your company requires the products to fulfill those functions)?

	<i>Functional requirement</i>						
	Water repellency	Dirt repellency	Fire retardancy	Odour-inhibiting	Anti fouling	Color retention	UV-proof
Car safety seats (Danish: Autostole)	☐	☐	☐	☐	☐	☐	☐
Buggies (Danish: Barnevogn)	☐	☐	☐	☐	☐	☐	☐
Cradle seats (Danish: Skråstole)	☐	☐	☐	☐	☐	☐	☐
Carriers (Danish: Bærestole)	☐	☐	☐	☐	☐	☐	☐
Changing mats (Danish: Skifteunderlag)	☐	☐	☐	☐	☐	☐	☐
Buggy sleeping bags (Danish: Køreposer)	☐	☐	☐	☐	☐	☐	☐
Travel- or camping beds	☐	☐	☐	☐	☐	☐	☐
Children's beanbag chair (Danish: Sækkestole)	☐	☐	☐	☐	☐	☐	☐

4. Do you know if any of the following substances are used **intentionally** in the above mentioned products? Please tick both YES and NO if some products in a category contain the substance while others do not.

	YES	NO	Don't know	If yes, please indicate which product(s) (only textile and foam parts) and brand(s):
Organotin compounds	☐	☐	☐	
Formaldehyde	☐	☐	☐	
Triclosan	☐	☐	☐	
Other biocides:	☐	☐	☐	
Pesticides	☐	☐	☐	
Flame retardants	☐	☐	☐	
UV filters and/or stabilizers	☐	☐	☐	
Ftalates	☐	☐	☐	
Azo dyes	☐	☐	☐	

Alkylphenols and alkylphenolethoxylates	☐	☐	☐	
Solvents (e.g. NMP, DMAc, DMF)	☐	☐	☐	
Per- or polyfluorinated substances	☐	☐	☐	
Chlorinated phenols, benzenes or toluenes	☐	☐	☐	
Polycyclic aromatic hydrocarbons (PAH)	☐	☐	☐	
Heavy metals	☐	☐	☐	
Aniline	☐	☐	☐	
Other:	☐	☐	☐	

5. Do you know if any of the following substances are contained **unintentionally** in the above mentioned products? The substances may be contained *e.g.* as residues from textile processing.

	YES	NO	Don't know	If yes, please indicate which product(s) (only textile and foam parts) and brand(s):
Organotin compounds	☐	☐	☐	
Formaldehyde	☐	☐	☐	
Triclosan	☐	☐	☐	
Other biocides:	☐	☐	☐	
Pesticides	☐	☐	☐	
Flame retardants	☐	☐	☐	
UV absorbers and/or stabilizers	☐	☐	☐	
Phthalates	☐	☐	☐	
Azo dyes	☐	☐	☐	
Alkylphenols and alkylphenolethoxylates	☐	☐	☐	
Solvents (e.g. NMP, DMAc, DMF)	☐	☐	☐	
Per- or polyfluorinated substances	☐	☐	☐	
Chlorinated phenols, benzenes or toluenes	☐	☐	☐	
Polycyclic aromatic hydrocarbons (PAH)	☐	☐	☐	
Heavy metals	☐	☐	☐	
Aniline	☐	☐	☐	
Other:	☐	☐	☐	

6. Does your company have any requirements restricting the use or content of certain substances in your products? If yes, to which products do the requirements apply?

<i>Requirement</i>	YES	NO	Don't know	If yes, please indicate which product(s) and brand(s):
OEKO-TEX® Standard 100	☐	☐	☐	
OEKO-TEX® Standard 1000	☐	☐	☐	
EU flower	☐	☐	☐	
Nordic Ecolabel – Swan	☐	☐	☐	
Other:	☐	☐	☐	

7. Has any analysis of chemical substances in the textile products sold in your company been performed? Can you provide any results from textiles analyses on possible concentration ranges of the substances?

	Analysis	<i>Compound detected</i>			If yes, please indicate product(s) (textile and foam parts) and concentration range:
		YES	NO	Don't know	
Organotin compounds	☐	☐	☐	☐	
Formaldehyde	☐	☐	☐	☐	
Triclosan	☐	☐	☐	☐	
Other biocides:	☐	☐	☐	☐	
Pesticides	☐	☐	☐	☐	
Flame retardants	☐	☐	☐	☐	
UV absorbers and/or stabilizers	☐	☐	☐	☐	
Ftalates	☐	☐	☐	☐	
Azo dyes	☐	☐	☐	☐	
Alkylphenols and alkylphenolethoxylates	☐	☐	☐	☐	
Solvents (e.g. NMP, DMAc, DMF)	☐	☐	☐	☐	
Per- or polyfluorinated substances	☐	☐	☐	☐	
Chlorinated phenols, benzenes or toluenes	☐	☐	☐	☐	
Polycyclic aromatic hydrocarbons (PAH)	☐	☐	☐	☐	
Heavy metals	☐	☐	☐	☐	
Aniline	☐	☐	☐	☐	
Other:	☐	☐	☐	☐	

8. Other comments:

Appendix 2: List of chemical substances from the Danish Consumer Council survey of car safety seats

PAH
Naphthalene
Acenaphthylene
Acenaphthene
Fluorene
Phenanthrene
Anthracene
Fluoranthene
Pyrene
Benzo[a]anthracene
Chrysene
Benzo[b]fluoranthene
Benzo[j]fluoranthene
Benzo[k]fluoranthene
Benzo[e]pyrene
Benzo[a]pyrene
Indeno[1,2,3-c,d]pyrene
Dibenzo[a,h]anthracene
Benzo[g,h,i]perylene
Phthalates
Dimethylphthalate
Diethylphthalate
Diisobutylphthalate
Dibutylphthalate
Benzyl-Butylphthalate
DEHP
Diisononylphthalate
Diisodecylphthalate
Di-n-Octylphthalat
Diethylhexyladipate
Acetyltributylcitrate (Citroflex)
Plasticizers/flameretardants
TCEP (Tris-Chlorethylphosphate)
TCPP (Tris-Chlorpropylphosphate)
TDCPP (Tris-Dichlorpropylphosphate)
Tris-Butylphosphate
Tri-Phenylphosphate
TKP (Tris-Kresylphosphate)
TBEP (Tris-(Butoxyethyl)phosphate)
Phenolic compounds
Bisphenol A (frei und freigesetzt)
Phenol (frei und freigesetzt)
Nonylphenol (frei und freigesetzt)
Antimicrobial
Triclosan
o-Phenylphenol
Chlorkresol
PCP
Premethrin
Organo tin
MBT
DBT
TBT
MOT
DOT
TPhT

Brominated flameretardants
Hexabrombiphenyl
Pentabromdiphenylether
Octabromdiphenylether
Decabromdiphenylether
Hexabromoxyclododecan
Tetrabrombisphenol A
Metals
Antimon
Arsen
Barium
Blei
Cadmium
Chrom
Quecksilber
Selen
Colourants
Acid Red 26
Basic Red 9
Basic Violet 14
Direct Black 38
Direct Blue 6
Direct Red 28
Disperse Blue 1
Disperse Orange 11
Disperse Yellow 3
Disperse Blue 3
Disperse Blue 7
Disperse Blue 26
Disperse Blue 35
Disperse Blue 102
Disperse Blue 106
Disperse Blue 124
Disperse Brown 1
Disperse Orange 1
Disperse Orange 3
Disperse Orange 37
Disperse Red 1
Disperse Red 11
Disperse Red 17
Disperse Yellow 1
Disperse Yellow 9
Disperse Yellow 39
Disperse Yellow 49
Solvent Yellow 1
Solvent Yellow 2
Solvent Yellow 3
Basic Violet 1
Basic Violet 3

4-Chloranilin
Benzidin
o-Toluidin
4,4'-Diamino-3,3'-dichlorodiphenylmethan
2-Naphthylamin
3,3'-Dimethylbenzidin
4,4'-Diamino-3,3'-dimethyldiphenylmethan
3,3'-Dimethoxybenzidin
4-Chloro-o-toluidin
Anilin
2-Methyl-5-Nitroanilin (2-Amino-4-nitrotoluen)
4,4'-Thiodianilin
4-Aminobiphenyl
2,4-Diaminotoluol (2,4-Toluyldiamin)
o-Anisidin (2-Methoxyanilin)
4-Aminoazobenzol (4-Aminoazobenzene)
2,4,5-Trimethylanilin
4,4'-Diaminodiphenylmethan
o-Aminoazotoluol
p-Kresidin
4,4'-Oxydianilin
3,3'-Dichlorbenzidin
2,4-Diaminoanisoole
2,4-Xylidine
2,6-Xylidine

Appendix 3: Health screening of a range of substances previously identified in textile products for children (in Danish)

De følgende afsnit giver en oversigt over sundhedseffekter af de kemiske stoffer, der jf. kortlægningen er påvist i relevante produkter med tekstil til børn i forskellige tidligere undersøgelser. Beskrivelserne er baseret på klassificering, forekomst på EU's liste over hormonforstyrrende stoffer⁴ og informationer fra tidligere forbrugerprojekter fra Miljøstyrelsens hjemmeside samt forbrugerkemi.dk. Derudover er miljøprojektet "Kemikalier i tekstiler" (Larsen et al., 2000) brugt som opslagsværk for identifikation af stoffer fra de enkelte stofgrupper, som anvendes i tekstilproduktionen.

Forklaringer på de enkelte faresætningskoder kan ses bagest i oversigten, der også indeholder en tabel med de generiske afskæringsværdier, som angiver den koncentration, hvor der skal tages hensyn til tilstedeværelsen af et stof med henblik på klassificeringen. For nogle af stofferne gælder dog specifikke afskæringsværdier. Hvis det er tilfældet, er dette angivet ved de enkelte fareklasser. De specifikke afskæringsværdier kan slås op i CLP-forordningens Bilag VI og er ikke gengivet her.

Stofferne på EU's liste over hormonforstyrrende stoffer er blevet inddelt i tre kategorier for hhv. mennesker (hum.) og dyr (wildlife) afhængigt af påvisning af hormonforstyrrende effekter:

- Kategori 1 - bevis for hormonforstyrrende aktivitet i mindst én art ved hjælp af intakte dyr
- Kategori 2 - i det mindste nogle *in vitro* tegn på biologisk aktivitet relateret til hormonforstyrrelser
- Kategori 3 - ingen tegn på hormonforstyrrende aktivitet eller ingen data tilgængelige

Hvis stofferne eller nogle stoffer fra stofgruppe er klassificeret iht. kategori 1 eller 2 for mennesker, er dette gengivet her.

Phenoler, f.eks: pentachlorphenol (PCP), tetrachlorphenol (TeCP), trichlorphenol (TCP), orto-fenylphenol (OPP)

Nogle af de chlorerede phenoler er mistænkt for at være kræftfremkaldende, mens stort set alle virker irriterende ved hudkontakt. Andre sundhedsegenskaber fremgår af klassificeringen i nedenstående tabel.

På forbrugerkemi angives nogle af stofferne også for at være mistænkt for at virke hormonforstyrrende, dog er kun 2,4-dichlorophenol på EU's liste over stoffer, der mistænkt for at være hormonforstyrrende (Kat. 2).

⁴ Tilgængelig på: http://ec.europa.eu/environment/chemicals/endocrine/strategy/substances_en.htm#priority_list

TABEL B3- 1

HARMONISERET KLASSIFICERING AF NOGLE PHENOLERFORBINDELSER (CLP FORORDNINGEN 1272/2008).

Index Nr	Stof	CAS Nr	Klassificering	
			Fareklasse- og kategorikode(r)	Faresætningskode(r)
604-002-00-8	pentachlorophenol	87-86-5	Acute Tox. 3 * Acute Tox. 3 * Skin Irrit. 2 Eye Irrit. 2 Acute Tox. 2 * STOT SE 3 Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H301 H311 H315 H319 H330 H335 H351 H400 H410
604-013-00-8	2,3,4,6-tetrachlorophenol	58-90-2	Acute Tox. 3 * Skin Irrit. 2 Eye Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1	H301 H315 H319 H400 H410
604-018-00-5	2,4,6-trichlorophenol	88-06-2	Acute Tox. 4 * Skin Irrit. 2 Eye Irrit. 2 Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H302 H315 H319 H351 H400 H410
604-020-00-6	2-phenylphenol	90-43-7	Skin Irrit. 2 Eye Irrit. 2 STOT SE 3 Aquatic Acute 1	H315 H319 H335 H400

* Der findes specifikke afskæringsværdier for stoffet mht. til de markerede fareklasser.

Organotinforbindelser

For gruppen af tributyltin-forbindelser og triphenyltin hydroxid er der vedtaget en harmoniseret klassificering, mens der for andre stoffer fra den stofgruppe ikke er vedtaget en harmoniseret klassificering.

De største bekymringer mht. tinorganiske stoffer gælder effekter i miljøet, da nogle stoffer fra gruppen har vist sig at virke hormonforstyrrende i miljøet og at kunne påvirke immunforsvaret på længere sigt. Tributyltin og tributyltin oxid er klassificerede som kat. 2, mens triphenyltin (stoffer) er klassificeret som kat. 3 på EU's liste over hormonforstyrrende stoffer.

TBT angives desuden til at kunne forårsage moderat til svær hud- og øjenirritation, ligesom der også er set tegn på irritation af luftveje efter inhalation⁵.

⁵ Datablad om TBT forbindelser: <http://mst.dk/media/mst/83373/TBT%20datablad%20final%20version%20apr%202007.pdf>

TABEL B3- 2

HARMONISERET KLASSIFICERING AF NOGLE ORGANOTINFORBINDELSER (CLP FORORDNINGEN 1272/2008).

Index Nr*	Stof	CAS Nr	Klassificering	
			Fareklasse- og kategorikode(r)	Faresætningskode(r)
050-008-00-3	Tributyltin compounds with the exception of those specified elsewhere in this Annex	-	Acute Tox. 3 * Acute Tox. 4 * Skin Irrit. 2 Eye Irrit. 2 STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H301 H312 H315 H319 H372 H400 H410
050-004-00-1	Triphenyltin hydroxid	76-87-9	Acute Tox. 3 * Acute Tox. 3 * Skin Irrit. 2 Eye Dam. 1 Acute Tox. 2 * STOT SE 3 Carc. 2 Repr. 2 STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H301 H311 H315 H318 H330 H335 H351 H361d H372 H400 H410

* Der findes specifikke afskæringsværdier for stoffet mht. til de markerede fareklasser.

Dimethylfumarat (DMFu)

For DMFu er der ikke vedtaget en harmoniseret klassificering. Skadelig ved hudkontakt, hudirriterende, kan forårsage en allergisk hudreaktion og øjenirriterende er de hyppigste effekter nævnt under selvklassificeringen af stoffet.

I EU er der konstateret tusindvis af tilfælde af allergi overfor DMFu. DMFu har vist sig at kunne trænge igennem tøj og ind på huden, hvor det kan medføre en hudallergisk reaktion, med kløe, rødme og væskende hævelser, som kan være vanskelig at behandle⁶.

Formaldehyd

Formaldehyd anvendes som biocid og kan derudover fraspaltes fra andre konserveringsmidler (eksempler findes på [forbrugerkemi](#)). Formaldehyd kan give risiko for kræft hos mennesker. Desuden er stoffet klassificeret som akut giftig ved indtagelse, indånding eller hudkontakt og kan forårsage allergisk hudreaktion og irritere hud og øjnene (tabel B3-3).

TABEL B3- 3

HARMONISERET KLASSIFICERING AF FORMALDEHYD (CLP FORORDNINGEN 1272/2008).

Index Nr	Stof	CAS Nr	Klassificering	
			Fareklasse- og kategorikode(r)	Faresætningskode(r)
605-001-00-5	Formaldehyde, % *	50-00-0	Acute Tox. 3 * Acute Tox. 3 * Skin Corr. 1B	H301 H311 H314

⁶ <http://mst.dk/virksomhed-myndighed/kemikalier/regulering-og-regler/faktaark-om-kemikalierreglerne/dmf/>

Index Nr	Stof	CAS Nr	Klassificering	
			Fareklasse- og kategorikode(r)	Faresætningskode(r)
			Skin Sens. 1 Acute Tox. 3 * Carc. 2	H317 H331 H351

* Der findes specifikke afskæringsværdier for stoffet mht. til de markerede fareklasser.

Triclosan

Triclosan er mistænkt for at være hormonforstyrrende og mistænkes derudover for at kunne øge risikoen for, at børn udvikler allergi over for andre stoffer på grund af triclosans virkning på bakterier og dermed immunsystemet (forbrugerkemi.dk). Stoffet er for nylig blevet forbudt som biocid i tekstiler⁷ og er desuden reguleret i kosmetiske produkter.

TABEL B3- 4

HARMONISERET KLASSIFICERING AF TRICLOSAN (CLP FORORDNINGEN 1272/2008).

Index Nr	Stof	CAS Nr	Klassificering	
			Fareklasse- og kategorikode(r)	Faresætningskode(r)
604-070-00-9	triclosan 2,4,4'-trichloro-2'-hydroxy-diphenyl-ether 5-chloro-2-(2,4-dichlorophenoxy)phenol	3380-34-5	Skin Irrit. 2 Eye Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1	H315 H310 H400 H410

Sølv og sølvforbindelser

Sølv eller sølvforbindelser kan ligesom Triclosan være tilsat som antibakterielt middel. Sølv på nanoform er ligeledes blevet fundet i tøj, især sportstøj, hvor det skal modvirke dårlig lugt (Poulsen et al., 2011). Sundhedseffekterne af nanosølv er ikke velundersøgte, men udover at være meget giftig for vandmiljøet (Tabel B3-5) kan sølv og sølvforbindelser bidrage til bakteriernes resistensdannelse overfor antibiotika (Klif, 2010).

TABEL B3- 5

HARMONISERET KLASSIFICERING AF TRICLOSAN (CLP FORORDNINGEN 1272/2008).

Index Nr	Stof	CAS Nr	Klassificering	
			Fareklasse- og kategorikode(r)	Faresætningskode(r)
047-001-00-2	silver nitrate	7761-88-8	Ox. Sol. 2 Skin Corr. 1B Aquatic Acute 1 Aquatic Chronic 1	H272 H314 H400 H410

⁷ Beslutning i EU om ikke-optagelse af triclosan under Biocidforordningen i produkttype 2, 7 og 9 blev vedtaget den 14. marts i år og er således allerede trådt i kraft. Forbuddet gælder al brug af triclosan som konserveringsmiddel i tekstiler (som er PT9, deriblandt fibre, læder, gummi og polymeriserede materialer) (Kilde: Marianne Schmidt, Miljøstyrelsen, 17.06.2014).

Bromerede flammehæmmere - polybromerede biphenyler (PBB) og polybromerede diphenyl ether (PBDE)

Nogle af de bromerede flammehæmmere mistænkes for bl.a. at kunne skade fostre, fremkalde kræft, forstyrre hormonbalancen og nedsætte fertiliteten. Gruppen af polybromerede biphenyler og 4 af de bromerede diphenyl ether er ligeledes på EU liste over hormonforstyrrende stoffer (hvh. kat. 1 og 2). Kun to af stofferne har en harmoniseret klassificering.

TABEL B3- 6

HARMONISERET KLASSIFICERING AF NOGLE BROMEREDE FLAMMEHÆMMERE (CLP FORORDNINGEN 1272/2008).

Index Nr	Stof	CAS Nr	Klassificering	
			Fareklasse- og kategorikode(r)	Faresætningskode(r)
602-094-00-4	diphenylether; octabromo derivate	32536-52-0	Repr. 1B	H360Df
602-083-00-4	diphenyl ether, pentabromo derivative pentabromodiphenyl ether	32534-81-9	STOT RE 2 * Lact. Aquatic Acute 1 Aquatic Chronic 1	H373 H362 H400 H410

* Klassificeringen skal betragtes som et minimum klassifikation.

Fosforbaserede flammehæmmere

De i Tabel B3-7 listede stoffer er de mest almindelige fosforbaserede flammehæmmere i tekstilprodukter (Andersen et al. ,2014). Flere af stofferne anvendes kun til skum og fyldmateriale og ikke til selve tekstilet. To af stofferne har en klassificering som kræftfremkaldende (TDCP og TCEP), mens TCEP er også klassificeret som reproduktionstoksisk.

TABEL B3- 7

HARMONISERET KLASSIFICERING AF NOGLE FOSFORBASEREDE, CHLOREREDE FLAMMEHÆMMERE (CLP FORORDNINGEN 1272/2008).

Index Nr	Stof	CAS Nr	Klassificering	
			Fareklasse- og kategorikode(r)	Faresætningskode(r)
015-199-00-X	tris[2-chloro-1-chloromethyl)ethyl] phosphate (TDCP)	13674-87-8	Carc. 2	H351
015-102-00-0	tris(2-chloroethyl)phosphate (TCEP)	115-96-8	Acute Tox. 4 * Carc. 2 Repr. 1B Aquatic Chronic 2	H302 H351 H360F H411
015-188-00-X	(1-methylethylidene)di-4,1-phenylenetetraphenyl diphosphate	5945-33-5	Aquatic Chronic 4	H413

* Der findes specifikke afskæringsværdier for stoffet mht. til de markerede fareklasser.

UV-absorbere og -stabilisatorer

Der anvendes overordnet 3 grupper af UV beskyttende stoffer i tekstiler: Benzophenoner, triazoler og triaziner. Nogle benzophenoner er mistænkt for at være kræftfremkaldende, hormonforstyrrende og/eller kunne skade barnet under graviditeten, mens andre ikke anses for at være problematiske i samme grad. I mange tilfælde mangler der også data for at foretage en vurdering (forbrugerkemi.dk).

I forbindelse med en endnu ikke afsluttet kortlægning af UV-filtre i forskellige produkter igangsat af Miljøstyrelsen, har tekstilvirksomheden Gabriel oplyst, at der benyttes et sulfoneret benzotriazolderivat i eksempelvis møbeltekstiler, og at leverandøren af benzotriazolen ligeledes leverer triazin til polyesterfibre i bilindustrien.

I forbindelse med samme kortlægningsprojekt er det fundet, at data fra litteraturen peger på, at typiske UV absorbere anvendt til tekstiler (herunder auto-tekstiler) omfatter blandt andet: 2-hydroxybenzophenon (117-99-7) (tekstil af bomuld), 2,4-dihydroxybenzophenon (131-56-6) (tekstil af polypropylen), 2,2'-dihydroxy-4,4'-dimethoxybenzophenon (131-54-4) (tekstil af poly(m-phenylen-terephthalamid-fibre). Andre absorbere, som nævnes er: 2-hydroxyphenylbenzotriazoler og 2-hydroxyphenyl-s-triaziner. Til polyester anvendes råvarerne Tinovin 326 (Bumetrisol/3896-11-5) og Uvinul D-49 (131-54-4). Ingen af de nævnte stoffer har en harmoniseret klassificering.

Ftalater

Denne stofgruppe omfatter en lang række stoffer, hvor 6 af dem (DEHP, DBP, BBP, DINP, DIDP, DNOP) er reguleret under REACH. For DEHP, DBP og BBP må koncentrationen ikke overstige 0,1% i alt legetøj og alle småbørnsartikler, mens den for de tre øvrige ikke må overstige 0,1% i legetøj og småbørnsartikler, som kan puttes i munden. Ud over disse regler, som gælder i hele EU, er det i Danmark i følge bekendtgørelse om forbud mod ftalater i legetøj og småbørnsartikler (BEK nr 855 af 05/09/2009) forbudt at producere, importere eller sælge legetøj og småbørnsartikler eller dele deraf, der indeholder ftalater i koncentrationer over 0,05%.

Flere af ftalaterne påvirker evnen til at få børn og/eller skader det ufødte barn. Derudover er mange mistænkt for at være hormonforstyrrende, mens nogle ftalater anses for at være uproblematisk. Forbrugerkemi.dk har vurderet [18 ftalater](#) mht. deres sundheds- og miljøeffekter.

DEHP, DBP og BBP (butylbenzylphthalat) er klassificeret som kat. 1, DINP og DIDP som kat. 2 på EU's liste over stoffer, der er mistænkt for at være hormonforstyrrende.

TABEL B3- 8

HARMONISERET KLASSIFICERING AF NOGLE FTALATER (CLP FORORDNINGEN 1272/2008).

Index Nr	Stof	CAS Nr	Klassificering	
			Fareklasse- og kategorikode(r)	Faresætningskode(r)
607-317-00-9	di-(2-ethylhexyl) ftalat (DEHP)	117-81-7	Repr. 1B	H360FD
607-318-00-4	dibutyl ftalat (DBP)	84-74-2	Repr. 1B Aquatic Acute 1	H360Df H400
607-430-00-3	benzyl butyl ftalat (BBP)	85-68-7	Repr. 1B Aquatic Acute 1 Aquatic Chronic 1	H360-Df H400 H410

Azofarvestoffer /aromatiske aminer

Azofarvestoffer kan give anledning til forekomst af en række aromatiske aminer i tekstiler, som f.eks. biphenyl-4-amin eller benzidin. Mange af de aromatiske aminer virker kræftfremkaldende, herunder de 22 navngivne aminer, som er listet under tillæg 8 i REACH Bilag XVII. Endvidere er en række af stofferne klassificeret med en eller flere af følgende risikosætninger: kan give overfølsomhed ved hudkontakt, giftig ved hudkontakt, fare for varige alvorlige skader på helbred ved hudkontakt, alvorlige sundhedsfare ved længere tids påvirkning ved hudkontakt, mulighed for varige skader på helbred, kan forårsage arvelige genetiske skader, og mulighed for skade på forplantningsevnen.

TABEL B3- 9

HARMONISERET KLASSIFICERING AF NOGLE AZOFARVESTOFFER OG AROMATISKE AMINER (CLP FORORDNINGEN 1272/2008).

Index Nr	Stof	CAS Nr	Klassificering	
			Fareklasse- og kategorikode(r)	Faresætningskode(r)
612-072-00-6	biphenyl-4-amine	92-67-1	Acute Tox. 4 * Carc. 1A	H302 H350
612-042-00-2	benzidine	92-87-5	Acute Tox. 4 * Carc. 1A Aquatic Acute 1 Aquatic Chronic 1	H302 H350 H400 H410
612-008-00-7	aniline	62-53-3	Acute Tox. 3 * Acute Tox. 3 * Skin Sens. 1 Eye Dam. 1 Acute Tox. 3 * Muta. 2 Carc. 2 STOT RE 1 Aquatic Acute 1	H301 H311 H317 H318 H331 H341 H351 H372 H400

* Der findes specifikke afskæringsværdier for stoffet mht. til de markerede fareklasser.

Alkylphenoler og alkylphenoethoxylater

Alkylphenoethoxylaterne kan nedbrydes til alkylphenolerne, blandt disse er nonylphenol den bedst undersøgte. Nonylphenol kan skade forplantningsevnen samt barnet under graviditet. Desuden er stoffet mistænkt for at kunne give allergi og anses for at være hormonforstyrrende.

Af de 5 listede alkylphenoler på EU's liste over mistænkte hormonforstyrrende stoffer er nonylphenol og 4-ter-octylphenol klassificeret som kat. 1, mens 4-tert-Butylphenol er klassificeret som kat. 2.

TABEL B3- 10

HARMONISERET KLASSIFICERING AF ENKELTE ALKYLPHENOLER OG ALKYLPHENOETHOXYLATER (CLP FORORDNINGEN 1272/2008).

Index Nr	Stof	CAS Nr	Klassificering	
			Fareklasse- og kategorikode(r)	Faresætningskode(r)
601-053-00-8	nonylphenol	25154-52-3	Acute Tox. 4 * Skin Corr. 1B Repr. 2 Aquatic Acute 1 Aquatic Chronic 1	H302 H314 H361fd H400 H410

* Der findes specifikke afskæringsværdier for stoffet mht. til de markerede fareklasser.

N-Methyl-2-pyrrolidone (NMP)

Sundhedseffekterne af NMP er tidligere blevet beskrevet i et forbrugerprojekt om rengøringsmidler (Andersen et al., 2010). Blandt sundhedseffekterne af NMP er irritation af slimhinder i øjne og luftveje hos mennesker, sløvhed og uregelmæssig vejrtrækning, og effekter på fosterudviklingen. Derudover trænger NMP nemt igennem huden og kan dermed fremme hudgennemtrængeligheden af andre stoffer.

TABEL B3- 11

HARMONISERET KLASSIFICERING AF NMP (CLP FORORDNINGEN 1272/2008).

Index Nr	Stof	CAS Nr	Klassificering	
			Fareklasse- og kategorikode(r)	Faresætningskode(r)
606-021-00-7	N-methyl-2-pyrrolidone	872-50-4	Skin Irrit. 2 Eye Irrit. 2 STOT SE 3 Repr. 1B	H315 H319 H335 H360D

N,N-Dimethylacetamid (DMAc)

N,N-Dimethylacetamid er klassificeret som reproduktionstoksisk (Kan skade det ufødte barn) og som farligt ved indånding og hudkontakt (Tabel B3-12). Blandt kroniske effekter er der fundet mulige "kroniske lever- og nyreskader". Stoffet kan give systemiske skader ved indånding og optagelse over hud i tilstrækkelige mængder over en længere periode (Svendsen et al., 2004).

TABEL B3- 12

HARMONISERET KLASSIFICERING AF DMAc (CLP FORORDNINGEN 1272/2008).

Index Nr	Stof	CAS Nr	Klassificering	
			Fareklasse- og kategorikode(r)	Faresætningskode(r)
616-011-00-4	N,N-dimethylacetamide	127-19-5	Acute Tox. 4 * Acute Tox. 4 * Repr. 1B	H312 H332 H360D

* Der findes specifikke afskæringsværdier for stoffet mht. til de markerede fareklasser.

N,N-dimethylformamid; dimethyl formamid (DMF)

Dimethylformamid er klassificeret som farligt ved hudkontakt og indånding, samt øjenirriterende og reproduktionstoksisk (Kan skade det ufødte barn).

Selvom dyreforsøg ikke kunne påvise, at stoffet er mutagen eller kræftfremkaldende, har adskillige test af personer som har været udsat for dimethylformamid indikeret, at stoffet måske medvirker til udvikling af testikelkræft. Det er derudover vist, at stoffet medfører leverskader (Hansen et al., 2004).

TABEL B3- 13

HARMONISERET KLASSIFICERING AF DIMETHYL FORMAMID (CLP FORORDNINGEN 1272/2008).

Index Nr	Stof	CAS Nr	Klassificering	
			Fareklasse- og kategorikode(r)	Faresætningskode(r)
616-001-00-X	N,N-dimethylformamide	68-12-2	Acute Tox. 4 * Eye Irrit. 2 Acute Tox. 4 * Repr. 1B	H312 H319 H332 H360D

* Der findes specifikke afskæringsværdier for stoffet mht. til de markerede fareklasser.

Polyfluoroalkylforbindelser (PFAS)

PFAS kan inddeles i en lang række undergrupper, såsom perfluoralkylsulfonsyrer (PFS) og salte (sulfonater; PFSA), sulfonamiderne (FOSA, FOSE etc.), perfluoralkylcarboxylsyrer (PFCA), og telomererne – fluortelomeralkoholer (FTOH), fluortelomersulfonater (FTS), fluortelomerphosphater (FTP), samt fluortelomeriodider og fluortelomeralkener. Alle disse telomere kan i flere trin og med tiden nedbrydes til PFCA.

Stoffernes sundhedsegenskaber er i højere grad knyttet til længden af perfluoralkylkæden (C_nF_{2n+1} - eller $F(CF_2)_n$ -) end til den funktionelle gruppe. En perfluoralkylkæde af 6-8 carbon (fx PFHxS, PFOA, PFOS og PFNA) er tilsyneladende de mest sundhedsskadelige.

Det er kun C8-stofferne PFOS og PFOS derivater som har en harmoniseret klassificering i henhold til CLP-forordningen, som angivet i Tabel B3-14.. Ifølge klassificeringen har stofferne både kræftfremkaldende og reproduktionstoksiske egenskaber.

TABEL B3- 14
HARMONISERET KLASSIFICERING AF PFAS (CLP FORORDNINGEN 1272/2008).

Index Nr	Stof	CAS Nr	Klassificering	
			Fareklasse - og kategorikode(r)	Faresætningskode(r)
607-624-00-8	perfluorooctane sulfonic acid (PFOS);	1763-23-1	Carc. 2	H351
	potassium perfluorooctane sulfonate (K-PFOS);	2795-39-3	Repr. 1B	H360D
	diethanolamine perfluorooctane sulfonate ;	70225-14-8	STOT RE 1	H372
	ammonium perfluorooctane sulfonate (NH ₄ -PFOS);	29081-56-9	Acute Tox. 4 *	H332
	lithium perfluorooctane sulfonate (Li-PFOS)	29457-72-5	Acute Tox. 4 *	H302
			Lact.	H362
			Aquatic	H411
			Chronic 2	

* Der findes specifikke afskæringsværdier for stoffet mht. til de markerede fareklasser.

Tungmetaller

Blandt tungmetaller som primært kan forekomme i tekstiler, enten pga. deres anvendelse i farvning, trykning eller som urenhed, er chrom, nikkel, kobber og kobolt.

Trivalente chromforbindelser betragtes som relativt ugiftige, chrom anses således også som et essentielt næringsstof. Chrom(VI)forbindelser er meget giftige og kan ved hudkontakt forårsage allergi. Ved lang tids eksponering for chrom(VI)-forbindelser ses forskellige former for mavetarmgener og øget forekomst af lungekræft⁸.

Nikkel kan forårsage hudallergi, og en sensibilisering over for nikkel kan føre til kontaktdermatitis. Derudover har eksponering i arbejdsmiljøet ført til øget risiko for lungekræft (Strandesen og Poulsen, 2008).

Længerevarende, oral eksponering for kobber kan føre til leverskader. Kobber og kobbersalte kan frembringe allergiske reaktioner ved kontakt med huden hos følsomme individer (Strandesen og Poulsen, 2008). En harmoniseret klassificering af kobber, kobber nitrat eller kobber acetat er ikke tilgængelig.

Kobolt kan virke sensibiliserende og er klassificeret som ”Kan forårsage en allergisk hudreaktion” og ”Kan forårsage allergi-eller astmasymptomer og åndedrætsbesvær ved indånding”.

⁸ <http://mst.dk/media/mst/67609/Chrom%20dec2002.pdf>

TABEL B3- 15

HARMONISERET KLASSIFICERING AF UDVALGTE METALLER (CLP FORORDNINGEN 1272/2008).

Index Nr	Stof	CAS Nr	Klassificering	
			Fareklasse- og kategorikode(r)	Faresætningskode(r)
024-017-00-8	Chromium (VI) compounds, with the exception of barium chromate and of compounds specified elsewhere in the Annex	-	Skin Sens. 1 Carc. 1B Aquatic Acute 1 Aquatic Chronic 1	H317 H350i H400 H410
028-002-01-4	Nikkel (pulver)	7440-02-0	Skin Sens. 1 Carc. 2 STOT RE 1 Aquatic Chronic 3	H317 H351 H372 H412
027-001-00-9	Kobolt	7440-48-4	Skin Sens. 1 Resp. Sens. 1 Aquatic Chronic 4	H317 H334 H413

Polycykliske aromatiske hydrocarboner (PAH)

PAH dækker over en stofgruppe på mere end 100 stoffer, som er sammensat af benzenringe.

Benzo[a]pyren (BaP) bruges tit som en indikator for forekomst og sundhedseffekter for PAH og er samtidig det bedst undersøgte stof fra gruppen.

BaP og nogle andre PAH har vist sig at være giftige ved indånding, indtagelse eller hudoptagelse. Nogle er kræftfremkaldende, mutagene og reproduktionstoksiske. Langvarig indånding kan medføre et fald i lungefunktion, bryst smerter og irritation, samt langvarig hudkontakt kan forårsage dermatitis og vorter. BaP menes at forårsage lungekræft og hudkræft hos mennesker (PHE, 2008). Nogle af sundhedseffekterne fremgår også af klassificeringerne i Tabel B3-16.

TABEL B3- 16

HARMONISERET KLASSIFICERING AF PAH'ER (CLP FORORDNINGEN 1272/2008).

Index Nr	Stof	CAS Nr	Klassificering	
			Fareklasse- og kategorikode(r)	Faresætningskode(r)
601-032-00-3	benzo[a]pyrene	50-32-8	Skin Sens. 1 Muta. 1B Carc. 1B Repr. 1B Aquatic Acute 1 Aquatic Chronic 1	H317 H340 H350 H360FD H400 H410

Index Nr	Stof	CAS Nr	Klassificering	
			Fareklasse- og kategorikode(r)	Faresætningskode(r)
601-049-00-6	benzo[e]pyrene	192-97-2	Carc. 1B Aquatic Acute 1 Aquatic Chronic 1	H350 H400 H410
601-033-00-9	benz[a]anthracene	56-55-3	Carc. 1B Aquatic Acute 1 Aquatic Chronic 1	H350 H400 H410
601-048-00-0	chrysene	218-01-9	Muta. 2 Carc. 1B Aquatic Acute 1 Aquatic Chronic 1	H341 H350 H400 H410
601-034-00-4	benz[e]acephenanthrylene	205-99-2	Carc. 1B Aquatic Acute 1 Aquatic Chronic 1	H350 H400 H410

GHS-Faresætninger og generiske afskæringsværdier for klassificeringskategorier jf. CLP-forordningen (EF Nr. 1272/2008)

TABEL B3- 17

Sundhedsfaresætninger	
H300	Livsfarlig ved indtagelse.
H301	Giftig ved indtagelse.
H302	Farlig ved indtagelse.
H304	Kan være livsfarligt, hvis det indtages og kommer i luftvejene.
H310	Livsfarlig ved hudkontakt.
H311	Giftig ved hudkontakt.
H312	Farlig ved hudkontakt.
H314	Forårsager svære forbrændinger af huden og øjenskader.
H315	Forårsager hudirritation.
H317	Kan forårsage allergisk hudreaktion.
H318	Forårsager alvorlig øjenskade.
H319	Forårsager alvorlig øjenirritation.
H330	Livsfarlig ved indånding.
H331	Giftig ved indånding.

H332	Farlig ved indånding.
H334	Kan forårsage allergi- eller astmasymptomer eller åndedrætsbesvær ved indånding.
H335	Kan forårsage irritation af luftvejene.
H336	Kan forårsage sløvhed eller svimmelhed.
H340	Kan forårsage genetiske defekter <i><angiv eksponeringsvej, hvis det er endeligt påvist, at faren ikke kan frembringes ad nogen anden eksponeringsvej></i> .
H341	Mistænkt for at forårsage genetiske defekter <i><angiv eksponeringsvej, hvis det er endeligt påvist, at faren ikke kan frembringes ad nogen anden eksponeringsvej></i> .
H350	Kan fremkalde kræft <i><angiv eksponeringsvej, hvis det er endeligt påvist, at faren ikke kan frembringes ad nogen anden eksponeringsvej></i> .
H351	Mistænkt for at fremkalde kræft <i><angiv eksponeringsvej, hvis det er endeligt påvist, at faren ikke kan frembringes ad nogen anden eksponeringsvej></i> .
H360	Kan skade forplantningsevnen eller det ufødte barn <i><angiv specifik effekt, hvis kendt> <angiv eksponeringsvej, hvis det er endeligt påvist, at faren ikke kan frembringes ad nogen anden eksponeringsvej></i> .
H361	Mistænkt for at skade forplantningsevnen eller det ufødte barn <i><angiv specifik effekt, hvis kendt> <angiv eksponeringsvej, hvis det er endeligt påvist, at faren ikke kan frembringes ad nogen anden eksponeringsvej></i> .
H362	Kan skade børn, der ammes.
H370	Forårsager organskader <i><eller angiv alle berørte organer, hvis de kendes> <angiv eksponeringsvej, hvis det er endeligt påvist, at faren ikke kan frembringes ad nogen anden eksponeringsvej></i> .
H371	Kan forårsage organskader <i><eller angiv alle berørte organer, hvis de kendes> <angiv eksponeringsvej, hvis det er endeligt påvist, at faren ikke kan frembringes ad nogen anden eksponeringsvej></i> .
H372	Forårsager organskader <i><eller angiv alle berørte organer, hvis de kendes></i> ved længerevarende eller gentagen eksponering <i><angiv eksponeringsvej, hvis det er endeligt påvist, at faren ikke kan frembringes ad nogen anden eksponeringsvej></i> .
H373	Kan forårsage organskader <i><eller angiv alle berørte organer, hvis de kendes></i> ved længerevarende eller gentagen eksponering <i><angiv eksponeringsvej, hvis det er endeligt påvist, at faren ikke kan frembringes ad nogen anden eksponeringsvej></i> .
Miljøfaresætninger	
H400	Meget giftig for vandlevende organismer.
H410	Meget giftig med langvarige virkninger for vandlevende organismer.
H411	Giftig for vandlevende organismer, med langvarige virkninger.
H412	Skadelig for vandlevende organismer, med langvarige virkninger.
H413	Kan forårsage langvarige skadelige virkninger for vandlevende organismer.

Fareklasse	Generiske afskæringsværdier, som der skal tages hensyn til

Akut toksicitet:	
— Kategori 1–3	0,1 %
— Kategori 4	1 %
Hudætsning/-hudirritation	1 % (1)
Alvorlig øjenskade/øjenirritation	1 % (2)
Farlig for vandmiljøet	
— Akut kategori 1	0,1 % (3)
— Kronisk kategori 1	0,1 % (3)
— Kronisk kategori 2–4	1 %
(1) Eller < 1 %, når dette er relevant, jf. 3.2.3.3.1. (2) Eller < 1 %, når dette er relevant, jf. 3.3.3.3.1. (3) Eller < 0,1 %, når dette er relevant, jf. 4.1.3.1. i CLP forordningen (EF Nr. 1272/2008)	

Appendix 4: Results from GC/MS screening analyses for volatile and semi-volatile organic substances (in Danish)

4A. Screeningsanalyseresultater - autostole

TABEL B4- 1

RESULTATER AF GC/MS-SCREENING AF PRØVE A1, AUTOSTOL 0-13 KG, SORT BETRÆK INKLUSIV SKUM

Komponent	CAS-nr.	Indhold i mg/kg
N-Ethyl-N-(2-cyanoethyl)-anilin	148-87-8	9,0
2,6-Dichlor-4-nitroanilin	99-30-9	3,0
Tris(2-chlorisopropyl)-phosphat (TCPP)	13674-84-5	130
Bis(2-chlor-1-methylethyl) 3-chlorpropyl-phosphat	137909-40-1	82
Bis(3-chlor-1-propyl)(1-chlor-2-propyl)-phosphat	137888-35-8	26
6-Chlor-2,4-dinitroanilin	3531-19-9	30
Ikke identificeret komponent	-	32
Ikke identificeret komponent	-	17
Bisphenol A	80-05-7	9
3,3',5,5'-Tetramethylbiphenyl	25570-02-9	10
Bis(1,3-dichlor-2-propyl)-2,3-dichlor-1-propyl-phosphat	68460-03-7	600
Tris-(1,3-dichlorisopropyl)-phosphat (TDCP)	13674-87-8	50
Ikke identificeret komponent	-	13
Methyl-6-methoxy-2-anthracencarboxylat	297144-04-8	8
Ikke identificeret komponent	-	38
Ikke identificeret komponent	-	42
Ikke identificeret komponent	-	120

TABEL B4- 2

RESULTATER AF GC/MS-SCREENING AF PRØVE A2A, AUTOSTOL 9-36 KG, GRÅ TEKSTIL OG SKUM

Komponent	CAS-nr.	Indhold i mg/kg
3,5-di-tert-Butyl-4-hydroxybenzaldehyd	1620-98-0	24
Tris-(2-chlorisopropyl)-phosphat (TCPP)	13674-84-5	390
Bis-(1-chlor-2-propyl)(3-chlor-1-propyl)-phosphat	137909-40-1	270
Bis-(3-chlor-1-propyl)(1-chlor-2-propyl)-phosphat	137888-35-8	81
Ikke identificeret komponent	-	130
Ikke identificeret komponent	-	41
Ikke identificeret komponent	-	32

Bis-(1,3-dichlor-2-propyl)-2,3-dichlor-1-propyl-phosphat	68460-03-7	2300
Tris-dichlorpropylphosphat	78-43-3	160
Ikke identificeret komponent	-	120
Ikke identificeret komponent	-	110

TABEL B4- 3

RESULTATER AF GC/MS-SCREENING AF PRØVE A2B, AUTOSTOL 9-36 KG, SORT PLAST

Komponent	CAS-nr.	Indhold i mg/kg
Indeholder primært siloxaner	-	Påvist

TABEL B4- 4

RESULTATER AF GC/MS-SCREENING AF PRØVE A3A, AUTOSTOL 9-36 KG, SORT BETRÆK INKLUSIV SKUM OG BAGSIDE

Komponent	CAS-nr.	Indhold i mg/kg
2,4-Toluendiisocyanat (2,4-TDI)	584-84-9	120
Butyleret hydroxytoluen (BHT)	128-37-0	8
Tris-(2-chlorisopropyl)-phosphat (TCPP)	13674-84-5	650
Bis-(1-chlor-2-propyl)(3-chlor-1-propyl)-phosphat	137909-40-1	490
Bis-(3-chlor-1-propyl)(1-chlor-2-propyl)-phosphat	137888-35-8	130
Ikke identificeret komponent		1040
p-(p-Ethoxyphenyliminomethyl)-benzonitril	34128-02-4	49
Ikke identificeret komponent	-	97
Ikke identificeret komponent	-	140
Ikke identificeret komponent	-	67
Bis-(1,3-dichlor-2-propyl)-2,3-dichlor-1-propyl-phosphat	68460-03-7	6700
Bis-(1,3-dichlor-2-propyl)-2,3-dichlor-1-propyl-phosphat	68460-03-7	780
Ikke identificeret komponent	-	67
Ikke identificeret komponent	-	70
Ikke identificeret komponent	-	140

TABEL B4- 5

RESULTATER AF GC/MS-SCREENING AF PRØVE A3B, AUTOSTOL 9-36 KG, GRÅT BETRÆK INKLUSIV SKUM OG BAGSIDE

Komponent	CAS-nr.	Indhold i mg/kg
2,4-Toluendiisocyanat (2,4-TDI)	584-84-9	98
Tris-(2-chlorisopropyl)-phosphat (TCPP)	13674-84-5	1450
Bis-(1-chlor-2-propyl)(3-chlor-1-propyl)-phosphat	137909-40-1	500
Bis-(3-chlor-1-propyl)(1-chlor-2-propyl)-phosphat	137888-35-8	64
Ikke identificeret komponent	-	1200
Ikke identificeret komponent	-	290
Ikke identificeret komponent	-	86
Ikke identificeret komponent	-	56
Ikke identificeret komponent	-	68
Ikke identificeret komponent	-	37
Bis-(1,3-dichlor-2-propyl)-2,3-dichlor-1-propyl-phosphat	68460--03-7	7450
Tris-(1,3-dichlorisopropyl)-phosphat (TDCP)	13674-87-8	560
Ikke identificeret komponent	-	250
Ikke identificeret komponent	-	52
Ikke identificeret komponent	-	180
Ikke identificeret komponent	-	300
Ikke identificeret komponent	-	130
Ikke identificeret komponent	-	35
Ikke identificeret komponent	959-26-2	220

TABEL B4- 6

RESULTATER AF GC/MS-SCREENING AF PRØVE A4, AUTOSTOL 0-13 KG, BLÅ TEKSTIL OG SKUM PLUS BAGSIDE

Komponent	CAS-nr.	Indhold i mg/kg
Ikke identificeret komponent	-	1290
Ikke identificeret komponent	-	27
Tris-(2-chlorisopropyl)-phosphat (TCPP)	13674-84-5	25
Ikke identificeret komponent	-	82
6-Chlor-2,4-dinitroanilin	3531-19-9	140
Ikke identificeret komponent	-	180
Ikke identificeret komponent	-	61
Triphenoxyphosphin	101-02-0	110
Ikke identificeret komponent	-	130

Bis-(1,3-dichlor-2-propyl)-2,3-dichlor-1-propyl phosphat	68460-03-7	10100
Tris-(1,3-dichlorisopropyl)-phosphat (TDCP)	13674-87-8	760
Ikke identificeret komponent	-	190
Ikke identificeret komponent	-	45
Ikke identificeret komponent	-	190
Ikke identificeret komponent	-	180
Ikke identificeret komponent	-	430
Ikke identificeret komponent	-	54
Ikke identificeret komponent	-	43
Diisodecylftalat, isomer	-	88
Ikke identificeret komponent	-	37
Ikke identificeret komponent	-	82
Ikke identificeret komponent	-	1500
Ikke identificeret komponent	-	890

TABEL B4- 7

RESULTATER AF GC/MS-SCREENING AF PRØVE A5A, AUTOSTOL 0-18 KG, RØD TEKSTIL OG SKUM MED BÅND

Komponent	CAS-nr.	Indhold i mg/kg
2,4-Toluendiisocyanat (2,4-TDI)	584-84-9	54
2,4-Di-tert-butylphenol	96-76-4	51
Ikke identificeret komponent	-	780
Tris-(2-chlorisopropyl)-phosphat (TCPP)	13674-84-5	18
Ikke identificeret komponent	-	69
Ikke identificeret komponent	-	130
Ikke identificeret komponent	-	13
Bis-(1,3-dichlor-2-propyl)-2,3-dichlor-1-propyl-phosphat	68460-03-7	9400
Tris-(1,3-dichlorisopropyl)-phosphat (TDCP)	13674-87-8	580
Triphenylphosphat	115-86-6	84
Ikke identificeret komponent	-	150
Ikke identificeret komponent	-	120
Ikke identificeret komponent	-	320
Ikke identificeret komponent	-	1540

TABEL B4- 8

RESULTATER AF GC/MS-SCREENING AF PRØVE A5B, AUTOSTOL 0-18 KG, SORT BAGSIDE AF GUMMI

Komponent	CAS-nr.	Indhold i mg/kg
Alifatiske kulbrinter	-	140000

TABEL B4- 9

RESULTATER AF GC/MS-SCREENING AF PRØVE A6A, AUTOSTOL 0-4 ÅR, GRÅ TEKSTIL YDERSIDE MED SKUM

Komponent	CAS-nr.	Indhold i mg/kg
Dibutylftalat	84-64-0	20
Ikke identificeret komponent	-	22
4,4'-diphenylmethan-diisocyanat (MDI)	101-68-8	Påvist
4,4'-Diaminostilben	621-96-5	43
Ikke identificeret komponent	-	89
Ikke identificeret komponent	-	59
Ikke identificeret komponent	-	190
Bumetrizol	3896-11-5	74
Ikke identificeret komponent	-	59
Ikke identificeret komponent	-	40
4,4'-Dioctyldiphenylamin	101-67-7	32

TABEL B4- 10

RESULTATER AF GC/MS-SCREENING AF PRØVE A6B, AUTOSTOL 0-4 ÅR, BAGSIDE

Komponent	CAS-nr.	Indhold i mg/kg
Hexamethylen-diisocyanat	822-06-0	79
Isophoron-diisocyanat	4098-71-9	79
Ikke identificeret komponent	-	190
Ikke identificeret komponent	-	400
Ikke identificeret komponent	-	140
Ikke identificeret komponent	-	360
Ikke identificeret komponent	-	88
Mono-(2-ethylhexyl)-ftalat	4376-20-9	200
Ikke identificeret komponent	-	110
Ikke identificeret komponent	-	260
Diisodecylftalat, isomer	-	>12100
Diisodecylftalat, isomer	-	>3320
Diisodecylftalat, isomer	-	1520

Ikke identificeret komponent	-	180
Ikke identificeret komponent	-	300
Ikke identificeret komponent	-	120
Ikke identificeret komponent	119-07-3	58

> Betyder større end. Systemet er overbelastet.

TABEL B4- 11

RESULTATER AF GC/MS-SCREENING AF PRØVE A7A, AUTOSTOL 0-13 KG, SORT TEKSTIL MED SKUM

Komponent	CAS-nr.	Indhold i mg/kg
2,4-Toluendiisocyanat (2,4-TDI)	584-84-9	920
7-Hydroxy-1-indanon	6968-35-0	200
Ikke identificeret komponent	-	210
Diethyl isofthalat	636-53-3	59
Ikke identificeret komponent	-	220
Ikke identificeret komponent	-	220
Ikke identificeret komponent	-	590
Ikke identificeret komponent	-	530
4,4'-Diocetyldiphenylamin	101-67-7	56
Ikke identificeret komponent	-	610
Ikke identificeret komponent	-	370
Irganox 1076	2082-79-3	110

TABEL B4- 12

RESULTATER AF GC/MS-SCREENING AF PRØVE A7B, AUTOSTOL 0-13 KG, PRINT/SKRIFT

Komponent	CAS-nr.	Indhold i mg/kg
2,4-Toluendiisocyanat (2,4-TDI)	584-84-9	940
7-Hydroxy-1-indanon	6968-35-0	210
Ikke identificeret komponent	-	250
Diethyl-isoftalat	636-53-3	60
Ikke identificeret komponent	-	230
Ikke identificeret komponent	-	24
Ikke identificeret komponent	-	220
Ikke identificeret komponent	-	51
Ikke identificeret komponent	-	150
Ikke identificeret komponent	-	210
Ikke identificeret komponent	-	70

Ikke identificeret komponent	-	650
4,4'-Dioctyldiphenylamin	101-67-7	56
Ikke identificeret komponent	-	590
Ikke identificeret komponent	-	100
Ikke identificeret komponent	-	450
Irganox 1076	2082-79-3	110

TABEL B4- 13

RESULTATER AF GC/MS-SCREENING AF PRØVE A8A, AUTOSTOL 0-13 KG, SORT TEKSTIL OG SKUM FRA SELEN

Komponent	CAS-nr.	Indhold i mg/kg
2,4-Toluendiisocyanat (2,4-TDI)	584-84-9	28
Ikke identificeret komponent	-	16
Ikke identificeret komponent	-	300
1,3,5-Triazin-2,4,6-triamin	108-78-1	160
Ikke identificeret komponent	-	40
Tri-(2-chlorethyl)-phosphat	115-96-8	580
3,5-di-tert-Butyl-4-hydroxybenzaldehyd	1620-98-0	81
Tris-(2-chlorisopropyl)-phosphat (TCPP)	13674-84-5	6600
Bis-(1-chlor-2-propyl)(3-chlor-1-propyl)-phosphat	137909-40-1	2490
Bis-(3-chlor-1-propyl)(1-chlor-2-propyl)-phosphat	137888-35-8	320
Ikke identificeret komponent	-	270
Ikke identificeret komponent	-	520
Bis-(1,3-dichlor-2-propyl)-2,3-dichlor-1-propyl phosphat	68460-03-7	2800
Tris-(1,3-dichlorisopropyl)-phosphat (TDCP)	13674-87-8	250
Ikke identificeret komponent	-	170

TABEL B4- 14

RESULTATER AF GC/MS-SCREENING AF PRØVE A8B, AUTOSTOL 0-13 KG, RØD TEKSTIL OG SKUM

Komponent	CAS-nr.	Indhold i mg/kg
2,4-Toluendiisocyanat (2,4-TDI)	584-84-9	21
Ikke identificeret komponent	-	130
Ikke identificeret komponent	-	290
Ikke identificeret komponent	-	41
1,3,5-Triazine-2,4,6-triamin	108-78-1	51
Ikke identificeret komponent	-	66

Tri(2-chlorethyl) phosphat	115-96-8	61
3,5-di-tert-Butyl-4-hydroxy-benzaldehyd	1620-98-0	53
Tris(2-chlor-isopropyl)-phosphat (TCPP)	13674-84-5	6230
Bis(1-chlor-2-propyl)(3-chlor-1-propyl)phosphat	137909-40-1	2100
2,4-Dinitroanilin	97-02-9	25
Ikke identificeret komponent	-	440
4,4'-Diaminostilbene	621-96-5	55
Bis-(1,3-dichlor-2-propyl)-2,3-dichlor-1-propyl-phosphat	68460-03-7	2590
Tris-(1,3-dichlorisopropyl)-phosphat (TDCP)	13674-87-8	260

TABEL B4- 15

RESULTATER AF GC/MS-SCREENING AF PRØVE A29, AUTOSTOL 0-13 KG, SORT TEKSTIL OG SKUM SAMT HJERTE

Komponent	CAS-nr.	Indhold i mg/kg
Benzo-[f]-1,5-diazabicyclo[3.2.2]nonen	7092-76-4	25
Ikke identificeret komponent	-	660
Tributylprop-1-en-1,2,3-tricarboxylat	7568-58-3	28
Tributylacetylcitrat	77-90-7	1900
Triphenylphosphat	115-86-6	330
Diphenyl-isopropylphenyl-phosphat	28108-99-8	460
Ikke identificeret komponent	2240-47-3	84
Ikke identificeret komponent	-	120
Ikke identificeret komponent	-	190
Ikke identificeret komponent	-	62
Ikke identificeret komponent	-	124
Ikke identificeret komponent	-	48
Tris-(2-isopropylphenyl)-phosphat	64532-95-2	14
Ikke identificeret komponent	-	16
Ikke identificeret komponent	-	11
Ikke identificeret komponent	-	1060

TABEL B4- 16

RESULTATER AF GC/MS-SCREENING AF PRØVE A30, AUTOSTOL 0-13 KG, GRÅ TEKSTIL OG SKUM

Komponent	CAS-nr.	Indhold i mg/kg
Benzo-[f]-1,5-diazabicyclo[3.2.2]nonen	7092-76-4	47
Butyleret hydroxytoluen (BHT)	128-37-0	22
Ikke identificeret komponent	-	540

4B. Screeningsanalyseresultater - bæreseler

TABEL B4- 17

RESULTATER AF GC/MS-SCREENING AF PRØVE B9A, BABYBÆRESELE, 0-3 ÅR, LYS TEKSTIL MED SKUMFIBRE

Komponent	CAS-nr.	Indhold i mg/kg
Ekstraherbare organiske komponenter	-	-

- : Ikke påvist.

TABEL B4- 18

RESULTATER AF GC/MS-SCREENING AF PRØVE B9B, BABYBÆRESELE, 0-3 ÅR, SKUMPLADE MELLEM TEKSTILER OG INDVENDIGE TEKSTILER

Komponent	CAS-nr.	Indhold i mg/kg
2,4-Toluendiisocyanat (2,4-TDI)	584-84-9	220
1-[2-(Isobutyryloxy)-1-methylethyl]-2,2-dimethylpropyl-2-methylpropanoat	74381-40-1	48
Ikke identificeret komponent	-	131
Methyl (2E,4E,6E)-2-cyano-7-(dimethylamino)-2,4,6-heptatrienoat	58064-21-4	260
4,4'-diphenylmethan-diisocyanat (MDI)	101-68-8	700
Ikke identificeret komponent	-	240
Ikke identificeret komponent	-	97
Tris-(2,4-di-tert-butylphenyl)-phosphat	95906-11-9	68
Ikke identificeret komponent	-	31

TABEL B4- 19

RESULTATER AF GC/MS-SCREENING AF PRØVE B9C, BABYBÆRESELE, 0-3 ÅR, SMAL STROP MED PLASTINDLÆG OG LYS TEKSTIL

Komponent	CAS-nr.	Indhold i mg/kg
Alifatisk kulbrinte	-	21
Kodaflex/txib (2,2,4-Trimethylpentanediol diisobutyrat)	6846-50-0	62
Alifatisk kulbrinte	-	50
Alifatisk kulbrinte	-	18
Alifatisk kulbrinte (284)	-	20
Ikke identificeret komponent	-	33
Ikke identificeret komponent	-	22
Ikke identificeret komponent	-	42
Tris-(2,4-di-tert-butylphenyl)-phosphat	95906-11-9	82

TABEL B4- 20

RESULTATER AF GC/MS-SCREENING AF PRØVE B10A, BÆRESELE, 3- 24 MDR., SKUM FRA RØD, GRÅ OG GRÅ SMÆK

Komponent	CAS-nr.	Indhold i mg/kg
Dibutylftalat	84-74-2	74
Ikke identificeret komponent, evt. amin	-	110
Ikke identificeret komponent, evt. amin	-	140
Ikke identificeret komponent, evt. amin	-	65
Ikke identificeret komponent	-	150
Ikke identificeret komponent, evt. en thiazolforbindelse	-	140
Ikke identificeret komponent	-	67
Irganox 1076	2082-79-3	270

TABEL B4- 21

RESULTATER AF GC/MS-SCREENING AF PRØVE B10B, BÆRESELE, 3- 24 MDR., RØD TEKSTIL MED FILT, GRÅ TEKSTIL OG HAGESMÆK

Komponent	CAS-nr.	Indhold i mg/kg
Ikke identificeret komponent	-	41
Ikke identificeret komponent	-	16

TABEL B4- 22

RESULTATER AF GC/MS-SCREENING AF PRØVE B10C, BÆRESELE, 3- 24 MDR., HVID SKUMPLADE I SMÆKKEN

Komponent	CAS-nr.	Indhold i mg/kg
Alifatisk kulbrinte	-	16
Alifatisk kulbrinte	-	23
Methyl-hexadecanoat	112-39-0	28
Alifatisk kulbrinte	-	24
Methyl 7-octadecenoat	57396-98-2	26
Alifatisk kulbrinte	-	150
Erucylamid	112-84-5	53
2,6,10-Trimethyl-1,5,9-undecatrien	62951-96-6	34

TABEL B4- 23

RESULTATER AF GC/MS-SCREENING AF PRØVE B11, BÆRESELE, 3,5 – 15 KG, GRÅ TEKSTIL OG SKUM

Komponent	CAS-nr.	Indhold i mg/kg
Ikke identificeret komponent	-	54

TABEL B4- 24

RESULTATER AF GC/MS-SCREENING AF PRØVE B12A, BÆRESELE 3,5-6 KG, SKUM FRA SELE STROP, SKUM SAMT FILT

Komponent	CAS-nr.	Indhold i mg/kg
2-Ethylhexansyre	149-57-5	110
Tris-(2-chlorisopropyl)-phosphat (TCPP)	13674-84-5	4670
Bis-(1-chlor-2-propyl)(3-chlor-1-propyl)-phosphat	137909-40-1	2340
Bis-(3-chlor-1-propyl)(1-chlor-2-propyl)-phosphat	137888-35-8	510
Ikke identificeret komponent	-	550
Ikke identificeret komponent	-	630
Ikke identificeret komponent	-	580
Ikke identificeret komponent	-	440

TABEL B4- 25

RESULTATER AF GC/MS-SCREENING AF PRØVE B12B, BÆRESELE 3,5-6 KG, GRÅ, SORT OG HVID TEKSTIL FRA HAGESMÆK

Komponent	CAS-nr.	Indhold i mg/kg
Tris-(2-chlorisopropyl)-phosphat (TCPP)	13674-84-5	310
Bis-(1-chlor-2-propyl)(3-chlor-1-propyl)-phosphat	137909-40-1	140
Bis-(3-chlor-1-propyl)(1-chlor-2-propyl)-phosphat	137888-35-8	25
Ikke identificeret komponent	-	90
Bumetrizol	3896-11-5	150
Ikke identificeret komponent	-	96
Ikke identificeret komponent	-	46

TABEL B4- 26

RESULTATER AF GC/MS-SCREENING AF PRØVE B12C, BÆRESELE 3,5-6 KG, PLAST PLADE I HAGESMÆK

Komponent	CAS-nr.	Indhold i mg/kg
Tris-(2-chlorisopropyl)-phosphat (TCPP)	13674-84-5	270
Bis-(1-chlor-2-propyl)(3-chlor-1-propyl)-phosphat	137909-40-1	97
Ikke identificeret komponent	-	760
Ikke identificeret komponent	-	550
Ikke identificeret komponent	-	88
Ikke identificeret komponent	-	330

TABEL B4- 27

RESULTATER AF GC/MS-SCREENING AF PRØVE B13A, BÆRESELE 4-48 MDR., SKUM I SELESTROP MED PLASE OG FILT I SMÆKKEN

Komponent	CAS-nr.	Indhold i mg/kg
Benzophenon	119-61-9	14
Acetophenon-azin	729-43-1	33
Irganox 1076	2082-79-3	170

TABEL B4- 28

RESULTATER AF GC/MS-SCREENING AF PRØVE B13B, BÆRESELE 4-48 MDR, HULLET TEKSTIL FRA SELESTROP MED FIBRE, SORT TEKSTIL FRA SELESTROP SAMT SORT TEKSTIL FRA INDERSIDE AF SMÆK

Komponent	CAS-nr.	Indhold i mg/kg
Benzophenon	119-61-9	7
2-Chlor-4,6-dinitroanilin	3531-19-9	46
6-Brom-2,4-dinitroanilin	1817-73-8	5
Ikke identificeret komponent	-	17
Spinacen	111-02-4	18
Ikke identificeret komponent	-	18
Ikke identificeret komponent	-	110

TABEL B4- 29

RESULTATER AF GC/MS-SCREENING AF PRØVE B14A, BÆRESELE 3,5-9 KG, SKUM I SMÆKKEN

Komponent	CAS-nr.	Indhold i mg/kg
Diethylftalat	84-66-2	67
Isopropyllaurat	10233-13-3	46
Ikke identificeret komponent	-	82
Ikke identificeret komponent, evt. siloxan-forbindelse	-	340
Ikke identificeret komponent	-	460
Ikke identificeret komponent	-	220
4,4'-Diocetylphenylamin	101-67-7	88

TABEL B4- 30

RESULTATER AF GC/MS-SCREENING AF PRØVE B14B, BÆRESELE 3,5-9 KG, HVID TEKSTIL PÅ SMÆKENS BAGSIDE
SAMT GRÅ OG SORT TEKSTIL PÅ STROPPE

Komponent	CAS-nr.	Indhold i mg/kg
4,4,5,7,8-Pentamethyldihydrocoumarin	39170-97-3	54
Diethylftalat	84-66-2	17
Isopropyl-laurat	10233-13-3	23
Benzyl-benzoat	120-51-4	46
Ikke identificeret komponent		190
Ikke identificeret komponent		61

TABEL B4- 31

RESULTATER AF GC/MS-SCREENING AF PRØVE B15A, BÆRESELE 3,5-15 KG, SKUM MED FILT PÅ SMÆKEN

Komponent	CAS-nr.	Indhold i mg/kg
3,3,5,7-Tetramethyl-1-indanon	54789-23-0	49
2-Ethyl-2-methyl-1,3-indandion	70292-52-3	96
1-[2-(Isobutyryloxy)-1-methylethyl]-2,2-dimethylpropyl 2-methylpropanoat	74381-40-1	390
Methylhexadecanoat	112-39-0	1210
Bis(2-ethylhexyl)-tereftalat	6422-86-2	190

TABEL B4- 32

RESULTATER AF GC/MS-SCREENING AF PRØVE B15B, BÆRESELE 3,5-15 KG, SORT REM/SELE SAMT MØRKEBLÅ TEKSTIL

Komponent	CAS-nr.	Indhold i mg/kg
Dodecansyre	143-07-7	56
Methyl-hexadecanoat	112-39-0	100
Hexadecansyre	57-10-3	45
6-Chlor-2,4-dinitroanilin	3531-19-9	140
2,4-Dinitroanilin	97-02-9	17
Ikke identificeret komponent	-	57
Ikke identificeret komponent	-	77
Ikke identificeret komponent	-	570

TABEL B4- 33

RESULTATER AF GC/MS-SCREENING AF PRØVE B16A, BÆRESELE 3,5-18 KG, SKUM I SELEN

Komponent	CAS-nr.	Indhold i mg/kg
Ikke identificeret komponent	-	490
Ikke identificeret komponent	-	120
Ikke identificeret komponent	-	120
4,4'-Bis-(α,α -dimethyl-benzyl)-diphenylamin	10081-67-1	250

TABEL B4- 34RESULTATER AF GC/MS-SCREENING AF PRØVE B16B, BÆRESELE 3,5-18 KG, SORT OG GRÅ TEKSTIL FRA SELEN
INDVENDIG

Komponent	CAS-nr.	Indhold i mg/kg
Spor af kulbrinter	-	Påvist

TABEL B4- 35

RESULTATER AF GC/MS-SCREENING AF PRØVE B17A, BÆRESELE 6 MDR. – 12 KG, SKUM I SMÆK, HAGESMÆK OG I SELEN

Komponent	CAS-nr.	Indhold i mg/kg
Alifatiske kulbrinter	-	Højt indhold
Ikke identificeret komponent	-	450
2-Monopalmitin	23470-00-0	850
2-Monostearin	621-61-4	250

TABEL B4- 36RESULTATER AF GC/MS-SCREENING AF PRØVE B17B, BÆRESELE 6 MDR. – 12 KG, HVID OG SORT TEKSTIL FRA SMÆK
OG HAGESMÆK

Komponent	CAS-nr.	Indhold i mg/kg
2-chloro-4,6-dinitro-benzamin	3531-19-9	26
Ikke identificeret komponent	-	5
Ikke identificeret komponent	-	5
Ikke identificeret komponent	-	14

TABEL B4- 37

RESULTATER AF GC/MS-SCREENING AF PRØVE B18A, BÆRESELE 3,6-18,2 KG, SKUM FRA SELE SAMT FILT FRA SMÆKKEN

Komponent	CAS-nr.	Indhold i mg/kg
Tris-(chloroethyl)-phosphat	115-96-8	2690
Tris-(1-chloro-2-propyl)-phosphat (TCPP)	13674-84-5	6800
Bis-(2-chloro-1-methylethyl)-3-chloropropyl phosphate	137909-40-1	3000
2-Chloro-1-methylethyl-bis-(3-chloropropyl)-phosphat	137888-35-8	450
Ikke identificeret komponent	-	1770
Bis-(1,3-dichloro-2-propyl)-2,3-dichloro-1-propyl phosphat	68460-03-7	7010
Tris-(1,3-dichloro-2-propyl)-phosphat (TDCP)	13674-87-8	460
Ikke identificeret komponent	-	110
Ikke identificeret komponent	-	3400

TABEL B4- 38

RESULTATER AF GC/MS-SCREENING AF PRØVE B18B, BÆRESELE 3,6-18,2 KG, GRÅ TEKSTIL FRA REM PÅ SELE OG SORT TEKSTIL FRA KANTBÅND

Komponent	CAS-nr.	Indhold i mg/kg
Tris-(chloroethyl)-phosphat	115-96-8	51
Tris-(1-chloro-2-propyl)-phosphat (TCPP)	13674-84-5	150
Bis-(2-chloro-1-methylethyl)-3-chloropropyl-phosphat	137909-40-1	43
Alifatiske kulbrinter	-	Påvist

TABEL B4- 39

RESULTATER AF GC/MS-SCREENING AF PRØVE B18C, BÆRESELE 3,6-18,2 KG, HVIDT NET-TEKSTIL I SELEN SAMT HVID TEKSTIL I SMÆKKEN

Komponent	CAS-nr.	Indhold i mg/kg
Flygtige og semiflygtige organiske stoffer	-	-

- Kun meget lave indhold af uidentificerede komponenter

4C. Screeningsanalyseresultater - babymadrasser

TABEL B4- 40

RESULTATER AF GC/MS-SCREENING AF PRØVE M19A, LIFTMADRAS, TEKSTIL

Komponent	CAS-nr.	Indhold i mg/kg
1,4-Butandiol	110-63-4	4,4
Dibutylftalat, DIBP	84-69-5	11
4,4'-diphenylmethan-diisocyanat (MDI)	101-68-8	58
Ikke identificeret komponent	-	20
Ikke identificeret komponent	-	26
Ikke identificeret komponent	-	63
Ikke identificeret komponent	-	53
Ikke identificeret komponent	-	12
Ikke identificeret komponent	-	30
Ikke identificeret komponent	-	33
Ikke identificeret komponent	-	24
Ikke identificeret komponent	-	14

TABEL B4- 41

RESULTATER AF GC/MS-SCREENING AF PRØVE M19B, LIFTMADRAS, SKUM

Komponent	CAS-nr.	Indhold i mg/kg
Benzylalkohol	100-51-6	14
2-Ethylhexansyre	149-57-5	66
2,4-Toluendiisocyanat (2,4-TDI)	584-84-9	360
3,4-Toluendiamin	496-72-0	Påvist
5-Methylbenzimidazol	5400-75-9	450
5-Methylbenzimidazol	5400-75-9	411
1,6-Dioxacyclododecane-7,12-dion	777-95-7	23
4,4'-Vinylendianilin	621-96-5	62
Tert-octyldiphenylamin	NIST 370313 *	180
Ikke identificeret komponent	-	310
Ikke identificeret komponent	-	210
Ikke identificeret komponent	-	700
Ikke identificeret komponent	-	160
Ikke identificeret komponent	-	20
4,4'-Diocetyldiphenylamin	101-67-7	82
Ikke identificeret komponent	-	29

* Stoffet har ikke noget CAS-nr. i NIST biblioteket

TABEL B4- 42

RESULTATER AF GC/MS-SCREENING AF PRØVE M20A, LIFTMADRAS, TEKSTIL

Komponent	CAS-nr.	Indhold i mg/kg
Methyl hexadecanoat	112-39-0	13
Ikke identificeret komponent	-	19
Methyl 9-octadecenoat	2462-84-2	67
Methyloctadecanoat	112-61-8	13
Alifatiske kulbrinter	-	Påvist*
Ikke identificeret komponent	-	61
Ikke identificeret komponent	-	47

* Påvist i høje koncentrationer

TABEL B4- 43

RESULTATER AF GC/MS-SCREENING AF PRØVE M20B, LIFTMADRAS, SKUM

Komponent	CAS-nr.	Indhold i mg/kg
2,4-Toluendiisocyanat (2,4-TDI)	584-84-9	42
1,1,3-Trimethyl-3-phenylindan	3910-35-8	230
Dicumyl	1889-67-4	360
2,4-Diphenyl-4-methyl-2(E)-penten	22768-22-5	610
4-Cumylphenol	599-64-4	530
Methylhexadecanoat	112-39-0	76
13-Epimanool	1438-62-6	39
Biformen	5957-33-5	47
Isopimaradien	1686-66-4	100
Methyloleat	112-62-9	260
Abetinsyre (usikker identifikation)	514-10-3	Påvist

TABEL B4- 44

RESULTATER AF GC/MS-SCREENING AF PRØVE M20C, LIFTMADRAS, FLAMINGO-SKUM

Komponent	CAS-nr.	Indhold i mg/kg
1-Phenyl-1,3,3-trimethylindan	3910-35-8	17
2,3-Dimethyl-2,3-diphenylbutan	1889-67-4?	26
(E)-bis-1,1'-(1,3,3-Trimethyl-1-propen-1,3-diyl)-benzen	22768-22-5	51
Palmitinsyre (hexadecansyre)	57-10-3	280

Komponent	CAS-nr.	Indhold i mg/kg
Ikke identificeret komponent	-	17
Ikke identificeret, evt palmitinsyre	57-10-3	950
Ikke identificeret komponent	-	68
Ikke identificeret komponent	-	43
Ikke identificeret komponent	-	100
Ikke identificeret komponent	-	43
Ikke identificeret komponent	-	60
Ikke identificeret komponent	-	43
Ikke identificeret komponent	-	17
Ikke identificeret komponent	-	17
Ikke identificeret komponent	-	17
Ikke identificeret komponent	-	9
Ikke identificeret komponent	-	17
4,4'-Butylen-bis-(6-tert-butyl-methylphenol)	85-60-9	26
cis-13-Docosenoamid	112-84-5	140
Oleamid	301-02-0	34

Der ses en meget stor, bred top i chromatogrammet, som forstyrrer identifikationen af komponenter fra efter palmitinsyren. Det kan muligvis stor mængde af abetinsyre, CAS 514-10-3.

TABEL B4- 45
RESULTATER AF GC/MS-SCREENING AF PRØVE M21A, LIFT, TEKSTIL

Komponent	CAS-nr.	Indhold i mg/kg
Acetophenon	98-86-2	4,1
Ikke identificeret komponent	-	4
Ikke identificeret komponent	-	6

TABEL B4- 46
RESULTATER AF GC/MS-SCREENING AF PRØVE M21B, LIFT, SKUM

Komponent	CAS-nr.	Indhold i mg/kg
Ikke identificeret component	-	69
Ikke identificeret component	-	13
Ikke identificeret component	-	1170
Ikke identificeret component	-	140
Ikke identificeret komponent	-	36

TABEL B4- 47
RESULTATER AF GC/MS-SCREENING AF PRØVE M22A, LIFT, TEKSTIL

Komponent	CAS-nr.	Indhold i mg/kg
Alifatiske kulbrinter (C20-C30)	-	2250
Ikke identificeret komponent	-	140
gamma-Sitosterol	83-47-6	71
Ikke identificeret komponent	-	23
Ikke identificeret komponent	-	43

TABEL B4- 48
RESULTATER AF GC/MS-SCREENING AF PRØVE M22B, LIFT, SKUM

Komponent	CAS-nr.	Indhold i mg/kg
Benzylalkohol	100-51-6	24
2,4-Toluendiisocyanat (2,4-TDI)	584-84-9	32
3,4-Toluendiamin	496-72-0	12
5-Methylbenzimidazolon	5400-75-9	62
5-Methylbenzimidazolon	5400-75-9	62
Uidentificeret ftalat	-	44
4,4'-Vinylendianilin	621-96-5	33
4,4'-Vinylendianilin	621-96-5	69
Ikke identificeret komponent	-	100
Ikke identificeret komponent	-	68
Ikke identificeret komponent	-	230
Ikke identificeret komponent	-	77
4,4'-Dioctyldiphenylamin	101-67-7	48
Irganox 1076	2082-79-3	480

TABEL B4- 49
RESULTATER AF GC/MS-SCREENING AF PRØVE M23A, BLØD LIFT, TEKSTIL

Komponent	CAS-nr.	Indhold i mg/kg
Benzylalkohol	100-51-6	7
Acetophenon	98-86-2	18
2-Phenyl-butanol-2	1565-75-9	4
Alifatiske kulbrinter (C20-C30)	-	207
Ikke identificeret komponent	-	70
Ikke identificeret komponent	-	130

TABEL B4- 50
RESULTATER AF GC/MS-SCREENING AF PRØVE M23B, BLØD LIFT, SKUM

Komponent	CAS-nr.	Indhold i mg/kg
Alifatiske kulbrinter	100-51-6	75
Ikke identificeret component	98-86-2	78
Ikke identificeret component	1565-75-9	625
Ikke identificeret component	-	127
Ikke identificeret komponent	-	34

TABEL B4- 51
RESULTATER AF GC/MS-SCREENING AF PRØVE M24A, LIFT, TEKSTIL

Komponent	CAS-nr.	Indhold i mg/kg
Benzaldehyd	100-52-7	3
Benzylalkohol	100-51-6	3
Acetophenon	98-86-2	14
2-Hydroxy-N-methyl-2-phenylpropanamid	2019-70-7	2
2,4-Toluendiisocyanat (2,4-TDI)	584-84-9	9
5-Methylbenzimidazolon	5400-75-9	3
5-Methylbenzimidazolon	-	4
Bis-(2-hydroxyethyl)-terefthalat	959-26-2	6
Tris-(1,3-dichlorisopropyl)-phosphat (TDCP)	13674-87-8	65
Alifatiske kulbrinter	-	64
Ikke identificeret component	-	71
Ikke identificeret component	-	6
Ikke identificeret silan	-	72

TABEL B4- 52

RESULTATER AF GC/MS-SCREENING AF PRØVE M24B, LIFT, SKUM

Komponent	CAS-nr.	Indhold i mg/kg
2,4-Toleundiisocyanat (2,4-TDI)	584-84-9	170
5-Methylbenzimidazolon	5400-75-9	150
5-Methylbenzimidazolon	5400-75-9	130
Ikke identificeret komponent	-	72
Ikke identificeret komponent	-	37
4,4'-Vinylendianilin	621-96-5	50
Ikke identificeret komponent, chlorholdig	-	100
Ikke identificeret komponent, chlorholdig	-	750
tert-Octyldiphenylamin	NIST 370313*	390
Tris-(1,3-dichlorisopropyl)-phosphat (TDCP)	13674-87-8	29700
Tris-(1,3-dichlorisopropyl)-phosphat (TDCP)	13674-87-8	310
Ikke identificeret komponent	-	150
Ikke identificeret komponent	-	170
Ikke identificeret komponent	-	340
Ikke identificeret komponent	-	300
Ikke identificeret komponent	-	210
Ikke identificeret komponent	-	100
Ikke identificeret komponent	-	3190
Ikke identificeret komponent	-	1570
Ikke identificeret komponent	-	190
4,4'-Dioctyldiphenylamin	101-67-7	72

* Stoffet har ikke noget CAS-nr. i NIST-biblioteket

TABEL B4- 53
RESULTATER AF GC/MS-SCREENING AF PRØVE M25A, LIFTMADRAS, TEKSTIL

Komponent	CAS-nr.	Indhold i mg/kg
Ikke identificeret komponent	-	6
N,N-Dimethyldodecylamin	112-18-5	9
Evt. Siloxan	-	6
Ikke identificeret komponent	-	24
Ikke identificeret komponent	-	220
Ikke identificeret komponent	-	71
Ikke identificeret komponent	-	42
Ikke identificeret komponent	-	42
Tris-(2,4-di-tert-butylphenyl)-phosphat	95906-11-9	40

TABEL B4- 54
RESULTATER AF GC/MS-SCREENING AF PRØVE M25B, LIFTMADRAS, SKUM

Komponent	CAS-nr.	Indhold i mg/kg
Glycerin	56-81-5	200
Evt. Siloxan	-	100
Evt. Siloxan	-	70
4,4'-Vinylendianilin	621-96-5	94
Evt. Siloxan	-	64
4,4'-Diphenylmethane diisocyanat	101-68-8	310
4,4'-Diphenylmethane diisocyanat	101-68-8	420
Evt. Siloxan	-	120
Tributylacetylcitrat	77-90-7	2900
Tert-octyldiphenylamin	-	170
Ikke identificeret komponent	-	110
Ikke identificeret komponent	55255-72-6	610
Ikke identificeret komponent	-	77
Ikke identificeret komponent	-	1200
Ikke identificeret komponent	-	97
Evt. Equilenin	517-09-9	160
4,4'-Dioctyldiphenylamin	101-67-7	140
4,4'-Dioctyldiphenylamin	-	33
Irganox 1076	2082-79-3	1300

TABEL B4- 55
 RESULTATER AF GC/MS-SCREENING AF PRØVE M26A, LIFTMADRAS, TEKSTIL

Komponent	CAS-nr.	Indhold i mg/kg
1,6-Hexamethylene-diisocyanat (HDI)	822-06-0	6
Ikke identificeret komponent	-	49
Ikke identificeret komponent	-	9
Ikke identificeret komponent	-	10
Ikke identificeret komponent	-	47

TABEL B4- 56
 RESULTATER AF GC/MS-SCREENING AF PRØVE M26B, LIFTMADRAS, SKUM

Komponent	CAS-nr.	Indhold i mg/kg
Phenylisocyanat	103-71-9	62
Anilin	62,53-3	210
Benzothiazol	95-16-9	61
1H-Indazol	271-44-3	190
N,N'-Diphenylcarbodiimid	622-16-2	67
cis-9-Hexadecensyre	373-49-9	58
Haxadecansyre	57-10-3	110
Oleinsyre	112,80,1	1860
N,N'-Diphenylguanidin (accelerator)	102-06-7	390
Ikke identificeret component	-	54
Ethylziram	14324-55-1	130
N-phenyl-9-oktadecenamid	5429-85-6	157
Ikke identificeret komponent	-	53
Ikke identificeret komponent	-	160
Ikke identificeret komponent	-	160
Ikke identificeret komponent	-	650

TABEL B4- 57
RESULTATER AF GC/MS-SCREENING AF PRØVE M27A, LIFTMADRAS, TEKSTIL

Komponent	CAS-nr.	Indhold i mg/kg
1,8-Diazacyclotetradecan-2,9-dion	5776-79-4	8
Dodecylpalmitat	42232-29-1	22
Ikke identificeret komponent	-	38
Oleinsyre, eicosylester	22393-88-0	410
Ikke identificeret komponent	-	31
Ikke identificeret komponent	-	110
Ikke identificeret komponent	-	44

TABEL B4- 58
RESULTATER AF GC/MS-SCREENING AF PRØVE M27B, LIFTMADRAS, SKUM

Komponent	CAS-nr.	Indhold i mg/kg
2-Ethylhexansyre	149-57-5	110
2,4-Toleundiisocyanat (2,4-TDI)	584-84-9	130
2,4-Toluendiamin	95-80-7	15
5-Methylbenzimidazolon	5400-75-9	130
5-Formyl-2,4-dimethyl-1H-pyrrole-3-carbonitril	32487-71-1	120
4,4'-Vinylenedianilin	621-96-5	49
4,4'-Vinylenedianilin	621-96-5	83
Ikke identificeret komponent	-	44
Ikke identificeret komponent	-	66
Ikke identificeret komponent	-	45
Ikke identificeret komponent	-	179
Evt. 1,4-Bis(aminomethyl)-anthra-9,10-quinon	77862-13-6	130
4,4'-Dioctyldiphenylamin	101-67-7	56
Irganox 1076	2082-79-3	310

TABEL B4- 59

RESULTATER AF GC/MS-SCREENING AF PRØVE M28A, LIFTMADRAS, TEKSTIL

Komponent	CAS-nr.	Indhold i mg/kg
Dimethylvinylmethanol	115-18-4	11
3-Penten-2-on	625-33-2	34
3-Penten-2-ol	1569-50-2	20
1-Octen-4-ol	40575-42-6	110
1-Octen-4-ol	40575-42-6	100
Styren	100-42-5	6
Ikke identificeret komponent	-	22
1,1,2,2-Tetrachlorethan	79-34-5	47
Benzylalkohol	100-51-6	12
Hexaglycerol	77-99-6	16
2,4-Toluendiisocyanat (2,4-TDI)	584-84-9	410
5-Formyl-2,4-dimethyl-1H-pyrrole-3-carbonitril	32487-71-1	13
Ikke identificeret komponent	-	67
Ikke identificeret komponent	-	87
Ikke identificeret komponent	-	20
Ikke identificeret komponent	-	240
Ikke identificeret komponent, evt. isocyanat	-	38
Ikke identificeret komponent, evt. isocyanat	-	5
Diphenylmethane 4,4'-diisocyanat	101-68-8	17
Ikke identificeret komponent	-	3
Isocyanat	-	3
Benzo[a]fluoren	238-84-6	1
Diethylhexylftalat (DEHP)	117-81-7	22
Ikke identificeret komponent	-	44
C.I. Disperse red 60	17418-58-5	42
Ikke identificeret komponent	-	44

TABEL B4- 60
 RESULTATER AF GC/MS-SCREENING AF PRØVE M28B, LIFTMADRAS, SKUM

Komponent	CAS-nr.	Indhold i mg/kg
Acetophenon	98-86-2	17
2,4-Toluendiisocyanat (2,4-TDI)	584-84-9	25
5-Methylbenzimidazolon	5400-75-9	51
5-Formyl-2,4-dimethyl-1H-pyrrole-3-carbonitril	32487-71-1	51
Ikke identificeret komponent	-	15
Diisobutylftalat	84-69-5	57
Dibutylftalat	84-74-2	22
4,4'-Vinylenedianilin	621-96-5	47
4,4'-Vinylenedianilin	621-96-5	78
Ikke identificeret komponent	-	35
Ikke identificeret komponent	-	53
Ikke identificeret komponent	-	47
Ikke identificeret komponent	-	170
Fx 1,4-Bis(aminomethyl)anthra-9,10-quinon	77862-13-6	130
4,4'-Dioctyldiphenylamin	101-67-7	65

Appendix 5: Analysis results – formaldehyde (in Danish)

TABEL B5- 1

RESULTATER FOR FORMALDEHYD I TEKSTILER FRA AUTOSTOLE

		Formaldehyd
Prøve mrk.	Produktnavn	mg/kg
A1	Tekstil	<2
A2A	Tekstil	<2
A3A	Tekstil	< 2
A3B	Tekstil	< 2
A4	Tekstil	< 2
A5A	Tekstil	< 2
A5B	Tekstil	< 2
A6A	Tekstil	< 2
A6B	Tekstil	< 2
A7A	Tekstil	< 2
A7B	Tekstil	< 2
A8A	Tekstil	< 2
A8B	Tekstil	< 2
A29	Tekstil	< 2
A30	Tekstil	< 2

TABEL B5- 2

RESULTATER FOR FORMALDEHYD I TEKSTILER FRA BÆRESELER

		Formaldehyd	
Prøve mrk.	Produktnavn	mg/kg	Gennemsnit mg/kg
9A	Tekstil	<2	< 2
9C	Tekstil	<2	
10B	Tekstil	< 2	< 2
11	Tekstil	< 2	
12A	Tekstil	< 2	< 2
12B	Tekstil	< 2	

13A	Tekstil	< 2	< 2
13B	Tekstil	< 2	
14B	Tekstil	< 2	< 2
15B	Tekstil	< 2	
16B	Tekstil	< 5	< 2
17B	Tekstil	< 2	
18B	Tekstil	< 5	< 2
18C	Tekstil	< 5	

<: Betyder mindre end den angivne detektionsgrænse.

TABEL B5- 3
RESULTATER FOR FORMALDEHYD I TEKSTILER FRA MADRASSER

		Formaldehyd	
Prøve mrk.	Produktnavn	mg/kg	Gennemsnit mg/kg
19A	Tekstil	<2	< 2
20A	Tekstil	<2	
21A	Tekstil	< 2	< 2
22A	Tekstil	< 2	
23A	Tekstil	< 2	< 2
24A	Tekstil	< 2	
25A	Tekstil	< 2	< 2
26A	Tekstil	< 2	
27A	Tekstil	< 2	< 2
28A	Tekstil	< 2	

<: Betyder mindre end den angivne detektionsgrænse.

Appendix 6: List of azo dyes and other amines included in the analyses for azo dyes (in Danish)

TABEL B6- 1
OVERSIGT OVER STOFFER UNDERSØGT VED ANALYSEN FOR AZOFARVESTOFFER

Farvestof	CAS-nr.
4-Aminobiphenyl	92-67-1
Benzidin	92-87-5
4-Chloro-o-toluidin	95-69-2
2-Naphthylamin	91-59-8
o-Aminoazotoluen	97-56-3
5-Nitro-o-toluidin	99-55-8
4-Chloroanilin	106-47-8
4-Methoxy-m-phenylenediamin	615-05-4
4,4'-Diaminodiphenylmethan	101-77-9
3,3'- Dichlorobenzidin	91-94-1
3,3'- Dimethoxybenzidin	119-90-4
3,3'- Dimethylbenzidin	119-93-7
4,4'-Methylenedi-o-toluidin	838-88-0
p-Cresidin	120-71-8
4,4'-Methylen-bis-(2-chloranilin)	101-14-4
4,4'-Oxydianilin	101-80-4
4,4'-Thiodianilin	139-65-1
o-Toluidin	95-53-4
4-Methyl-m-phenylenediamin	95-80-7
2,4,5-Trimethylanilin	137-17-7
o-Anisidin	90-04-0
2,4-Xylidin / 2,6-Xylidin	95-68-1/87-62-7
4-Amino-azobenzen*	60-09-3
Øvrige aromatiske aminer	
Anilin	62-53-3
p-Phenylendiamin	106-50-3
2,6-Dichlor-p-phenylendiamin/4,5-Dichlor-o-phenylendiamin	609-20-1/5348-42-5

* Analyse af 4-aminoazobenzen blev ikke udført, da der ikke blev påvist anilin eller p-phenylendiamin i betydelige mængder.

Appendix 7: List of polybrominated biphenyls (PBB) and polybrominated diphenyl ethers (PBDE) included in the analyses for brominated flame retardants (in Danish)

TABEL B7- 1
OVERSIGT OVER STOFFER UNDERSØGT VED ANALYSEN FOR PBB OG PBDE

Flammehæmmer
Polybromerede biphenyler (PBB), enkelt stoffer:
2,2',5,5'-TetraBB # 52
2,2',4,5,5'-PentaBB # 101
2,2',4,4',5,5'-HexaBB # 153
2,2',3,4,4',5,5'-HeptaBB # BB 180
2,2',3,3',4,4',5,5'-OctaBB # BB 194
2,2',3,3',4,4',5,5',6-NonaBB # BB 206
DecaBB # 209
Polybromerede diphenylethere (PBDE), enkelt stoffer:
2,2',4-TriBDE (BDE-17)
2,4,4'-TriBDE (BDE-28)
2,2',4,4'-TetraBDE (BDE-47)
2,2',4,5'-TetraBDE (BDE-49)
2,3',4,4'-TetraBDE (BDE-66)
2,3',4',6-TetraBDE (BDE-71)
3,3',4,4'-TetraBDE (BDE-77)
2,2',3,4,4'-PentaBDE (BDE-85)
2,2',4,4',5-PentaBDE (BDE-99)
2,2',4,4',6-PentaBDE (BDE-100)
2,3',4,4',6-PentaBDE (BDE-119)
3,3',4,4',5-PentaBDE (BDE-126)
2,2',3,4,4',5'-HexaBDE (BDE-138)
2,2',4,4',5,5'-HexaBDE (BDE-153)
2,2',4,4',5,6'-HexaBDE (BDE-154)
2,3,3',4,4',5-HexaBDE (BDE-156)
2,2',3',4,4',5',6-HeptaBDE (BDE-183)
2,2',3,4,4',6,6'-HeptaBDE (BDE-184)
2,2',3,4,4',6,6'-HeptaBDE (BDE-184)
2,3,3',4,4',5',6-HeptaBDE (BDE-191)
2,2',3,4,4',5,5',6-OctaBDE (BDE-196)
2,2',3,3',4,4',6,6'-OctaBDE (BDE-197)
2,2',3,3',4,4',5,5',6-NonaBDE (BDE-206)
2,2',3,3',4,4',5,6,6'-NonaBDE (BDE-207)
DecaBDE (BDE-209)

Chemical substances in car safety seats and other textile products for children

The objective with this report is to examine and assess whether there are chemical substances in car safety seats and other textile products for children that may pose a risk, and to evaluate whether the regulations on the area are met. The analyzed product groups are car safety seats, baby slings, and baby mattresses and 10 products from each product groups are investigated.

The specific substances selected for quantitative chemical analysis based on the results of the screening analyses and initial hazard/exposure assessment were phosphorus-based flame retardants, the phthalate DIDP, azo dyes (through certain aromatic amines as indicators), isocyanates and brominated flame retardants. The substances found in most samples in significant concentrations were the phosphorus-based flame retardants TCEP, TCPP and TDCP and two isomers of diisodecyl phthalate (DIDP). Furthermore, an illegal level of an aromatic amine, indicating use of azodyes, was found in one product.

Migration tests were performed for the phosphorus-based flame retardants. The seven products having the highest content of one or more of these substances – 4 car safety seats, 2 baby slings and 1 baby mattress – were tested for migration to artificial sweat over a period of 3 hours. Migration was observed in all tests, varying from <1 % up to 30% of the material's content (by weight). Three products were shown to pose a risk under foreseeable use of the product.



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**Ministry of Environment
and Food of Denmark**
Environmental
Protection Agency

Analysis and risk assessment of fragrances and other organic substances in squishy toys

Survey of chemical
substances in consum-
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Sources must be acknowledged.

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Preface

This report presents a study and risk assessment of squishy toys carried out for the Danish Environmental Protection Agency in 2018.

This study includes a description of typical sales points, prices, countries of production, target group for squishy toys and the expected contents of chemical substances in the toy. In addition, information on the expected usage of the toy has been collected in this study. This information creates the foundation for this report's description of real-life exposure scenarios regarding children's exposure to substances while using squishy toys.

Chemical analysis of selected squishes was carried out and is reported here. The chemical analysis has been performed in two phases: a screening phase and a phase with quantitative analysis.

The analysis results create background for risk assessment of selected chemical substances from squishies selected in cooperation with the Danish Environmental Protection Agency.

The analyzed products have been purchased by the Danish Environmental Protection Agency during the period from February to March 2018, and the project was carried out from March 2018 to June 2018.

This project is prepared for the Danish Environmental Protection Agency by the Danish Technological Institute with DHI as subcontractor of hazard assessment, exposure scenarios and risk assessment.

Summary and conclusions

Background

Soft foam figures, also called squishies or squishy toys, are popular among children (2017-2018) and are available in many different sizes, colors and models. Playing with the toy involves children e.g. hugging and shaping the foam figure in their hands. When the grip is released, the toy folds out and returns to its original shape, after which the figure can be squeezed and shaped again as a type of stress ball. Squishies are also considered a collectible, e.g. displayed in the child's room, but to a lesser extent used for playing with. Squishies often have a perfumed or chemical smell and are expected to be made of polyurethane foam (PUR), which may potentially contain a number of problematic chemical substances. Until now, only limited information is available on which chemical substances, e.g. fragrances and other organic compounds, are contained in and released from squishy toys.

Aim

The aim of this project is to obtain knowledge about which organic substances, including fragrances, are released from the products and the possible release of substances that give a chemical smell. It must also be assessed whether the substances identified in the products present a risk to children.

Result of the survey

A survey on squishy products for children, which are considered available on the Danish market, has been performed. The mapping includes a description of typical sales points, prices, production countries and the target group for squishy toys. A study on the chemical substances possibly present in the products, including substances originating from the polymer material or added to the toy to obtain a particular fragrance, has also been carried out. Further, information on the expected use of the toys has been collected through a few Danish distributors and their suppliers.

The range of squishies available on the Danish market is large, and the products were found at many retailers in physical Danish stores, Danish webshops and non-EU webshops. China is indicated as the country of production for the vast majority of the identified products. On some of the purchased products it is stated that they are scented, and, therefore, some squishies for children on the Danish market are expected to contain fragrance. Products with comparable designs are found on the market from different providers and with diverse odor/smell. The contacted distributors and importers pay particular attention to the odor of as well as the specific content of fragrance in products, and, in some cases, they deselect products with added fragrance.

Distributors and importers have, in addition to the fragrance substances, been focusing on the content of phthalates, while none of the contacted operators have expressed concerns about the content of substances typically used in the production of the polymeric material, for example, any residues of the monomer which the material consists of or other additives, such as solvents or foaming agents that give the material the properties that characterize squishy toys.

The products are considered collectibles, and it is normal for children to own several different squishies (5-10). Children who collect eagerly can be expected to own even more (between 30 and up to 200 pieces are indicated by distributors / importers for those with the largest collections). The typical use of squishies is described as active play where the products are squeezed, and the restoration to the original shape is observed and compared to other squish-

ies. One of the contacted distributors therefore considers it likely that in case of squishies skin contact occurs longer than with other types of toys. The target audience for the products is generally determined by the contacted importers and distributors from approx. 6 years and upwards. Some types of squishies are expected to be used for role playing, which can last for longer periods.

The information collected in the survey forms the basis for the selected analysis program and this report's description of realistic exposure scenarios of children to substances during their use of squishy toys.

Description of exposure scenarios

Based on information regarding children's play and their use of squishies, a typical scenario was described for the target group of children over 6 years of age. In a scenario for inhalation of vapors, data from emission measurements are used to calculate the concentrations of the substances in a standard children's room of 7 m² (17.4 m³). As a worst-case scenario, it is assumed that up to 40 squishies have been collected in the room and that the child stays in the room for 15 hours. Regarding skin exposure, it is assumed that the child has a skin contact with a squishy for 2 hours daily, and during this time is exposed to substances released from the material over time (through migration).

As a worst-case scenario for an unintentional but possible use, a scenario was described where a 3-year-old child is sleeping 10 hours with a squishy in his arms. In this scenario, the child may breathe relatively concentrated vapors comparable to those measured in the small climate chambers used in the emission experiments. For skin contact, a contact time of 10 hours is used.

Purchasing and selecting samples for analyses

The Danish Environmental Protection Agency purchased 43 different squishies (2-3 of each product) prior to the initiation of this study. The price of products ranged between DKK 7 and DKK 150 with an average price of approx. 60 DKK. Purchasing of products was highly influenced by the high demand, and some products were sold out during the purchase period, while new ones were offered during the same period. Purchases have therefore been limited to the available products during the purchase period (February to March 2018).

The goal for purchasing these samples was that the products should be targeted to children and that most products should be purchased in Danish webshops and non-EU webshops, while purchases in physical Danish stores should constitute a minor part. Due to long delivery times from non-EU webshops, purchases from these types of points of sale became fewer than intended, although still with a majority of products purchased in webshops. Several other criteria were also used in purchasing and selection of samples for analysis, such as popularity, price, material characteristics, packaging, product odor/smell and design.

Eight squishies were selected for initial screening of content and substances that emit to provide an overview of substances that could be relevant for further investigation. The eight samples were selected so that four squishies were purchased in physical Danish stores, two squishies were purchased in Danish webshops and two squishies were bought in non-EU webshops (Chinese producers). In the sample selection, there is an excess of squishies from physical Danish stores compared to the set goals (50% against 10-20%), which is due to the fact that in the selection of samples, priority was also given to samples that smell and their price, as well as it was chosen to include two comparable products from different dealers.

Initial analysis - screening of eight selected samples

Analysis program

The eight products selected for initial screening were analyzed by GC/MS for chemical content and for the emission of volatile chemical substances in a climate chamber test. This method of screening is chosen due to focus in the project on volatile organic compounds, and the same eight products are analyzed for content and emission for comparison of results.

Three out of the eight products are also selected for identification of the material type (IR characterization). The three selected samples are also analyzed for isocyanates which may be present as residual monomer in the PUR material and are highly respiratory irritant.

Results of screening of chemical content.

Approximately 100 chemical substances have been found in each of the eight squishies, and 35 of these substances could be identified with great certainty. Several of the other substances were also identified, but with a higher uncertainty.

The 35 substances identified with the highest degree of certainty are probably predominantly chemical substances originating in the polymer material from which squishies are made of. This applies, for example, to amine compounds (dimethylaminoethanol, dimethylformamide, octadecenamid, triethyldiamine and pentamethyldiethyltriamine) used in the preparation of the polymeric material. There is also triethyl phosphate, which is probably used as a catalyst in the polymerization process. Also, phthalates (DEP and DEHP), cyclohexanone and octadecyl di-tert-butyl-4-hydroxyhydrocinnamate are identified in the products, as well as 4,4'-methylenebis benzeneamine, which is probably a monomer residue.

Among the substances identified with a higher uncertainty are e.g. the fragrances versalid and galaxolide, the organophosphorous-based fire retardant tris(2-ethylhexyl) phosphate (TEHP) and traces of the reactive radical initiator azobisisobutyronitrile, which may indicate that the chemical reaction that is taking place during the manufacturing process is not fully completed (incomplete polymerization). Traces of glycol and silicon compounds were also found in an amount indicating that they may be residues from the polymer chain.

Screening analysis by extraction and subsequent GC/MS analysis results in a dissolution and partial degradation of the polymer which may result in finding substances bound in the product under normal use and therefore not found in emission or migration analyzes. It cannot be denied that some substances could have been added in free form to the material to obtain special material properties, and hence are not chemically bound to the polymer network. Therefore, there may be a risk that some release of the substances during use may occur depending on the physicochemical properties of the individual substances.

Results of screening by emission tests

The emission analyses of the eight selected squishies show that a number of volatile organic compounds, carbonyls and VOCs can be identified in the individual samples. Several fragrances have been found in the emissions: D-limonene, benzyl alcohol, L-linalool, delta-3-carene, alpha-pinene and beta-pinene. In addition, N,N-dimethylformamide (DMF), N,N-dimethylaminoethanol, triethylenediamine, bis(2-(dimethylamino)ethyl)ether, 1,2-ethanediamine and N-[2-(dimethylamino)ethyl]-N,N',N'-trimethyl-cyclohexanone found in high concentrations. Many of these substances are also identified in the screening of chemical content.

In six out of eight squishies, concentrations of formaldehyde, acetaldehyde, propanal, butanal, acrolein and crotonaldehyde are below 10 µg/m³ of air in the performed emission screenings. This is considered low in comparison to existing limit values for e.g. formaldehyde in indoor climate, which is 100 µg/m³.

Results of material determination

Material determination has been made using infra-red spectroscopy, which unambiguously shows that the three analyzed squishies consist mainly of polyurethane foam, confirming the knowledge obtained in mapping.

Results of isocyanate screening

Three selected squishies are analyzed for isocyanates, which is one of the monomers that the material is made of. These residues of monomers are found in all three squishies. The complexity of the material interferes with the analysis, but an estimated worst case total value of 1.5 mg/kg isocyanate is found in the squishy having the highest content.

Initial hazard assessment

For the identified substances found in the screening of content analyzes and emission analyzes, hazard classification data were found in the European Chemicals Agency's database (EU harmonized CLP classification and / or classification used in the substance REACH registration).

Subsequently, special attention was paid to substances with serious hazard classifications such as:

- CMR 1A, 1B, 2
- STOT RE 1.2
- STOT SE 1.2
- Acute tox. 1,2,3
- Skin Sens 1
- Resp. Sens 1

Substances with very high emissions or high content levels were also included in the prioritization, although the substances were not prioritized based on their classifications.

For further emission analyzes, 14 substances (including 3 fragrances) were identified, while 26 declarable fragrances in addition to the substances N,N-dimethylformamide (DMF) and N,N-dimethylaminoethanol (DMAE), both found in much high amounts of content, were identified as relevant for migration analysis. The substance N,N-dimethylformamide is furthermore a substance that appears on REACH's candidate list of substances of very high concern due to its classification as toxic for reproduction, Repr. 1B.

Quantitative emission and migration analysis on selected samples

Analysis program

It has not been possible to include all substances with a relevant classification with respect to children's exposure from squishies. An analysis program containing quantitative analyses of emission in a climate chamber and quantitative determination of migration of selected substances was, therefore, designed.

Substance-specific quantification of selected substances has been completed for a total of twelve squishies, the eight were tested during the performed screening analyses and four additional squishies (one purchased in a physical Danish shop, two purchased in Danish webshop and one purchased in a non-EU webshop). For emission tests in the climate chamber and subsequent quantification of volatile substances, attention is put on fragrances, carcinogens, amines and chlorinated substances found in the initial screening. The emission to air from all the squishies of the selected substances after a short period of time (1 hour) and after 3 days (3 days) in the climate chamber is measured to determine how emission is reduced

during the first days after unpacking and to provide information for the risk assessment of children's exposure.

Migration to artificial sweat was also performed with the same 12 squishies with subsequent substance-specific analysis of DMF, 26 fragrances and DMAE in the migration fluid.

Results of migration analysis for artificial sweat

Migration analysis was conducted with a one-hour and 100 mL migration fluid per 10 cm² sample as defined in EN 71-10: 2005. The migration is done to artificial sweat at body temperature and on a sample with as high a proportion of the outside surface of the product as possible.

For DMF and DMAE, migration has not been detected under the conditions used for any of the samples tested. The detection limit per surface is 100 µg/cm² for this analysis, which corresponds to 1000 µg/g sample and with an estimated uncertainty of 20% RSD.

None of the 26 fragrances tested were found in a concentration above the detection limit in the 12 selected products. Detection limit per surface area for this analysis is 2 µg/cm², which corresponds to 50 µg/g and with an estimated uncertainty of 20% RSD.

Results of emission testing

The emission of three fragrances is confirmed in the 12 analyzed squishies; limonene and linalool, which must be declared if the content in the toy exceeds 100 mg/kg, and benzyl alcohol, which is not allowed in toys. Limonene is found in concentrations up to 1900 µg/m³ in emission tests after one hour, which, however, is reduced to 1100 µg/m³ after 3 days. As the declaration obligation is set for content and no content analysis of the limonene in these exact samples has been performed, it can neither be confirmed nor denied if the limit of 100 mg/kg has been exceeded. Benzyl alcohol has been found in low concentrations (< 5 µg/m³) in the emissions from four squishies after 1 hour and 3 days, respectively. The Danish Statutory Order on toys permits substances that are not allowed in toys to be present if these are technically unavoidable and below 100 mg/kg. As the low values indicate, this is probably the case. In all squishies, other fragrance-relevant terpenes have also been found: alpha-pinene, beta-pinene and delta-3-carene. Both the alpha-pinene and beta-pinene are among 82 recognized contact allergens identified in cosmetics.

Various chlorinated substances have been found in emission tests: dichloromethane (methylene chloride), chloroform, 1,2-dichloroethane and 1,2-dichloropropane. From 1 hour to 3 days, the concentrations of most substances are reduced in the emission from the squishies.

Three amines have been found: N,N-dimethylaminoethanol (DMAE), triethylenediamine and bis (2- (dimethylamino) ethyl) ether where the concentration either rises or has approximately the same concentration in the air from 1 hour to 3 days. A fourth amine; 1,1,4,7,7-Pentamethyl-diethylenetriamine is found in the emission test of eight of the tested samples. Unlike the other seven samples analyzed after 1 hour of emission, the substance for squishy No. 033 is not seen after one hour, but only after 3 days of emission. Overall, it is thus estimated that amines are released more slowly from the material over time than the other volatile organic compounds measured, which means that children are potentially exposed to this type of substance over a long period of time.

The concentrations of the individual substances vary with the type of squishy and it also indicates that the weight and the corresponding surface area of the squishies are important for the concentrations of volatile organic compounds released in the climate chamber test. It also suggests that the type of substances that are released varies with the manufacturer. Four of the analyzed samples have comparable appearance in sets of two (i.e., look similar with re-

spect to figure, color and design); sample 038 and 061 are similar to each other and sample 034 and 063 are similar to each other. Sample 061 and 063 are from the same retailer (online store in non-EU country) but from two different manufacturers, while samples 034 and 038 are purchased in a Danish online store (manufacturer not specified). There are substances that are identified in all or many of the tested samples, but in general there are great differences in the substances that are released by emission in the climate chamber. At a first glance, there are no apparent trends in content and no conclusions can be reached as to which substances are typically used in production and which fragrances are used. Even for products manufactured by the same producer (samples 051 and 054), significant differences are observed in the chemical content of the two squishies, both in terms of substances and emission concentrations.

Hazard and risk assessment

In the hazard assessment of the emitted substances, data were retrieved on tolerable exposure levels for the substances (the REACH legislation uses the term DNEL, Derived No Effect Level) for the 17 substances considered most problematic. As a starting point, DNEL values derived by expert groups in EU or DNEL values developed in previous environmental management projects were used. For four of the substances where no DNEL value was found, these were derived in this project (Appendix 7).

In the actual risk assessment process, the measured exposure levels were compared to the DNEL values for the substances and a RCR (Risk Characterization Ratio) was calculated:

$$\text{RCR} = \text{Exposure } (\mu\text{g}/\text{m}^3) / \text{DNEL } (\mu\text{g}/\text{m}^3)$$

RCR values above 1 mean that the exposure level exceeds the tolerable DNEL value, which in turn means that the level of protection is too low, and there may be a risk associated with the use.

In the case of emission measurements of 12 different selected squishies, emission of a number of substances was detected at levels that may cause irritation of the eyes and respiratory tract, and which, in the case of repeated exposure, may cause damage to the mucous membranes. This included the following substances where RCR values were found to be significantly greater than 1:

- dimethylaminoethanol, (RCR = 28 as the highest value)
- N,N-dimethylformamide, (RCR = 73 as the highest value)
- cyclohexanone, (RCR = 15 as the highest value)
- triethylenediamine, (RCR = 206 as the highest value)
- bis(2-(dimethylamino)ethyl)ether, (RCR = 200 as highest value)
- 1,1,4,7,7-pentamethyldiethylenetriamine, (RCR = 46 as the highest value)

In an exposure scenario for a small child sleeping with a squishy in his arms, unacceptably high levels of the substances N,N-dimethylformamide, triethylenediamine and cyclohexanone were reached for all 12 squishies based on the emission measurements one hour after unpacking of the toy. For four squishies for which 3-day measurements were also made, the concentrations remained unacceptably high for the substances N,N-dimethylformamide and triethylenediamine.

For other mucosal irritants: bis(2-(dimethylamino)ethyl)ether; 1,1,4,7,7-pentamethyldiethylenetriamine and dimethylaminoethanol the emission level from 6 of the squishies exceeding a tolerable level 1 hour after the unpacking. After three days, the level of bis 2-(dimethylamino)ethyl)ether remained unacceptably high for three out of four squishies.

For substances with other effects, e.g. methylene chloride (carcinogenic) and xylenes (chronic neurotoxic), the tolerable exposure level was only exceeded slightly at 1 hour after unpacking (RCR up to 2.3 for methylene chloride and RCR up to 4.9 for xylenes). Since these adverse effects are associated with long-term exposure above the DNEL value, a short-term exceeded RCR value is considered to be less significant in a risk assessment context.

In a scenario where more squishies are stored in a child's room, unacceptably elevated levels were found in the child's room after three days for the substances N,N-dimethylformamide, N,N-dimethylaminoethanol, triethylenediamine, (bis(2-(dimethylamino)ethyl)ether), 1,1,4,7,7-pentamethyldiethylenetriamine and cyclohexanone.

In migration tests for artificial sweat fluid, no migration of any of the selected substances was found, so a quantitative risk assessment for skin exposure to these substances could not be performed. However, in case of skin contact, the skin will also be exposed to the emitted substances, so the risk of skin irritation cannot be excluded. Similarly, the risk of skin allergy cannot be ruled out due to the emission of substances that can cause skin allergy, which emitted in smaller amounts. Particularly, repeated skin contact may promote the development of skin allergy. If small children are sucking on parts of the squishy, the possible risk of skin irritation and skin allergy is greatest for areas where the skin is thinnest, e.g. on the lips.

1. Introduction

1.1 Background

Soft foam figures, also called squishies or squishy toys, have become very popular among children during the recent years (2017-2018). Squishy toys can be found in many different sizes, colors and models and are made to resemble, among others, animals, different food products, e.g. ice cream, cakes and fruit, and as emojis. Squishies can be purchased in e.g. toy stores, supermarkets, book stores and webshops.

Toys are, for one thing, used by a child squeezing and forming foam figures in her hands. After releasing the grip around the soft toy, the toy unfolds and returns to its initial shape and then the toy can be squeezed and shaped once again. Furthermore, squishies are also considered a collectible. This means that some children own many different toy designs that are, e.g., displayed in children's rooms and only to a lesser extent used as toys. Many toy distributors, among others BR, Toys'R'Us and Squishyshoppen, describe squishy toys as being easy to put in a bag, pocket or the car, and the toy is often found in classrooms, where pupils use the soft squishies as a sort of stress ball.

Squishies are often fragranced or have a chemical smell and are expected to be produced from polyurethane foam (PUR) due to the product's ability to reestablish the initial structure after deformation. The type of material is, however, not decisively confirmed prior to this project, and there is a limited knowledge of the chemical ingredients. PUR, as well as other polymer materials, may contain many different chemical substances (see e.g. Danish Environmental Protection Agency 2014A) such as:

- fragrances
- solvents
- preservatives
- residual monomers (for PUR e.g. isocyanates)
- fire retardants (phosphates as well as chlorinated and brominated compounds)
- coloring agents (e.g. heavy metals)
- softening agents (e.g. phthalates)
- other additives, such as foaming agents, catalysts, stabilizers, antioxidants and flavoring agents (to prevent animals and children from eating them).

Some websites provide information that certain squishies have an odor/smell upon delivery, but that this odor/smell decreases with time and during use, and that the odor/smell is not hazardous. Currently, only limited information is available on which fragrances and other organic compounds are included in and emitted from squishy toys.

1.2 Aim

The aim of this project is to gain knowledge on which organic substances, including fragrances, are emitted from these products as well as possible emission of substances with a chemical smell. It will also be assessed, whether substances identified in products constitute a risk for children.

2. Survey of squishy toys

This survey consists of collecting information on typical sales points, prices, countries of production, target group for squishy toys and expected content of chemical substances in squishy toys on the Danish market. Information on the expected use of toys has also been collected. This information creates background for the description of real-life exposure scenarios regarding children's exposure to substances during use of squishy toys and choice of methods for chemical analysis of selected products.

2.1 Legislation in relation to squishy toys

Squishies sold on the Danish market must comply with the Danish Statutory Order on safety requirements for toys (BEK no. 309 of 3/4/2017, *Legetøjsbekendtgørelsen*), which is the implementation of the Directive of the European Parliament and the Council of 18 June 2009 on the safety of toys (2009/48/EC). Furthermore, certain limitations are in force regarding the specific chemical substances according to EU chemical regulation REACH (1907/2006) and a few other special Danish regulations. In the following sections, limitations for chemical substances expected to be relevant for squishies are described.

Limitations of Danish Statutory Order on safety requirements for toys

According to Danish Statutory Order on safety requirements for toys, the toys must be produced in such a way that they do not constitute a risk for hazardous effect on human health as a result of exposure to chemical substances in toys. The requirements in the statutory order include toys used for the intended purpose or in ways children may be expected to use them.

CMR substances

Generally, substances classified as carcinogenic, mutagenic or toxic for reproduction (CMR substances) may only be used in toy parts that are not accessible to children, including via inhalation. If substances with CMR classification are used in parts accessible to children, their concentration must be lower than the respective classification limit of these substances.

Fragrances

Of particular relevance for squishy toys are the limitations included in the Directive 2009/48/EC regarding the use of many allergenic fragrances. In total, 55 fragrances are prohibited in concentrations exceeding 100 mg/kg, and 11 other fragrances must be declared if added above this concentration. A list of these substances can be found in Appendix 1.

Migration limits for some chemical elements

For toys, there are established migration limits for a long range of chemical elements such as cadmium, nickel and chromium. Migration limits depend on the material of toys, and squishies would mainly be categorized as scraped-off toy material. The complete list of migration limits is given in Appendix 1.

Limitations in toys for children under the age of three and toys intended to be placed in the mouth

Specific limit values are in force for substances in toys for children under the age of three and in toys intended to be placed in the mouth. Substances such as phenol, bisphenol A, formaldehyde, the flame retardants TCEP, TCPP and TDCP as well as the preserving agents benzisothiazolinone, methylchloroisothiazolinone (MCI) and methylisothiazolinone (MI). The specific limit values are given in Appendix 1. In addition to these limitations, there is a national limitation in Denmark on all phthalates in toys and products for small children under the age of three

(BEK no. 855 of 5/9/2009). The limit is 0.05 % and applies to all phthalates except phthalates already regulated in EU. Currently, it is uncertain how squishy toys will be age-determined, since there is no consensus between member states at EU level. According to Danish Statutory Order on toy safety, toys must be safe for children under the age of three unless the toy carries a warning that it is not safe for children under the age of three or that it is clearly not intended for small children. Since it has not been clearly indicated, whether squishy toys are not intended for small children, it may be expected that the product is safe for all age groups unless the products are marked with a warning.

Limitations in REACH

In addition to the mentioned restrictions in Danish Statutory Order on safer toys, the use of chemicals in toys is also subject to limitations in the REACH regulation, Appendix XVII. Limitations in toys include six specific phthalates (entry 51 and 52), benzene (entry 5) and eight PAHs (entry 50). The exact limitations are given in Appendix 1.

2.2 Aim and method

The aim of the screening is to shed light on which materials squishies typically consist of and which possible content substances are expected to be found and emitted from these toys. The Danish Environmental Protection Agency has carried out a screening of toys on the market and have purchased a selection of products prior to project start. The survey takes its departure in information collected by the Danish Environmental Protection Agency when purchasing the toys, and, on this basis, a short description on the following has been made:

- sales points
- price differences
- country of production
- product materials
- ingredient expected to be found in products
- typical age of target group for this product
- reflections of children's play with products regarding exposure, e.g. skin contact, play time and time spent in children's room indicated in hours per day.

Knowledge of sales points and prices has been collected by the Danish Environmental Protection Agency prior to project start and has been processed by the Danish Technological Institut during the survey. Additional knowledge of above-mentioned aspects, e.g. from label insert or description of the purchased products, was also included in the survey and was supplemented to a lesser degree with information found by an internet search.

Since the toy industry constitutes a very important information source, a telephone interview with selected importers and distributors from the toy industry has been conducted with the aim to collect information on material types, potential problematic ingredients and description of how children are expected to play with these types of toys (see questionnaire for phone interview in Appendix 2). During telephone interviews, the contents of fragrances, which constitute a risk in relation to development of allergy (SCCS 2012), and contents of volatile organic compounds that emit smell, such as solvents, were specifically addressed.

2.3 Information collected from distributors and importers

During the survey, four stakeholders within the industry were contacted: two toy distributors and two toy importers (the one via their distributor, who has forwarded the answers). Products purchased from both of the contacted distributors are included in the analysis. All questions have been answered either by phone or via e-mail in March 2018.

Distributor 1

A Danish squishy distributor confirms that their marketed products are made of polyurethane foam (PUR). The products are made in China, but companies responsible for the production are based outside China (Canada and UK). The distributor is always very attentive when dealing directly with Chinese manufacturers and is always dependent on their trust towards suppliers.

In general, the distributor does not see a risk for a content of problematic substances as being more significant for this type of toys compared to other toy products and thus has not posed stricter requirements for squishies. The documentation for compliance with existing legislation has been collected, and, for instance, test results for phthalates have been documented, where—according to legislation—there are limitations to the substance contents. Among the marketed products from this distributor, there may be products containing declarable fragrances within the permitted concentrations according to Directive 2009/48/EC, i.e. below 100 mg/kg, while other products are entirely without fragrance. In other squishy products, which the distributor has screened during purchase, some products have been found with declarable fragrances that are considered allergenic.

The distributor has received a few inquiries from consumers regarding their concern about the smell typically emitted by squishies. The products have a “chemical” smell, and the distributor considers it highly possible that the inquiries are primarily connected to media coverage of these products. No inquiries have been registered with specific health-related effects. These inquiries are mainly connected to concerns about the experienced smell, where probably one or two customers have indicated that they have been directly annoyed by the smell. There are no inquiries related to allergic reactions. The distributor assessed that the number of inquiries reflects the sales figures and the media coverage of the products during the recent period, and can generally be compared to similar toy products as e.g. fidget spinners.

The distributor considers children between the age of 6-10 years, maybe up to 12 years, to be the typical target group for squishy products. Marketing through e.g. YouTube stories are mostly directed towards older children in this age group. The distributor's sales figures indicate that children between the age of 6-10 years purchase and use these products (sales peak with 10-year-olds), and the distributor generally recommends the products from the age of 6 and above.

The distributor expects that playing with these products is centered around squeezing and observing the toy while returning to its original shape. These products are usually rubbed in hands and may be included in roleplays, where usually several squishies are involved. Due to the squeezing function, longer skin contact is highly possible compared to other types of toys. However, the toys are mainly expected to be used in waking state and cannot be compared to soft toys or teddy bears, since the material surface does not feel pleasant against skin as e.g. textile materials.

Squishies are available in series, where several designs can be collected, as well as so-called blind bags, where consumers have an opportunity to acquire rare versions. The products remain at the top-ten list of sold products, and no decrease in interest has been observed yet. It is highly unusual to have only one squishy, but 100-200 pcs. as sometimes reported in media, is likewise highly unusual. The trend is that more squishies are purchased and collected, but this has not been investigated by analyzing sales figures.

Distributor 2 and Importer 1

The second Danish distributor of squishies informs that technical documentation on squishies is procured in the same way as for similar types of toy products, and the distributor in general has strict requirements for chemical content in products, and he performs random sample control himself. The distributor has a list of specific substances that he does not allow in toys, and he works closely together with importers to ensure that their products meet all requirements. This distributor has via his importers been confirmed that the squishy toys marketed by this specific distributor consist of PUR and are produced in China. During purchase of squishies, a special focus has been on the potential content of fragrances and phthalates. Squishies with added fragrance have actively been deselected, and, hence, the distributor has no knowledge of which fragrances are typically used for squishies. According to one importer, fragrances, that provide the odor of cream, pineapple and peach are often used.

No inquiries from consumers have been registered regarding health-related complaints when using these products. Only a few inquiries have been registered regarding the hazard of the smell of these products.

The typical target group, as indicated by this distributor, are children between the age of 6-12 years, while their importer has chosen to recommend these products for children between the age of 5 and 14 years. The distributor has assessed that the product does not appeal to small children, since the product cannot be activated, and small children (under the age of 3) do not sit and observe the appearance and expansion of squeezed object. The product does not emit any sound, does not have any effects or moving parts that could catch a small child's attention. The distributor has assessed that the color and design may possibly make a difference and expects that squishies looking like food products could appeal to small children, if the toys are visible to a child under the age of 3. According to the importer, the selection of material for the product may result in the formation of small parts, which is why the use by small children advised against as indicated on the packaging ("not suitable for children under 3 years).

Playing with these products is expected to be of long duration for the target group, i.e. play for hours by comparing the product designs and observing the squeezing and reformation of the original form. The speed for reformation is of main importance for the play, since products taking longer to return to their original shape are preferred. According to importers, roleplay with squishies, which is expected have a longer duration, are also a widespread activity.

Products are undoubtedly seen by both the distributor and its importers as collectibles, and the distributor is aware of a customer who purchased 65 squishies. The Danish distributor still observes a demand for these products, but it seems to be declining. According to the respective distributor's importer, there is still growth on the international market.

Importer 2

A Danish importer of squishies, who covers the Nordic market, screened a long list of possible producers when introducing the product to the market. In the screening, the producers were especially assessed according to the quality of the documentation, they forward, and their professionalism when handling requirements regarding chemical contents. This also relates to requirements that exceed the requirements determined by the legislation. The importer has been in contact with 10-12 producers, which all have their products produced in China, but only a few delivered the required documentation, among others on chemical contents, and could be approved as suppliers. This importer has currently suspended its import as the Danish Safety Technology Authority investigates the products, including investigations regarding possible special safety requirements for this type of products.

The producers have confirmed the importer that the products meet the relevant regulations, e.g. REACH and the special regulations to toys, including the contents of phthalates. Further-

more, it is confirmed that products comply with standards EN71-1 to 71-3. The producers have confirmed that squishies consist of PUR, and that the material contains typical additives that are commonly expected for this type of material, as well as different amine-based catalysts and foaming agents. This importer distributes only squishies with no added fragrance due to customer demands. Thus, the importer does not have a specific knowledge of fragrances that are typically used in squishies.

According to the importer's opinion, the omission of fragrance in the products has resulted in squishies with a strong chemical smell, which—according to the producers—is characteristic for the material (PUR). However, the smell does raise concern among consumers, which is why the importer has received several inquiries regarding this, either directly from the consumers or via distributors. Furthermore, the importer has received 5-7 inquiries regarding concerns about smell via the consumer app "Tjek kemien". Never before has this importer received inquiries via "Tjek kemien", but the importer has concluded that it is due to the chemical smell, and he has not experienced other inquiries relating to specifically health-related complaints. In this relation, the importer suggests degassing the products before use.

According to this importer, a typical target group for squishies is between the age of 8-14. The trend started among older children in the age group, and the younger children are now following the trend, which is a typical pattern. The products are marked with a label 6+, and the importer will not deny the possibility that also smaller children would play with squishies. However, it is not suggested by the importer, whose own experience further indicate that the recommended age group matches the market trend.

The importer has no direct experience with children's play with products, but considers it likely that children play with each squishy for about 15 min. at a time by squeezing, observing and comparing them to other squishies. New forms and designs of squishies are being produced continuously, and the current trends, according to this importer, is more detailed squishy designs, which are more attractive to older children, e.g. for using squishies in roleplays. The importer's products are definitely seen to be collectibles, and it is considered that each child could easily have 5-10 in their possession, and that is not unusual that a child may have up to 30-40 squishies.

New squishy figures are introduced all the time, e.g. as collectibles, and the number of sold products currently exceeds 100,000 pcs. in total. The demand among some of the importer's customers is considered to be declining. The sales of squishies started at toy retailers, but has now spread to many types of retailers and, hence, the total sales figures could still be on the increase.

2.4 Availability of squishies and indicated target group

In relation to this study, squishies have been found at nine out of more than 15 investigated distributors selling products in one or more physical shops in Denmark (toy shops and other shops expected to sell toys) and/or online shops in both Denmark and non-EU countries, where squishies can e.g. be purchased directly from China. The aim of this study has not been to provide a full overview of sales points for squishies, and it is expected that, besides these nine shops, there is a number of other retailers, especially online. At the time of purchase for this survey, the demand for some types of squishies was very high (February – March 2018), meaning it was found not uncommon that products marketed on online shops were soon sold out.

This project has only focused on those squishies aimed at children, i.e. which typically appeal to children due to their design. Squishies can be found in many different sizes, colors and models. They come in different series, which can be collected, and in editions that are rare or

hard to find. For instance, squishies can be formed as cakes, fruit and cute animals, and they may have been added fragrance.

Squishies with country of production given all originate in China, which confirms the information received from the interviewed importers and distributors. Thus, the products of Chinese origin and of low price are highly prioritized in relation to purchase of samples. Some websites provide information that certain squishies have a specific smell upon delivery, but that the smell disappears with time and use and that the smell is not hazardous¹. In addition, it is stated that squishies are easy to use and that the product can, thus, be used by small children, except children under the age of 3, since the product may contain small parts².

2.5 Material type and expected content of fragrance and other volatile organic compounds

Squishy toys are described as being produced from PUR³, which can be manufactured with the desired properties for *memory foam*, i.e. materials that regain their initial form after being compressed. This is confirmed by the interviewed importers and distributors, who do not have the knowledge of the use of other material types for squishies. However, it cannot be excluded that other forms of polymer materials may be used by other manufacturers.

In the following, monomers, initiators, catalysts and typical additives will be reviewed with focus on substances in the categories of fragrances or other volatile organic compounds.

Monomers, initiators and catalysts

Polyurethane (PUR) generally consists of di- or triisocyanate, which has reacted with a polyol. Both isocyanates and polyols applied in the production of PUR contain on average two or more functional groups in order to create the correct three-dimensional network and cross-linking of the material. The reaction is typically catalyzed by tertiary amines such as 1,4-diazabicyclooctane (CAS no. 280-57-9), dimethylcyclohexylamine (CAS no. 98-94-2), dimethylethanolamine (DMEA) and bis-(2-dimethylaminoethyl) ether (CAS no. 3033-62-3) (Adam et al., 2005). Some amines have in their pure form problematic health-related properties, e.g. they may cause heavy skin burns and eye damage (H314). The choice of catalysts determines the properties of PUR foam, and besides the traditional catalysts, a range of specialized catalysts have been developed (Soto et al., 2014). It is expected that these amines are physically and/or chemically linked in polymer material after termination of the reaction. The release of these substances upon normal usage is expected to be limited; however, dependent on the physicochemical properties of the respective amine.

The properties of PUR foam are to a high degree affected by the type of isocyanates and polyols that are applied in production. There are many accessible isocyanates and polyols as well as additives and process requirements that may vary in relation to PUR production and which contribute to the wide range of properties of PUR, e.g. foam which retakes its form (so-called memory foam or LR/Low-resilience PU).

The mostly applied isocyanates are the aromatic isocyanates diphenylmethane diisocyanate (MDI) or toluene diisocyanate (TDI), which are more reactive than aliphatic isocyanates such as hexamethylene diisocyanate (HDI) or isoforon diisocyanate (IPDI) (Randal and Lee, 2002). Most isocyanates are difunctional, meaning they have two isocyanate groups per molecule.

¹ E.g. Squishyshoppen.dk – *Hvad er en Squishy eller Squishies* (What is a Squishy or Squishies) [ed.: The page and contents have been removed].

² According to descriptions on the retailers' web pages that have later been removed.

³ <https://www.tabletcovers.dk/products/squishy-gadget-large-blue-koala-lysebla> states "material: elastic polyurethane" – reached 2018.04.23.

Isocyanates are severely irritant to the respiratory tract, and their possible existence in squishies may cause a potential risk in relation to their use.

Polyols are polymers that on average have two or more hydroxyl groups per molecule. The length of the polyol chain and its functionality contribute to the properties of the final polymer material. The initiators, such as ethylenediamine and triethanolamine, are applied in the production of some polyols. In the same way, diacids of high purity and glycols are used, such as adipic acid (CAS no. 124-04-9) and 1,4-butanediol (CAS no. 110-63-4), to produce some conventional polyols for PUR.

Typical additives

The production of PUR foam requires the formation of a gas simultaneously with the process of polymerization to inflate the material. The gas can be e.g. carbon dioxide either generated by reacting isocyanate with water (a process catalyzed by tertiary amines) or added as gas. The required gas for inflation may also form by volatile compounds evaporating due to increased temperatures caused by the exothermic polymerization reaction. It has been observed that such volatile compounds have been used for foam formation, e.g. 1,1,1,3,3-pentafluoropropane and 1,1,1,2-tetrafluoroethane or carbon hydrides, such as n-pentane (Feske, 2004).

Other additives are typically added as well, e.g. surface-active substances designed to create foam with the preferred properties. The examples surface-active substances used in such processes include polydimethylsiloxane-polyoxyalkylene block copolymers, silicone oils and nonylphenolethoxylates, which are, however, not typically volatile compounds.

Traditionally, flame retardants are added to many types of PUR foam, and in an analysis of bicycle helmets from 2018, the chlorine-/phosphorous-based flame retardants TCPP, TDCP, TIBP, TCEP and TPHP were identified in small amounts (Danish Environmental Protection Agency, 2018). Previous studies have also indicated the use of brominated flame retardants (Danish Environmental Protection Agency, 2014A).

Cross-linking agents and substances that prolong polymer chains are often hydroxyl- and amine-terminated compounds with a low molecular weight, and, hence, they have a significant role determining the properties of PUR. The most important are ethylene glycol, 1,4-butanediol (1,4-BDO or BDO), 1,6-hexandiol, cyclohexanedimethanol and hydroquinone bis(2-hydroxyethyl) ether (HQEE). Diethanolamine and triethanolamine are used in foam to build firmness and add catalytic activity.

Several distributors and suppliers express their concerns regarding the possible contents of phthalates in squishy toys. Phthalates are also listed by suppliers as possible plasticizing agents for PUR (Eastman, 2014). Phthalates, such as bis(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DINP), dibutyl phthalate (DBP) and bis(2-ethylhexyl) isophthalate (iso-DEHP), have been previously found in PUR mattresses for children (Brandon et al., 2015). The presence of phthalates has also been reported earlier in surveys of carpets by the Danish Environmental Protection Agency, where they could originate from the foam material on the backside, which could e.g. be PUR (Danish Environmental Protection Agency, 2016B). Health-related effects of some phthalates include CMR and hormone-disrupting effects.

Other volatile organic compounds (VOCs) that may be present in PUR foam include e.g. aldehydes, ketones, terpenes and solvents such as xylene, styrene, toluene, dimethylformamide, dichloromethane and benzene. Previously, these compounds/compound groups have been identified in emissions from carpets with foam backsides (Danish Environmental Protection Agency, 2016B). According to the survey of carpets, some these compounds (e.g. aldehydes) may cause obnoxious smells to the annoyance of consumers with varying odor threshold, i.e.

the substance concentration in air, where it is possible to register the smell. Some of the VOCs may also result in health-related effects (e.g. styrene, which is a CMR substance).

On many of the identified products, the content of fragrances (e.g. 'scented') and flavor (e.g. 'contains non-toxic additives designed to taste bad') has been indicated, but no specific substances in products have been stated. In a survey of allergens in products for children carried out by the Danish Environmental Protection Agency, several cosmetic products and toys were analyzed. The analyses showed contents of, among others, terpineol, vanillin, benzaldehyde and camphor as well as limonene and linalool (Danish Environmental Protection Agency, 2016C). Due to the characteristic odor of squishy products, it is expected that some types do contain fragrances that may cause obnoxious smells and odors to the annoyance of consumers. Further, for many fragrances there is a risk that they are allergens (SCCS, 2012).

2.6 Purchase of samples

As a preparation to this study, 43 different squishies (2-3 of each product) were purchased. Two of these were, however, deselected by initiation of the project; one sample deselected due to the purchased product examples not being identical (the squishy itself was inside a plastic cup, which could contain different types of squishies), and the other sample deselected since the purchased product examples had considerably different colors.

The price range for products was between 7 - 150 DKK with an average price of approx. 60 DKK. The purchase of products has been affected by the great demand for squishies, and a few of the identified products on the market were sold-out during the purchase period, while new products have emerged. Thus, the purchase has been limited to products available during the purchase period. The main aim has been to ensure that the number of products purchased from Danish and foreign webshops selling low-price products from Chinese manufacturers constitute a majority of all purchased product samples. The actual proportion was slightly different, but still with the majority of products was purchased in webshops, see TABLE 1.

Criteria for selection of samples for purchase:

- *representativity referring to point of purchase*: products have been purchased to match the proportions described in TABLE 1.
- *representativity referring to price*: products represent different price levels.
- *material*: the purchased products have been made of slow-rising foam, also called memory foam, which is deformed upon pressure and then slowly regains its original form. It is assumed that the material is PUR foam. Thus, products of e.g. PVC or silicone are not part of the survey.
- *packaging*: as far as possible, it is prioritized that products are inside a packaging upon purchase, since it ensures the highest possible presence of volatile or semi-volatile chemical substances and can, thus, be expected to constitute the worst-case scenario regarding children's exposure to the substances.
- *scent/odor*: it is prioritized that products have a scent/odor, since fragrances and other chemical substances that emit odor are the main priority in this study.
- *appealing to children and popularity*: squishies that appeal to children, i.e. products with e.g. cute appearance, faces with large eyes and/or forms that are appealing for playing or hugging, are selected. Further, popular products, i.e. indicated as for example "very popular/best-selling" on company's website, are sought to be prioritized.
- *"equal appearance"*: In foreign webshops distributing low-price products from Chinese manufacturers, squishies that in form and appearance seem comparable to squishies available in Danish webshops or physical shops are sought to be prioritized to investigate if the products may also be comparable regarding content and emission of chemical substances.

TABLE 1. Overview of distribution of purchased products in percentage for different distributor types.

Type of retailer	Aim for sample representation by purchase	Actual sample representation
Physical Danish shops (e.g. supermarkets, toy shops, other toy distributors, e.g. book shops)	10-20 %	30 %
Danish webshops	45-55 %	47 %
Webshops from non-EU countries (Chinese manufacturers)	30-35 %	23 %

* Due to very long delivery times, the sample proportion from non-EU webshops is lower than the targeted proportion (23 % vs. 30-35 %).

The packaging and labelling of the purchased products vary considerably. A number of the products are delivered in unlabeled plastic bags (especially those received directly from China) with e.g. no indication of a possible CE label of the product and if the product is suitable for children under the age of 3.

2.7 Summary of the survey

The selection of squishies available on the Danish market is vast, and for the largest part of the identified products, China is indicated as the producing country.

According to importers and distributors, the required documentation on compliance to legislation is delivered through the supplier chain. Some of the contacted players actively set requirements regarding which fragrances may be used in their products, and some even require that the products contain no fragrances, which the manufacturers have complied with. On some of the purchased products, it has been indicated that they are scented, and, thus, some of the squishies on the Danish market are expected to contain fragrances. Products with comparable design have been found on the market from different retailers and with a widely varying odor/smell.

Besides the fragrances, the focus among many suppliers has been on the contents of phthalates, while none of the contacted players have mentioned concerns regarding the potential contents of residue monomers or additives such as solvents or foaming agents used in polymer production. The presence of foaming agents and catalysts used in the polymerization process are, however, indicated in the technical documentation from one manufacturer.

The smell from products has caused several inquiries to importers/distributors from concerned consumers; however, no specific examples of health-related discomfort thereof. The smell is allegedly characteristic for the used polymer material according to a Chinese manufacturer. Based on the collected information and the material properties, the material is highly likely to be PUR.

The products are considered collectibles, and it is normal that children own many different squishies (5-10). Children that are eager collectors are expected to have an even higher number (between 30 and up to 200 pcs. for the largest collectors, as indicated by distributors/importers). The most typical use of squishies is described to be active play, where products are squeezed, observed and compared to other squishies while returning to their original shape. Therefore, one of the contacted distributors considers it possible that playing with squishies involves long-term skin contact compared to other types of toys. The contacted

importers and distributors have determined the target group for products in general to be from approx. 6 years and above, which has been confirmed by the actual sales figures of one Danish distributor; and the nature of the marketing material also indicates this target group. Some types of squishies are expected to be used for roleplays, which also can last for longer periods of time. A use time from 15 minutes at a time and a total use time of up to several hours a day is, thus, considered to be realistic for the target group.

3. Exposure scenarios

Based on the information collected in this survey on the expected use of squishy toys, the following chapter offers a description of the exposure scenarios for children's exposure to substances emitted from toys during use. A range of different scenarios can be presented. The scenarios described here are considered the most relevant in relation to the accessible knowledge before execution of chemical analyses of content and emission of substances.

3.1 Presentation of exposure scenarios

The description of an exposure scenario for squishy toys is based on a real-life worst-case scenario for a child's use of a squishy (see chapter 2.3) and on the principles described in ECHA (2016) regarding consumer exposure. When assessing the products' safety, it is important to ensure that also unforeseen use is included in considerations and assessments. Hence, it is considered relevant as a worst-case scenario to include the possible small children's usage of the toys. It is not deemed impossible that children under the age of 3 years, especially younger siblings, would use the toys due to their appealing appearance and/or due to the sensory experiences they offer.

Besides this scenario, a typical scenario is also described for the target group of squishies that primarily includes children at the age of 6 and above.

3.1.1 Assessment of worst-case scenario

The scenario with the highest possible direct exposure from squishy toys is assessed to be a young 3-year-old child, who sleeps while hugging the squishy toy, and where the toy is very close to the child's nose and mouth. This scenario has the highest possible potential partly for skin contact (migration to child's palms) and partly for inhalation of vapors emitted from the toy.

A scenario for a 3-year-old is assessed to lead to considerably higher exposure to migrating and emitting substances compared to a scenario for e.g. exposure of an older child (see scenario for typical use below).

A realistic worst-case scenario for a young child's exposure may be described using the following relevant parameters:

Body weight, child 3 years old: 14 kg

ECHA (2016) refers to RIVM (2014) that indicate a body weight of 12.4 kg for a 2-3-year-old and 15.7 kg for 3-6-year-olds based on the 25 percentile in the two age groups, which is why an average for these two age groups of 14 kg is applied for a 3-year-old child.

Inhalation volume during rest/sleep: 0.18 m³/hour

ECHA (2016) refers to RIVM (2014) that indicate an inhalation volume during resting at 0.12 m³ air/hour for 1-3-year-old children and 0.24 m³ air/hour for 4-6-year-olds, which is why an average for these two age groups of 0.18 m³ air/hour is applied for a 3-year-old child.

Duration of exposure: 10 hours/day

This is considered a pragmatic worst-case estimate for a child lying and hugging the toy close to her body during sleep.

Surface area of two palms in contact with the squishy: 150 cm²

RIVM (2016) indicates 270 cm² and 330 cm² as palm surface area for 2-3-year-olds and 3-6-year-olds, respectively. The average of 300 cm² is thus applied to 3-year-olds. 50 % of this value is applied for palms.

From this, the child's exposure may be calculated as follows:

$$\text{Inhalation (systemic intake, mg/kg lgv/d)} = \text{measured chamber concentration mg/m}^3 \times 0.18 \text{ m}^3/\text{hour} \times 10 \text{ hours/d}/14 \text{ kg}$$

$$\text{Inhalation (local exposure eyes/respiratory tract, mg/m}^3\text{)} = \text{measured chamber concentration mg/m}^3$$

$$\text{Skin exposure + sucking (mg/kg lgv/d)} = \text{migration/cm}^2 \text{ during 10 hours} \times 150 \text{ cm}^2/14 \text{ kg}$$

In this worst-case context, it is not considered relevant to specifically separate skin exposure and sucking, as it is assumed that the entire migrating amount from the squishy toy is transferred to hands, from where the substance is absorbed through skin or may be absorbed by sucking of hands.

The worst-case scenario will be used for risk assessment of the analysis results from the selected squishy toys, i.e. emission and migration of chemical substances from the toys. With the results of risk assessments, the uncertainties and limitations for applying this worst-case scenario will be assessed together with other uncertainties that are part of the risk assessment, including the uncertainties regarding hazard assessment of each substance.

3.1.2 Scenario for typical use

The information acquired from importers and distributors in chapter 2.2 states that the typical target group is children between the age of 6-12. Generally, the product is considered not to be targeted young children (under 3 years).

Typical playing with the products is expected to involve squeezing and observing the reestablishment of its original form. The products are often squeezed in hands and may be used for roleplays, where many squishies are often involved. Due to the squeezing function, longer-term skin contact is considered to be realistic for this type of product compared to other types of toys.

Some children consider the products collectibles, and the squishies are available both in series, where more designs can be collected, and the so-called blind bags, where one has a chance to acquire rare designs. Thus, it is not considered unusual that children own up to 30-40 squishies.

As a worst-case scenario for the typical use (for target group down to 6 years) based on emission measurements, that/those squishies with the highest emission will be selected, and an equilibrium concentration in the room will be calculated with an assumption that there are 40 squishies in the room. A residence time of 15 hours in the room is applied. For skin contact, a daily play of 2 hours with squishy toys is assumed.

A realistic worst-case scenario for a typical use of squishies may be described with the following relevant parameters:

Body weight, child – 6 years: 20 kg

ECHA (2016) refers to RIVM (2014) that indicate a body weight of 15.7 kg for 3-6-year-olds and 24.3 kg for 6-11-year-olds based on the 25 percentile in the two age groups, which is why an average for these two age groups of 20 kg is applied for a 6-year-old child.

Child's Room, volume: 17.4 m³

The Danish Environmental Protection Agency (2016) has previously analyzed exposure in children's rooms based on this size of a child's room (corresponding to a floor space of 7 m²).

Air change: 0.5 times per hour (corresponding to 8.7 m³ per hour)

According to guidelines in Nordtest Metode NT Build 482.

Number of squishies emitting in the room: 40 pcs.

Child's inhalation volume per day: 12.5 m³

ECHA (2016) refers to RIVM (2014) that has indicated an inhalation volume during 24 hours of 11 m³ air for 4-6-year-olds and 14 m³ air for 7-9-year-olds, which is why an average for these two age groups of 12.5 m³ is applied for a 6-year-old child.

Duration of exposure: 15 hours/day by inhaling
 2 hours by skin contact

Surface area of two palms in contact with the squishy: 230 cm²

(460 cm² is indicated as the surface area for two hands for 6-11-year-old children (RIVM 2016). 50 % of this value is applied for palms).

From this, a child's exposure may be calculated as follows:

Inhalation (systemic intake, mg/kg lgv/d) =
calculated room concentration mg/m³ x 12,5 m³/day/20 kg

Inhalation (local exposure eyes/respiratory tract, mg/m³) =
calculated room concentration mg/m³

Skin exposure + sucking (mg/kg lgv/d) =
migration/cm² during 2 hours x 214 cm²/20 kg

4. Analyses of squishy toys

The aim of the analysis of squishies is to determine the contents and emission of relevant chemical substances for the subsequent risk assessment.

Different squishies were selected for the chemical analyses. Many squishies have been declared having added fragrance, which is why the products are analyzed with a focus on the fragrances considered to be allergenic by the EU Scientific Committee for Consumer Safety (SCCS, 2012). If children are exposed to allergenic substances, there is a risk that they will develop allergy, which is why there has been a special focus on this type of substances. The specific fragrances, which are detected by the methods selected in this analysis, have been chosen since they are substances typically used industrially, are easily commercially available and there are reference standards available for the analysis. In the introductory survey of squishy toys, other substances that may cause a perfumed chemical odor have been identified. For this reason, other emitting and migrating substances from squishies are of interest, e.g. solvents, foaming agents, flame retardants, preservatives and residue monomers, which may contribute to the odor/smell of squishies and the risk of exposure.

Based on introductory chemical analyses and screenings, it is decided, which quantitative analyses are to be performed in order to assess the risk of playing with the purchased products. A quantitative determination of emitting and migrating fragrances and other substances identified in the introductory screenings and considered to be problematic for a child's health is carried out. The analyses are undertaken, so that migration and emission times are realistic in relation to the exposure scenarios, so that they can be used for risk assessments.

4.1 Analysis program

In this study, an analysis program has been determined for selected squishies based on the established exposure scenarios and the knowledge acquired in the survey regarding material type and expected substance content in squishy toys.

The analysis program was created in cooperation with Danish Environmental Protection Agency and consists of two steps: 1) a screening including analysis of substance content and emission in climate chambers, and 2) a supplementing material identification. Based on the results of the introductory screening, quantitative analyses on substances that emit in the climate chamber and are released by migration were performed. The analysis program scheme is illustrated in FIGURE 1.

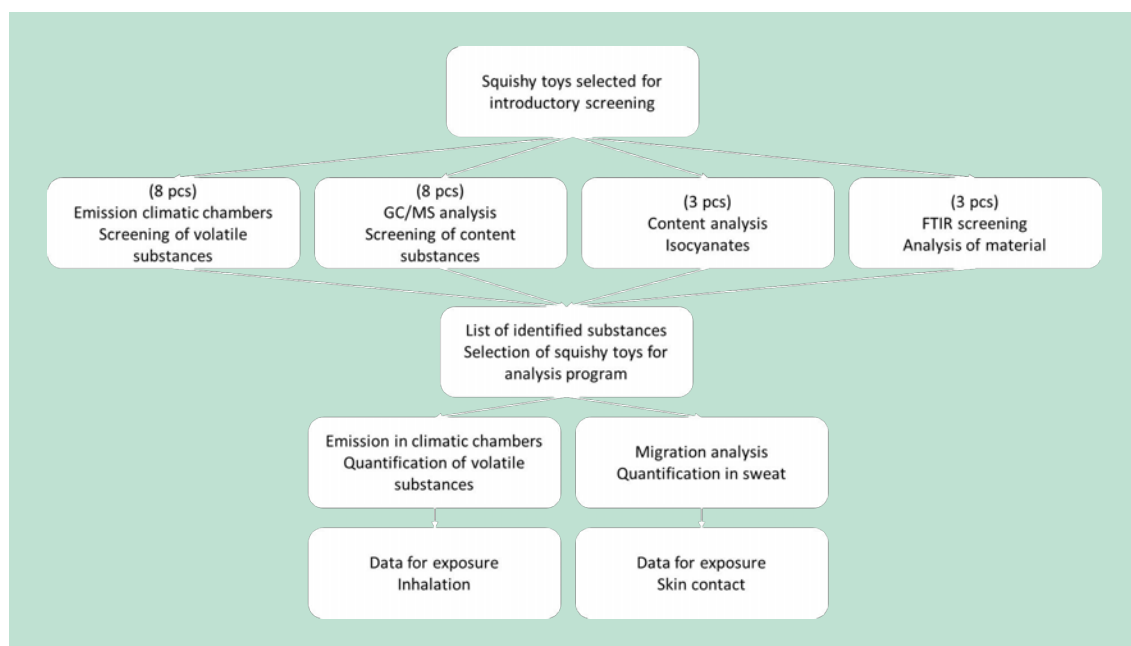


FIGURE 1. Graphic representation of proposals to analysis program in two stages.

4.2 Selection of samples and analyses for introductory screening

By initiation of the analysis, eight products were selected using the same criteria as for purchase of squishies for the introductory analysis of relevant substances present in the products (screening with GC/MS) and emission of volatile chemical substances (climatic chamber test). These analysis methods were chosen due to the project's focus on volatile organic compounds, and it has been chosen that the same eight products will be analyzed for content and emission in order to be able to compare results and assess the emission from products, as far as possible.

The screening analyses by GC/MS cover an extensive number of volatile and semi-volatile organic substances. The method is suitable for aromatic and generally non-polar organic substances with boiling points between 60°C and 500°C, depending on the specific substance. The list of banned fragrances in toys (Appendix 1, TABLE 15) shows that substances belong to many different chemical substance categories, including monoterpenes, aldehydes, alcohols, acids and esters, which all are volatile organic compounds (VOC).

Many fragrances are categorized as allergenic. 26 fragrances, which, among others, are declarable in cosmetic products due to allergenic effects (SCCS, 2012) are likewise included in the 55 regulated substances in Danish Statutory Order on Toys and are either banned or declarable in toys (BEK no. 309 of 3/4/2017). 15 of these substances are banned in toys, and the remaining 11 substances must be declared if they are present in concentrations above 100 mg/kg (see Appendix 1, TABLE 15 with *). SCCS have identified in total 82 fragrance substances as known contact allergens based on clinical experience (SCCS, 2012).

Low-molecular weight aldehydes with 1 to 4 carbon (C1-C4) are very volatile (VVOC) and can, thus, not be analyzed with the same method as VOCs. It has been decided to include analysis of C1-C4-aldehydes in the introductory analysis, since they are both relevant to smell and have a classification of relevance to exposure to children.

Eight squishies have been selected for introductory screening of both substances present in the products and substances that emit in order to create an overview of, which substances could be relevant for further studies. These eight samples have been selected to best possible

represent the sample representation, i.e. point of sales, as decided when purchasing the products, chapter 2.6. Four squishies purchased in physical Danish shops, 2 squishies purchased in Danish webshops and 2 squishies purchased in webshops from non-EU countries (Chinese manufacturers) were selected. In the sample selection, more squishies are from physical Danish shops than the original aim prescribed (50 % against 10-20 %), which is partly due to the very long delivery times from webshops in non-EU countries and partly due to other priority parameters, including scent and price of samples. The eight products and the analyses performed are listed in TABLE 2. Three of the eight products were selected for identification of material type (IR characterization). These three selected samples were further analyzed for isocyanates, which may occur as residue monomers in PUR material and are highly irritant to the respiratory tract.

One product is marked with 'Contains non-toxic additive designed to taste bad', but has no indication of the specific substance (024). Four products are marked with a warning that they may not be eaten or are not food products (048, 051, 054, 056), and two products indicate that they are 'scented' (051, 054). Additionally, three products have been marked to have unique properties such as 'slow rising' (051, 054) and 'improved formula' (024).

TABLE 2. Selected products for screening analyses.

Product No.	Analysis completed	Purchase price in DKK	Weight (grams)	Distributor type
024	Climatic chamber test GC/MS-screening	150.00	94	Physical Danish shop
038	Climatic chamber test GC/MS-screening IR Isocyanate analysis	69.00	63	Danish webshop
043	Climatic chamber test GC/MS-screening	49.00	116	Non-EU webshop
048	Climatic chamber test GC/MS-screening	79.00	102	Danish webshop
051	Climatic chamber test GC/MS-screening	60.00	110	Physical Danish shop
054	Climatic chamber test GC/MS-screening	70.00	52	Physical Danish shop
056	Climatic chamber test GC/MS-screening IR Isocyanate analysis	49.95	45	Physical Danish shop
061	Climatic chamber test GC/MS-screening IR Isocyanate analysis	13.30	76	Non-EU webshop

4.3 Introductory analyses

4.3.1 Material identification with FTIR

Objective

The objective of the analysis is to confirm that the applied material is PUR.

Sample preparation

A piece of foam without painting has been cut out of each of the three squishies (038, 056 and 061). Measurements were performed directly on foam pieces without additional sample preparation.

Analysis method

FTIR spectroscopy was carried out on a 4500a FTIR instrument from Agilent Technologies. Measurements were performed in three locations on the foam on each of the three samples. The spectra were recorded using the ATR (attenuated total reflectance) method with 32 repetitions and a spectral resolution of 8 cm^{-1} . Search has been conducted in reference spectra from databases from Agilent, Sigma Aldrich and Smiths Detection.

Results

The spectra from the different locations on each sample are comparable, which makes it probable that the samples are relatively homogeneous. Furthermore, the spectra from the three different samples are almost identical and have a good consistency with a reference spectrum of polyurethane foam, see Figure 3 to Figure 5 in Appendix 3.

4.3.2 Determination of isocyanates

Objective

Isocyanates are used for production of PUR foam. Thus, there is risk that this type of organic compounds may be found as residue monomers in squishy toys made of PUR foam. Three selected squishies are by FTIR confirmed to contain PUR foam. The three squishies have been analyzed for five specific isocyanates that, according to experience, may appear in PUR foam materials.

Sample preparation

Partial samples of the material were weighed out and subsequently extracted with dichloromethane with added internal standard. Partial samples were chosen to contain both the surface of squishy figure and the internal material. Next, extracted isocyanates were derivatized with 9-(N-methylamino-methyl)anthracene (MAMA), which activates the substances for fluorescence detection (FLD). Spiking has been carried out on sample material to examine the presence of positive and negative interferences in the extraction liquid as a result of sample preparation, so-called matrix effects.

Analysis method

The applied analysis method is based on the standard ISO 13130-8 (2004), which has been developed for the analysis of foam materials, e.g. such as PUR foam. The analysis of extracts of squishy toys is performed with HPLC-FLD and quantification of calibration curves by derivatization of the examined isocyanates.

The detection limit for each isocyanate is 0.02 mg/kg. The relative standard deviation for determination in triplicate is normally < 5 % for homogeneous materials and < 20 % for products consisting of several materials and/or decorative surface coating. For analysis of squishy toys, the relative standard deviation is observed from 7 to 51 %, which assign unknown matrix effects from the sample material.

Results

The contents of five different isocyanates in three selected squishies are given in TABLE 3. The results given are the average of three measurements. For two of the isocyanates, the content has been indicated as a sum, as the chromatographic separation of the two substances was unsatisfactory.

TABLE 3. Results from the analysis of selected isocyanates in three squishies. LOD = limit of detection.

Unit: mg/kg		Product no.		
Isocyanate	CAS no.	038	056	061
Sum of 2,6-TDI and HDI	91-08-7 and 822-06-0	0.18	<LOD	<LOD
2,4-TDI	584-84-9	0.59	<LOD	<LOD
4,4-MDI	101-68-8	0.06	0.13	0.08
4,4-HMDI	5124-30-1	0.65	0.24	0.56
SUM of isocyanates	-	1.5	0.4	0.6

During the analysis, it was noticed that the extract was cloudy. After centrifugation, the extract a separate, liquid phase was isolated, but not analyzed further. According to experience, extracts from pure PUR foam materials are clear. The isolated phase abated was located below the dichloromethane phase and was fluid. Upon visual observation, it was determined that this phase constituted less than 5% of the total volume.

Retrieval tests for individual isocyanates have been carried out for all analyzed squishies by adding a known concentration of the individual isocyanates during the extraction. The results showed a great variation in the analyzed contents of isocyanates. This variation refers to the high complexity of the analyzed material. Phase separation may lead to an undetermined insecurity in the indicated results. The insecurity cannot be determined within this project, and the phase separation may result in the underestimation of the content of isocyanate, since the partition relation between phases is not fixed.

4.3.3 Screening of content by extraction and GC/MS

Objective

GC/MS screening is a suitable tool for the introductory assessment of a range of different material and product types, including squishies. Screening has been performed with an aim to identify substances that are present at a level relevant for exposure via skin contact or oral intake of the products. GC/MS screening gives an overview of the contents of many organic substances that may have a physiological effect. This could be e.g. fragrances, organic solvents, flame retardants and monomers.

Sample preparation

Partial samples of approx. 1.0 grams (precisely weighed) were divided and extracted during mechanical shaking with 40 ml extraction liquid consisting of a 1:4 mixture of methanol and dichloromethane with added internal standards. The samples were degraded by the extraction except for a small amount of “dust”.

Analysis method

The extract was analyzed using gas chromatography combined with mass-selective detection (GC/MS) with a DB-XLB 60m GC column. The content of volatile and semi-volatile organic substances in products may be identified using this method, as the actual mass spectra are compared with mass spectra in the NIST library, i.e. a database with mass spectra for more than 500,000 chemical compounds. For the examination of consistency between a given spectrum and the NIST library, a hit rate is indicated between the unknown substances and the library reference. The hit rate is a mathematical calculation on how close the consistency is between a reference spectrum of a substance in the NIST library and the spectrum of the respective substance in the analysis. The hit rate is calculated based on the relative intensity between the ten most intensive masses measured by mass spectrometry and the ten most intensive masses for all substances in the library. The identification from the NIST library is considered indicative, and, for this reason, substance-specific analysis with reference substances for definitive positive identification of content substances should be performed. Identification based on the NIST library may form the basis for an assessment of whether it is relevant to proceed with a migration test for specific volatile and/or semi-volatile organic substances.

The content of the identified content substances has been calculated as naphthalene equivalents, and the quantification of each component must, thus, be considered as being semi-quantitative, since the component response factor during the analysis may vary from the response factor of naphthalene.

The detection limit has been estimated to 5 - 100 mg/kg, depending on the substance. Some substances will not respond to the chosen analysis equipment. The uncertainty of the analysis has been estimated to a general 30% RSD and is very dependent on the substance.

Results

Gas chromatography analysis has been carried out after dissolving squishy toys in methanol/dichloromethane. Squishies were dissolved, but gave a solid residue product of particles in the liquid. Since PUR is not entirely dissolved in the methanol/dichloromethane mixture, this is an indication that the polymer material used for squishies does not only contain PUR elements, but may also contain side- or backbones of other polymer types.

By extraction, the polymer dissolves and is degraded. This may cause detection of substances, e.g. the amine-containing compounds possibly used as additives to achieve a certain mechanical property, but which are not accessible for release for used migration substances, as these do not degrade the material as do the solvent used for extraction.

The results for contents analysis are presented in Appendix 4.1 – 4.3. Based on a hit rate and assessment, all identifications are categorized under category 1 to 3. Category 1 are substances that are identified with certainty from the NIST library (high hit rate) and confirmed by specialist evaluation. Category 2 are substances evaluated to be identified with certainty by a specialist despite minor unconformity with the NIST library compared to category 1 substances. Category 3 are substances with an uncertain identification, i.e. the listed substances are most probably not identified correctly, but are substances with similar molecular structure and chemical functionality as the listed substances.

Approx. 100 peaks from each of the eight squishies has been evaluated, and it was possible with great certainty to identify (category 1) 35 substances. 16 substances could be evaluated as probably being identified with certainty (category 2). The uncertainty of category 2 could be due to e.g. co-elution with other content substances or a need for manual identification of the degradation. Thus, it was not possible to achieve direct conformity with the MS library. 22 substances could not be identified, but for each the MS spectrum was comparable with the spectrum of another substance. In other words, these are unidentified substances that have a high chemical resemblance to the listed substance (category 3).

The 35 category 1 substances are general substances that most probably have origin in the polymer system, where there is a large amount of amine compounds that may have been used as copolymer. This includes dimethylaminoethanol, dimethylformamide, octadecenamide, triethyldiamine and pentamethyldiethyltriamine. Also, triethylphosphate is identified, which is presumably a catalyst residue from the polymerization process. In addition to amine compounds, it can be mentioned that there are phthalates (DEP and DEHP), cyclohexanone and octadecyl di-tert-butyl-4-hydroxyhydrocinnamate (Irganox 1076) in the products. Further, 4,4'-methylenebis benzenamine is identified, which is most probably a monomer residue.

The 16 substances in category 2, which have been determined with no direct consistency with the MS library, differ from the above-mentioned by not being just as widely represented in these eight products. This may be due to them disappearing in the co-elution in some of the samples. There are, among others, fragrances such as versalide and galaxolide, where none of these are among the 55 banned or 11 declarable fragrances regulated in the Directive on toy safety (EC 2009/48/EF), and the organophosphor-based flame retardant tris(2-ethylhexyl) phosphate (TEHP) could be found in one of the products. Furthermore, low concentrations (<10-16 mg/kg) of the reactive radical initiator azobisisobutyronitrile (CAS no. 78-67-1) were found in three of the products, which may indicate an incomplete polymerization during production.

The 22 substances not identified (category 3), but whose chemical functionality could be recognized, are of similar chemical character as the substances mentioned above. An interesting substance that could be mentioned is a substance that resembles 4,4'-methylenebis benzenamine, but which has differences in the MS spectrum that indicate that it must be another substance. Presumably, this is also a monomer residue. Other noteworthy substances are an organophosphorus compound that is presumably a flame retardant found in one of the products, a substance that may be a DBP-phthalate, and a substance that could be an ethyl form of DMAE.

No traces of chlorinated substances have been found, and no decay of the characteristic isotope distribution of chlorine has been observed. Thus, it is assessed that there is probably no chlorinated paraffins in the products (SCCP/MCCP).

Traces of glycol and silicone compounds were found in mass spectra from the contents screenings. These can be e.g. parts of the polymer degraded by dissolution, or they could be free solvents added to achieve specific physical properties for the material.

4.3.4 Screening for emission of volatile substances in climatic chamber

Objective

The objective of the screening is to identify which volatile substances are emitted from entire squishies squeezed during playing. The method finds those substances that are released in indoor climate and, thus, identifies the substances that children may be exposed to.

Method

Emission of volatile substances was carried out in 113 L climatic chambers according to modified version of well-established testing methods (ISO 16000-9 and EN 16516) for emission from materials at 23°C, 50 % RH and an air change of ½ times per hour (0.5 h^{-1}). Sampling and analysis of VOC was performed according to ISO 16000-6, while sampling and analysis of aldehydes was performed in accordance with ISO 16000-3.

An air sample was drawn on the relevant sampling medium with calibrated pumps from the empty chamber, and, moreover, air samples were drawn from the chamber with sample material. The sampling volume has been indicated for each substance group and method. Air samples were collected on calibrated GilAir Plus pumps.

One hour after the squishy had been squeezed 10 times inside the climatic chamber, 0.4 liters and 4 liters of air were collected on Tenax tubes with a flow of 80 mL/min. for VOC analysis as well as 30 liters of air on 2,4-dinitrophenylhydrazine-filter (DNPH) with a flow of 650 mL/min. for carbonyl analysis.

Modified ISO 16000-6 Screening analysis for emitted substances from climatic chamber test with TDS-GC/MS: The collected VOCs on Tenax were thermally desorbed (TDS) and analyzed with gas chromatography combined with mass-selective detection (GC/MS) on a Gerstel-Agilent system with a 60 m DB-5 GC column.

The identity of VOCs was determined by searching Wiley W9N by conformity of the MS spectra of more than 80%. The identified VOCs are quantified and reported as toluene equivalents according to EN 16516 for concentrations above $5 \mu\text{g}/\text{m}^3$. The actual concentrations of the identified substances and the corresponding toluene equivalents will be close to each other if the response of the identified substances to MS signal resembles the response of toluene. This means that e.g. toluene equivalents for aromatic hydrocarbons, BTEX (benzene, toluene, xylene), which resemble toluene in their response to MS signal, would be close to the actual concentration. Limit of detection (LOD) is dependent on the particular substance, but is typically 1 - 10 ng on Tenax medium, which corresponds to $0.25 - 2.5 \mu\text{g}/\text{m}^3$ in air by collecting 4 liters of air. By collecting 0.4 liters of air, the detection limit would be $2.5 - 25 \mu\text{g}/\text{m}^3$ air. Air samples of low volume are also collected to quantify substances in high concentrations to avoid exceeding the upper quantification limit. In general, polar substances have higher detection limits, and in special cases, as for e.g. dimethyl aminoethanol, it is even above $24 \mu\text{g}/\text{m}^3$. The analysis uncertainty for VOC depends on the particular substance, but is within 30% (RSD, relative standard deviation).

Modified ISO 16000-3 Detection of volatile carbonyls (aldehydes and ketones) collected on 2,4-dinitrophenylhydrazine-filter (DNPH): the collected carbonyls are derivatized on the filter and eluted with acetonitrile. Following, they are analyzed with HPLC with UV detection, identified by retention time and UV spectrum, and quantified using calibrated reference substances. The detection limits (LOD) are $0.03 \mu\text{g}$ for formaldehyde, acetaldehyde, butyraldehyde and acrolein and $0.05 \mu\text{g}$ for propanal and crotonaldehyde on DNPH medium. Analysis uncertainty for carbonyls is 7.5 % (RSD, relative standard deviation).

Results

The results for emission analysis of volatile organic compounds, carbonyls and VOCs are presented in Appendix 5.1 and Appendix 5.2.

Several fragrances have been found in emissions: D-limonene, benzyl alcohol, L-linalool, 3-carene, alpha-pinene and beta-pinene.

N,N-Dimethylformamide (DMF), N,N-dimethylaminoethanol, triethylenediamine, bis(2-(dimethylamino)ethyl) ether, 1,2-ethandiamine and N-[2-(dimethylamino)ethyl]-N,N',N'-trimethyl-cyclohexanone have been found in high concentrations.

The result of the detection of volatile aldehydes is given in TABLE 4. In addition to the established content of aldehyde, 2-butanone has been found in two squishies (043 and 054), which is detected by aldehyde analysis and TDS-GC/MS analysis (see Appendix 5.2) in very high concentrations. 2-Butanone has a carbonyl group that attaches itself to the collection medium (DNPH), and can thus hamper the collection of other aldehydes on DNPH (samples marked with * in TABLE 4).

TABLE 4. Results of aldehyde analysis in air samples.

Unit: µg/m ³		Product number							
Substance	CAS no.	024	038	043*	048	051	054*	056	061
Formaldehyde	50-00-0	11	23	8,2	3,1	19	4,6	9,7	6,6
Acetaldehyde (ethanal)	75-07-0	6,6	17	27	8,0	30	8,1	18	10
Propanal	123-38-6	1,5	6,4	19	1,9	5,3	5,2	3,3	2,0
Butanal	123-72-8	1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0
Acrolein	107-02-8	< 1,0	< 1,0	< 1,0	4,0	< 1,0	< 1,0	< 1,0	< 1,0
Crotonaldehyde (butenal)	4170-30-3	< 1,7	< 1,7	< 1,7	< 1,7	< 1,7	< 1,7	< 1,7	< 1,7

*The DNPH capacity of the tube is exhausted, meaning the true content may be higher.

4.4 Summary of results from introductory analysis

The material identification shows that squishies consist mainly of PUR foam (Appendix 3). GC/MS screening analysis shows substances that may indicate the presence of a modified PUR that ensures the characteristic texture of squishies. It is supposedly the PUR type LRPu, which is known from e.g. memory-foam products. The material type may be squeezed together, and it will hold its form for a certain period, where after it will slowly return to its original form. The viscoelastic property of the material may be achieved by having fewer cross-linkages and more non-covalent linkages.

Squishies do not exhibit the characteristics of normal PUR products neither at extraction for isocyanate analysis, nor at content screening. The screening analysis at extraction results in a solution and partly degradation of the polymer, which may give rise to detection of substances that are bound physically and/or chemically in the product under normal use and are thus not possible to find in emission or migration analyses. It cannot be excluded that substances have been added to the material in a free form, and, thus, cannot be chemically bound to the polymer network (covalent binding), in order to achieve the specific material properties. Hence, there can be a risk that a certain degree of substances may occur during use depending on the physicochemical properties of each substance.

The results of GC/MS screening are found in Appendix 4. Approx. 100 tops in each of the eight squishies have been analyzed, which allowed identification (category 1) of 35 substances with great certainty, while 16 substances could be considered being definitely identified (category 2). 22 substances could not be identified, but the respective MS spectra were comparable to the spectrum of another substance, i.e. presumably close chemical resemblance with this listed substance (category 3).

The 35 substances in category 1 are mainly substances originating in the polymer system, e.g. amine compounds (dimethylaminoethanol, dimethylformamide, octadecenamide, triethyldiamine and pentamethyldiethyltriamine), which are used in the production of polymer material. Triethylphosphate was also found, which is supposedly a catalyst residue from the polymerization process. There are also phthalates (DEP and DEHP), cyclohexanone and octadecyl di-tert-butyl-4-hydroxyhydrocinnamate (Irganox 1076) in these products, as well as 4,4'-methylenbis benzenamine, which may be a monomer residue.

Among the 16 substances in category 2, there are, among others, the fragrances versalide and galaxolide, the organophosphorus-based flame retardant tris(2-ethylhexyl) phosphate (TEHP) and traces of the reactive radical initiator azobisisobutyronitrile, which may indicate an incomplete polymerization during production. Additionally, traces of glycol and silicone compounds were found, which elute in volumes indicating that them as residue of the polymer chain.

The 22 substances not identified (category 3), but for which it was possible to make a guess about their chemical functionality, were of similar chemical character as substances described above, among others, amines, organophosphorus-compounds and phthalates.

No traces of chlorinated substances have been found, and no decay with the characteristic isotope distribution of chlorine could be observed. Thus, it can be assumed that there are no chlorinated paraffins in these products (SCCP/MCCP).

Three selected squishies have been analyzed for five specific isocyanates. The results showed that these residue monomers are found in the analyzed squishies. The complexity of the material influences the analyses, but an estimated worst-case total value of 1.5 mg/kg of isocyanate has been found in the squishy with the highest content.

In six of eight squishies, concentrations of formaldehyde, acetaldehyde, propanal, butanal, acrolein and crotonaldehyde, analyzed by emission, are under 10 µg/m³ air in the performed screenings for emission of volatile substances (Appendix 5.1).

4.5 Introductory hazard assessment of screening results

To focus the following analytical chemical analysis, it is essential to prioritize measurements and assessments on substances identified in the screening analysis that are of the greatest concern. Thus, an introductory hazard assessment was performed to identify those substances that have the most problematic effects. These substances are pointed out based on their harmonized classification or, if this is not possible, on the substance classification indicated in the REACH registry of substances.

The following classifications are prioritized:

- CMR 1A, 1B, 2
- STOT RE 1,2
- STOT SE 1,2
- Acute tox 1,2,3
- Skin sens 1
- Resp sens 1

Thereafter, the relevance of substances is assessed for further prioritization based on the measured levels as substances with very high levels should also be prioritized even if they do not meet the prioritization for classification. Finally, fragrances are prioritized, as these were chosen as a focus in this project beforehand.

With these criteria for prioritization, the results for content analyses and emission analyses have been evaluated. Appendix 4 indicates the results from content analysis and Appendix 5 includes the results for emission analyses, and in both tables the last column shows the result of the prioritization of each identified substance.

TABLE 5. Prioritized substances and concentration estimate (in naphthalene equivalents) based on GC/MS screening in Appendix 4.

Substance	CAS no.	Hazard class	H-statement	Prioritization	Conc. in screening for contents by GC/MS (mg/kg)
Dioxane	123-01-1	Eye Irrit. 2, STOT SE 3, Carc. 2	H319 H335 H351	Yes, classification	10-29
N,N,- Dimethylaminoethanol	108-01-0	Acute Tox. 4 Acute Tox. 4 Skin Corr. 1B Acute Tox. 4	H302 H312 H314 H332	Yes, high concentration	62-421***
N,N-Dimethylformamide	68-12-2	Acute Tox. 4, Eye Irrit. 2, Acute Tox. 4, Repr. 1B	H312 H319 H332 H360d	Yes, high concentration and classification	24-274
Triethylenediamine	280-57-9	Acute Tox. 4 Skin Irrit. 2 Eye Dam. 1	H302 H315 H318	Yes, high concentration, also emission	119-1198***
Bis(2-(dimethylamino)ethyl)ether	3033-62-3	Acute Tox. 4, Acute Tox. 3, Skin Corr. 1B, Eye Dam. 1, Acute Tox 4	H302 H311 H314 H318 H332	Yes, high concentration	288-448***
Phenol*	108-95-2	Acute Tox. 3, Acute Tox. 3, Skin Corr. 1B, Acute Tox. 3, Muta. 2, STOT RE 2	H301 H311 H314 H331 H341 H373	Yes, classification	10-50***
Linalool	78-70-6	Skin sens 1B	H317	Yes, classification	40-60***
Pentamethyldiethylenetriamine	3030-47-5	Acute Tox. 4, Acute Tox. 3, Skin Corr. 1B	H302 H311 H314	Yes, high concentration	44-754***
2-Ethylhexylacid	149-57-5	Repr 2	H361d	Yes, classification	17-24
4-tert-butylcyclohexyl acetate	32210-23-4	Skin sens 1B	H317	Yes, classification	19-47
3-(4-Isopropylphenyl)-2-methylpropionaldehyde	103-95-7	Skin Irrit. 2, Skin Sens. 1B	H315 H317	Yes, classification	22
BHT (butylated Hydroxytoluene)	128-37-0	-	-	Yes, endocrine-disrupting	23-91
4,4'-Methylenebis benzenamine	101-77-9	Skin sens 1, Muta 2, Carc 1B, STOT SE 1, STOT RE 2	H317 H341 H350 H370 H373	Yes, classification	64-271
2-(2H-Benzotriazol-2-yl)-5-methylphenol or Drometrizol	4998-48-5 or 2440-22-4	Skin sens 1	H317	Yes, classification	44
Bis(2-ethylhexyl) phthalate (DEHP)**	117-81-7	Repr 1B	H360d	Yes, classification	243
Versalide (fragrance)	88-29-9	-	-	Yes, fragrance	138
Galaxolide (fragrance)	1222-05-5	-	-	Yes, fragrance	20-174

* Phenol has been found in very low concentrations and with interference.

*** Phthalates have extremely low migration rate to aqueous migration liquids*

****also detected by emission, see Appendix 5*

All these substances are interesting with respect to migration due to relatively low volatility. Especially, substances classified for skin allergy (among others, fragrances) are considered relevant, because even a very low exposure to this type of substances can cause a risk for skin sensitization.

N,N-Dimethylformamide (DMF) and N,N-dimethylaminoethanol (DMAE) were identified in relatively high concentrations in both emission and content analyses, and can, therefore, be of interest regarding migration.

Several amines have been detected, among others, triethylenediamine and pentamethyldiethylenetriamine in screening of the extract from squishies. Amines may be problematic in relation to extraction to organic solvent, as amines may become protonated and, thus, less prone to extraction to an organic solvent. The detection of amine signals that it is present and that it can be analyzed using the method, but it is unknown which matrix effect is present and whether the result is undervalued compared to the measured response. Finally, the substances have been prioritized for emission analyses, where the substances will most likely be more critical due to possible irritation effects of the vapors.

DEHP is considered of less relevance, since the substance, due to its high fat solubility, does not migrate to artificial sweat during migration test.

According to an overall assessment based on classification, content level identified in screening and taking the analytic method into consideration, the project group agreed with the Danish Environmental Protection Agency to focus on the following substances in the subsequent migration test:

- N,N-dimethylformamide (DMF)
- N,N-dimethylaminoethanol (DMAE)
- fragrances (the 26 declarable in cosmetics)

From the emission analysis in Appendix 5, the substances in TABLE 6 have, thus, been prioritized for further assessment.

TABLE 6. Prioritized emission substances and concentration estimate (in toluene equivalents) according to screening results in Appendix 5.

Substance	CAS no.	Classification	H-state-ment	Prioritization	Conc. in screening by emission (μm^3)
Methylene chloride	75-09-2	Carc. 2	H351	Yes, classification	<5-10
N,N-Dimethylaminoethanol	108-01-0	Acute Tox. 4 Acute Tox. 4 Skin Corr. 1B Acute Tox. 4	H302 H312 H314 H332	Yes, high concentration	96-840*
Toluene	108-88-3	Skin Irrit. 2 Asp. Tox. 1 STOT SE 3 STOT RE 2 Repr. 2	H315 H304 H336 H373 H361d	Yes, classification	7-95
N,N-dimethylformamide	68-12-2	Acute Tox. 4 Eye Irrit. 2	H312 H319	Yes, classification SVHC due to Repr	210-3700*

		Acute Tox. 4 Repr. 1B	H332 H360D	1B.	
Benzene, ethyl-	100-41-4	Acute Tox. 4 Asp. Tox. 1 STOT RE 2	H332 H304 H373 (hearing organs)	Yes, classification	16-340
m-Xylene	108-38-3	Acute Tox. 4 Skin Irrit. 2 Acute Tox. 4	H312 H315 H332	Yes, high concentration	<5-510
p-Xylene	106-42-3	Acute Tox. 4 Skin Irrit. 2 Acute Tox. 4	H312 H315 H332	Yes, high concentration	<5-120
Styrene	100-42-5	Skin Irrit. 2 Eye Irrit. 2 Acute Tox. 4 STOT RE 1 Repr. 2	H315 H319 H332 H372 (hearing organs) H361d	Yes, classification	20-40
o-Xylene	95-47-6	Acute Tox. 4 Skin Irrit. 2 Acute Tox. 4	H312 H315 H332	Yes, high concentration	14-460
Cyclohexanone	108-94-1	Acute Tox. 4	H332	Yes, high concentration	630-6800
Alpha-pinene, (-)-	80-56-8	Acute Tox. 4 Asp. Tox. 1 Skin Irrit. 2 Skin Sens. 1	H302 H304 H315 H317	Yes, classification and odorous substance	7-14
Cyclotetrasiloxane, octamethyl-	556-67-2	Repr. 2	H361f	Yes, classification	1-15
2-Beta-pinene	127-91-3	Asp. Tox. 1 Skin Irrit. 2 Skin Sens. 1B	H304 H315 H317	Yes, classification and odorous substance	<5
Delta 3-Carene	13466-78-9	Asp. Tox. 1 Skin Irrit. 2 Skin Sens. 1	H304 H315 H317	Yes, classification and odorous substance	9-19
Phenol	108-95-2	Acute Tox. 3 Acute Tox. 3 Skin Corr. 1B Acute Tox. 3 Muta. 2 STOT RE 2	H301 H311 H314 H331 H341 H373	Yes, classification	1-10*
Triethylenediamine	280-57-9	Acute Tox. 4 Skin Irrit. 2 Eye Dam. 1	H302 H315 H318	Yes, high concentration	480-920*

*Also detected in GC/MS screening of contents, see Appendix 4.

It must be noted that formaldehyde has not been included in further analysis and assessment. This is related to the fact that this substance has been found in relatively low concentration compared to the existing indoor climate value for formaldehyde of 100 µg/m³. Furthermore, the analysis of formaldehyde and other very volatile aldehydes require a special analysis method compared to the analysis of the remaining substances.

Fragrances have been found in both emission and extraction analyses in different amounts. Linalool has been found by both extraction and emission, while pinene, carene and limonene have been primarily found in emission analyses. The latter has been found in low concentrations, which is most likely the main reason why they are not found by extraction.

4.6 Choice of samples and methods for quantitative analysis of selected substances

It has not been possible to include all substances with the prioritized classification. Thus, an analysis program including quantitative analyses of emissions in climatic chamber and qualitative detection of migration of selected substances has been set up.

A substance-specific quantification of selected substances for 12 squishies in total has been performed; the eight squishies tested in climatic chamber during screening and additional four squishies. For the emission test in climatic chamber and subsequent quantification of volatile substances, the focus is on fragrances (see list of quantified fragrances in Appendix 6.1), carcinogenic substances, amines and chlorinated substances that were found in the introductory screenings (see quantified substances in Appendix 6.2). In total, 12 squishies that have been emission tested.

Migration to artificial sweat is performed with the same 12 squishies with subsequent substance-specific analysis of DMF, 26 fragrances as well as DMAE in migration liquid.

Eventually, the migration liquid is analyzed with GC/MS, while emission tests are performed with collection on Tenax® and subsequent analysis using TD-GC/MS.

Sample selection

Migration analysis is carried out for the eight squishies already tested in both the content screening as well as for emission of volatile substances (VOC and aldehydes) in climatic chamber, so that data can be compared for the exposure assessment.

Further, four products from the remaining purchased squishies are selected according to the same criteria applied for selection of the initial eight squishies. Two products have been marked with warning that they are not edible/are not food products (33, 44), and one product indicates that it is 'scented' (44). These 12 selected products are listed in TABLE 7. The same squishies are selected for emission and migration, so that the results can be compared, and the two analyses of exposure can provide an overall picture of each individual product.

TABLE 7. Products selected for stage 2 in analysis program. Samples that were not analyzed during screening have been marked with [].

Product no.	Price in DKK	Weight (g)
024	150	94
038 ^{*(A)}	69	63
043 ^{**}	49	116
048 ^{*(B)}	79	102
051	60	110
054	70	52
056	49.95	45
061 ^{**}	13.3	76
[033]	49	20
[034] ^{*(A)}	79	134
[044] ^{*(B)}	79	86
[063] ^{**}	54	142

*from Danish webshop; samples are purchased in different shops, A and B, respectively.

** from US webshop that sells low-priced products primarily from Chinese manufacturers.

4.7 Quantitative detection of emissions in climatic chamber

Emission of selected substances to air from entire squishies after a short-term play (1 hour) and after being in a chamber for 3 days (3 nights/days) is measured to determine how emission can be expected to fall during the first days after unpacking and provide the possibility to assess the risk in an exposure scenario, where emission in a large room over a longer period of time is assumed.

Quantification of the selected volatile substances from the 12 selected samples is carried out according to method description in section 4.3.4. VOC compounds have been identified and quantified against reference substances. Carcinogenic substances in category 1 are quantified and reported in concentrations of $> 1 \mu\text{g}/\text{m}^3$, and the other problematic substances that were selected based on the screening have been quantified and reported as " < 5 " if measured in a concentration below $5 \mu\text{g}/\text{m}^3$.

Substances that have been shown and quantified in chamber tests are indicated in TABLE 8. All fragrances (according to Appendix 1), except the two last Evernia-extracts, are calibrated and quantified, and the proven fragrances are illustrated in TABLE 8. The presence of Evernia Furfuracea Extract and Evernia Prunastri Extract could not be confirmed or denied, since it was not possible to find reference substances.

Emission has been confirmed for three fragrances (mentioned in Appendix 1) in these 12 squishies; limonene and linalool that are declarable if the content in toys exceeds 100 mg/kg as well as benzyl alcohol that is prohibited in toys. Limonene has been found in very high concentrations during emission from squishy no. 034 and 063, where emission from no. 063 decreases from approx. 1900 to 1100 $\mu\text{g}/\text{m}^3$ between 1 hour and 3 nights/days. Since the obligation of declaration is related to content, and no content analysis of limonene in the respective samples has been performed, it is not possible to either confirm or deny whether the limit of 100 mg/kg has been exceeded. For linalool, the highest emission was found from samples 034 and 063. Just as for limonene, no content analysis for linalool has been performed in the two samples, but linalool has been found in content analysis of samples 048 and 054 at a concentration level of 60 and 40 mg/kg, respectively (stated as naphthalene equivalents). A calculation of the concentration using a rather uncertain one-point calibration using reference substance indicates that the actual concentration may be higher than the result when using naphthalene equivalents, which is why the actual emission may possibly exceed 100 mg/kg. Benzyl alcohol has been found in low concentrations ($< 5 \mu\text{g}/\text{m}^3$) in emissions from squishy no. 034, 044, 048 and 061 after 1 hour and 3 days and nights, respectively. The Danish Statutory Order on toys (Legetøjsbekendtgørelsen) opens an opportunity that substances not allowed in toys can be present if it is technically inevitable and below 100 mg/kg, which may be the case as indicated by the low values. In all squishies, additional odor-relevant terpenes have been found: alpha-pinene, beta-pinene and delta-3-carene. Both alpha-pinene and beta-pinene are among 82 known contact allergens identified within cosmetics (SCCS 2012).

Various chlorinated substances were found by emission: dichloromethane (methylene chloride), chloroform, 1,2-dichloroethane and 1,2-dichloropropane. Furthermore, high concentrations of different amines were found: N,N-dimethylaminoethanol, triethylenediamine, bis(2-(dimethylamino)ethyl)ether and 1,1,4,7,7-pentamethyl-diethylenetriamine, which corresponds to the information acquired in the prior survey.

Very high concentrations of several of substances in TABLE 8 have been found, and even despite using Tenax tubes with the lowest air volume for quantification, there is so much substance that the concentration exceeds the tube capacity and the upper linear measuring range. This is marked for each substance and measured concentrations. From 1 hour to 3 days and nights, the concentrations in emissions of most of the substances from squishies

decrease.⁴ Three amines have been found: N,N-dimethylaminoethanol (DMAE), triethylenediamine and bis(2-(dimethylamino)ethyl)ether, where concentrations either increase or have somewhat the same concentration in air from 1 hour to 3 days and nights. 1,1,4,7,7-Pentamethyl-diethylenetriamine is found in emissions in eight of the examined samples. In contrast to other seven samples, which all were analyzed after 1 hour of emission, the substance for squishy no. 033 is not observed after an hour, but only in emissions after 3 days and nights.⁵ Overall, it is thus evaluated that amines are released from the material slower over time compared to other measured VOCs.

The concentrations of each substance vary according to the type of squishy, and it also indicates the fact that the weight and the consequent surface area of the squishy has an impact on VOC concentrations released in climate chamber test. Further, it indicates that the type of substances that emit varies between manufacturers. Measurements have been carried out on four samples with comparable appearances (i.e. similar to each other regarding form, colors and design by pairs); sample 038 and 061 that resemble each other and sample 034 and 063 that resemble each other, respectively. Samples 061 and 063 are from the same distributor (webshop in non-EU country), but from different manufacturers, while samples 034 and 038 are purchased in a Danish webshop (manufacturer not indicated). It has been evaluated that samples have totally different emission profiles despite the same substances being identified in many of the examined samples. There are no clear tendencies as to the content, and no conclusions can be made on the type of substances typically used for manufacturing or which added fragrances are used. Even products produced by the same manufacturer (sample 051 and 054) exhibit distinct differences in the chemical content of the two squishies both regarding the substances and emission concentration.

⁴ When measuring emission from materials, concentrations will initially increase to a given level and then decrease to a constant level, typically over 28 days. I.e., the emissions can in the intervening time from 1 hour to 3 days have been of higher concentrations than the concentrations measured after 1 hour and 3 days, respectively.

⁵ The diffusion rate of a substance varies depending on e.g. chemical structure and size of the given molecule, just as materials from which the substance emits can be physically and chemically different, thereby changing the affinity between emitting substance and the host material. For reasons like this, the concentration of slow-emitting substances can increase to a higher concentration after three days than the concentration after 1 hour, even despite a given air renewal. For e.g. triethylenediamine and 1,1,4,7,7-pentamethyl-diethylenetriamine, the emission results may be due to the amines originating from synthesis of the PUR foam, i.e. they are localized inside the polymer material and are expected to have a higher affinity to the material. For this reason, they are not emitted as fast as e.g. limonene, which is added—possibly after termination of the foam formation process. The "added substances", i.e. fragrances and solvents, originating from application of colors to the surface of a squishy are not trapped inside in the polymer matrix and are, therefore, able to emit faster, resulting in lower concentrations after 3 days. DMF emits faster due to e.g. a lower boiling point and lower affinity to the PUR foam. This means that several parameters (boiling point, affinity, size of the emitting molecule and porosity of the hosting material, etc.) in a complex interaction determine the rate of emission.

TABLE 8. Climatic chamber test emission of 12 squishies with measured concentrations of volatile compounds (VOC) after 1 hour and 3 days and nights (4 selected products).

Unit: µg/m ³		Product number (weight)															
Substance, CAS no. and classification**		024 (94 g)	038 (63 g)	043 (116 g)	048 (102 g)	051 (110 g)	054 (52 g)	056 (45 g)	061 (76 g)	033 (20 g)	034 (134 g)	044 (86 g)	063 (142 g)				
	Time	1h	1h	1h	1h	1h	1h	1h	1h	1 h	3 d	1 h	3 d	1 h	3 d	1 h	3 d
Methylene chloride (CAS no.75-09-2) CARC 2			6	22			<5		<5	<5		560		11			
2-Butanone (CAS no.78-93-3) EYE IRRIT 2		<5	1100	3800*	130	85	220	5	66	12	22	2100*	<5	410			
Chloroform (CAS no.67-66-3) CARC 2, REPR 2, STOT RE1			9	8		1	4		1	7		12		8			
1,2-Dichloroethane (CAS no.107-06-2) CARC 1B			3	2			8				3			3			
Propane, 1,2-dichloro- (CAS no.78-87-5) CARC 1B						6			6			3					
N,N-Dimethylaminoethanol (CAS no.108-01-0) (SKIN CORR 1B)		250	1600		34	2900*		970	240	91	1800	1000		6800*	3200*		
Toluene (CAS no.108-88-3) REPR 2, STOT RE2		9	54	41	3	120	44	16	120	22	330	22	96	4	230	25	

Unit: µg/m3		Product number (weight)															
Substance, CAS no. and classification**		024 (94 g)	038 (63 g)	043 (116 g)	048 (102 g)	051 (110 g)	054 (52 g)	056 (45 g)	061 (76 g)	033 (20 g)	034 (134 g)	044 (86 g)	063 (142 g)				
	Time	1 h	1 h	1 h	1 h	1 h	1 h	1 h	1 h	1 h	3 d	1 h	3 d	1 h	3 d	1 h	3 d
N,N-dimethylformamide (CAS no.68-12-2) REPR 1B		720	2000	14000*	4900*	3000*	4100*	1500	520	4300*	1200	6000*	3600*	4000*	2300	6500*	3000*
Ethylbenzene (CAS nr.100-41-4) STOT RE2		<5	12	270	<5	18	350	15	22	<5		12	<5	44	7	19	<5
m,p-Xylene (CAS no.179601-23-1)		<5	27	770	7	33	920	42	44	24		19	<5	120	19	50	14
o-Xylene (CAS no.95-47-6)		<5	9	490	<5	13	560	25	18	16	<5	7	<5	50	8	15	6
Styrene (CAS no.100-42-5) REPR 2, STOT RE1		2	51	10		21	25		13	2		3	1	11	2	8	3
Cyclohexanone (CAS no.108-94-1)		3500*	3500*	15000*	5200*	5000*	8200*	3900*	1200	4600*	1100*	9100*	5600*	7700*	3900*	5300*	5600*
alpha-Pinene (CAS no.7785-26-4) SKIN SENS 1		8	11	9	8	7	8	16	11	13		11	<5	5		14	<5
Cyclotetrasiloxane, octamethyl (CAS no.556-67-2) REPR 2				3						1		2	2	7	2	2	1
beta-Pinene (CAS no.18172-67-3)		<5	<5	<5	<5	<5	<5	5	<5	<5		6	<5	<5		<5	

Unit: µg/m3		Product number (weight)															
Substance, CAS no. and classification**		024 (94 g)	038 (63 g)	043 (116 g)	048 (102 g)	051 (110 g)	054 (52 g)	056 (45 g)	061 (76 g)	033 (20 g)	034 (134 g)	044 (86 g)	063 (142 g)				
Time		1 h	1 h	1 h	1 h	1 h	1 h	1 h	1 h	1 h	3 d	1 h	3 d	1 h	3 d	1 h	3 d
SKIN SENS 1																	
delta 3-Carene (CAS no.13466-78-9) SKIN SENS 1		6	7	7	5	<5	6	10	8	4	<5	6	<5	6	<5	9	6
Triethylenediamine (CAS no.280-57-9) (EYE DAM 1)		1300	1400	2700*	2400	3200*	2600	2000	1900	450	710	2600	2700*	1900	2700*	3500*	2900*
Limonene (CAS no.5989-27-5) SKIN SENS 1B		<5	<5	<5	6	17	<5		47	<5		310	260			1900*	1100*
Benzyl alcohol (CAS no.100-51-6)					<5				<5			<5	<5	<5			
Bis(2-(dimethylamino)ethyl)ether (CAS no.3033-62-3) (SKIN CORR 1B)		7	390	<5	22	1000		<5		<5	6	260	400			280	340
Linalool (CAS no.78-70-6) SKIN SENS 1		12	44	<5	20	38		6	31			210	200	18	11	480	340
1,1,4,7,7-Pentamethyl-diethylenetriamine (CAS no.3030-47-5) (SKIN CORR 1B)		870	150	220	<5	90	85	100				210					

Unit: µg/m3		Product number (weight)															
Substance, CAS no. and classification**		024 (94 g)	038 (63 g)	043 (116 g)	048 (102 g)	051 (110 g)	054 (52 g)	056 (45 g)	061 (76 g)	033 (20 g)		034 (134 g)		044 (86 g)		063 (142 g)	
	Time	1h	1h	1h	1h	1h	1h	1h	1h	1 h	3 d	1 h	3 d	1 h	3 d	1 h	3 d
Butylated hydroxytoluene (CAS no.128-37-0)		<5	<5	6	7	<5	<5	<5		<5	<5		<5	10	8	8	10
Sum of all quantified VOCs		6684	10375	37358	12742	15551	17130	8611	4249	9541	3226	20697	13787	16641	8961	25547	16545

*Due to high concentration of substance, the capacity of collection medium (Tenax) has been exceeded, and the real concentration may be higher.

**Selected classification: CMR Cat. 1A, 1B, 2; STOT RE 1, 2; STOT SE 1, 2; Acute Tox. 1, 2, 3; Skin Sens. 1, Resp. sens 1. Other classifications that are considered relevant have been included in brackets (), see chapter 4.5 with introductory hazard assessment.

< 5 indicates that the substances have been quantified in a concentration below 5 µg/m³. Substances with Carc. 1A and Carc. 1B are quantified down to 1 µg/m³ according to EN 16516.

4.8 Quantitative analysis results for migration to sweat

Objective

The objective of the analysis is to determine the amount of DMF and DMAE that can migrate from product to artificial sweat under controlled conditions as defined in EN 71-10:2005.

Sample preparation

A piece of sample was cut in the size of 10 - 15 cm² material with as high a part of outer surface as possible and transferred to a Schott Duran bottle containing 10 mL/cm² artificial sweat heated to 37°C, after which the bottle is closed with a red screw cap with PTFE insert. Thereafter, the migration is continued for 1 hour in a heating cabinet set to 37°C. Then, the migration liquid is transferred to another container.

The migration period was 1 hour and 100 mL of migration liquid per 10 cm² sample as defined in EN 71-10:2005. The migration liquid was thereafter taken out and a portion was extracted with dichloromethane, which can be analyzed with GC/MS for contents of the selected substances. The result is indicated in mg/dm². The migration according to EN 71-10:2005 has been modified with respect to migration liquid, temperature and sampling, which would be artificial sweat, physiological temperature and partial sample with as much outside surface as possible, respectively.

DMF and DMAE have an estimated detection limit of 1 mg/L in synthetic solution with an uncertainty of 10-20 % RSD. The quantification will take place against an external calibration curve. The matrix is examined by addition of a known amount of analysis that is, thereafter, attempted to be recovered with the analysis method. The 26 regulated fragrances have a detection limit of 0.1 mg/L in migration liquid except for two natural extracts, where a qualitative assessment will be performed to determine whether they are present or not. The uncertainty is 10-20 % RSD in relation to synthetic injections. The quantification is performed against an external calibration curve. Spike of the matrix shows that an uncertainty of up to 30% RSD may be expected.

Analysis method

For DMF and DMAE analysis, an alkaline regulation of pH 10 is performed, and a shaking of dichloromethane is performed with added internal standard. The shaking effectiveness is investigated by spike of samples. Spiking is carried out using volumes that are expected to be relevant based on results from the introductory tests. Thereafter, the shaken liquid is injected on GC/MS, where identification and quantification against authentic reference substances is carried out set up using a calibration curve in the interval around the expected concentration.

The shaking effectiveness is determined by spiking, and a recovery of 20-50% is present by identification of a detection limit level. The level of the recovery is possibly due to the water/dichloromethane partition of the substances in the samples, and the quantification with external calibration curve is performed in pure dichloromethane.

Spiking is performed on migration liquid from samples at the level of the detection limit, and a response from samples with spike was observed, but not from samples without spike. Thus, it can be concluded with certainty that the contents in migration liquid are below the stated detection limit.

For DMF and DMAE:

Detection limit per surface: 100 µg/cm² corresponding to 10 mg/dm²

Detection limit per mass: 1000 µg/g

Estimated uncertainty: 20 % RSD

For the analysis of fragrances, shaking with dichloromethane has been performed, and, thereafter, the extract was analyzed according to EN 16274:2012 *Methods for analysis of allergens. Quantification of suspected fragrance allergens in consumer products. Step 1: GC analysis of ready-to-inject sample*. The analysis has been performed with specific calibration for each individual fragrance. According to the standard, no quantification of the two natural extracts from *Evernia Furfuracea* and *Evernia Prunastri* has been performed, and these are merely indicated as detected or not detected as defined. For the 24 quantified fragrances, there was a recovery between 70-94% in the spike of migration liquid from samples, except for benzyl alcohol, where the recovery percentage was 50%.

For fragrances:

Detection limit per surface: 2 µg/cm² corresponding to 0.2 mg/dm²

Detection limit per mass: 50 µg/g

Estimated uncertainty: 20 % RSD

Results

None of the examined fragrances (Appendix 6.1) were identified in a concentration above detection limit in the 12 examined products.

For DMF and DMAE, no content above the detection limit in none of the samples has been indicated.

Chromatograms for the analyzed squishies were mainly the same. Since it has not been possible to review all chromatograms in detail, one chromatogram was selected for closer review (038). 36 tops were identified, where there are mainly tops that represent substances that could not be identified with using the NIST database. The mass spectra from the dominant part of the tops measured by area may be from glycol-like substances of unknown complexity. In total, 36 individual substances are identified, and in total 110 mg/L of organic substance were found in the migration liquid quantified as naphthalene equivalents. This corresponds to 25,000 mg/kg corresponding to 2.5 % of the total mass that has migrated to the liquid. If converted to migration per area, it corresponds to 110 mg/dm².

One substance listed in EN 71-9 + A1:2007 was identified. It was cyclohexanone, and calculated as naphthalene equivalents the concentration was 1 mg/L, which is below the listed limit of 46 mg/L.

According to the information above, it was attempted to weigh the test sample again to evaluate mass loss during the 24-hour migration, which was determined to be a loss of 15% of the test sample (squishy 038). The difference between weight loss and the chemical analysis is due to migration of components outside the measuring range for GC/MS as well as the uncertainty in detection as naphthalene equivalents. The migrated amount of glycol-like organic substances may be the phase that was observed and interrupted the analysis of isocyanates.

4.9 Substances found by emission and content comparison of selected squishies

In TABLE 9, only results for the substances with concentrations above 50 µg/m³ for emission or 50 mg/kg for content, respectively, and with one or more of the prioritized classifications is given.

As expected, the climatic chamber test shows a higher detection of low-boiling substances (VVOC)⁶, while the content analyses show more high-boiling substances (SVOC) and other

⁶ VVOC's cannot be detected by applied GC/MS for content screening.

less volatile substances. There is an overlap, where VOC in an adequately high concentration is found by both emission in chamber and in content analyses, e.g. from N,N-dimethylaminoethanol (DMAE) to 1,2-ethandiamine and N-[2-(dimethylamino)ethyl]-N,N',N'-trimethyl as well as N,N-dimethylformamide (DMF). There is a long range of substances found in both products, but also distinctive differences between the two squishies. Sample 038 (purchased in a Danish webshop) has, e.g., higher emission of N,N-dimethylformamide and linalool, but a lower emission of toluene and limonene.

Regarding the contents, differences are observed as well, since 038 contains more 4,4'-methylene-bis benzenamine than 061 purchased in a non-EU webshop. In the screening analysis of content substances in 061, there is an indication of finding a phthalate in the sample (possibly DEHP), but no quantitative analysis has been performed to confirm its presence.

Limonene and linalool are the most widely used fragrances in toys (Danish Environmental Protection Agency, 2016C), which means that their presence in many of the examined squishies is not surprising. Limonene and linalool are observed by emission from 10 out of 12 examined squishies at levels of up to 1900 µg/m³ for limonene and 480 µg/m³ for linalool. In the content screening, linalool was identified in two squishies at levels between 40-60 mg/kg. This corresponds well with previously reported values for linalool in toys (e.g. 63 mg/kg in erasers; Danish Environmental Protection Agency 2006).

TABLE 9. Comparison of selected screening results from emissions in climatic chamber (concentration indicated as toluene equivalents) and content analyses with GC/MS (concentration indicated as naphthalene equivalents). Substances identified in concentrations above either 50 µg/m³ or 50 mg/kg as well as with at least one of the following classifications are included in this table: CMR 1A, 1B, 2, STOT RE 1,2, STOT SE 1,2, Acute tox 1,2,3, Skin sens 1, Resp sens 1.

Substance	CAS no.	038*		061**		Classification***
		Emission (µg/m ³) ¹	Content (mg/kg) ²	Emission (µg/m ³) ¹	Content (mg/kg) ²	
Toluene	108-88-3	57		95		Flam. Liq. 2 Skin Irrit. 2 Asp. Tox. 1 STOT SE 3 STOT RE 2 Repr. 2
N,N-Dimethylformamide	68-12-2	710	60	210	94	Acute Tox. 4 Eye Irrit. 2 Repr. 1B
D-Limonene	5989-27-5	9		52		Liq. 3 Skin Irrit. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1
L-Linalool	78-70-6	84		47		Skin Sens. 1B
4,4'-Methylenebis benzenamine	101-77-9		136		64	Skin Sens. 1 Muta. 2 Carc. 1B STOT SE 1 STOT RE 2 Aquatic Chronic

		038*		061**		
Substance	CAS no.	Emission ($\mu\text{g}/\text{m}^3$) ¹	Content (mg/kg) ²	Emission ($\mu\text{g}/\text{m}^3$) ¹	Content (mg/kg) ²	Classification***
						2
Bis(2-ethylhexyl) phthalate (DEHP) coeluted with (possibly DINCH)	117-81-7 + (166412-78-8, if DINCH)				243	Repr 1B

*squishy purchased in a Danish webshop

** squishy purchased in an US webshop that sells low-priced products primarily from Chinese manufacturers

***harmonized classification or classification according to REACH registration of substances

5. Risk assessment

In this chapter, risk screening is performed for the exposure scenarios described in chapter 3, and the measurement results described in chapter 4 with a focus on the prioritized substances.

5.1 Risk assessment of inhalation scenarios

5.1.1 Hazard assessment of prioritized substances

For the substances prioritized in the emission analysis (see TABLE 6), data on tolerable exposure limits have been collected from the following sources:

- Recent consumer projects/limit value projects from the Danish Environmental Protection Agency
- The EU-LCI list “Agreed LCI values”
- WHO’s limit values for indoor and outdoor air
- Opinions from ECHA’s risk assessment committee, RAC
- The US EPA IRIS database (given as reference concentrations, RfC).

The EU-LCI list constitutes an important source, since it provides exposure levels for a number of individual substances. Lowest Concentration of Interest (abbrev. LCI) is a concentration determined to limit emission of chemical substances from building materials. In a report from the European Commission from 2013, LCI values have been composed for a total of 21 substances based on the same method as is used in the REACH regulation for establishment of DNEL values. The EU LCI list “Agreed LCI values” is continuously updated and can be downloaded on their website (EU-LCI 2016).

According to a search in the above-mentioned sources, TABLE 10 shows relevant DNEL values for the prioritized substances in relation to emission. DNEL values have been established to protect against the most critical effects of substances, i.e. effects that occur at the lowest exposure levels to the substances. This way, the DNEL value, which is based on mucous membrane irritation as the most critical effect, will also protect against other possible severe effects, which may be caused by the substance at higher exposure levels. It must be noted that after the follow-up emission analyses, new priority substances have been added to the list in TABLE 10 compared to TABLE 6, since some substances from TABLE 6 are excluded due to relatively low emission levels (e.g. octamethylcyclotetrasiloxane and phenol).

TABLE 10. DNEL values and critical effects for prioritized substances*.

Substance	CAS no.	Classification**	DNEL µg/m ³	Reference, DNEL	DNEL, critical effect if inhaled
Methylene chloride	75-09-2	Carc. 2	100	US EPA 2011	Estimate for 10 ⁻⁶ cancer risk, affects liver
			600	US EPA 2011	
2-Butanone	78-93-3	Eye Irrit. 2	5.000	US EPA 2003	Affects the development of the unborn child Unspecified
			20.000	EU-LCI 2016	
N,N-Dimethyl- aminoethanol	108-01-0	Skin Corr. 1B Acute Tox. 4	100	Appendix 7	Affects the cornea and irritates respiratory tract

Substance	CAS no.	Classification**	DNEL µg/m ³	Reference, DNEL	DNEL, critical effect if inhaled
Toluene	108-88-3	Skin Irrit. 2 Asp. Tox. 1 STOT SE 3 STOT RE 2 Repr. 2	2.900 725	EU-LCI 2013 Danish EPA 2016D	Neurotoxicity Neurotoxicity
N,N-Dimethyl- formamide	68-12-2	Acute Tox. 4 Eye Irrit. 2 Repr. 1B	100** 80	CICAD 2001 Danish EPA 2014B	Affects liver Mucous membrane irritation
Ethylbenzene	100-41-4	Acute Tox. 4 Asp. Tox. 1 STOT RE 2	850 200	EU-LCI 2013 Danish EPA 2016D	Neurotoxicity + affects liver Neurotoxicity
Xylenes	1330-20-7	Acute Tox. 4 Skin Irrit. 2	500 125	EU-LCI 2013 Danish EPA 2016D	Neurotoxicity + mucous membrane irritation Neurotoxicity
Styrene	100-42-5	Skin Irrit. 2 Eye Irrit. 2 Acute Tox. 4 STOT RE 1 Repr. 2	250 175	EU-LCI 2013 Danish EPA 2016D	Neurotoxicity + mutagenicity Neurotoxicity
Cyclohexanone	108-94-1	Acute Tox. 4 Skin Irrit. 2 Eye damage 1	410	EU-LCI 2013	Mucous membrane irritation
Triethylenedia- mine	280-57-9	Acute Tox. 4 Skin Irrit. 2 Eye Dam. 1	7	Appendix 7	Mucous membrane irritation
Bis(2-(dimethyl- amino)ethyl)ether	3033-62-3	Acute Tox. 4 Acute Tox. 3 Skin Corr. 1B Eye Dam. 1 Acute Tox. 4	2	Appendix 7	Mucous membrane irritation
1,1,4,7,7- Pentamethyl- diethylenetri- amine	3030-47-5	Acute Tox. 3 Skin Corr. 1B	8	Appendix 7	Mucous membrane irritation
Terpenes:					
delta 3-Carene	13466-78-9		1.500	EU-LCI 2013	Unspecified
Linalool	78-70-6	<i>all:</i> Asp. Tox. 1 Skin Irrit. 2	-	-	-
Limonene	5989-27-5	Skin Sens. 1B	4.500	Danish EPA 2013	Mucous membrane irritation
alpha-Pinene	7785-26-4		2.500	EU-LCI 2013	Affects kidneys
beta-Pinene	18172-67-3		-	-	-

* Harmonized classification/classification in REACH-registration of substance.

** an EU LCI-value of 15 µg/m³ is included in the proposal, but is not approved yet. Background documentation for this value is not available.

All DNEL values are based on 24-hour daily, continuous exposure. However, it was not possible to find data on the tolerable exposure limit for four substances. For these substances, data was searched in the REACH registrations in order to establish a tolerable exposure limit. The substances are:

- N,N-dimethylaminoethanol (DMAE)
- triethylenediamine
- bis(2-(dimethylamino)ethyl)ether
- 1,1,4,7,7-pentamethyldiethylenetriamine

Evaluation of data and calculation of DNEL values for the four substances has been performed in Appendix 7. The calculation has been performed using guidelines in the ECHA guideline for calculation of DNEL values, and, thus, do not necessarily reflect the DNEL values reported by the registrant in the REACH registration.

The critical effects of the four substances are their effect on the respiratory tract or eye mucous, and, for all substances, very low DNEL values have been reached, as given in TABLE 15.

It must be noted that the substance N,N-dimethylformamide has been included on the REACH candidate list as a substance of very high concern (SVHC) due to the classification of the substance as reprotoxic. However, this effect is not the basis for the indicated tolerable exposure limits, since the effects on liver and mucous membrane irritation occur at lower exposure levels compared to reprotoxic effects.

In the risk screening of squishy toys, the lowest DNEL value identified, if more values for a substance are found, has been used for precautionary reasons.

5.1.2 Risk assessment of exposure scenario for small children

As stated in chapter 3, small children lying and sleeping with a squishy toy could be exposed to the highest concentration of the emitting substances, since the toy is within the child's inhalation zone.

Therefore, emission tests in the small climatic chambers (113 L) were set up in a way, so that measured concentrations in chambers could simulate inhalation concentrations of a sleeping child.

By risk screening of this scenario, the measured levels for emission are compared to the stated tolerable exposure limits and a risk characterization coefficient is calculated:

$$\text{RCR} = \text{Exposure } (\mu\text{g}/\text{m}^3) / \text{DNEL } (\mu\text{g}/\text{m}^3)$$

RCR values exceeding 1 mean that the exposure limit exceeds the tolerable DNEL value, which again means that the protection level is too low and that there may be a risk.

It should be noted that all DNEL values in TABLE 10 have been expressed in the unit $\mu\text{g}/\text{m}^3$. The values for the substances with neurotoxicity as the critical effect (ethylbenzene, xylenes, styrene) are established in the report Danish Environmental Protection Agency (2016d) based on the EU-LCI values for the substances. Further, for each of the substances, there has been paid regard to small children possibly being particularly sensitive to effects on the central nervous system and that small children at a given concentration in the air inhale a larger amount of substances compared to their body weight. For these substances, the described aspects have been included by applying an extra factor of 4 compared to the EU-LCI values (see Danish Environmental Protection Agency, 2016d). In relation to other effects, such as

mucous membrane irritation, children are not considered more sensitive than adults, as these effects depend on the dosage to which the mucous membrane is exposed.

Finally, there is no need, as mentioned in chapter 3, to include calculations regarding the child's weight and inhalation volume, when DNEL is expressed in $\mu\text{g}/\text{m}^3$, and this value is also considered to be valid for children. Weight and inhalation volume would have been relevant if the DNEL value was indicated in the unit mg/kg body weight per day, since this value is based on the child's weight, and inhalation volume should be converted to a DNEL value in the unit $\mu\text{g}/\text{m}^3$.

In TABLE 11, RCR values are calculated for the highest measured values for each of the prioritized substances to clarify which substances can constitute a risk when emitted from squishy toys.

Note that DNEL values in TABLE 11 have been translated from DNEL values for 24-hour daily exposure to 10-hour daily exposure by multiplying the 24-hour value with 2.4. This is considered of relevance, since calculation of the 24-hour DNEL values is performed by scaling proportionally from experiments with animals, where exposure duration is typically six hours daily, and where scaling for 24 hours is performed by multiplying the exposure level for six hours with 6h/24h.

TABLE 11. Risk assessment and calculation of RCR values for 10-hour daily exposure (RCR = max. exp./DNEL (10h)).

Substance	CAS no.	Max. measured exposure level $\mu\text{g}/\text{m}^3$ 1 hour/3 days	DNEL (24h) $\mu\text{g}/\text{m}^3$	DNEL (10 h) $\mu\text{g}/\text{m}^3$	RCR (10 h) Based on exposure level measured after 1 hour / 3 days	DNEL, critical effect
Methylene chloride	chlo- 75-09-2	560/0	100	240	2.3/0	Estimate for 10^{-6} cancer risk
2-Butanone	78-93-3	3,800/0*	5.000	12.000	0.32/0	Affects the development of the unborn child
N,N-Dimethylaminoethanol	108-01-0	6,800/3,200*	100	240	28/13	Affects the cornea and respiratory tract irritation
Toluene	108-88-3	330/25*	725	1.740	0.19/0.01	Neurotoxicity
N,N-Dimethylformamide	68-12-2	14,000/3,600*	80	192	73/19	Mucous membrane irritation
Ethylbenzene	100-41-4	350/7*	200	480	0.73/0.01	Neurotoxicity
Xylenes	1330-20-7	1,480/27*	125	300	4.9/0.09	Neurotoxicity
Styrene	100-42-5	51/3*	175	420	0.3/0.02	Neurotoxicity
Cyclohexanone	108-94-1	15,000/5,600*	410	984	15/5.7	Unspecified (most likely eye and respiratory tract irritation)
Triethylenediamine	280-57-9	3,500/2,900	7	17	206/171	Mucous membrane irritation

Substance	CAS no.	Max. measured exposure level $\mu\text{g}/\text{m}^3$ 1 hour/3 days	DNEL (24t) $\mu\text{g}/\text{m}^3$	DNEL (10 t) $\mu\text{g}/\text{m}^3$	RCR (10 h) Based on exposure level measured after 1 hour / 3 days	DNEL, critical effect
Bis(2-(dimethylamino)ethyl)ether	3033-62-3	1000/400*	2	5	200/80	Mucous membrane irritation
1,1,4,7,7-Pentamethyl-diethylenetriamine	3030-47-5	870/210*	8	19	46/11	Mucous membrane irritation
Terpenes:						
delta 3-Carene	13466-78-9	10/6*	1.500	3.600	~0/~0	Unspecified
Linalool	78-70-6	480/340	-	-	-	-
Limonene	5989-27-5	1,900/1,100	4.500	10.800	0.18/0.10	Mucous membrane irritation
alpha-Pinene	7785-26-4	16/0*	2.500	6.000	~0/~0	Affects liver
beta-Pinene	18172-67-3	6/0	-	-	-	-

* Indicates that 1-hour and 3-day measurement results are not associated, i.e. from the same squishy.

In the table, the RCR values above 1 are marked with **bold** letters. The table shows that the emissions of substances:

- N,N-dimethylformamide,
- N,N-dimethylaminoethanol
- triethylenediamine
- bis(2-(dimethylamino)ethyl)ether
- 1,1,4,7,7-pentamethyldiethylenetriamine
- cyclohexanone

may be seen as particularly critical, because RCR values after an hour are all in the interval 15 (cyclohexanone) - 206 (triethylenediamine), while values after three days are in the interval 5,7 (cyclohexanone) - 171 (triethylenediamine). These RCR values are very high, and, thus, it is possible that there may be a risk for other effects that the substances may have at higher exposure levels. This type of analysis is, however, outside the scope of this project.

A limited overstepping of RCR of 1 for methylene chloride is assessed to be less critical, since there is a fast emission of methylene chloride, and after 3 days the RCR is below 1. Since DNEL for methylene chloride is based on a cancer risk for daily exposure throughout the entire life, a very short-term and limited overrun is not considered serious. Also for xylenes the RCR > 1 for 1-hour measurements. However, the risk of chronic neurotoxic effects is related to long-term exposure above the DNEL level. For the squishies with 3-day measurements of xylenes, it is observed that xylenes emit rather fast, but certain knowledge of emission after 3 days for the respective squishy is not available.

Since the focus is on the six substances with the highest RCR values from TABLE 11, and emissions from each of the squishies are evaluated, the following overview has been obtained

for the squishies with emissions exceeding the DNEL value (i.e. RCR > 1) after 1 hour and after 3 hours (TABLE 12).

TABLE 12. Overview of squishies with RCR > 1 for selected substances

Substance	CAS no.	DNEL µg/m ³ (10 t)	Squishies with RCR > 1 after 1 hour	Squishies with RCR > 1 after 3 days
N,N-Dimethylaminoethanol	108-01-0	240	024, 034, 038, 051, 056, 061, 063	034, 063
N,N-Dimethyl-formamide	68-12-2	192	024, 033, 034, 038, 043, 044, 048, 051, 054, 056, 061, 063 (all squishies)	033, 034, 044, 063 (all squishies with 3-day-measurements)
Cyclohexanone	108-94-1	984	024, 033, 034, 038, 043, 044, 048, 051, 054, 056, 061, 063 (all squishies)	033, 034, 044, 063 (all squishies with 3-day-measurements)
Triethylenediamine	280-57-9	17	024, 033, 034, 038, 043, 044, 048, 051, 054, 056, 061, 063 (all squishies)	033, 034, 044, 063 (all squishies with 3-day-measurements)
Bis(2-(dimethylamino)ethyl)ether	3033-62-3	5	024, 034, 038, 048, 051, 063	033*, 034, 063
1,1,4,7,7-Pentamethyl-diethylenetriamine	3030-47-5	19	024, 038, 043, 051, 054, 056	033*

* Only overrun at 3-day measurements and not at 1-hour measurements (the reason for higher emission after 3 days is unknown).

For all substances, the critical effect is mucous membrane irritation related to eye and respiratory tract irritation, which is why emission of several substances simultaneously will contribute to an intensified and, most likely, additive effect.

To highlight the risk of a concrete squishy, a risk assessment is performed of squishy no. 063 below, see results in TABLE 13. This squishy represents a rather high emission with many substances, and both 1-hour and 3-day measurements are carried out for this squishy.

TABLE 13. Risk assessment of squishy no. 063.

Substance	Conc. (µg/m ³) 1 hour/3 days	DNEL (10 t) µg/m ³	RCR 1 hour/3 days	DNEL, critical effect
Methylene chloride	11/0	240	0.05/0	Estimate for 10 ⁻⁶ cancer-risk
2-Butanone	410/0	12,000	0.03/0	Affects the development of the unborn child
N,N-Dimethylaminoethanol	6,800*/3,200*	240	>28/>13	Affects the cornea and respiratory tract irritation
Toluene	230/25	1,740	0.13/0.01	Neurotoxicity

N,N-Dimethylformamide	6,500*/3,000*	192	>34/>16	Mucous irritation	membrane
Ethylbenzene	19/<5	480	0.04/<0.01	Neurotoxicity	
Xylenes	65/20	300	0.22/0.07	Neurotoxicity	
Styrene	8/3	420	0.02/0.01	Neurotoxicity	
Cyclohexanone	5,300*/5,600*	984	>5.4/>5.7	Unspecified (most likely eye and respiratory tract irritation)	
Triethylenediamine	3,500*/2,900*	17	>206/>171	Mucous irritation	membrane
Bis(2-(dimethylamino)ethyl)ether	280/340	5	56/68	Mucous irritation	membrane
1,1,4,7,7-Pentamethyl-diethylenetriamine	0/0	19	0/0	Mucous irritation	membrane
Terpenes:					
delta-3-Carene	9/6	3,600	~0/~0	Unspecified	
Linalool	480/340	-	-	-	
Limonene	1,900*/1,100*	10,800	0.18/0.10	Mucous irritation	membrane
alpha-Pinene	14/<5	6,000	~0/~0	Affects kidneys	
beta-Pinene	<5/0	-	-	-	

* Minimal values, because sampling tubes were saturated with VOCs.

The table shows that RCR exceeds the value 1 in the interval 5.4 – 206 after 1 hour and in the interval 5.7 – 171 after 3 days for the substances:

- cyclohexanone
- N,N-dimethylformamide
- N,N-dimethylaminoethanol
- triethylenediamine
- bis(2-(dimethylamino)ethyl)ether

For all these substances, the critical effect is mucous membrane irritation related to the eye and respiratory tract, which is why emission of several substances at the same time will contribute to an enhanced and, most likely, additive effect. No RCR values above 1 are observed for substances with other effects, e.g. carcinogenic or neurotoxic effects.

Thus, it can be concluded that there is a significant risk for eye and respiratory tract irritation/effects in a scenario, where a young child sleeps with squishy 063.

5.1.3 Risk assessment of scenario for older children

As the worst-case scenario for the typical use (for target group down to six years and others, who may stay in the room), a squishy with a high emission will be selected from the emission

measurements, and an equilibrium concentration in the room will be calculated under the assumption that there are 40 squishies in the room and that the child stays in the room for 15 hours daily for periods of time.

The scenario with measuring a squishy in the small climatic chamber (with ventilation rate of 0.5 times per hour) with volume of 0.113 m³ can be scaled up to a “standard” child’s room of 17.4 m³ (with ventilation rate of 0.5 times per hour), because a factor of 0.113 m³ / 17.4 m³ = 0.0065 is applied against the measurement results in the small climatic chamber. If 40 squishies are put in the room, the factor becomes 40 x 0.0065 = 0.26.

Note that DNEL values in TABLE 14 are translated from DNEL values for 24-hour daily exposure to 15-hour daily exposure by multiplying the 24-hour value with 1.6.

TABLE 14. Risk assessment and calculation of RCR for 40 squishies corresponding to no. 063 in a child’s room.

Substance	Conc. (µg/m ³) Small climatic chamber 1 hour/3 days	Conc. (µg/m ³) 40 squishies in child’s room 1 hour/3 days	DNEL (24 h) (µg/m ³)	DNEL (15 h) (µg/m ³)	RCR Based on exposure levels measured after 1 hour / 3 days	DNEL, Critical effect
Methylene chloride	11/0	2.9/0	100	160	0.02/0	Estimate for 10 ⁻⁶ cancer risk
2-Butanone	410/0	107/ 0	5,000	8,000	0.01/0	Affects the development of the unborn child
N,N-Dimethylaminoethanol	6,800*/3,200*	1,768/832	100	160	>11/>5.2	Affects the cornea and respiratory tract irritation
Toluene	230/25	60/ 6.5	725	1.160	0.05/0.01	Neurotoxicity
N,N-Dimethylformamide	6,500*/3,000*	1,690/780	80	128	>13/>6.1	Mucous membrane irritation
Ethylbenzene	19/<5	4.9/1.3	200	320	0.02/~0	Neurotoxicity
Xylenes	65/20	17/ 5.2	125	200	0.09/0.03	Neurotoxicity
Styrene	8/3	2.1/0.8	175	280	0,01/~0	Neurotoxicity
Cyclohexanone	5,300*/ 5,600*	1,378/1,456	410	656	>2.1/>2.2	Unspecified (most likely eye and respiratory tract irritation)
Triethylenediamine	3,500*/ 2,900*	910/754	7	11	>83/>69	Mucous membrane irritation
Bis(2-(dimethylamino)ethyl)ether	280/340	73/88	2	3.2	23/28	Mucous membrane irritation

Substance	Conc. (µg/m ³) Small climatic chamber 1 hour/3 days	Conc. (µg/m ³) 40 squishies in child's room 1 hour/3 days	DNEL (24 h) (µg/m ³)	DNEL (15 h) (µg/m ³)	RCR Based on exposure levels meas- ured after 1 hour / 3 days	DNEL, Critical effect
1,1,4,7,7-Pentamethyl-diethylenetriamine	0/0	0/0	8	13	0/0	Mucous mem- brane irritation
Terpenes:						
delta-3-Carene	9/6	2.3/1.6	1,500	2,400	~0/~0	Unspecified
Linalool	480/340	125/88	-	-	-	-
Limonene	1,900*/1,100*	494/286	4,500	7,200	0.07/0.04	Mucous mem- brane irritation
alpha-Pinene	14/<5	3.6/<1.3	2,500	4,000	~0/~0	Affects kidneys
beta-Pinene	<5/0	<1.3/0	-	-	-	-

* Minimal values, because sampling tubes were saturated with VOCs.

The table indicates that RCR exceeds the value 1 in the interval 2.1 – 83 after 1 hour and in the interval 2.2 – 69 after 3 days for the substances:

- cyclohexanone
- N,N-dimethylformamide,
- N,N-dimethylaminoethanol
- triethylenediamine
- bis(2-(dimethylamino)ethyl)ether

For all five substances, the critical effect is mucous membrane irritation related to eye and respiratory tract, which is why emission of several substances at the same time will contribute to an enhanced and, most likely, additive effect. Thus, it can be concluded that there is a significant risk for eye and respiratory tract irritation in a scenario, where 40 squishies are stored in a child's room, and where the child resides in the room a full day.

Even with only one squishy, the RCR value would equal 1.7 for the substance triethylenediamine after 3 days' storage in a child's room.

5.2 Risk assessment of scenario with skin contact

No substances have been detected above the indicated detection limits in the performed migration tests. It is, thus, not possible to perform a quantitative risk assessment on the basis of migration data with calculation of RCR values.

As tables for emission analysis indicate, emission of several substances hazardous to health takes place, which is why a child will be exposed to these vapors by skin contact with a squishy. It is assessed that particularly substances that can cause skin irritation and skin allergy will be able to constitute a potential risk.

The following eight substances, which can cause skin irritation, have been found in relatively high concentrations in the emission test and can, thus, have an impact on potential risk by skin contact:

- cyclohexanone
- N,N-dimethylaminoethanol

- triethylenediamine
- bis(2-(dimethylamino)ethyl)ether
- 1,1,4,7,7-Pentamethyldiethylenetriamine
- toluene
- xylenes
- styrene

Furthermore, a risk for skin allergy cannot be excluded, since the examined squishy products can emit or contain the following seven skin allergy-causing substances:

- delta-3-carene
- linalool
- limonene
- alfa-pinene
- beta-pinene
- 3-(4-isopropylphenyl)-2-methylpropionaldehyde
- 4-tert-butylcyclohexylacetate

In general, relatively low emissions have been detected for the allergy-causing substances except for the substances limonene and linalool, which in a single squishy (no. 063) emitted in relatively high levels (limonene: 1900 µg/m³ and linalool: 480 µg/m³ at 1-hour measurements).

Even though the risk of these effects cannot be quantified, it is assessed that the greatest risk of these effects may occur in case of a scenario where a young child lies down and hugs a squishy toy during sleep (up to 10 hours). The young child's contact can be of significantly longer duration compared to an older child's skin contact during play with squishies (approx. 2 hours).

A possible risk of skin irritation/allergy is evaluated to be greatest in areas, where the skin is particularly thin, which means that if young children suck on parts of the squishy, the area around lips will be particularly exposed.

5.3 Limitations and uncertainties

5.3.1 Exposure scenario

The basis for the inhalation scenario for a 3-year-old child is that the child lies down and sleeps holding the squishy in the inhalation area, and that measurement results performed after 1 hour and 3 days in a small climatic chamber of 113 liters reflect an inhalation concentration for a child (partly directly after unpacking of the toy and partly after 3 days). The experiment design itself with a small chamber is considered to reflect the concentration in the child's inhalation area in a realistic way.

At the same time, the exposure estimates based on 1-hour and 3-day measurements will as a rule overestimate the exposure over a longer period of time, since emission of a wide range of substances decreases fast after unpacking, which can be seen, among others, after 1-hour measurements and 3-day measurements for e.g. hydrocarbons (toluene, xylenes, ethylbenzene, styrene). It is also worth mentioning that particularly critical substances (cyclohexanone, N,N-dimethylformamide, N,N-dimethylaminoethanol and triethylenediamine), which produce high RCR values, preserve a rather high concentration from the 1-hour to 3-days measurements, which indicates that the emission of these substances will last over a longer period of time.

To acquire better knowledge of the period of time at which RCR values are above 1, follow-up measurements over a longer period of time will be necessary.

It is assessed that there is a greater uncertainty regarding the exposure scenario for older children, since there are more variables that must be taken into consideration, including the number and choice of squishies actually stored in the child's room, the size of the child's room, ventilation and the length of stay in the room. Initially in the emission analyses, each squishy is squeezed 10 times inside the climatic chamber. In case of a scenario with exposure to 40 squishies, the likelihood that all squishies are squeezed ten times would be low, which is why the concentration may be overestimated. It is also worth indicating that some substances retain a rather high concentration after 1-hour to 3-day measurements, which indicates that the emission of these substances will last over a longer period of time with no additional physical action. It is, thus, assessed that even the storage of relatively few squishies within the established scenarios will result in RCR values above 1.

5.3.2 Establishment of DNEL values and risk assessment

It must be mentioned that for some of the most critical substances: N,N-dimethylaminoethanol, triethylenediamine, bis(2-(dimethylamino)ethyl)ether and 1,1,4,7,7-pentamethyldiethylenetriamine, no expert assessments were available indicating a tolerable exposure limit. For this reason, DNEL values were estimated from inhalation experiments on animals reported in REACH registration of the substances. Especially, DNEL values for triethylenediamine vapors may be considered as uncertain, since the value is calculated on the basis of a study, where animals were exposed to the substance in the form of an aerosol, which will most likely create a stronger response in animals in comparison to vapors.

Furthermore, it has not been possible within this project to perform a more detailed assessment of these studies or to find additional data for these substances, which might potentially have an impact on determining the DNEL value.

Hence, there is some uncertainty regarding the determination of DNEL values for these substances, and in order to reach a more precise risk assessment, a more thorough assessment of these substances is required. Likewise, this applies to the substance cyclohexanone, which emitted in large volumes, and for which the background for the used LCI value is unknown.

In the risk assessment, focus is put on the risk of substance effects that occur at the lowest exposure levels (the critical effects for the DNEL value). However, in case of very high RCR values it cannot be excluded that there is a risk for other, possibly more critical, effects that may occur at higher levels. An analysis of this aspect is not included in this project, as no assessment of each substance regarding the dosage-response and DNEL values for any other possible effects has been carried out.

5.4 Final conclusion, risk assessment

In emission measurements of 12 selected, different squishies, emission was measured for a range of substances at levels that—based on the risk screening performed in the report—may cause irritation of eyes and respiratory tract, and that may cause damage to mucous membranes during exposure of a longer period of time.

This included the following substances that showed RCR values considerably higher than 1:

- dimethylaminoethanol, (RCR = 28 as highest value)
- N,N-dimethylformamide, (RCR = 73 as highest value)
- cyclohexanone, (RCR = 15 as highest value)
- triethylenediamine, (RCR = 206 as highest value)
- bis(2-(dimethylamino) ethyl) ether), (RCR = 200 as highest value)
- 1,1,4,7,7-pentamethyldiethylenetriamine, (RCR = 46 as highest value)

In an exposure scenario of a young child lying and sleeping while hugging a squishy, on the basis of measurements performed 1 hour after unpacking the toy, for *all 12 squishies* unac-

ceptably high levels of the substances N,N-dimethylformamide, triethylenediamine and cyclohexanone were measured. For four squishies, which also underwent follow-up 3-day measurements, the concentrations remained at unacceptably high levels for the substances N,N-dimethylformamide and triethylenediamine.

For other mucous membrane, irritant substances: bis(2-(dimethylamino)ethyl)ether; 1,1,4,7,7-pentamethyldiethylenetriamine and dimethylaminoethanol, emissions from six squishies resulted in exceeding the tolerable limit 1 hour after unpacking. After three days, the levels of bis(2-(dimethylamino)ethyl)ether were still unacceptably high for three out of four squishies.

For substances with other effects, e.g. methylene chloride (carcinogenic) and xylenes (chronically neurotoxic), minor overstepping of the tolerable exposure level was detected 1 hour after unpacking (RCR up to 2.3 for methylene chloride and RCR up to 4.9 for xylenes). Since these hazardous effects are related to a long-term exposure above the DNEL value, a short-term overstepping of the RCR value in a risk assessment context is considered to be less significant.

In a scenario, where several squishies are stored in a child's room, unacceptably increased levels were detected in the child's room of the substances: N,N-dimethylformamide, N,N-dimethylaminoethanol, triethylenediamine, bis(2-(dimethylamino)ethyl)ether, 1,1,4,7,7-pentamethyldiethylenetriamine and cyclohexanone.

After execution of migration tests to an artificial sweat liquid, no migration of any of the selected substances was detected, which is the reason why no quantitative risk assessment for skin exposure for these substances can be carried out. Upon skin contact, the skin will also be exposed to the emitted substances, which does not exclude the risk of skin irritation. In the same way, the risk of skin allergy cannot be excluded due to the emission of skin allergy-causing substances, though emissions in smaller amounts, but in the case of recurrent skin contact can cause development of skin allergy. If young children suck on part of the squishy toy, a potential risk of skin irritation and skin allergy would be greatest in areas with the thinnest skin, e.g. lips.

6. List of abbreviations

CAS	Chemical Abstracts Service
DBP	Dibutyl phthalate
DEHP	Diethylhexyl phthalate
DEP	Diethyl phthalate
DMAE	Dimethylaminoethanol
DMF	Dimethylformamide
DNPH	2,4-Dinitrophenylhydrazine
DOP	Dioctyl phthalate
ECHA	European Chemicals Agency
FLD	Fluorescence detector
FTIR	Fourier Transformed Infrared Spectroscopy
GC/MS	Gas Chromatography / Mass Spectrometry
HDI	Hexamethylene diisocyanate
HMDI	Hydrogenated methylene diphenyl diisocyanate
HPLC	High-pressure liquid chromatography
LRPu	Low-resilience polyurethane
MAMA	9-(N-Methylamino-methyl)anthracene
MCCP	Medium-chained chlorinated paraffins
MDI	Methylene diphenyl diisocyanate
NIST	National Institute of Standards and Technology
OPFR	Organophosphorous flame retardants
pH	$-\log([H_3O^+])$
PTFE	Polytetrafluoroethylene
PU	Polyurethane
PUR	Polyurethane
PVC	Polyvinylchloride
RCR	Risk characterization ratio
REACH	Registration, Evaluation, Authorisation and Restriction of Chemical Substances
RH	Relative humidity
RSD	Relative standard deviation
SCCP	Short-chained chlorinated paraffins
STOT RE	Specific Target Organ Toxicity Repeated Exposure
STOT SE	Specific Target Organ Toxicity Single Exposure
SVHC	Substances of very high concern
SVOC	Semi-volatile organic compound, boiling point > approx. 287°C (n-hexadecane)
TDI	Toluene diisocyanate
TDS	Thermal desorption system
TEHP	Tris(2-ethylhexyl) phosphate
VOC	Volatile organic compound
VVOC	Very volatile organic compound, boiling point < 68°C (n-hexane)

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Appendix 1. Substances restricted in toys by existing legislation

Appendix 1.1 Allergenic fragrances listed in EU's Toy Safety Directive 2009/48/EC

According to EU's Toy Safety Directive 2009/48/EC, toys may not contain the 55 allergenic fragrances listed in TABLE 15.

TABLE 15. 55 allergenic fragrances banned in toys according to EU Toy Safety Directive 2009/48/EC.

No.	Name of the allergenic fragrance	CAS no.
1	Alanroot oil (Inula helenium)	97676-35-2
2	Allylthiocyanate	57-06-7
3	Benzyl cyanide	140-29-4
4	4-tert-Butylphenol	98-54-4
5	Chenopodium oil	8006-99-3
6	Cyclamen alcohol	4756-19-8
7	Diethyl maleate	141-05-9
8	Dihydrocumarin	119-84-6
9	2,4-Dihydroxy-3-methylbenzaldehyde	6248-20-0
10	3,7-Dimethyl-2-octen-1-ol (6,7-Dihydrogeraniol)	40607-48-5
11	4,6-Dimethyl-8-tert-butylcoumarin	17874-34-9
12	Dimethyl citraconate	617-54-9
13	7,11-Dimethyl-4,6,10-dodecatrien-3-on	26651-96-7
14	6,10-Dimethyl-3,5,9-undecatrien-2-on	141-10-6
15	Diphenylamine	122-39-4
16	Ethyl acrylate	140-88-5
17	Fig leaf, fresh and preparations	68916-52-9
18	trans-2-Heptenal	18829-55-5
19	trans-2-Hexenal diethyl acetal	67746-30-9
20	trans-2-Hexenal dimethyl acetal	18318-83-7
21	Hydroabietyl alcohol	13393-93-6
22	4-Ethoxy-phenol	622-62-8
23	6-Isopropyl-2-decahydronaphthalenol	34131-99-2
24	7-Methoxycoumarin	531-59-9
25	4-Methoxyphenol	150-76-5
26	4-(p-Methoxyphenyl)-3-buten-2-one	943-88-4
27	1-(p-Methoxyphenyl)-1-penten-3-one	104-27-8

No.	Name of the allergenic fragrance	CAS no.
28	Methyl trans-2-butenolate	623-43-8
29	6-Methylcumarin	92-48-8
30	7-Methylcumarin	2445-83-2
31	5-Methyl-2,3-hexanedione	13706-86-0
32	Costus root oil (<i>Saussurea lappa Clarke</i>)	8023-88-9
33	7-Ethoxy-4-methylcumarin	87-05-8
34	Hexahydrocumarin	700-82-3
35	Peru balsam, crude (Exudation of <i>Myroxylon pereirae</i> (Royle) Klotzsch)	8007-00-9
36	2-Pentylidene-cyclohexanone	25677-40-1
37	3,6,10-Trimethyl-3,5,9-undecatrien-2-one	1117-41-5
38	Verbena oil (<i>Lippia citriodora Kunth</i>)	8024-12-2
39	Musk ambrette (4-tert-Butyl-3-methoxy-2,6-dinitrotoluene)	83-66-9
40	4-Phenylbut-3-en-2-on	122-57-6
41*	Amyl cinnamal	122-40-7
42*	Amylcinnamyl alcohol	101-85-9
43*	Benzyl alcohol	100-51-6
44*	Benzyl salicylate	118-58-1
45*	Cinnamyl alcohol	104-54-1
46*	Cinnamal	104-55-2
47*	Citral	5392-40-5
48*	Coumarin	91-64-5
49*	Eugenol	97-53-0
50	Geraniol	106-24-1
51*	Hydroxy-citronellal	107-75-5
52*	Hydroxy-methylpentylcyclohexencarboxaldehyde	31906-04-4
53*	Isoeugenol	97-54-1
54*	Oakmoss extracts	90028-68-5
55*	Treemoss extracts	90028-67-4

* Declarable substance in cosmetics according to Regulation on cosmetic products EC 1223/2009.

However, the presence of traces of these 55 fragrances shall be allowed provided that such presence is technically unavoidable under good manufacturing practice and does not exceed 100 mg/kg.

In addition, the names of the following allergenic fragrances shall be listed on the toy, on an affixed label, on the packaging or in an accompanying leaflet, if added to a toy, as such, at concentrations exceeding 100 mg/kg in the toy or components thereof. See TABLE 16.

TABLE 16. 11 allergenic fragrances, which according to EU's Toy Safety Directive 2009/48/EC shall be listed if they are used in toys in concentrations above 100 mg/kg.

No.	Name of the allergenic fragrance	CAS no.
1*	Anisyl alcohol	105-13-5
2*	Benzyl benzoate	120-51-4
3*	Benzyl cinnamate	103-41-3
4*	Citronellol	106-22-9
5*	Farnesol	4602-84-0
6*	Hexyl cinnamaldehyde	101-86-0
7*	Lilial	80-54-6
8*	d-Limonene	5989-27-5
9*	Linalool	78-70-6
10*	Methyl heptine carbonate	111-12-6
11*	3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	127-51-5

* Declarable substance in cosmetics according to Regulation on cosmetic products EC 1223/2009.

Appendix 1.2 Restrictions in Appendix II, Annex C in Toy Safety Directive: toys intended for children under 3 years and toys intended to be placed in mouth

TABLE 17. Specific limit values for chemicals used in toys intended for use by children under 36 months or in other toys intended to be placed in mouth.

Substance	CAS no.	Limit value
TCEP	115-96-8	5 mg/kg (limit value for content)
TCPP	13674-84-5	5 mg/kg (limit value for content)
TDCP	13674-87-8	5 mg/kg (limit value for content)
Bisphenol A	80-05-7	0.04 mg/l (migration value) according to methods established in EN 71-10:2005 and EN 71-11:2005
Formamide	75-12-7	20 µg/m ³ (limit value for emission) after at most 28 days from the beginning of emission analysis of foam materials in toys that contain more than 200 mg/kg (limit value based on contents)
1,2-Benzisothiazol-3(2H)-one	2634-33-5	5 mg/kg (limit value for content) in water-based toy materials according to methods EN 71-10:2005 and EN 71-11:2005
Reaction mixture of: 5-chlor-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1)	55965-84-9	1 mg/kg (limit value for content) in water-based toy materials
5-Chlor-2-methyl-isothiazolin-3(2H)-one	26172-55-4	0.75 mg/kg (limit value for content) limit value for content in water-based toy materials
2-methylisothiazolin-3(2H)-one	2682-20-4	0.25 mg/kg (limit value for content) in water-based toy materials
Phenol	108-95-2	5 mg/l (limit value for migration) in polymer materials according to methods established in EN 71-10:2005 and EN 71-11:2005. 10 mg/kg (limit value for content) as preservative according to methods in EN 71-10:2005 and EN 71-11:2005

Appendix 1.3 Restrictions according to REACH

PAHs

- (a) Benzo[a]pyrene (BaP)
CAS No 50-32-8
- (b) Benzo[e]pyrene (BeP)
CAS No 192-97-2
- (c) Benzo[a]anthracene (BaA)
CAS No 56-55-3
- (d) Chrysene (CHR)
CAS No 218-01-9
- (e) Benzo[b]fluoranthene (BbFA)
CAS No 205-99-2
- (f) Benzo[j]fluoranthene (BjFA)
CAS No 205-82-3
- (g) Benzo[k]fluoranthene (BkFA)
CAS No 207-08-9
- (h) Dibenzo[a,h]anthracene (DBAhA)
CAS No 53-70-3

Toy plastic and rubber components that have a direct long-term or short-term repeated skin contact shall not contain any of the above-mentioned PAHs in concentrations above 0.5 mg/kg.

Benzene

Toys and toy components shall not contain benzene in concentrations above 5 mg/kg.

Phthalates

DEHP, DBP and BBP shall not be present in toys at concentrations above 0.1 %. DINP, DNOP and DIDP shall not be found in concentrations above 0.1 % in toys that can be placed in mouth.

National restrictions of phthalates: concentration of phthalates in toys for children under 3 years shall be lower than 500 mg/kg. Phthalates, which are regulated by EU-regulations, are not included in the national restrictions.

Appendix 2. Questionnaire guide used for industry contacts

Example of initial contact:

I am calling from Danish Technological Institute, and we are conducting an analysis of squishy toys for the Danish Environmental Protection Agency. Hence, we would like to interview selected Danish distributors and importers of this type of toys to gain knowledge about these products. Danish Environmental Protection Agency have selected you for this purpose. Would you be willing to participate? The interview will take approximately 15 minutes. Do you have time now, or should we find another time, where I could call you?

We will sum up the information in a report to be published by the Danish Environmental Agency, but it will be in an anonymous form. We will send a draft of the text to you for review prior its publication, so that you have an opportunity to provide comments.

Question guide during the interview (is forwarded before, if desired):

1. Have you been and/or are you concerned or particularly aware of any substances when purchasing squishies? Which ones?
2. Do you have knowledge of specific substances in squishies, about which you have asked questions or acquired information (legislative regulation and requirements for businesses)? Which information have you asked about and did you receive confirmation of the contents of squishies?
3. Have you acquired information on the content of fragrances, other substances with an odor or which originate from surface coating or similar? What answers did you receive?
4. Squishies are expected to be made of polyurethane foam, which has been confirmed in an analysis of samples. Are you able to provide supplementary knowledge on material types used in squishies? Are e.g. other types of polymer materials used and which?
5. Where are squishies that you sell produced?
6. Have you received inquiries about squishies from consumers regarding health-related effects or risks? What are consumers' inquiries usually about?
7. Who do you consider the primary target group (age-related)?
8. Would you consider it possible that also young children (e.g. <3 years of age) play with squishies?
9. Knowledge of usage among consumers, e.g. how long and how do children usually play with squishies?
10. How many squishies do children typically purchase? Are they seen as collectables, so that it can be expected that each child owns several squishies?
11. Are you able to provide some information in the demand – is it still increasing/levelled out/decreasing? Do you see changes or new derived trends?

It must be noted, whether the participant is a distributor or an importer.

Appendix 3. FTIR-analysis of foam in squishies

Sample preparation

A piece of foam without paint was cut out of each of the three squishies (802708-38, 802708-56 and 802708-61). Measurements have been conducted directly on foam pieces with no additional sample preparation.

Analysis method

FTIR spectroscopy is conducted on a 4500a FTIR-instrument from Agilent Technologies. Spectra are produced with ATR method (attenuated total reflectance) with 32 repetitions and a spectral resolution of 8 cm⁻¹.

Search in reference spectra has been performed from databases by Agilent, Sigma Aldrich and Smiths Detection.

Results

Measurements have been performed in three places on the foam from each of the three samples. Spectra from different areas are comparable, which confirms that these samples are relatively homogenous. Furthermore, spectra from these three samples are close to identical. All spectra are shown in Figure 2.

For all three samples, the best match is a reference spectrum from polyurethane – see Figure 3 to Figure 5.

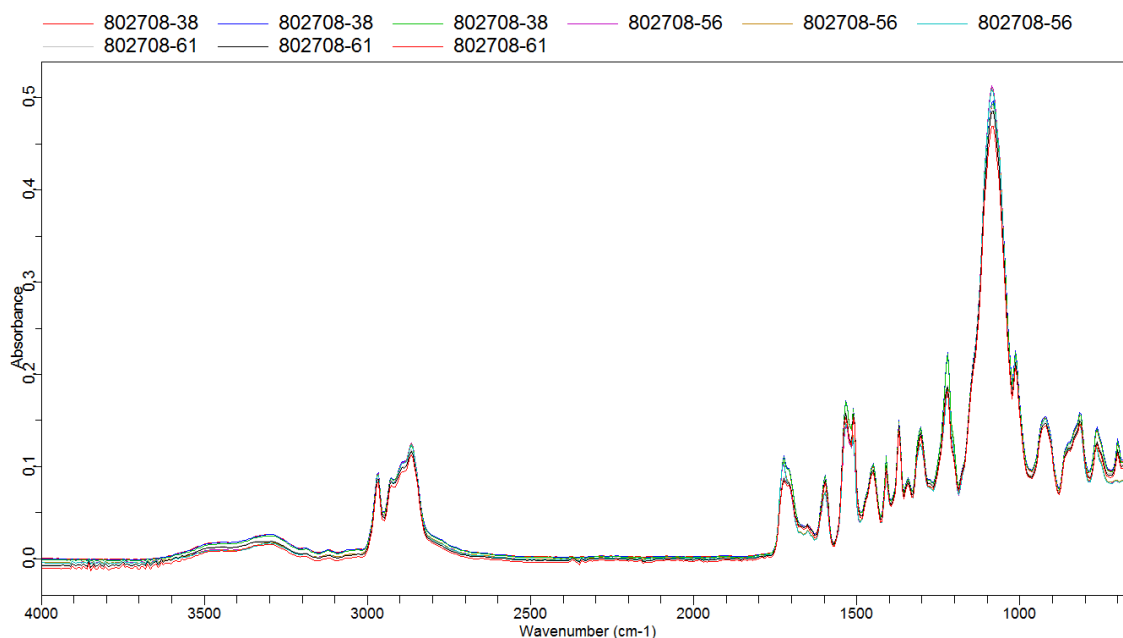


Figure 2: FTIR spectra of the three samples.

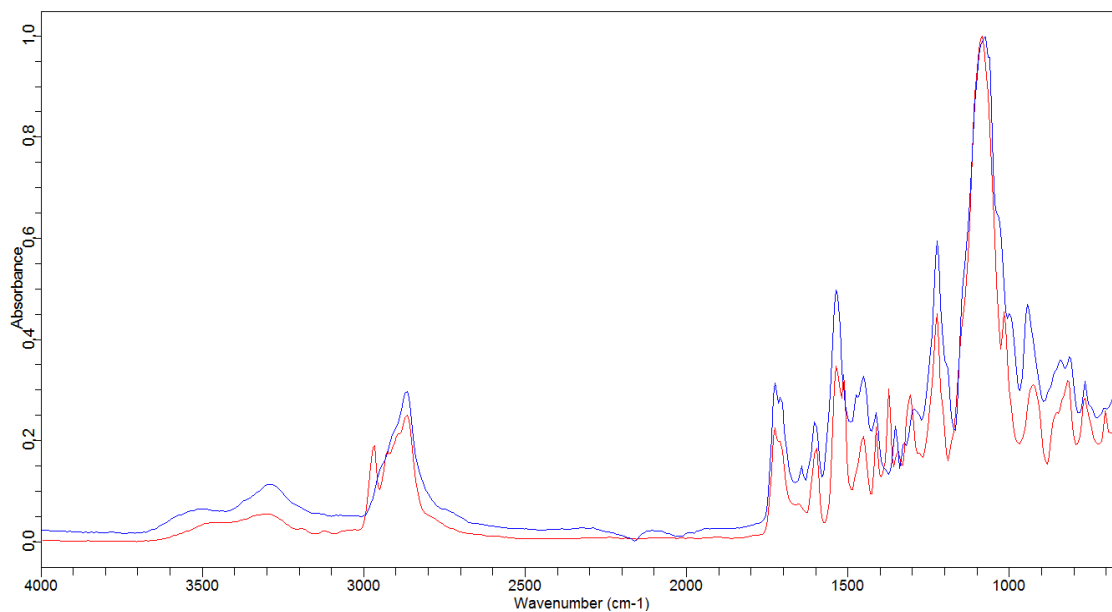


Figure 3: FTIR spectrum of 802708-38 (red) compared with database spectrum of polyurethane (blue).

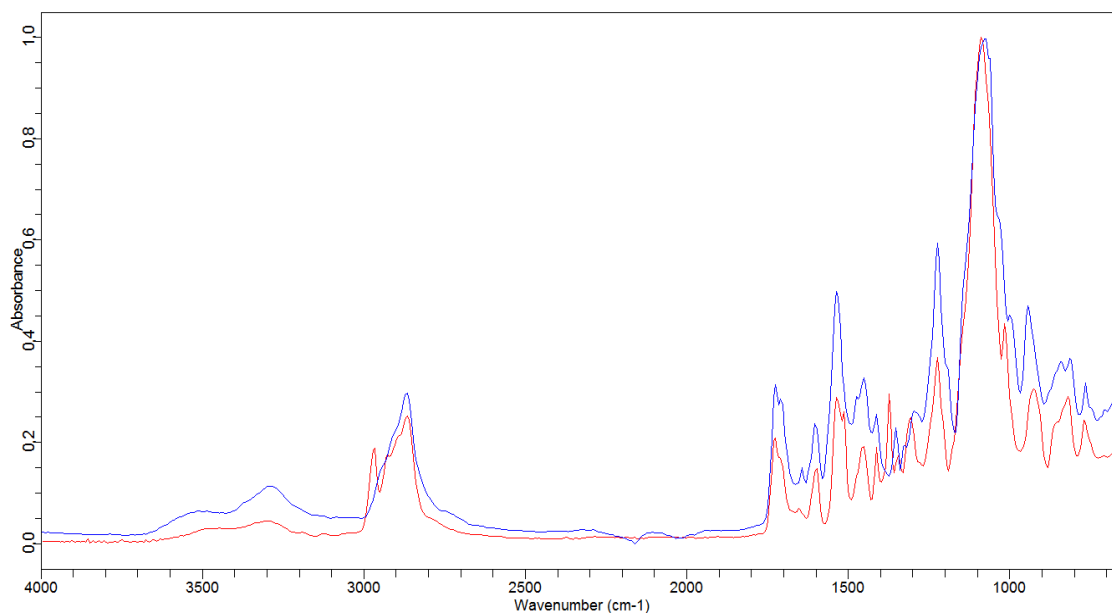


Figure 4: FTIR spectrum of 802708-56 (red) compared with database spectrum of polyurethane (blue).

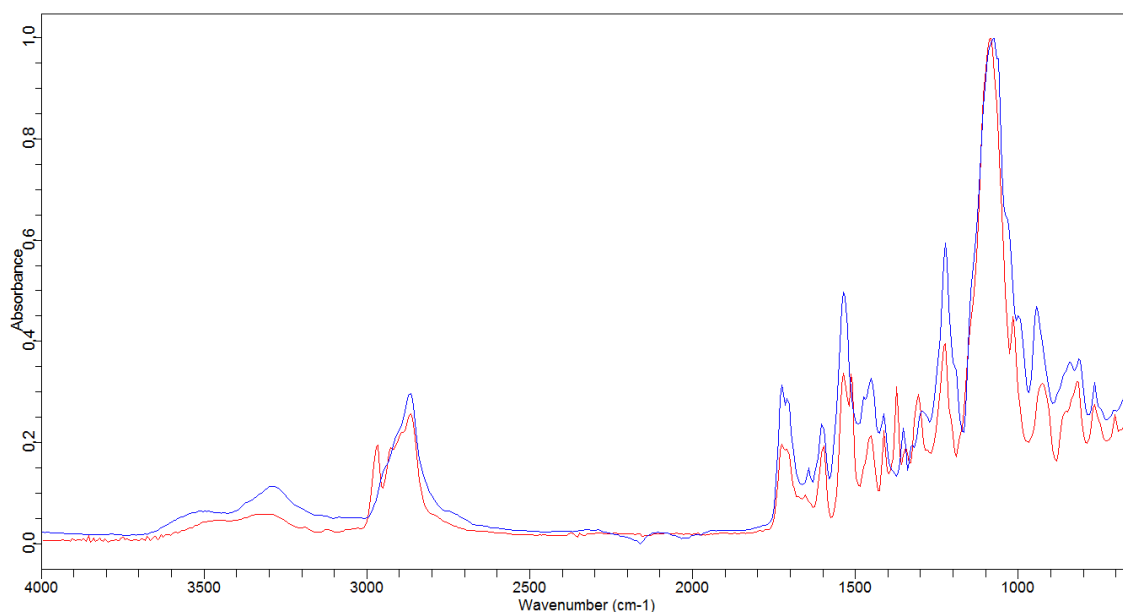


Figure 5: FTIR spectrum of 802708-61 (red) compared with database spectrum of polyurethane

Appendix 4. Screening results of content substances in partial samples from eight squishies with GC/MS

Appendix 4.1 Content substances with certain identification from library (category 1)

Unit: mg/kg	Product number											
Substance	CAS no.	024	038	043	048	051	054	056	061	Hazard class	Hazard state- ment	Prioritization
Ethylene glycol	107-21-1	-	-	-	-	-	-	40	-	Acute Tox. 4	H307	no
Dioxane	123-91-1	-	-	12	-	-	-	29	< 10	Flam. Liq. 2 Eye Irrit. 2 STOT SE 3 Carc. 2	H225 H319 H335 H351	Yes Class.
N,N-Dimethylaminoethanol	108-01-0	62	330	421	-	400	105	89	-	Flam. Liq. 3 Acute Tox. 4 Acute Tox. 4Skin Corr. 1B Acute Tox. 4	H226 H302 H312 H314 H332	Ja High concentration
Propylene glycol	57-55-6	-	-	-	-	-	-	-	16	Not Classified	-	No
(2-Chloroethyl)dimethylamine	107-99-3	-	19	-	-	<10	-	-	-	Acute Tox. 3 Skin Irrit. 2 Skin Sens. 1	H301 H315 H317	No Low concentration

Unit: mg/kg	Product number											
Substance	CAS no.	024	038	043	048	051	054	056	061	Hazard class	Hazard state- ment	Prioritization
										Eye Irrit. 2 Muta. 2 Carc. 2 Aquatic Chronic 3	H319 H341 H351 H412	
Dimethylformamide	68-12-2	-	60	-	274	24	-	53	94	Acute Tox. 4 Eye Irrit. 2 Acute Tox. 4 Repr. 1B	H312 H319 H332 H360D	Yes Class. High concentration
Xylene	106-42-3	-		17	-	-	-		49	Flam. Liq. 3 Acute Tox. 4 Skin Irrit. 2 Acute Tox. 4	H226 H312 H315 H332	No
Cyclohexanone	108-94-1	89	140	868	896	115	59	241	683	Flam. Liq. 3 Acute Tox. 4	H226 H332	No
1,4-Butanediol	110-63-4	171	-	-	-	-	-	-	-	Acute Tox. 4 STOT SE 3	H302 H336 (Central nervous) (Inhalation)	No
Diethylene glycol	111-46-6	-	44	-	-	29	150	-	-	Acute Tox. 4	H302	No
Bis(2-hydroxypropyl) ether	110-98-5	-	-	-	-	-	36	-	-	-	-	No
Triethylenediamine	280-57-9	119	620	522	1085	606	1198	878	738	Flam. Sol. 1 Acute Tox. 4 Skin Irrit. 2 Eye Dam. 1	H228 H302 H315 H318	Yes High concentration
Bis(2-(Dimethylamino)ethyl) ether	3033-62-3	-	288	-	-	448	-	-	-	Acute Tox. 4 Acute Tox. 3	H302 H311	Yes

Unit: mg/kg	Product number											
Substance	CAS no.	024	038	043	048	051	054	056	061	Hazard class	Hazard state- ment	Prioritization
										Skin Corr. 1B Eye Dam. 1 Acute Tox. 4	H314 H318 H332	High Conc.
Linalool	78-70-6	-	-	-	60	-	40	-	-	Skin Sens. 1B	H317	Yes Class.
2-ethylhexyl acid	149-57-5	17	-	-	-	-	-	24	23	Repr. 2	H361d	Yes Class.
Triethyl phosphate	78-40-0	-	115	111	-	120	-	-	104	Acute Tox. 4	H302	No
Methyl 2-oxocyclopentanecarboxylate	10472-24-9	-	-	-	42	79	49	-	-	-	-	No
Pentamethyldiethylenetriamine	3030-47-5	693	89	558	-	44	-	754	672	Acute Tox. 4 Acute Tox. 3 Skin Corr. 1B	H302 H311 H314	Yes High concentration
Acetic acid, 2-phenylethyl ester	103-45-7	-	-	-	-	-	60	-	-	Eye Dam. 1	H318	No
1,2-Diacetone	102-62-5	-	838	-	191	782	-	-	-	-	-	No No data on class
Triethanolamine	102-71-6	-	-	-	192	-	-	-	-	Not classified	-	No
3-(4-Isopropylphenyl)-2-methylpropionaldehyde	103-95-7	-	-	-	22	-	-	-	-	Skin Irrit. 2 Skin Sens. 1B Aquatic Chronic 3	H315 H317 H412	Yes Class.
BHT (Butylated Hydroxytoluene)	128-37-0	31	-	38	91	-	-	72	23	Aquatic Chronic 1	H410	Yes Endocrine-disrupting
γ-n-Heptylbutyrolactone (peach scent)	104-67-6	-	104	-	-	71	-	-	-	Aquatic Chronic 3	H412	No

Unit: mg/kg	Product number											
Substance	CAS no.	024	038	043	048	051	054	056	061	Hazard class	Hazard state- ment	Prioritization
Diethylphthalate	84-66-2	-	-	1271	761	-	192	-	582	Not Classified	-	No
Triethanolamine borate	283-56-7 + 15277-97-1	-	-	-	165	-	-	-	-	-	-	No Missing class.
Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate	6386-38-5	< 20	-	-	-	-	-	-	-	Aquatic Chronic 2	H411	no
2-(2H-Benzotriazol-2-yl)-5-methylphenol eller Drometrizole	4998-48-5 or 2440-22-4	-	-	-	44	-	-	-	-	Skin Sens 1B Aquatic Chronic 1	H317 H410	Yes Class.
Tri(2-ethylhexyl) phosphite	301-13-3	-	-	67	-	-	-	-	-	-	-	No
4,4'-methylenebis benzenamine	101-77-9	262	136	271	-	133	114		64	Skin Sens. 1 Muta. 2 Carc. 1B STOT SE 1 STOT RE 2 Aquatic Chronic 2	H317 H341 H350 H370 ** H373 ** H411	yes Class.
Octadecenamide	301-02-0	622	853	645	1001	902	507	748	438	-	-	No No data on class.
Bis(2-ethylhexyl) phthalate (DEHP) co-eluted with (probably DINCH)	117-81-7 + (166412-78-8 if DINCH)	-	-	-	-	-	-	-	243	Repr 1B	H360FD	yes class.
2-((2H-benzotriazo)-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol (UV absor- bent)	3147-75-9	-	-	-	42	-	-	-	13	Not classified	-	No
Sucrose Octaacetate	126-14-7	166	-	-	-	-	-	-	-	-	-	No

Unit: mg/kg	Product number											
Substance	CAS no.	024	038	043	048	051	054	056	061	Hazard class	Hazard state- ment	Prioritization
Irganox 1076	2082-79-3	69	61	< 20	-	40	74	<30	< 20	Not classified	-	no

Appendix 4.2 Content substances assessed with relatively large certainty to be the component, but no direct match from library (category 2)

Unit: mg/kg		Product number										
Substance	CAS no.	024	038	043	048	051	054	056	061	Hazard class	Hazard statement	Prioritization according to chapter 4.5
Ethylene glycol and dioxan co-elution	123-91-1	-	-	-	74	-	-	-	-	Flam. Liq. 2 Eye Irrit. 2 STOT SE 3 Carc. 2	H225 H319 H335 H351	Yes (dioxane classification)
Phenol	108-95-2	-	-	10-50	-	-	-	-	-	Acute Tox. 3 Acute Tox. 3* Skin Corr. 1B Acute Tox. 3 Muta. 2 STOT RE 2	H301 H311 H314 H331 H341 H373	yes class
Azodi(isobutyronitrile)	78-67-1	< 10	-	16	-	-	-	-	37	Self-react. C Acute Tox. 4 Acute Tox. 4 Aquatic Chronic 3	H242 H302 H332 H412	no
α-Monoacetine	106-61-6	-	-	-	-	32	-	-	-	-	-	no
4-tert-Butylcyclohexyl acetate	32210-23-4	-	-	-	47	-	19	-	-	Skin Sens. 1B	H317	yes class
γ-n-Amylbutyrolactone	104-61-0	-	-	-	-	-	15	-	-	Not classified	-	No
Ethylene glycol diphenyl ether	104-66-5	-	-	-	-	-	-	-	21	Aquatic Chronic 2	H411	No
Versalide (fragrance)	88-29-9	-	-	-	-	-	138	-	-	-	-	Yes fragrance
Galaxolide (fragrance)	1222-05-5	-	-	-	20-100	174	-	-	-	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	Yes fragrance
Salicylic acid, benzyl ester	118-58-1	-	-	-	-	-	-	-	< 12	Skin Sens. 1B	H317	no

Unit: mg/kg		Product number										
Substance	CAS no.	024	038	043	048	051	054	056	061	Hazard class	Hazard statement	Prioritization according to chapter 4.5
										Eye Irrit. 2 Aquatic Chronic 3	H319 H412	
Tetradecanamide	1120-16-7	< 20	26	26	31	35	-	-	-	-	-	no
Docosane	629-97-0	-	89	-	-	81	95	-	-	-	-	no
Hexadecanamide	629-54-9	28	42	39	62	74	65	57	30	-	-	no
Octadecanamide	124-26-5	122	175	-	206	59	-	28	-	Not classified	-	no
Phosphoric acid, tris(2-ethylhexyl) ester	78-42-2	-	-	63	-	-	-	-	-	Not classified	-	no
p,p-Dioctyldiphenylamine or Benzenamine, 4-(1,1,3,3-tetramethylbutyl)-N-[4-(1,1,3,3-tetramethylbutyl)phenyl]-	101-67-7 or 15721-78-5	-	-	28	-	-	-	-	25	Aquatic Chronic 3 or Not classified	H412 or none	no

Appendix 4.3 Content substances with uncertain assessment of identification. Possibly incorrect ID. May be a similar molecule or other (category 3)

Unit: mg/kg		Product number									
Substance	CAS no.	024	038	043	048	051	054	056	061	Hazard class	Hazard statement
N,N-Diethyl-2-aminoethanol	100-37-8	101	110	40	72	129	190	190	102	-	-
Terpeniol	10482-56-1	-	-	-	21	-	-	-	-	-	-
Tripropylene glycol	1638-16-0	32	47	-	35	47	113	34	35	-	-
γ-Decalactone	706-14-9	-	-	-	-	-	37	-	-	Not classified	-
Tripropylene glycol	1638-16-0	61	71	0,5	57	95	102	67	39	-	-
Tripropylene glycol	1638-16-0	128	77	4	58	115	125	109	40	-	-
Phthalate (possibly DBP)	84-74-2	-	-	-	-	-	30-80	Positive, but unknown amount	Positive, but unknown amount	-	-
4-[Ethyl(phenyl)amino]benzaldehyde	?	14	-	49	16	-	-	-	-	-	-
Glycol compound with possible silane functionality	-	-	28	34	-	21	44	251	18	-	-
Not identified	-	-	-	-	-	305	452		309	-	-
Not identified	-	-	-	-	-	455	-	-	476	-	-
Not identified (amine compound)	-	-	-	-	63	-	-	-	-	-	-
4,4'-methylenebis benzenamine	-	153	103	197	-	75	75	-	91	-	-

Unit: mg/kg		Product number									
Substance	CAS no.	024	038	043	048	051	054	056	061	Hazard class	Hazard statement
Hexadecenamide (unknown db)	-	32	45	39	78	69	31	52	30	-	-
p,p'-Diphenylmethane diisocyanate	101-68-8	-	-	-	-	-	-	Positive, but unknown amount	8,9	-	-
Organophosphorus compound	-	-	-	93	-	-	-	-	-	-	-
Octadecenamide (possibly another db or trans)	301-02-0	102	39	< 124	166	108	83	90	37	-	-
4,8,12,16-tetraoxaeicosan-1-ol (glycol)	?	148	-	-	477	-	-	-	-	-	-
Organophosphorus compound	-	-	-	51	-	-	-	-	-	-	-
Dodecanoic acid, undecyl ester	3658-44-4	-	35	-	-	30	37	-	-	-	-
Not identified (DINCH??)	(166412-78-8 if DINCH)	592	197	193	475	204	179	169	-	-	-
2-[2-Methoxy-5-(1,1,3,3-tetramethylbutyl)phenyl]-2H-benzotriazole	?	90	-	144	41	-	-	-	126	-	-

Appendix 5. Volatile substances from eight whole squishies measured in climatic chamber test

Appendix 5.1 Concentration of carbonyls: C1-C4 aldehydes in air: ISO 16000-3

Unit: $\mu\text{g}/\text{m}^3$		Product number										
Substance	CAS no.	024	038	043*	048	051	054*	056	061	Classification	H-statement	Prioritization according to chapter 4.5
Formaldehyde	50-00-0	11	23	8,2	3,1	19	4,6	9,7	6,6	Carc. 1B Muta. 2 Skin Sens. 1	H350 H341 H317	No Low concentration
Acetaldehyde (ethanal)	75-07-0	6,6	17	27	8,0	30	8,1	18	10	Carc. 2	H351	No Low conc.
Propanale	123-38-6	1,5	6,4	19	1,9	5,3	5,2	3,3	2,0	-	-	No Low conc.
Butanale	123-72-8	1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	-	-	No Low conc.
Acrolein	107-02-8	< 1,0	< 1,0	< 1,0	4,0	< 1,0	< 1,0	< 1,0	< 1,0	Acute tox. 1	H330	No Low conc.
Crotonaldehyde (butenal)	4170-30-3	< 1,7	< 1,7	< 1,7	< 1,7	< 1,7	< 1,7	< 1,7	< 1,7	Muta. 2	H341	No Low conc.

Appendix 5.2 Screening of volatile organic compounds (VOCs) in air: ISO 16000-6

Substance screening on the basis of criteria stated in chapter 5.5

Unit: $\mu\text{g}/\text{m}^3$ as toluene equivalents		Product number										
Substance	CAS no.	024	038	043*	048	051	054*	056	061	Classification	H-statement	Prioritization according to chapter 4.5
Ethanol	000064-17-5	nd		<5					9	Flam. Liq. 2	H225	no
1,1-Dichloro-1-fluoroethane **	001717-00-6		11	25		6	14		23	Aquatic Chronic 3 Ozone 1	H412 H420	no
Ethane, 1,2-dichloro-1-fluoro- **	000430-57-9		23									no
2-Propanone	000067-64-1	<5		67						Flam. Liq. 2 Eye Irrit. 2 STOT SE 3	H225 H319 H336	no
Acetic acid, methyl ester	000079-20-9		42	100			6			Flam. Liq. 2 Eye Irrit. 2 STOT SE 3	H225 H319 H336	no
Methylene chloride	000075-09-2		<5	10			<5			Carc. 2	H351	Yes, class.

Unit: µg/m ³ as toluene equivalents		Product number										
Substance	CAS no.	024	038	043*	048	051	054*	056	061	Classification	H-statement	Prioritization according to chapter 4.5
Ethan, 1,1-dichloro-	000075-34-3			<5						Flam. Liq. 2 Acute Tox. 4 * Eye Irrit. 2 STOT SE 3 Aquatic Chronic 3	H225 H302 H319 H335 H412	no
2-butanon	000078-93-3		230	490	61	34	80		31	Flam. Liq. 2 Eye Irrit. 2 STOT SE 3	H225 H319 H336	no
Ethene, 1,1-dichloro-	000075-35-4			<5						Flam. Liq. 1 Acute Tox. 4 * Carc. 2	H224 H332 H351	No Low concentration
Acetic acid ethyl ester	000141-78-6		<5	53			5			Flam. Liq. 2 Eye Irrit. 2 STOT SE 3	H225 H319 H336	no
Carbonic acid, dimethyl ester	000616-38-6			8		<5			<5	Flam Liq 2	H225	no
Trichloromethane	000067-66-3						<5			Acute Tox. 4 Skin Irrit. 2 Eye Irrit. 2 Acute Tox. 3 Carc. 2 STOT RE 1 Repr. 2	H302 H315 H319 H331 H351 H372 H361d	No Low concentration
1-Propanol, 2-methyl-	000078-83-1			19	<5		8			Flam. Liq. 3 Skin Irrit. 2 Eye Dam. 1 STOT SE 3 STOT SE 3	H226 H315 H318 H335 H336	no
Ethan, 1,2-dichloro-	000107-06-2		1				3			Flam. Liq. 2 Acute Tox. 4 * Skin Irrit. 2 Eye Irrit. 2 STOT SE 3	H225 H302 H315 H319 H335	No Low concentration

Unit: µg/m ³ as toluene equivalents		Product number										
Substance	CAS no.	024	038	043*	048	051	054*	056	061	Classification	H-statement	Prioritization according to chapter 4.5
										Carc. 1B	H350	
Benzene **	000071-43-2		4							Flam. Liq. 2 Skin Irrit. 2 Eye Irrit. 2 Asp. Tox. 1 Muta. 1B Carc. 1A STOT RE 1	H225 H315 H319 H304 H340 H350 H372 **	No Low concentration
1-Butanol	000071-36-3								5	Flam. Liq. 3 Acute Tox. 4 * Skin Irrit. 2 Eye Dam. 1 STOT SE 3 STOT SE 3	H226 H302 H315 H318 H335 H336	No
Ethanamine, N,N-diethyl-	000121-44-8					<5				Flam. Liq. 2 Acute Tox. 4 * Acute Tox. 4 * Skin Corr. 1A Acute Tox. 4 *	H225 H302 H312 H314 H332	No
Heptane	000142-82-5	<5	<5			<5	<5			Flam. Liq. 2 Skin Irrit. 2 Asp. Tox. 1 STOT SE 3 Aquatic Acute 1 Aquatic Chronic 1	H225 H315 H304 H336 H400 H410	No
Propane, 1,2-dichloro-	000078-87-5					3			2	Flam. Liq. 2 Acute Tox. 4 * Acute Tox. 4 * Carc. 1B	H225 H302 H332 H350	No Low concentration
N,N-Dimethylaminoethanol	000108-01-0		816			840			96	Flam. Liq. 3 Acute Tox. 4 * Acute Tox. 4 * Skin Corr. 1B Acute Tox. 4 *	H226 H302 H312 H314 H332	Yes High concentration
Toluene	000108-88-3	8	57	32	7	93	39	11	95	Flam. Liq. 2 Skin Irrit. 2	H225 H315	Yes Class.

Unit: µg/m ³ as toluene equivalents		Product number										
Substance	CAS no.	024	038	043*	048	051	054*	056	061	Classification	H-statement	Prioritization according to chapter 4.5
										Asp. Tox. 1 STOT SE 3 STOT RE 2 * Repr. 2	H304 H336 H373 ** H361d ***	
1,2-Ethanediol	000107-21-1				36					Acute Tox. 4*	H307	no
Formamide, N,N-dimethyl-	000068-12-2	250	710	3700	2000	790	1300	520	210	Acute Tox. 4 * Eye Irrit. 2 Acute Tox. 4 * Repr. 1B	H312 H319 H332 H360D ***	Yes Class.
1,2-Propanediol	000057-55-6						12			Not Classified	-	no
Butanoic acid, ethyl ester	000105-54-4		36			18			10	Flam. Liq. 3	H226	no
Cyclotrisiloxane, hexamethyl-	000541-05-9		11		<5	11	10	5		Flam. Sol. 1	H228	no
Acetic acid, butyl ester	000123-86-4	48								Flam. Liq. 3 STOT SE 3	H226 H336	no
Heptane, 2,4-dimethyl-	002213-23-2					6			9	-	-	no
2-Furancarboxaldehyde	000098-01-1				4					Tox. 3 * Acute Tox. 4 * Skin Irrit. 2 Eye Irrit. 2 Acute Tox. 3 * STOT SE 3 Carc. 2	H301 H312 H315 H319 H331 H335 H351	No Low concentration
2,4-Dimethyl-1-heptene	019549-87-2					<5			6	-	-	no
Benzene, chloro-	000108-90-7		<5				<5			Flam. Liq. 3 Skin Irrit. 2 Acute Tox. 4 Aquatic Chronic 2	H226 H315 H332 H411	no
2-Propanol, 1-(2-propenyloxy)-	021460-36-6				21							no
Piperazine, 1,4-dimethyl-	000106-58-1			63				14		Flam. Liq. 2 Acute Tox. 4 Skin Corr. 1C Eye Dam. 1	H225 H302 H314 H318	no
Benzene, ethyl-	000100-41-4		16	270		34	340	16	28	Flam. Liq. 2 Acute Tox. 4 * Asp. Tox. 1 STOT RE 2	H225 H332 H304 H373 (hear-	Yes Class.

Unit: µg/m ³ as toluene equivalents		Product number										
Substance	CAS no.	024	038	043*	048	051	054*	056	061	Classification	H-statement	Prioritization according to chapter 4.5
											ing organs)	
m-Xylene	000108-38-3	<5	11		7	32	510		12	Flam. Liq. 3 Acute Tox. 4 * Skin Irrit. 2 Acute Tox. 4 *	H226 H312 H315 H332	Yes High concentration
p-Xylene	000106-42-3	<5	25	85	<5	<5	120	35	28	Flam. Liq. 3 Acute Tox. 4 * Skin Irrit. 2 Acute Tox. 4 *	H226 H312 H315 H332	Yes High concentration
Cyclopentanone, 2-methyl-	001120-72-5				<5					-	-	no
Styrene	000100-42-5		40a			20a				Flam. Liq. 3 Skin Irrit. 2 Eye Irrit. 2 Acute Tox. 4 * STOT RE 1 Repr. 2	H226 H315 H319 H332 H372 (hearing organs) H361d	Yes Class.
o-Xylene	000095-47-6		19a	450		14a	460	26		Flam. Liq. 3 Acute Tox. 4 * Skin Irrit. 2 Acute Tox. 4 *	H226 H312 H315 H332	Yes High concentration
Cyclohexanone	000108-94-1	1500	1700	6800	2500	1900	4300	1700	630	Flam. Liq. 3 Acute Tox. 4 *	H226 H332	Yes High concentration
Acetic acid, 2-(dimethylamino)ethyl ester	001421-89-2	5	26			37		5	<5	-	-	no
Acetic acid, pentyl ester	000628-63-7		55			29				Flam Liq 3	H226	no
Alpha-pinene, (-)-	000080-56-8	9	11	9	8	7	10	14	10	Flam. Liq. 3 Acute Tox. 4 Asp. Tox. 1 Skin Irrit. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H226 H302 H304 H315 H317 H400 H410	Yes Class. Odorous substance
Benzene, (1-methylethyl)-	000098-82-8			<5			17			Flam. Liq. 3 Asp. Tox. 1 STOT SE 3	H226 H304 H335	no

Unit: µg/m ³ as toluene equivalents		Product number										
Substance	CAS no.	024	038	043*	048	051	054*	056	061	Classification	H-statement	Prioritization according to chapter 4.5
										Aquatic Chronic 2	H411	
Gamma. Valerolactone	000108-29-2				<5					-	-	no
Benzene, propyl-	000103-65-1					<5	<5		<5	Flam. Liq. 3 Asp. Tox. 1 STOT SE 3 Aquatic Chronic 2	H226 H304 H335 H411	no
Benzene, 1-ethyl-2-methyl-	000611-14-3		<5			<5	<5			-	-	no
2-Cyclopenten-1-one, 3-methyl-	002758-18-1							<5		-	-	no
Benzaldehyde	000100-52-7		6	<5	<5	7	<5		9	Acute Tox. 4 *	H302	no
Mesitylene **	000108-67-8								<5	Flam. Liq. 3 STOT SE 3 Aquatic Chronic 2	H226 H335 H411	no
Benzene, 1,2,3-trimethyl- **	000526-73-8					<5				-	-	no
Cyclotetrasiloxane, octamethyl-	000556-67-2		6	15	1	4	7	2		Aquatic Chronic 4 Repr. 2	H413 H361f ***	Yes Class.
2-Beta-pinene	000127-91-3	<5	<5		<5	<5	<5	<5	<5	Flam. Liq. 3 Asp. Tox. 1 Skin Irrit. 2 Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 1	H226 H304 H315 H317 H400 H410	Yes Class.
Propylene Carbonate	000108-32-7				<5					Eye Irrit. 2	H319	no
Benzene, 1,2,4-trimethyl- **	000095-63-6								5	0	0	no
Benzene, 1,2,3-trimethyl- **	000526-73-8		<5			5				-	-	no
Hexanoic acid, ethyl ester	000123-66-0				<5					Flam. Liq. 3 Skin Irrit. 2	H226 H315	no
Benzene, 1,3,5-trimethyl- **	000108-67-8						<5			Flam. Liq. 3 STOT SE 3 Aquatic Chronic 2	H226 H335 H411	no
Decan	000124-18-5		<5	<5		<5	<5	<5	<5	Flam. Liq. 3 Asp. Tox. 1	H226 H304	no
Dipropylene glycol monomethyl ether isomer	000000-00-0	14										no
3-Hexen-1-ol, acetate, (Z)-	003681-71-8								13	Flam. Liq. 3	H226	no
Delta 3-Carene	013466-78-9	9		9	9		19	11		Flam. Liq. 3 Asp. Tox. 1	H226 H304	Yes Class.

Unit: µg/m ³ as toluene equivalents		Product number										
Substance	CAS no.	024	038	043*	048	051	054*	056	061	Classification	H-statement	Prioritization according to chapter 4.5
										Skin Irrit. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H315 H317 H400 H410	Odorous substance
Acetic acid, hexyl ester	000142-92-7					36				Flam. Liq. 3	H226	no
Phenol	000108-95-2	1		10						Acute Tox. 3 * Acute Tox. 3 * Skin Corr. 1B Acute Tox. 3 * Muta. 2 STOT RE 2 *	H301 H311 H314 H331 H341 H373 **	Yes Class.
Triethylenediamine	000280-57-9	480	700	810	870	920	890	780	770	Flam. Sol. 1 Acute Tox. 4 Skin Irrit. 2 Eye Dam. 1	H228 H302 H315 H318	Yes High concentration
Propanenitril, 2,2'-azobis[2-methyl-]	000078-67-1	27								Self-react. C Acute Tox. 4 * Acute Tox. 4 * Aquatic Chronic 3	H242 H302 H332 H412	no
Tetramethylsuccinonitril	003333-52-6						88			-	-	no
D-Limonene	005989-27-5	<5	9	5	6	28	<5	<5	52	Liq. 3 Skin Irrit. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H226 H315 H317 H400 H410	Yes Class. Odorous substance
Triethylenediamine	000280-57-9		<5							Flam. Sol. 1 Acute Tox. 4 Skin Irrit. 2 Eye Dam. 1	H228 H302 H315 H318	no
Benzyl alcohol	000100-51-6					<5			5	Acute Tox. 4 * Acute Tox. 4 *	H302 H332	no
Butanoic acid, 3-methyl-, butyl ester	000109-19-3								27	-	-	no
2(3H)-Furanone, 5-ethyldihydro-	000695-06-7		21				10			-	-	no
Naphthalene, decahydro-, trans-	000493-02-7		<5					<5		-	-	no
1,4-Dioxaspiro[4.5]decane	000177-10-6			35						-	-	no

Unit: µg/m ³ as toluene equivalents		Product number										
Substance	CAS no.	024	038	043*	048	051	054*	056	061	Classification	H-statement	Prioritization according to chapter 4.5
9-Methylbicyclo[3.3.1]nonane	025107-01-1							<5		-	-	no
Hexanoic acid, 2-propenyl ester	000123-68-2		190		9				15	Acute Tox. 3 Acute Tox. 3 Acute Tox. 3 Aquatic Acute 1 Aquatic Chronic 3	H301 H311 H331 H400 H412	Yes Class.
Cyclopropane, 1,2-dibutyl-	041977-32-6		<5							-	-	no
Undecane	001120-21-4			<5					7	Asp. Tox. 1	H304	no
Bis(2-(Dimethylamino)ethyl) ether	003033-62-3		310		15	440				Acute Tox. 4 Acute Tox. 3 Skin Corr. 1B Eye Dam. 1 Acute Tox. 4	H302 H311 H314 H318 H332	Yes High concentration
L-Linalool	000078-70-6		84		16	68			47	Skin Sens. 1B	H317	Yes Class. Odorous substance
Nonanal	000124-19-6			<5						Aquatic Chronic 3	H412	no
Benzene, 1-bromo-2-chloro-	000694-80-4	<5								-	-	no
Triethyl phosphate	000078-40-0		100	100		78	84			Acute Tox. 4 *	H302	no
Cyclopentasiloxane, decamethyl-	000541-02-6	<5	29	11	<5	10	<5	<5	<5	Not Classified	-	no
1H-Pyrazole, 4,5-dihydro-5,5-dimethyl-4-isopropylidene-	106251-09-6	12								-	-	no
Naphthalene, decahydro-2-methyl-	002958-76-1		10							-	-	no
Propylene glycol trimer	000000-00-0								7			no
2-Propanol, 1-[1-methyl-2-(2-propenyloxy)ethoxy]-	055956-25-7		50	62	36	40		33				no
1,2-Ethanediamine, N-[2-(dimethylamino)ethyl]-N,N',N'-trimethyl-	003030-47-5	580	150	230	<5	120	250	200		Acute Tox. 4 * Acute Tox. 3 * Skin Corr. 1B	H302 H311 H314	Yes Class High concentration
Benzoic acid, ethyl ester	000093-89-0			<5		<5	<5			Registrering 1: Not Classified Registrering 2: Skin Irrit. 2 Eye Irrit. 2	H315 H319	no
Naphthalene, decahydro-2,3-dimethyl- **	001008-80-6								<5	-	-	no

Unit: µg/m ³ as toluene equivalents		Product number										
Substance	CAS no.	024	038	043*	048	051	054*	056	061	Classification	H-statement	Prioritization according to chapter 4.5
Naphthalene, decahydro-1,6-dimethyl- **	001750-51-2		11			<5				-	-	no
Dodecane	000112-40-3	<5	72	11	<5	19	18		10	Asp. Tox. 1	H304	no
Naphthalene, decahydro-2,6-dimethyl- **	001618-22-0		7			<5				-	-	no
Benzene, ethyl-1,2,4-trimethyl-	054120-62-6						<5			-	-	no
cis, cis-3-Ethylbicyclo[4.4.0]decane	066660-42-2		5			<5				-	-	no
Acetic acid, 2-phenylethyl ester	000103-45-7								14	Eye Dam. 1	H318	no
Caprolactam	000105-60-2							<5	<5	Acute Tox. 4 * Skin Irrit. 2 Eye Irrit. 2 Acute Tox. 4 * STOT SE 3	H302 H315 H319 H332 H335	no
Cyclohexasiloxane, dodecamethyl-	000540-97-6			6		<5	<5	<5	<5	Not Classified	-	no
Anethol	000104-46-1			18			6			-	-	no
Tridecane	000629-50-5		17	<5	<5	9	5		<5	Asp. Tox 1	H304	no
Naphthalene, 2-methyl- **	000091-57-6					<5	8			-	-	no
Naphthalene, 1-methyl- **	000090-12-0					<5	<5			-	-	no
Triacetin	000102-76-1		47			57				Not Classified	-	no
Tetradecane	000629-59-4		<5	<5	<5	<5	<5		<5	Asp. Tox 1	H304	no
Isolongifolene	001135-66-6			<5						Registrering 1: Aquatic Acute 1 Registrering 2: Not Classified	H400	no
Naphthalene, 1,7-dimethyl- **	000575-37-1						<5			-	-	no
2(3H)-Furanone, 5-hexyldihydro-	000706-14-9		<5			<5			<5	Not Classified	-	no
trans-.beta.-Ionone	000079-77-6		5			6				Aquatic Acute 2 Aquatic Chronic 2	H401 H411	no
pentadecane	000629-62-9				<5					Asp. Tox 1	H304	no
Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-	000128-37-0	6	<5	8	11	<5	<5	6		Aquatic Chronic 1	H410	no
Diethyl Phthalate	000084-66-2			36			9			Not Classified	-	no
Benzophenone	000119-61-9	<5								STOT RE 2 Aquatic Chronic 3	H373 (liver, kidney) (Oral) H412	No Low concentration
1,2-Benzenedicarboxylic acid,	000084-69-5		2							Repr. 1B	H360Df	No

Unit: µg/m ³ as toluene equivalents		Product number										
Substance	CAS no.	024	038	043*	048	051	054*	056	061	Classification	H-statement	Prioritization according to chapter 4.5
bis(2-methylpropyl) ester												Low concentration
Phenol, 4-(1-methyl-1-phenylethyl)-	000599-64-4		<5							Acute Tox. 4 Eye Dam. 1 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H302 H318 H373 (Kidney) H400 H410	No Low concentration
Sum of unidentified VVOC	Rt up to C6	10	24	19	25	33		16	20			
Sum of unidentified VOC	Rt C6 to C16	150	540	470	58	280	170	490	180			
Sum of unidentified SVOC	Rt after C16						2					
Sum of all measured VVOC*	Rt up to C6	10	100	220	25	38	20	16	53			
Sum of all measured VOC*	Rt C6 to C16	3100	6200	10000	5700	6000	8800	3800	2300			
Sum of all measured SVOC*	Rt after C16		2	36			11					
TVOC (Toluene equivalents)		3100	6200	10000	5700	6000	8800	3800	2300			

* Calibrated compounds + Toluene equivalents of unidentified

** Must be quantified

*** Toluene equivalents

□ Styrene + o-xylene coeludes

Prioritization according to chapter 4.5.

Yes: prioritized either due to classification and/or high concentration

No: not prioritized either due to missing classification, low concentration or relevant classification, but low concentration.

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Appendix 6. Substances quantified in emission test

Appendix 6.1 Fragrances

Name	CAS no.
Phenylacetaldehyde	122-78-1
Citral (Neral isomer) 1	5392-40-5
Citral (Geranial isomer) 2	5392-40-5
Cinnamal	104-55-2
3,7-Dimethyl-7-Hydroxyoctanal	107-75-5
Lilial	80-54-6
alpha-Amylcinnamaldehyde isomer 1	122-40-7
alpha-Amylcinnamaldehyde isomer 2	122-40-7
Lyr al isomer 1	31906-04-4
Lyr al isomer 2	31906-04-4
alpha-Hexylcinnamaldehyde isomer 1	101-86-0
alpha-Hexylcinnamaldehyde isomer 2	101-86-0
Benzyl alcohol	100-51-6
Linalool	78-70-6
Citronellol	106-22-99
Geraniol	106-24-1
4-Methoxybenzyl alcohol	105-13-5
Cinnamyl alcohol	104-54-1
Eugenol	97-53-0
Methyl eugenol	93-15-2
Isoeugenol	97-54-1
alpha-Amylcinnamic Alcohol	101-85-9
Farnesol isomer 1	4602-84-0
Farnesol isomer 2	4602-84-0
d-Limonene	5989-27-5
1,8-Cineole	470-82-6
Camphor	76-22-2
Methyl 2-octynoate	111-12-6
4-Allylanisole(Estragole)	140-67-0
Safrole	94-59-7
Methyl 2-nonyate	111-80-8
Coumarin	91-64-5
iso-alpha-Methylinone	127-51-5
Benzyl benzoate	120-51-4

Benzyl salicylate	118-58-1
Benzyl Cinnamate	103-41-3

Appendix 6.2 Other substances quantified in emission test

Name	CAS
Methylene chloride #	75-09-2
Ethane, 1,2-dichloro- #	107-06-2
Benzene ? (must be quantified) #	71-43-2
Propane, 1,2-dichloro- #	78-87-5
N,N-Dimethylaminoethanol #	108-01-0
Toluene #	108-88-3
Formamide, N,N-dimethyl- #	68-12-2
Benzene, ethyl- #	100-41-4
m-Xylene #	108-38-3
p-Xylene #	106-42-3
Styrene #	100-42-5
o-Xylene #	95-47-6
Cyclohexanone #	108-94-1
Alpha-pinene #	80-56-8
Cyclotetrasiloxane, octamethyl- #	556-67-2
2-Beta-pinene #	127-91-3
Delta 3-Carene #	13466-78-9
Phenol #	108-95-2
Triethylenediamine #	280-57-9
D-Limonene #	5989-27-5
Bis(2-(Dimethylamino)ethyl) ether #	3033-62-3
L-Linalool	78-70-6
1,2-Ethanediamine, N-[2-(dimethylamino)ethyl]-N,N',N'-trimethyl- #	3030-47-5

Appendix 7. Calculation of DNEL values

N,N-Dimethylaminoethanol

In the REACH registration for the substance, the following relevant data for DNEL release has been given: in an inhalation test rats were exposed to vapors of N,N-dimethylaminoethanol at concentrations 0; 29; 88 and 277 mg/m³. Animals were exposed for 6h/day 5 days per week for 13 weeks. NOAEC was 29 mg/m³. Cornea opacity was observed at the medium exposure level, and mucous membrane irritation and damage in mucous membrane cells in the respiratory tract were observed at the highest exposure level.

For consumers, a DNEL value is calculated at continuous exposure:
NOAEC is converted to 24h = 29 mg/m³ x 6/24 x 5/7 = 5.2 mg/m³

DNEL = NOAEC / (AF1 x AF2 x AF3 x AF4)

AF1 (interspecies animal to human) = 2,5 (for local irritation effects)

AF2 (intraspecies, difference in human sensitivity) = 10

AF3 (duration of experiments, subchronic to chronic exposure) = 2

DNEL = 5.2 mg/m³ / (2.5 x 10 x 2) = **0.1 mg/m³**

Triethylenediamine

In the REACH-registration for the substance, the following data for DNEL release have been given: in an inhalation experiment rats were exposed to aerosols of triethylenediamine at concentrations 0; 0.0058; 0.063 or 0.62 mg/L. Animals were exposed 6h/day 5 days per week for 4 weeks. NOAEC was 0.006 mg/L corresponding to 6 mg/m³, since at higher concentrations the irritation of respiratory tract, hazardous effects and wounds on the mucous membrane and pneumonia occurred.

For consumers, a DNEL value is calculated at continuous exposure:
NOAEC konverteres til 24h = 6 mg/m³ x 6/24 x 5/7 = 1.1 mg/m³

DNEL = NOAEC / (AF1 x AF2 x AF3 x AF4)

AF1 (interspecies animal to human) = 2.5 (for local irritation effects)

AF2 (intraspecies, difference in human sensitivity) = 10

AF3 (duration of experiments, subchronic to chronic exposure) = 6

DNEL = 1.1 mg/m³ / (2.5 x 10 x 6) = **0.007 mg/m³**

Exposure to vapors must be expected to be less critical than for aerosols, which is why this DNEL value may be seen as relatively restrictive, as it is based on effects achieved after exposure to aerosols. Further, the large difference between the exposure levels applied results in uncertainty regarding the NOAEC value.

Bis(2-(dimethylamino)ethyl)ether; N,N,N',N'-tetramethyl-2,2'-oxybis(ethylamine)

In the REACH-registration for the substance, the following data for DNEL release have been given: in an inhalation experiment rats were exposed to vapors of bis(2-(dimethylamino)ethyl)ether at concentrations 0; 1.5; 8.2 and 38 mg/m³. Animals were exposed 6h/day 5 days per week for 14 weeks. LOAEC was 1.5 mg/m³, since at this concentration signs of irritation in eyes and respiratory tract occurred.

For consumers, a DNEL value is calculated at continuous exposure:
LOAEC is converted 24h = 1.5 mg/m³ × 6/24 × 5/7 = 0.27 mg/m³

$$\text{DNEL} = \text{LOAEC} / (\text{AF1} \times \text{AF2} \times \text{AF3} \times \text{AF4})$$

AF1 (interspecies animal to human) = 2,5 (for local irritation effects)

AF2 (intraspecies, difference in human sensitivity) = 10

AF3 (duration of experiments, subchronic to chronic exposure) = 2

AF4 (LOAEC to NOAEC extrapolation) = 3

$$\text{DNEL} = 0.27 \text{ mg/m}^3 / (2.5 \times 10 \times 2 \times 3) = \mathbf{0.002 \text{ mg/m}^3}$$

1,1,4,7,7-Pentamethyldiethylenetriamine; bis(2-dimethylaminoethyl) (methyl)amine

In the REACH-registration for the substance, the following data for DNEL release have been given: in an inhalation experiment rats were exposed to vapors of 1,1,4,7,7-pentamethyldiethylenetriamine at concentrations 0; 21; 85; 340 mg/m³. Animals were exposed 6h/day, 5 days per week in 2 weeks. LOAEC was 21 mg/m³, since at this concentration irritation of and effect on the mucous membrane cells in the respiratory tract occurred.

For consumers, a DNEL value is calculated at continuous exposure:
LOAEC is converted to 24h = 21 mg/m³ × 6/24 × 5/7 = 3.75 mg/m³

$$\text{DNEL} = \text{LOAEC} / (\text{AF1} \times \text{AF2} \times \text{AF3} \times \text{AF4})$$

AF1 (interspecies animal to human) = 2.5 (for local irritation effects)

AF2 (intraspecies, difference in human sensitivity) = 10

AF3 (duration of experiments, subchronic to chronic exposure) = 6

AF4 (LOAEC to NOAEC extrapolation) = 3

$$\text{DNEL} = 3.75 \text{ mg/m}^3 / (2.5 \times 10 \times 6 \times 3) = \mathbf{0.008 \text{ mg/m}^3} [\text{Tekst}]$$

Analysis and risk assessment of fragrances and other organic substances in squishy toys

The aim of this project has been to investigate whether it is possible to detect the contents of fragrances and other organic substances, which can be hazardous to children, in squishy toys. In the project, a total of 12 squishies were analyzed. Screening analyses of content as well as emission showed several problematic substances, such as N,N-dimethylformamide and various fragrances and amines. Quantitative analysis was performed on selected substances by emission after 1 hour and 3 days after unpacking the squishy from packaging and by migration test to artificial sweat. Migration of selected problematic substances was not detected, while emission analysis once again showed high concentrations of some problematic substances both after 1 hour and 3 days. The analysis results for emission have been used for the assessment of health-related risks of selected substances if a child uses these products in two scenarios. In an exposure scenario, where a young child lies down and sleeps while hugging a squishy, the measurements performed 1 hour after unpacking the toy showed unacceptably high levels of the substances N,N-dimethylformamide, triethylenediamine and cyclohexanone in all 12 squishies. In a scenario, where several squishies are kept in a child's room, also unacceptably levels of substances N,N-dimethylformamide, N,N-dimethylaminoethanol, triethylenediamine, bis(2-(dimethylamino)ethyl)ether, 1,1,4,7,7-pentamethyldiethylenetriamine and cyclohexanone were detected in the child's room after 3 days. In case of skin contact, the skin will also be exposed to the emitted substances, which is why a risk for skin irritation cannot be excluded. At the same time, the risk of skin allergy cannot be excluded either due to emission of skin allergy-causing substances that emitted in lower volumes, but which can still, cause the development of skin allergy in case of recurrent skin contact.



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**Risk Management Scope
for
Alcohols Group
Methanol, 1-Butanol,
and Benzenemethanol (Benzyl Alcohol)
Chemical Abstracts Service Registry Numbers
(CAS RNs):
67-56-1, 71-36-3, and 100-51-6**

Environment and Climate Change Canada

Health Canada

March 2022

Summary of Proposed Risk Management

This document outlines the risk management options under consideration for methanol, 1-butanol, and benzenemethanol (hereinafter referred to as benzyl alcohol), included in the Alcohols Group of the Chemicals Management Plan (CMP). In particular, the Government of Canada is considering:

1. Methanol:
 - Regulatory or non-regulatory action to help reduce inhalation exposure to methanol from paint/varnish remover products available to consumers to levels that are protective of human health.
2. 1-Butanol:
 - Regulatory or non-regulatory actions to help reduce inhalation exposure to 1-butanol from lacquer products available to consumers to levels that are protective of human health. Actions may aim to lower the concentration of 1-butanol in lacquer products to 1%.
3. Benzyl alcohol:
 - Measures to help reduce dermal exposures to benzyl alcohol from certain natural health products and non-prescription drugs by revising the listing for benzyl alcohol in the Natural Health Products Ingredients Database (NHPID); and
 - Measures to help reduce dermal exposures to benzyl alcohol from certain cosmetics by describing benzyl alcohol as a prohibited or restricted ingredient on Health Canada's Cosmetic Ingredient Hotlist.

Information on the following items should be provided (on or before May 11, 2022, to the contact details identified in section 8 of this document, to inform risk management decision-making:

- Current quantities and concentrations of methanol used in paint/varnish remover products that are available to consumers;
- Potential alternative substances to methanol for use in paint/varnish remover products available to consumers;

- Current quantities and concentrations of 1-butanol used in lacquer products that are available to consumers;
- Potential alternative substances to 1-butanol for use in lacquer products available to consumers;
- Current concentrations of benzyl alcohol in sunscreens, body moisturizers/creams, deodorants, and face creams;
- Potential alternative substances to benzyl alcohol for use in sunscreens, body moisturizers/creams, deodorants, and face creams; and
- Socio-economic and technical impacts and benefits associated with the proposed risk management for methanol, 1-butanol, and benzyl alcohol.

The risk management options outlined in this risk management scope may evolve through consideration of assessments and risk management options published for other CMP substances as required to ensure effective, coordinated, and consistent risk management decision-making.

Note: The above summary is an abridged list of options under consideration to manage this substance and to seek information on identified information gaps and uncertainties. Refer to section 3 of this document for more complete details in this regard.

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1. Context

The *Canadian Environmental Protection Act, 1999* (CEPA) (Canada 1999) provides the authority for the Minister of the Environment and the Minister of Health (the ministers) to conduct assessments to determine if substances are harmful or dangerous to the environment or human health as set out in section 64 of CEPA^{1,2}; and if so, to manage the associated risks.

The substances methanol, 1-butanol, and benzenemethanol (hereinafter referred to as benzyl alcohol), Chemical Abstracts Service Registry Numbers (CAS RNs)³ 67-56-1, 71-36-3, and 100-51-6, respectively, are included in the Alcohols Group of the Chemicals Management Plan (CMP; Canada 2022).

2. Issue

2.1 Draft Screening Assessment Conclusion

Health Canada and Environment and Climate Change Canada conducted a joint scientific assessment relevant to the evaluation of 21 substances that are part of the Alcohols Group. A notice summarizing the scientific considerations of the draft screening assessment for these 21 substances was published in the *Canada Gazette*, Part I, on March 12, 2022 (Canada 2022). Based on the information available, the draft screening assessment proposes that methanol, 1-butanol, and benzyl alcohol are toxic under section 64(c) of CEPA because they

¹ Section 64 [of CEPA]: *For the purposes of [Parts 5 and 6 of CEPA], except where the expression “inherently toxic” appears, a substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions that*

- (a) have or may have an immediate or long-term harmful effect on the environment or its biological diversity;*
- (b) constitute or may constitute a danger to the environment on which life depends; or*
- (c) constitute or may constitute a danger in Canada to human life or health.*

² A determination of whether one or more of the criteria of section 64 of CEPA are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposures in the general environment. For humans, this includes, but is not limited to, exposures from ambient and indoor air, drinking water, foodstuffs, products used by consumers. A conclusion under CEPA is not relevant to, nor does it preclude, an assessment against the hazard criteria specified in the *Hazardous Products Regulations*, which are part of the regulatory framework for the Workplace Hazardous Materials Information System for products intended for workplace use. Similarly, a conclusion based on the criteria contained in section 64 of CEPA 1999 does not preclude actions being taken under other sections of CEPA or other Acts.

³ Chemical Abstracts Service Registry Number (CAS RN). The Chemical Abstracts Service information is the property of the American Chemical Society and any use or redistribution, except as required in supporting regulatory requirements and/or for reports to the Government of Canada when the information and the reports are required by law or administrative policy, is not permitted without the prior, written permission of the American Chemical Society.

may be entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health (Canada 2022).

It is proposed that methanol, 1-butanol, and benzyl alcohol are not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity, or that constitute or may constitute a danger to the environment on which life depends under paragraphs 64(a) or (b) of CEPA, respectively (Canada 2022). The draft screening assessment proposes that the other 18 substances in the Alcohols Group do not meet any of the criteria set out in section 64 of CEPA.

The draft screening assessment also proposes that methanol meets the persistence, but not the bioaccumulation criteria, and that 1-butanol and benzyl alcohol do not meet the criteria for persistence or bioaccumulation, as defined in the *Persistence and Bioaccumulation Regulations* made under CEPA (Canada 2000).

According to the draft screening assessment, the exposure sources of concern for the general population to methanol and 1-butanol are based on the release of methanol and 1-butanol into air from certain do-it-yourself (DIY) products (e.g., paint/varnish removers and lacquers, respectively) and dermal exposure to benzyl alcohol from certain cosmetics (e.g., body creams/moisturizers, deodorants/antiperspirants, and face creams), natural health products and non-prescription drugs (e.g., sunscreens). As such, this document will focus on these specific exposure sources of concern (refer to section 5).

Although one substance, 2-propanol, 1,3-dichloro (common name 1,3-dichloro, 2-propanol or 1,3-DCP) (CAS RN 96-23-1), is not considered to be harmful to human health or the environment at current levels of exposure, this substance is associated with health effects of concern on the basis of its International Agency for Research on Cancer (IARC) carcinogenic to humans Group 2B designation. There may be a potential risk to human health if use patterns were to change. For this reason, follow-up activities to track changes in exposure and/or commercial use patterns for 1,3-DCP are being considered.

Of note, the proposed risk management options described in this document and the proposed conclusion outlined in the draft screening assessment are preliminary and may be subject to change. For further information, refer to the [draft screening assessment for the Alcohols Group](#).

2.2 Proposed Recommendation Under CEPA

Based on the findings of the draft screening assessment conducted pursuant to CEPA, the ministers propose to recommend that methanol, 1-butanol, and benzyl alcohol be added to Schedule 1 of the Act⁴.

The ministers will take into consideration comments made by stakeholders during the 60-day public comment period on the draft screening assessment and risk management scope. If the ministers finalize the recommendation to add these substances to Schedule 1 of CEPA, risk management instruments must be proposed and finalized within a set period of time, as outlined in sections 91 and 92 of CEPA (refer to section 8 for publication timelines applicable to this group of substances).

3. Proposed Risk Management

3.1 Proposed Human Health Objective

Proposed human health objectives are quantitative or qualitative statements of what should be achieved to address human health concerns.

The proposed human health objective for methanol, 1-butanol, and benzyl alcohol is to reduce exposure of the general population to these substances to levels that are protective of human health.

⁴ When a substance is found to meet one or more of the criteria under section 64 of CEPA, the ministers can propose to take no further action with respect to the substance, add the substance to the Priority Substances List for further assessment, or recommend the addition of the substance to the List of Toxic Substances in Schedule 1 of the Act.

3.2 Proposed Risk Management Objective and Options under Consideration

Proposed risk management objectives set quantitative or qualitative targets to be achieved by the implementation of risk management regulations, instrument(s) and/or tool(s) for a given substance or substances. In this case, the proposed risk management objective is to reduce inhalation exposure of the general population to methanol and 1-butanol in paint/varnish removers and lacquers, respectively, and to reduce dermal exposure of the general population to benzyl alcohol in sunscreens and certain other leave-on natural health products, non-prescription drugs, and cosmetics.

To achieve the proposed risk management objective and to work towards achieving the proposed human health objective, the risk management options under consideration are:

1. Methanol:
 - Regulatory or non-regulatory action to help reduce inhalation exposure to methanol from paint/varnish remover products available to consumers to levels that are protective of human health.
2. 1-Butanol:
 - Regulatory or non-regulatory actions to help reduce inhalation exposure to 1-butanol from lacquer products available to consumers to levels that are protective of human health. Actions may aim to lower the concentration of 1-butanol in lacquer products to 1%.
3. Benzyl alcohol:
 - (1) Revising the listing for benzyl alcohol in the NHPID by lowering the maximum allowable concentration in leave-on products containing benzyl alcohol as a non-medicinal ingredient from 3 to 1% in order to help reduce dermal exposures to benzyl alcohol from sunscreens and certain other leave-on natural health products and non-prescription drugs; and
 - (2) Measures to help reduce dermal exposures to benzyl alcohol from certain cosmetics by describing benzyl alcohol as a prohibited or restricted ingredient on Health Canada's Cosmetic Ingredient Hotlist. The Hotlist is used to communicate that certain substances may not be compliant with requirements of the *Food and Drugs Act* or provisions of the *Cosmetic Regulations*.

Following the publication of this risk management scope, additional information obtained from the public comment period and from other sources will be

considered, along with the information presented in this document, in the instrument selection and development process⁵. The risk management options outlined in this document may evolve through consideration of assessments and risk management options published for other CMP substances to ensure effective, coordinated, and consistent risk management decision-making.

3.3 Risk Management Information Gaps

In order to make informed decisions on proposed risk management, more information is needed on the following:

- Current quantities and concentrations of methanol used in paint/varnish remover products that are available to consumers;
- Potential alternative substances to methanol for use in paint/varnish remover products available to consumers;
- Current quantities and concentrations of 1-butanol used in lacquer products that are available to consumers;
- Potential alternative substances to 1-butanol for use in lacquer products available to consumers;
- Current concentrations of benzyl alcohol in sunscreens, body moisturizers/creams, deodorants, and face creams;
- Potential alternative substances to benzyl alcohol for use in sunscreens, body moisturizers/creams, deodorants, and face creams; and
- Socio-economic and technical impacts and benefits associated with the proposed risk management for methanol, 1-butanol, and benzyl alcohol.

3.4 Performance Measurement and Evaluation

Performance measurement evaluates the ongoing effectiveness and relevance of the actions taken to manage risks from toxic substances⁶. The aim is to determine whether human health and/or environmental objectives have been met and whether there is a need to revisit the risk management approach for that substance to ensure that risks are managed effectively over time. To achieve this, the Government of Canada will review the effectiveness of the risk management action(s) for methanol, 1-butanol, and benzyl alcohol.

The Government of Canada plans to measure the effectiveness of the risk management action(s) by collecting and analyzing data to measure progress towards meeting the risk management objective(s).

⁵ The proposed risk management regulation(s), instrument(s) or tool(s) will be selected using a thorough, consistent and efficient approach and take into consideration available information in line with the Government of Canada's Cabinet Directive on Regulatory Management (TBS 2012a), Red Tape Reduction Action Plan (TBS 2012b) and the Red Tape Reduction Act (Canada, 2015).

⁶ Performance measurement strategy available online at <https://www.canada.ca/en/environment-climate-change/services/evaluating-existing-substances/performance-measurement-evaluation-strategy.html>

The results of performance measurement and evaluation will be used to inform whether further risk management action is warranted and will be made available to Canadians along with recommendations for further action, if applicable.

4. Background

Methanol is a naturally occurring substance that is commonly produced in anaerobic environments by bacteria. However, production of methanol is mainly from anthropogenic sources. Canada is estimated to produce 600 000 - 700 000 tonnes of methanol and import 250 000 tonnes annually. It is predominantly used to produce formaldehyde. It is also used in automotive products, cosmetics, natural health products, non-prescription and prescription drugs, cleaning agents, adhesives, and paint/varnish remover products. It is a permitted food additive and may be used as a component in the manufacture of certain food packaging materials, such as polymer-based materials and printing inks.

1-Butanol occurs in nature through fermentation processes, but consumers are largely exposed to the substance from anthropogenic sources. It is used in cosmetics, as a non-medicinal ingredient (NMI) in natural health products, non-prescription and prescription drugs, and several consumer products such as cleaning agents, lacquers, automotive care products, epoxy adhesives and as a solvent in paint and ink products. It may also be used as a component in the manufacture of food packaging materials and as a food flavouring agent. From a survey issued pursuant to section 71 of CEPA, there was no reported manufacture of 1-butanol and 68 000 kg of 1-butanol were imported in Canada in 2011 (Environment Canada 2013).

Benzyl alcohol is found in nature (e.g., derived from plants), but consumer exposure to the substance is mainly from anthropogenic sources. Based on information submitted in response to a CEPA section 71 survey, 5 000 kg of benzyl alcohol was manufactured in and 735 000 kg imported to Canada in 2011 (Environment Canada 2013). This substance is found in a wide variety of products available to consumers such as household cleaning products, automotive products, and home improvement products, as well as in cosmetics, as an NMI in natural health products, non-prescription and prescription drugs. Benzyl alcohol is also a permitted food additive, and may be used as a food flavouring agent.

5. Exposure Sources and Identified Risks

5.1 Methanol

The draft screening assessment considered the possible risks from long-term exposure to indoor air containing methanol. There were no health risks from long-term inhalation exposures from indoor or outdoor air as determined by the levels of methanol obtained by general air monitoring, which were lower than the reference concentration (RfC) for inhalation of methanol of 20 mg/m³ based on developmental effects derived by the United States Environmental Protection Agency (US EPA). There were also no health risks from oral exposures (e.g., from food, uses in natural health products, non-prescription and prescription drugs) or dermal exposures (e.g., from cosmetics, natural health products, and non-prescription and prescription drugs).

However, the assessment did identify short-term inhalation exposure scenarios associated with risks to human health for methanol. These scenarios considered exposures to paint/varnish remover products available to consumers containing 10 or 35% methanol concentrations for small (≤ 500 g of product) or large (> 4500 g of product) projects. These scenarios resulted in estimated 24-hour average concentrations of methanol from 37 to 72 mg/m³ from use of paint remover with 35% methanol in small projects or 92 to 611 mg/m³ for larger projects with products containing 10 to 35% methanol, respectively. The estimated mean concentration of methanol for bathtub resurfacing in a bathroom ranged from 130 to 458 mg/m³ for 10 and 35% methanol containing products, respectively. All of these exposure estimates exceeded the RfC of 20 mg/m³ based on developmental effects derived by the US EPA and were therefore identified as a concern for human health.

5.2 1-Butanol

The draft screening assessment considered the possible risks from long-term exposure to indoor air containing 1-butanol from all household sources (including cosmetics, natural health products, household cleaning products and other home use products which may lead to the release of 1-butanol in air). There were no health risks from long-term inhalation exposures from indoor air as determined by the levels of 1-butanol obtained by general indoor air monitoring. There were also no health risks from oral exposures (e.g., from food, natural health products, prescription and non-prescription drugs) or dermal exposures (e.g., cosmetics, natural health products, and prescription and non-prescription drugs).

The assessment, however, did identify one short-term inhalation exposure scenario associated with neurodevelopmental and developmental risk to human health for 1-butanol. This scenario considered inhalation exposure to lacquer products available to consumers that are used by adults in the home. Estimated

inhalation exposures were compared to a lowest-observed-adverse-effect level (LOAEL) for developmental effects and the resulting margin of exposure (MOE) was considered potentially inadequate.

5.3 Benzyl alcohol

The assessment considered possible risks to Canadians from the environment and food, but concluded that potential risk, if any, from exposure to these sources would be less than that from products available to the consumer since benzyl alcohol exposure via environmental media is expected to be less than its exposure from products available to consumers. There were no health risks from dermal or inhalation exposures due to the use of cleaning products or air fresheners. The assessment did identify health risks from dermal exposure to sunscreens, body creams/moisturizers, deodorants/antiperspirants, and face creams.

Chronic dermal exposure scenarios were considered for the use of cosmetics (e.g., body creams/moisturizers, deodorants/antiperspirants, and face creams) as well as natural health products and non-prescription drugs (e.g., sunscreens) by both children and adults. The estimated dermal exposures were compared to an oral no-observed-adverse-effect level (NOAEL) for neurotoxic effects resulting in potentially inadequate MOEs.

6. Risk Management Considerations

6.1 Alternatives and Alternate Technologies

No publicly available information on alternatives to methanol or 1-butanol was identified for paint/varnish remover products or lacquer products, respectively. There were also no known alternatives identified for benzyl alcohol in cosmetics, natural health products, and non-prescription drugs. Follow-up information from stakeholders is requested, if known.

The US EPA's Safer Choice Criteria for Specialized Industrial Products and a report from the State of California on alternatives identified benzyl alcohol as an acceptable substitute for other chemicals that function as a solvent in paint strippers (EPA 2012, Jacobs *et al.*, 2015).

6.2 Socio-economic and Technical Considerations

Socio-economic factors will be considered in the selection process for a regulation and/or instrument respecting preventive or control actions, and in the development of the risk management objectives(s). Socio-economic factors will also be considered in the development of regulations, instrument(s) and/or tool(s) as identified in the *Cabinet Directive on Regulation* (TBS 2018) and the guidance provided in the Treasury Board Document *Assessing, Selecting, and Implementing Instruments for Government Action* (TBS 2007).

7. Overview of Existing Risk Management

7.1 Related Canadian Risk Management Context

7.1.1 Methanol

Domestically, methanol is listed on the Pest Management Regulatory Agency (PMRA) formulant list for pest control products currently registered in Canada under the *Pest Control Products Act* and Regulations (PMRA, 2010).

It is also listed as a Volatile Organic Compound (VOC) by National Pollutant Release Inventory (NPRI).

In Canada, the safety of chemicals used in food packaging materials is subject to the provisions of Division 23 of the *Food and Drug Regulations* and section 4(1)(a) of the *Food and Drugs Act*.

Methanol is a permitted food additive on the *List of Permitted Carrier or Extraction Solvents*, incorporated by reference into its respective Marketing Authorization issued under the *Food and Drugs Act*.

Methanol is listed as an ingredient restricted for use in cosmetic products. No person can sell a cosmetic containing an amount of methanol equal to or greater than 5 mL unless it is packaged in a child-resistant container as a result of section 15.2 of the *Cosmetic Regulations*. Containers, that contain an amount of methanol equal to or greater than 5 mL, must meet the requirements set out in subsection 28.2 of the *Cosmetic Regulations*. The NHPID entry for methanol refers to the presence of this ingredient as restricted on the Cosmetic Ingredient Hotlist and indicates that this ingredient must be used in accordance with the restrictions set out on the Hotlist when included in natural health products, unless additional evidence for safety is submitted.

The *Volatile Organic Compound (VOC) Concentration Limits for Certain Products Regulations* were published in the *Canada Gazette*, Part II, on January 5, 2022 under CEPA and apply to Canadian manufacturers and importers. They establish total VOC concentration limits for approximately 130 product categories and subcategories, including paint or coating removers or strippers. The regulations set a VOC concentration limit for the total amount of VOCs in a certain product but do not include a specific limit for a single VOC. For further information visit: [Certain products and volatile organic compounds – Canada.ca](https://www.canada.ca/en/environment-climate-change/services/chemicals-management/voc-concentration-limits-certain-products-regulations.html).

In addition, a paint/varnish remover product containing methanol is considered a consumer chemical product, which is regulated by the *Consumer Chemicals and Containers Regulations, 2001* (CCCR, 2001) under the *Canada Consumer Product Safety Act* (CCPSA).

7.1.2 1-Butanol

Domestically, 1-butanol is listed on the Pest Management Regulatory Agency (PMRA) formulant list for pest control products currently registered in Canada under the *Pest Control Products Act* and Regulations (PMRA, 2010).

In Canada, the safety of chemicals used in food packaging materials is subject to the provisions of Division 23 of the *Food and Drug Regulations* and section 4(1)(a) of the *Food and Drugs Act*. In Canada, the safety of food flavouring agents is subject to the provisions of section 4(1)(a) of the *Food and Drugs Act*.

It is also listed as a VOC by the NPRI.

The *Volatile Organic Compound (VOC) Concentration Limits for Architectural Coatings Regulations* were published on September 30, 2009 in *Canada Gazette*, Part II. The regulations set mandatory VOC concentration limits on products such as paints, stains, varnishes, lacquers and other types of coatings applied to a wide variety of stationary structures in residential, commercial, institutional and industrial settings. The regulations apply to manufacturers, importers and sellers of architectural coatings, as well as to users of traffic marking coatings. Under these regulations, there are 53 product categories of architectural coatings, each having a specific VOC maximum concentration limit. The regulations set a VOC concentration limit for the total amount of VOCs in a product but do not include a specific limit for a single VOC, such as 1-butanol. For further information visit: [Architectural coatings products and volatile organic compounds - Canada.ca](http://Architectural%20coatings%20products%20and%20volatile%20organic%20compounds%20-%20Canada.ca)

In addition, a lacquer product containing 1-butanol is considered a consumer chemical product, which is regulated by the *Consumer Chemicals and Containers Regulations, 2001* (CCCR, 2001) under the CCPSA.

7.1.3 Benzyl alcohol

Benzyl alcohol is listed in the NHPID with an acceptable daily intake up to 5 mg/kg bw/day, expressed as benzoic acid equivalents. It is also listed with a non-medicinal role for use as flavour enhancer, fragrance ingredient, preservative antimicrobial, or solvent. It must be declared as a medicinal ingredient in throat lozenges at daily doses at or above 100 mg/day, and in anorectal products in concentrations at or above 1% w/w. For topical use as NMI, it is allowed up to 10% in rinse-off products and up to 3% in leave-on products.

Benzyl alcohol is a permitted food additive on the *List of Permitted Carrier or Extraction Solvents*, incorporated by reference into its respective Marketing Authorization issued under the *Food and Drugs Act* where it is permitted as a carrier solvent in unstandardized flavouring preparations and in one type of standardized flavouring preparation. It may also be used as a food flavouring agent, the safety of which is subject to the provisions under section 4(1)(a) of the *Food and Drugs Act*.

7.2 Pertinent International Risk Management Context

7.2.1 Methanol

United States

- Under the Federal Food, Drug, and Cosmetic Act, methanol is listed under Food Additives. Permitted in Feed and Drinking Water of Animals; Methyl Esters of Conjugated Linoleic Acid (Cis-9, Trans-11 and Trans- 10, Cis-

12-Octadecadienoic Acids) as a reactant with refined sunflower oil to produce fatty acid methyl esters.

- Under Title 21: Food and Drugs, Part 184—Direct Food Substances Affirmed as Generally Recognized as Safe, §184.1259 Cocoa butter substitute, residual methanol must be 5 ppm maximum.
- Under PART 172—Food Additives Permitted for Direct Addition to Food for Human Consumption, Subpart I—Multipurpose Additives, §172.859 Sucrose fatty acid esters, methanol cannot exceed 10 ppm in sucrose fatty acid esters.
- Listed under Title 21: Food and Drugs, Subpart B—Substances for Use Only as Components of Paper and Paperboard, Part 176—Indirect Food Additives: Paper and Paperboard Components §176.210 Defoaming agents used in the manufacture of paper and paperboard.

European Union

- Listed under Dangerous Substances in Directive 2012/18/EU of the European Parliament and of the Council of 4 July 2012 on the control of major-accident hazards involving dangerous substances, PART 2: Named dangerous substances.
- Under Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, 3.1.1. Packaging to be fitted with child-resistant fastenings, methanol has a concentration limit of $\geq 3\%$ in packaging.
- Under 2006/257/EC: Commission Decision of 9 February 2006 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products, methanol is listed as a cosmetic ingredient.
- Listed in Directive 2009/32/EC (Consolidated-2016-11-09): Extraction solvents used in the production of foodstuffs and food ingredients.

7.2.2 1-Butanol

United States

- *Food and Drug Act*, Title 21 of the Code of Federal Regulation (CFR):
 - Part 172 – Food additives permitted for direct addition to food, where 1-butanol may be safely used as a flavouring agent or as a

- modified hop extract flavouring to no more than 50 ppm (US eCFR, 2017a).
- Part 175 – Indirect food additives in adhesives and components of coatings, where 1-butanol is safely used in adhesives and coatings for polyolefin films in food packaging
 - Part 176 – Indirect food additives in paper and paperboard components, where the substance is safe to use in articles intended for packaging, transporting or holding food; and
 - Part 177 – Indirect food additives in polymers
- The United States Food and Drug Administration (US FDA)'s Inactive Ingredient Search for Approved Drug Products list a *maximum potency per unit dose* of 0.02 mg for tablet, extended release.
 - *Federal Insecticide, Fungicide, and Rodenticide Act* (FIFRA), US EPA Regulations. 1-Butanol is classified as List 4B - an inert ingredient in pesticides based upon the 'reasonable certainty of no harm' safety finding. It is cleared for use in food and non-food as a dye. This inert ingredient is used pre-harvest with exemptions from the requirement of a tolerance when used in accordance with good agricultural practice. To be exempt from the requirement of a tolerance, it must be limited to not more than 0.005% of the pesticide formulation. It is also an inert ingredient applied to animals (with exemption from the requirement of a tolerance) (US EPA, 2005; US eCFR 2017d).
 - *Clean Air Act*, EPA Regulations. 1-Butanol is designated a VOC subject to compliance with 40 CFR Part 60 – Standards of Performance for New Stationary Sources.

European Union

- 1-Butanol is included in Annex I, list of monomers authorized in plastics production intended for food contact, of European Commission Regulation No 10/2011 (EC, 2011)
- Listed in the Register of Flavouring Substances under European Commission Regulation No 2232/96 (EC, 1996), where 1-butanol is safe for use as a food flavouring substance
- Listed in Directive 2009/32/EC (Consolidated-2016-11-09): Extraction solvents used in the production of foodstuffs and food ingredients
- Listed as an existing active substance in biocidal products in accordance with European Commission Regulation No 1451/2007 (EC, 2007)

7.2.3 Benzyl alcohol

United States

- *Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)*, US EPA Regulations: classified as List 3 – inert ingredient of unknown toxicity
- *Food and Drug Act*, Title 21 of the Code of Federal Regulation (CFR):
 - Part 73 – allowed as a colour additive in drugs
 - Part 172 – Food additives permitted for direct addition to food where benzyl alcohol may be safely used as a synthetic flavouring agent (US eCFR, 2017a).
 - Part 175 – Indirect food additives in adhesives and components of coatings, where benzyl alcohol should not exceed 4% of the resin for coatings intended for repeated use in contact with food
 - Part 177 – Indirect food additives in polymers allowing up to 1% benzyl alcohol in closures for food containers
- The US FDA's Inactive Ingredient Search for Approved Drug Products gives a *maximum potency per unit dose* for benzyl alcohol (US FDA 2019).
- Listed as approved for fragrance use on the EPA Fragrance Ingredient List

European Union

- Listed in the Regulation No 872/2012 as allowed as a flavouring substance
- Listed in Regulation (EU) No 10/2011 (Consolidated-2018-02-08): Plastic materials and articles intended to come into contact with food
- Listed in Regulation (EU) No 1333/2008 on food additives and Regulation (EU) No 231/2012 (Consolidated-2018-08-12): Specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008
- Listed in the Regulation No 1223/2009 on cosmetic products where benzyl alcohol must be listed on the ingredient list if concentrations are >0.001% in leave-on products or >0.01% in rinse-off products (Annex III)
- Also under Regulation No 1223/2009, allowed as a preservative in cosmetic products up to 1% (Annex V)

8. Next Steps

8.1 Public Comment Period

Industry and other interested stakeholders are invited to submit comments on the content of this risk management scope or other information that would help to inform decision-making (such as outlined in sections 3.2 or 3.3). Please submit additional information and comments prior to May 11, 2022.

The risk management approach, which will outline and seek input on the proposed risk management instrument(s), will be published at the same time as the final screening assessment. At that time, there will be further opportunity for consultation.

Comments and information submissions on the risk management scope should be submitted to the address provided below:

Environment and Climate Change Canada
Chemicals Management Division
Gatineau Quebec K1A 0H3
Tel: 1-800-567-1999 | 819-938-3232
Email: substances@ec.gc.ca

Companies who have a business interest in methanol, 1-butanol, and/or benzyl alcohol are encouraged to identify themselves as stakeholders. Stakeholders will be informed of future decisions regarding methanol, 1-butanol, and/or benzyl alcohol and may be contacted for further information.

8.2 Timing of Actions

Electronic consultation on the draft screening assessment and risk management scope: March 12, 2022 to May 11, 2022. This should include the submission of public comments, additional studies, and/or information on methanol, 1-butanol, and/or benzyl alcohol.

Publication of responses to public comments on the draft screening assessment and risk management scope: concurrent to the publication of the screening assessment and, if required, the risk management approach.

Publication of responses to public comments on the risk management approach, if applicable and if required, the proposed instrument(s): At the latest, 24 months from the date on which the ministers recommended that methanol, 1-butanol, and/or benzyl alcohol be added to Schedule 1 of CEPA.

Consultation on the proposed instrument(s), if required: 60-day public comment period starting upon publication of each proposed instrument.

Publication of the final instrument(s), if required: At the latest, 18 months from the publication of each proposed instrument.

These are planned timelines, and are subject to change. Please consult the [schedule of risk management activities and consultations](#) for updated information on timelines.

9. References

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Draft Risk Management Scope

PMRA [modified 2010 Aug 31]. [PMRA List of Formulants: list of formulants that are found in pest control products currently registered in Canada under the Pest Control Products Act and Regulations](#). Ottawa (ON): Pest Management Regulatory Agency. [accessed 2018 Feb].

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Benzenemethanol: Human health tier II assessment

01 July 2016

CAS Number: 100-51-6



- Preface
- Chemical Identity
- Import, Manufacture and Use
- Restrictions
- Existing Work Health and Safety Controls
- Health Hazard Information
- Risk Characterisation
- NICNAS Recommendation
- References

Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted to address specific concerns that could not be resolved during the Tier II assessment.

These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted and published separately, using information available at the time, and may be undertaken at different tiers.

This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

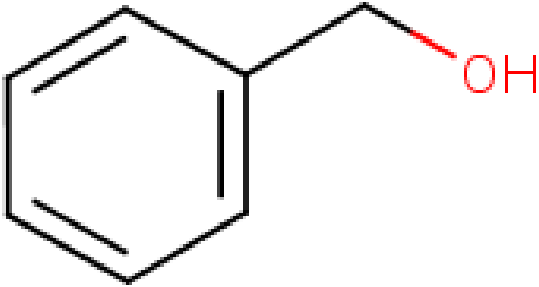
For more detail on this program please visit: www.nicnas.gov.au

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Acronyms & Abbreviations

Chemical Identity

Synonyms	benzyl alcohol phenylmethanol phenylcarbinol benzenecarbinol hydroxytoluene
Structural Formula	
Molecular Formula	C7H8O
Molecular Weight (g/mol)	108.14
Appearance and Odour (where available)	Colourless liquid with a faint aromatic odour.
SMILES	<chem>c1(CO)ccccc1</chem>

Import, Manufacture and Use

Australian

No specific Australian use, import, or manufacturing information has been identified.

The chemical is listed on the 2006 High Volume Industrial Chemicals List (HVICL) with a total reported volume of 10000–99999 tonnes.

International

The following international uses have been identified through:

- the European Union (EU) Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) dossier;
- the Organisation for Economic Co-operation and Development Screening information data set International Assessment Report (OECD SIAR);
- Galleria Chemica;
- the Substances and Preparations in Nordic countries (SPIN) database;
- the European Commission Cosmetic Ingredients and Substances (CosIng) database;
- the United States (US) Personal Care Product Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary; and
- the US National Library of Medicine's Hazardous Substances Data Bank (HSDB).

The chemical has reported cosmetic uses including as:

- a fragrance ingredient;
- a solvent and preservative;
- a viscosity decreasing agent; and
- an oral health care agent.

The chemical has reported cosmetic uses in the United States of America (USA), with identified use in 2042 products (Personal Care Products Council, 2011).

The chemical has reported domestic uses including in:

- adhesives, binding agents;
- bleaching agents;
- cleaning/washing agents;
- colouring agents;
- corrosion inhibitors;
- fillers;
- odour agents;

- surface treatments;
- surface-active agents; and
- paints, lacquers and varnishes.

The US Household Products Database states a concentration of up to: 30 % (paste) and 20 % (fluid) for home maintenance use; 10 % (paste) for inside the home use; and 1.5 % (cream, liquid and wipes) for personal care uses. One personal care product (Aveeno Moisturizing Shower and Bath Oil, liquid form) contains the chemical up to 5 % and another personal care product (Old Spice cologne, liquid form) contains the chemical up to 30 % (US Household Products Database).

The chemical has reported commercial uses including:

- in absorbents and adsorbents;
- in anti-freezing and anti-static agents;
- in construction and impregnation materials;
- as a fixing agent;
- in lubricants and additives;
- as a solvent and viscosity adjustor;
- in welding and soldering agents;
- in reprographic agents; and
- in photo chemicals and process regulators.

The chemical has reported site-limited uses including as intermediates.

The following non-industrial uses have been identified internationally:

- in food/feedstuff flavourings and nutrients;
- in non-agricultural pesticides and preservatives; and
- in pharmaceuticals.

Restrictions

Australian

No known restrictions have been identified.

International

The chemical is listed on the following (Galleria Chemica):

- EU Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products—Annex III—List of Substances which cosmetic products must not contain except subject to the restrictions laid down;
- New Zealand Cosmetic Products Group Standard—Schedule 5—Table 1: Components cosmetic products must not contain except subject to the restrictions and conditions laid down;

- The Association of Southeast Asian Nations (ASEAN) Cosmetic Directive Annex III—Part 1 List of substances which cosmetic products must not contain except subject to restrictions and conditions laid down;
- EU Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products—Annex V List of preservatives allowed in cosmetic products;
- New Zealand Cosmetic Products Group Standard—Schedule 7: Preservatives cosmetic products may contain with restrictions—Table 1: List of preservatives allowed; and
- ASEAN Cosmetic Directive Annex VI—Part 1—List of preservatives allowed for use in cosmetic products.

The US Cosmetic Ingredient Review (CIR) Expert Panel concluded that the chemical is safe for use in cosmetic formulations at concentrations up to 5 %. The chemical is also safe for use in hair dyes at concentrations up to 10 %. The available data are sufficient to support the safety of these ingredients in cosmetic products in which a primary route of exposure is inhalation (CIR, 2001).

Restrictions for the chemical for certain types of cosmetic products, according to Annex III of the REACH Regulations (List of restricted substances), are that they are not to be used. The presence of the substance must be indicated in the list of ingredients referred to in Article 19(1)(g) when its concentration exceeds:

- 0.001 % in leave-on products; and
- 0.01 % in rinse-off products.

Restriction for the chemical for certain types of cosmetic products, according to Annex V of the REACH Regulations (List of preservatives allowed in cosmetic products) is that a maximum concentration in ready-for-use preparations is 1 %.

Existing Work Health and Safety Controls

Hazard Classification

The chemical is classified as hazardous, with the following risk phrases for human health in the Hazardous Substances Information System (HSIS) (Safe Work Australia):

- Xn; R20/22 (acute toxicity)

Exposure Standards

Australian

No specific exposure standards are available.

International

No specific exposure standards are available

Health Hazard Information

Toxicokinetics

The chemical is readily and totally absorbed from the alimentary tract in humans following an oral application of 1.5 g; the chemical is metabolised within six hours and about 75–85 % is excreted in the urine. The absorbed chemical is readily oxidized to benzaldehyde as an intermediate, and then to benzoic acid, which is conjugated with glycine to hippuric acid and excreted via the kidneys. Higher doses of the chemical result in excretion as a glucuronide conjugate (NTP, 1989; SCF, 2002; REACH).

Acute Toxicity

Oral

The chemical is classified as hazardous with the risk phrase 'Harmful if swallowed' (Xn; R22) in the Hazardous Substances Information System (HSIS) (Safe Work Australia). The available data (median lethal dose (LD50) is 1620 mg/kg bw) support this classification.

Reported signs of toxicity were noted at all doses and included sedation, side and prone-position, bloody eyes and a reduced general condition. The noted mortality during the study was 1/10, 2/10, 6/10, 8/10 and 10/10 animals at 1254, 1463, 1467, 2090 and 2195 mg/kg bw, respectively (REACH). Similar conclusions have also been reported in other studies (NTP, 1989; WHO, 2001; SCF, 2002).

Dermal

Although no data are available for the chemical, chemicals in this category demonstrated low acute toxicity based on results from animal tests following dermal exposure. The reported LD50 in rats is >2000 mg/kg bw (WHO, 2001).

Inhalation

The chemical is classified as hazardous with the risk phrase 'Harmful by inhalation' (Xn; R20) in the HSIS (Safe Work Australia). While the available data do not support the classification, in the absence of more comprehensive information, amendment of the current HSIS classification is not warranted.

In an acute inhalation toxicity study, Wistar rats (five/dose/sex) were subjected to a single four-hour head/nose-only exposure to aerosol concentrations of the chemical at 3297 and 4178 mg/m³ and observed for 14 days post exposure. The concentration of 4178 mg/m³ was proven to be the maximum technically achievable concentration.

At 4178 mg/m³, transient clinical signs (piloerection, slight bradypnoea; recovery within one day) were seen as causally related to the slight irritant effect of the chemical to the upper respiratory tract. There was no indication of any lasting respiratory damage in this study. No mortality was noted in the study. The median lethal concentration (LC50) over four hours was concluded to be >4178 mg/m³ for male and female rats (REACH).

Observation in humans

There have been reports of premature infants, who had received benzyl alcohol in medications administered intravenously, suffering serious effects including central nervous system dysfunction, coma and death. Death occurred between 6–46 days in infants who had received 99–234 mg/kg bw of benzyl alcohol, while a matched control group of infants did not develop the syndrome, had received doses of 27–99 mg/kg bw of benzyl alcohol (SCF, 2002).

Corrosion / Irritation

Skin Irritation

The chemical is reported to slightly irritate the skin in animal studies. The effects were not sufficient to warrant hazard classification.

In a skin irritation study, the chemical (0.5 mL) was applied to the dorsal area of the trunk of three New Zealand White rabbits for four hours under observation for seven days. Slight irritation, which fully resolved within 72 hours, was seen in one animal (WHO, 2001; REACH).

Eye Irritation

Although the chemical produced only slight eye irritation in one animal study where the eye was washed out after application, the eye irritation effects reported in a second eye irritation study without washing after application support the chemical being classified (refer to **Recommendation** section).

In an eye irritation study, the chemical (0.1 mL) was applied on the conjunctival sac of one eye of each of three New Zealand White rabbits, with observation at 24, 48 and 72 hours after the administration and then daily until the ocular reactions were reversed. The average scores following grading at 24, 48 and 72 hours for three rabbits were 2 for corneal opacity, 1 for iritis, 2.67 for conjunctivitis, and 2.23 for chemosis. The average scores for all the end points are sufficient to support classification (REACH).

In an eye irritation study, the chemical (0.1 mL) was applied on the conjunctival sac of one eye of each of three New Zealand White rabbits. The treated eyes were washed 24 hours after instillation of the chemical. Irritation responses were observed at 24, 48 and 72 hours and at 14 and 21 days following application. The average scores following grading at 24, 48 and 72 hours for three rabbits were 1 for corneal opacity, 0.3 for iritis, 2 for conjunctivitis, and 0.7 for chemosis (REACH).

Sensitisation

Skin Sensitisation

While the chemical gave both positive and negative responses in animal studies, the maximisation test conducted, which was similar to the current test guidelines, was negative. The information presented here in humans also indicated that the chemical is unlikely to be a skin sensitizer (see **Sensitisation: observation in humans**). No sensitisation has been reported with the chemical among workers who used it over several decades. Therefore, the weight of evidence approach would indicate that the chemical is not a skin sensitizer in humans (WHO, 2001; REACH).

In a guinea pig skin sensitisation test, Himalayan guinea pigs (10/dose) were induced (intradermally) with doses of 0.05 mL of the undiluted chemical mixed with the same volume of Freund's complete adjuvant (FCA) on days 0, 2, 4, 7 and 9. The control animals were similarly treated with 0.05 mL of FCA alone. All the animals were challenged epicutaneously on days 21 and 35 in a patch test with a non-irritant concentration of the chemical. The chemical yielded a positive result for skin sensitisation.

In another guinea pig skin sensitisation test, Himalayan guinea pigs (4–6/group) were induced (epicutaneously) with 0.1 mL of undiluted chemical up to several diluted concentrations (up to 3 %) daily for 21 days. Animals were challenged epicutaneously on days 21 and 35, with the lowest irritant and a non-irritant concentration. Evaluations were performed at 42, 48, and 72 hours after the last challenged dose. The chemical tested positive for skin sensitisation in this test.

In an old skin sensitisation test (Draize test), Himalayan guinea pigs were induced (intradermally) with a dose of 0.05 mL of a 0.1 % solution of the chemical in isotonic saline. Further doses of 0.1 mL each were injected on alternate days for the following nine days. The treated animals and the untreated controls were challenged intradermally with 0.05 mL of a 0.1 % solution on days 35 and 49. The evaluation criterion was the mean diameter of the papular reactions. The guinea pigs showed no reaction in this test for skin sensitisation.

In a guinea pig maximisation test, Himalayan guinea pigs (10/dose) were induced (intradermally) with: 0.1 mL of a 5 % solution of the chemical, also with 0.1 mL of a 5 % emulsion of the chemical in FCA; each injection being given twice. In addition, 250 mg of the compound dissolved in petrolatum at a concentration of 25 %, which caused mild to moderate skin irritation under occlusion, was applied on day eight to the clipped skin area of the neck and was kept under occlusive bandage for two days

(total dose 20 mg intradermally plus 250 mg epicutaneously). On day 21, the chemical at a subirritant concentration of 25 % in petrolatum was applied to the flank epicutaneously for 24 hours. The reactions were read at 24 and 48 hours after removing the patch. The chemical tested negative for skin sensitisation.

Observation in humans

A repeat-insult patch test (HRIPT) was reported to be negative in 110 patients. In this study, patients were tested with two formulations containing 0.65 % of the chemical. A negative reaction was also reported for a 10 % concentration of the chemical in petrolatum using 25 male volunteers in a human maximisation study.

Three cutaneous reactions to 5 % of the chemical in petrolatum were noted among 713 cosmetic dermatitis patients over a period of six years from results compiled by 12 dermatologists. In another study, four positive patch tests to a 6.5 % concentration of the chemical were reported among 242 patients with histories of contact allergy of varying origin (CIR, 2001; REACH).

Repeated Dose Toxicity

Oral

The chemical is not considered to cause serious damage to health from repeated oral exposure. A no observed adverse effect level (NOAEL) of ≥ 200 mg/kg bw/day for mice and ≥ 400 mg/kg bw/day for rats can be established for the chemical from long-term studies.

In a repeated dose toxicity study, the chemical was administered (gavage) in corn oil to groups of B6C3F1 mice (10/sex/dose) and Fischer 344/N (F344/N) rats (10/sex/dose) at doses of 0, 50, 100, 200, 400, or 800 mg/kg bw/day, five days/week for 13 weeks. Eight out of ten male rats dosed with 800 mg/kg bw/day died during weeks seven and eight; four of these mortalities were related to gavage. While mortalities were scattered among all dose levels in mice, all mortalities (four males and six females), apart from one, were related to administration of the chemical through gavage. Clinical signs of neurotoxicity, including staggering, respiratory difficulty, and lethargy, were noted in rats dosed with 800 mg/kg bw/day. Haemorrhages around the mouth and nose were noted and there were pathological lesions in the brain, thymus, skeletal muscle, and kidney. Mice dosed with 800 mg/kg bw/day also showed staggering after dosing during the first two weeks of the study.

In the same study, reduced relative weight gain was noted in male rats dosed with 800 mg/kg bw/day; female rats dosed with 200 mg/kg bw/day or more; male mice dosed with 400 or 800 mg/kg bw/day; and female mice dosed with 200 mg/kg bw/day or more. Notable changes in body weight gain or chemically-related histopathologic lesions were not observed in rats or mice from the lower dose groups. Some of the deaths in the rats and mice could have been caused by a combination of the gavage procedure and chemical toxicity, since there was evidence that benzyl alcohol induced neurotoxic effects. An NOAEL of 200 mg/kg bw/day was established for mice, based on clinical signs and reduced body weight development in males and females. An NOAEL of 400 mg/kg bw/day was established for rats, based on clinical signs and reduced body weight development in males and females and histopathological changes in the brain at 800 mg/kg bw (NTP, 1989; REACH).

In a chronic toxicity/carcinogenicity study, the chemical was administered to groups of B6C3F1 mice (50/sex/dose) and F344/N rats (50/sex/dose) at a dose of 0, 100, or 200 mg/kg bw/day, and 0, 200, or 400 mg/kg bw/day, respectively, in corn oil on five days a week for 103 weeks (see **Carcinogenicity**). There were no effects on body weight gain and no chemically-related clinical signs were observed throughout the study in either mice or rats. Survival in both dose groups of female rats was reduced to 50 % compared with vehicle controls, primarily due to an increased number of gavage-related deaths. Gross necropsy and histopathology revealed no apparent chemically-related non-neoplastic responses in mice and rats. Therefore, an NOAEL of 200 and 400 mg/kg bw/day, respectively, in mice and rats was established from this study (NTP, 1989).

Dermal

No data are available.

Inhalation

Limited information indicates that the chemical is not likely to cause serious damage to health from repeated inhalation exposure.

In a repeated dose inhalation study, the aerosolised chemical was administered via the nose for six hours/day on five days/week for four weeks (a minimum of 20 exposures/animal) to Sprague Dawley (SD) rats (10/sex/dose) at target exposure concentrations (mean analytical concentrations) of 30 (41), 100 (102), 300 (290), and 1000 (1072) mg/m³. A concurrent control group was exposed to filtered air on a comparable regimen. All animals were euthanised on the day following the last exposure. There were no clinical test-substance related effects on mortality, body weight or body gain, food consumption, ophthalmoscopic examination, haematology, clinical chemistry, organ weight, gross pathology, or histopathology. As an adverse effect was not noted at any concentration, no observed adverse effect concentration (NOAEC) was considered to be 1072 mg/m³ (REACH).

Genotoxicity

The weight of the evidence of the in vitro and in vivo genotoxicity data indicates that the chemical does not have mutagenic or clastogenic potential (NTP, 1989; SCF, 2002; REACH).

The chemical was reported to be negative in several Ames tests, with and without metabolic activation, and also negative in an in vitro mammalian chromosome aberration test using Chinese hamster lung (CHL) cells without metabolic activation.

The chemical produced a significant increase in chromosomal aberrations with Chinese hamster ovary (CHO) cells in the presence, but not the absence, of S9. The chemical also produced an increase in sister chromatid exchanges (SCEs) in CHO cells in a sister chromatid exchange assay in mammalian cells, which was judged to be equivocal both with and without metabolic activation. While the chemical produced a positive response in mammalian cells in the mouse lymphoma thymidine kinase forward mutation assay without metabolic activation, negative results were reported for tests with metabolic activation.

The chemical was found to be negative in the in vivo micronucleus assay on mouse bone marrow cells, in an assay on replicative DNA synthesis in rats, and in the sex-linked recessive lethal assay in *Drosophila melanogaster*.

Carcinogenicity

The available information indicates that the chemical is not likely to have carcinogenic potential.

In a chronic toxicity/carcinogenicity study, the chemical was administered to groups of B6C3F1 mice (50/sex/dose) and F344/N rats (50/sex/dose) at a dose of 0, 100, or 200 mg/kg bw/day, and 0, 200, or 400 mg/kg bw/day, respectively, in corn oil for five days a week for 103 weeks (see **Repeat dose toxicity: oral**). Dose-related negative trends were noted in the incidences of anterior pituitary gland neoplasms in female rats (control 29/50; low dose 17/47; high dose 9/49) and of Harderian gland adenomas in male mice (8/50; 3/50; 2/50). Increased incidence of hyperplasia of the forestomach epithelium was seen (not statistically significant) in male rats (0/48; 0/19, 4/50). The increased incidence of adenomas of the adrenal cortex noted in the high-dose male mice (0/48; 0/44; 3/48) was within the historical range and not considered compound-related. The study concluded that the chemical showed no evidence of carcinogenic activity in male or female F344/N rats dosed with 200 or 400 mg/kg, or male or female B6C3F1 mice dosed with 100 or 200 mg/kg for two years (NTP, 1989).

Reproductive and Developmental Toxicity

The chemical does not show specific reproductive or developmental toxicity. Any reproductive and developmental effects were only observed secondary to maternal toxicity.

In a developmental toxicity study, 50 CD-1 mice were administered (gavage) the chemical at 550 mg/kg bw/day on gestation days (GD) 6–15. A further 50 mice received the corn oil vehicle only. No clinical signs were reported in the treatment group and maternal body weight and body-weight gain during treatment and up to day three days post-partum were not affected. Mortality was not significantly increased in treated animals compared with the control group. All other parameters examined, including the

gestation index, average number of live pups per litter, and postnatal survival and pup body weight on days zero and three post-partum were not significantly different from the control values. As no significant effects were noted in the study, the NOAEL was determined to be <550 mg/kg bw/day (CIR, 2001; OECD, 2001; REACH).

In another developmental toxicity study, 50 CD-1 mice were administered (gavage) the chemical at 750 mg/kg bw/day on GD 7–14. A control group of 50 animals received distilled water only. Clinical signs of toxicity were reported in up to 20 mice, and included hunched posture, tremors, inactivity, prostration, hypothermia, ataxia, dyspnoea, swollen or cyanotic abdomen, and piloerection. There were 19 deaths attributed to the treatment during the study. Maternal body weights were statistically significant as they reduced on day 18 of gestation and on day three post-partum. Significant reductions in pup body weight were reported on days one and three post-partum, including a lower mean pup weight per litter, mean litter weight change between day one and day three post-partum, and mean pup weight change between days one and three post-partum. No differences in pup survival were observed by day three post-partum. As effects were seen on dams and foetuses at the only dose used, the lowest observed adverse effect level (LOAEL) was 750 mg/kg bw/day for both maternal and foetal effects (CIR, 2001; WHO, 2001; REACH).

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include systemic acute effects (acute toxicity from oral and inhalation exposure).

The chemical is also irritating to the eyes.

Public Risk Characterisation

Although the use in cosmetic and domestic products in Australia is not known, the chemical has reported cosmetic and domestic uses overseas (see **Import, manufacture and use**). Considering the range of domestic and cosmetic products that could contain this chemical, the main route of public exposure is expected to be through the skin and eyes, inhalation from products applied as cosmetics, and from using domestic products.

While the chemical has various uses (as a fragrance ingredient, solvent, preservative, viscosity decreasing agent and oral health care drug), the chemical is not expected to be present in sufficiently high concentrations in these products to lead to toxic effects. The use of the chemical in personal care products is stated to be up to a concentration of 5 % as cream, liquid and wipes. A single personal care product reportedly has a much higher concentration of the chemical (up to 30 %) (see **Import, manufacture and use**), but this product is not expected to be used at high volumes on large skin areas.

It has been concluded that the chemical is safe for use in cosmetic formulations at concentrations up to 5 %, and in hair dyes at concentrations up to 10 % (CIR, 2001). Although a much higher concentration of chemical has been reported for a single personal care product (up to 30 %) and for home maintenance use (up to 30 %) (see **Import, manufacture and use**), provided that normal precautions are taken to avoid eye contact and inhaling chemical vapours, the risk from the use of domestic products is not considered to be unreasonable.

Therefore, the risk to public health is not considered to be unreasonable and further risk management is not considered necessary for public safety.

Occupational Risk Characterisation

During product formulation, ocular and inhalation exposure might occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to the chemical at lower concentrations could also occur while using formulated products containing the chemical. The level and route of exposure will vary depending on the method of application and work practices employed.

Given the critical systemic acute local health effects, the chemical could pose an unreasonable risk to workers unless adequate control measures to minimise ocular and inhalation exposure are implemented. The chemical should be appropriately classified and labelled to ensure that a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) has adequate information to determine the appropriate controls.

The data available support an amendment to the hazard classification in the HSIS (Safe Work Australia) (refer to **Recommendation** section).

NICNAS Recommendation

Assessment of the chemical is considered to be sufficient, provided that the recommended amendment to the classification is adopted, and labelling and all other requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory.

Regulatory Control

Work Health and Safety

The chemical is recommended for classification and labelling under the current approved criteria and adopted GHS as below. This assessment does not consider classification of physical and environmental hazards.

Hazard	Approved Criteria (HSIS) ^a	GHS Classification (HCIS) ^b
Acute Toxicity	Harmful if swallowed (Xn; R22)* Harmful by inhalation (Xn; R20)*	Harmful if swallowed - Cat. 4 (H302) Harmful if inhaled - Cat. 4 (H332)
Irritation / Corrosivity	Irritating to eyes (Xi; R36)	Causes serious eye irritation - Cat. 2A (H319)

^a Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)].

^b Globally Harmonized System of Classification and Labelling of Chemicals (GHS) United Nations, 2009. Third Edition.

* Existing Hazard Classification. No change recommended to this classification

Advice for consumers

Products containing the chemical should be used according to the instructions on the label.

Advice for industry

Control measures

Control measures to minimise the risk from ocular and inhalation exposure to the chemical should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate, or minimise risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemical is used. Examples of control measures which could minimise the risk include, but are not limited to:

- using closed systems or isolating operations;

- using local exhaust ventilation to prevent the chemical from entering the breathing zone of any worker;
- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemical.

Guidance on managing risks from hazardous chemicals are provided in the *Managing risks of hazardous chemicals in the workplace—Code of practice* available on the Safe Work Australia website.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Obligations under workplace health and safety legislation

Information in this report should be taken into account to help meet obligations under workplace health and safety legislation as adopted by the relevant state or territory. This includes, but is not limited to:

- ensuring that hazardous chemicals are correctly classified and labelled;
- ensuring that (material) safety data sheets ((M)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemical are prepared; and
- managing risks arising from storing, handling and using a hazardous chemical.

Your work health and safety regulator should be contacted for information on the work health and safety laws in your jurisdiction.

Information on how to prepare an (M)SDS and how to label containers of hazardous chemicals are provided in relevant codes of practice such as the *Preparation of safety data sheets for hazardous chemicals—Code of practice* and *Labelling of workplace hazardous chemicals—Code of practice*, respectively. These codes of practice are available from the Safe Work Australia website.

A review of the physical hazards of the chemical has not been undertaken as part of this assessment.

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Re-evaluation of benzyl alcohol (E 1519) as food additive

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Abstract

The Panel on Food Additives and Flavourings (FAF) provided a scientific opinion re-evaluating the safety of benzyl alcohol (E 1519) when used as a food additive. The Panel considered that adequate exposure and toxicity data were available. Benzyl alcohol (E 1519) is authorised as a food additive in the EU in accordance with Annex III to Regulation (EC) No 1333/2008. The Panel considered benzyl alcohol of low acute toxicity with no concern with respect to genotoxicity and carcinogenicity and established an acceptable daily intake (ADI) of 4 mg/kg body weight (bw) per day based on a no observable adverse effect level (NOAEL) of 400 mg/kg bw per day from the carcinogenicity study in rats. The mean and high exposure estimates in the refined exposure scenarios were maximally 0.27 and 0.81 mg/kg bw per day in toddlers, respectively. The exposure estimates to benzyl alcohol (E 1519) were below the ADI of 4 mg/kg bw per day in all population groups. The Panel noted that also the exposure in the *regulatory maximum level exposure assessment scenario* is below the ADI in all population groups. The Panel concluded that the exposure to benzyl alcohol (E 1519) does not raise a safety concern at the reported uses and use levels.

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Keywords: benzyl alcohol, E 1519, food additive, safety, risk assessment, dietary exposure, acceptable daily intake

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Summary

The present opinion deals with the re-evaluation of benzyl alcohol (E 1519) when used as a food additive.

Benzyl alcohol (E 1519) is authorised as a food additive in the European Union (EU) in accordance with Annex III to Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in Commission Regulation (EU) No 231/2012.

Benzyl alcohol was evaluated by JECFA in 1980 (JECFA, 1980) and concluded a group acceptable daily intake (ADI) (for benzoic acid together with benzyl alcohol) of 0–5 mg/kg body weight (bw), subsequently agreed by the Scientific Committee for Food (SCF) in 2002.

Benzyl alcohol is also included in the EU Union list (Commission Implementing Regulation (EU) No 872/2012) of authorised food flavourings substance with FL-no: 02.010, following its evaluation by the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) in 2008 in flavouring group evaluation 54 (FGE.54Rev1). In 2012, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) prepared a scientific opinion on safety and efficacy of benzyl alcohols (including benzyl alcohol) when used as flavouring in all animal species (EFSA FEEDAP Panel, 2012).

The European Medicines Agency (EMA) has registered the use of benzyl alcohol as an excipient in medical products.

In humans and animal species, benzyl alcohol is metabolised to benzoic acid which reacts with glycine in the liver and the resulting hippuric acid is excreted quickly in urine.

The Panel noted that benzyl alcohol was of low acute toxicity.

Oral short-term or subchronic studies with benzyl alcohol showed neurotoxicity manifested as lethargy in mice (from 500 mg/kg per day) and lethargy or staggering (from 800 mg/kg bw per day) in rats.

Benzyl alcohol was tested with negative results in the base set of genotoxicity tests and the Panel concluded that benzyl alcohol does not raise safety concern with respect to genotoxicity.

Benzyl alcohol is not carcinogenic in mice and rats up to 200 and 400 mg/kg bw per day, respectively, which were highest doses tested. The no observable adverse effect level (NOAEL) in the carcinogenicity study in rats was 400 mg/kg bw per day.

The Panel noted that reproductive studies were not available and that two developmental screening studies in mice were of limited design.

Considering the available data set, the Panel established an ADI of 4 mg benzyl alcohol/kg bw per day based on a NOAEL of 400 mg/kg bw per day from the carcinogenicity study in rats, based on no effect in the highest dose tested.

The dietary exposure to benzyl alcohol (E 1519) from its use as a food additive was calculated based on (1) maximum permitted levels (MPLs) set out in the EU legislation (defined as the regulatory maximum level exposure assessment scenario) and (2) the reported use levels (defined as the refined exposure assessment scenario).

The mean and high exposure estimates in the refined exposure scenarios were maximally 0.27 and 0.81 mg/kg bw per day in toddlers, respectively. Considering the food categories for which the presence of benzyl alcohol (E 1519) can be due to carry over via its authorisation according to Annex III to Reg (EC) No 1333/2008, the exposure estimates to benzyl alcohol (E 1519) were below the ADI of 4 mg/kg bw per day in all population groups. The Panel noted that also the exposure in the *regulatory maximum level exposure assessment scenario* is below the ADI in all population groups.

In conclusion, based on the toxicological database available, the Panel established an ADI of 4 mg/kg bw per day for benzyl alcohol (E 1519) and concluded that the exposure to benzyl alcohol (E 1519) does not raise a safety concern at the reported uses and use levels.

The Panel recommend that the European Commission considers introducing the maximum levels of chlorinated organic compounds as impurities in the specifications for purity of E 1519 in EU Regulation 231/2012.

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1. Introduction

The present opinion deals with the re-evaluation of benzyl alcohol (E 1519) when used as a food additive.

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background

Regulation (EC) No 1333/2008¹ of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under the Regulation (EU) No 257/2010.² This Regulation also foresees that food additives are re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU³ of 2001. The report "Food additives in Europe 2000"⁴ submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with a highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010 the 2003 Terms of References are replaced by those below.

1.1.2. Terms of Reference

The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

1.2. Information on existing authorisations and evaluations

Benzyl alcohol (E 1519) is authorised as a food additive in the EU in accordance with Annex III to Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012⁵.

In the EU, Benzyl alcohol (E 1519) has been evaluated by the Scientific Committee for Food (SCF) in 2002 (SCF, 2002) who concluded that *'the metabolism of benzyl alcohol to benzoic acid by man is well established. In special studies, biochemical effects have been investigated. The toxicity has been studied extensively, including acute, short-term and long-term toxicity, carcinogenicity, genotoxicity and developmental toxicity'*. The Committee considers that the studies on carcinogenicity in rats and mice

¹ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

² Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19–27.

³ COM(2001) 542 final.

⁴ Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord 2002, 560.

⁵ Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) no 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1.

did not show compound-related adverse effects at dose levels up to 200 mg/kg body weight (bw) in the mouse and up to 400 mg/kg in the rat, in both cases the highest dose levels tested. Data from subchronic studies on rats and/or mice show no observable adverse effect levels (NOAELs) of 400 mg/kg bw or more. Taking into account the toxicity data and the fact that benzyl alcohol is metabolised via benzaldehyde to benzoic acid, the Committee confirms the inclusion of benzyl alcohol in the group acceptable daily intake (ADI) of 0–5 mg/kg bw for benzoic acid and benzoates, as agreed in the SCF opinion of 1981. It should be noted that the total intake of benzyl alcohol and benzoic acid can result from different sources including the use of additives and flavourings as well as the natural occurrence in food. It is therefore possible that in some instances the intake of these substances may exceed the group ADI. Better data are required on use/residual levels following use of benzyl alcohol as carrier solvent in different food categories against the background of overall exposure in order to facilitate a more precise intake assessment.

Benzyl alcohol was evaluated by JECFA in 1980 (JECFA, 1980) and concluded a group ADI (for benzoic acid together with benzyl alcohol) of 0–5 mg/kg bw. In 1997, JECFA re-evaluated benzyl alcohol. JECFA concluded, based on long-term studies in rats and mice, that it is not carcinogenic. It was not mutagenic in the Ames test. A four-generation study in rats showed no effect on growth, fertility, lactation or survival. A group ADI of 0–5 mg per kg of body weight expressed as benzoic acid was confirmed.

In 2016, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) delivered a scientific opinion re-evaluating benzoic acid (E 210), sodium benzoate (E 211), potassium benzoate (E 212) and calcium benzoate (E 213) when used as food additives and derived a group ADI of 5 mg/kg bw per day (expressed as benzoic acid) in accordance with the ADI previously derived by SCF and JECFA.

Benzyl alcohol is also included in the EU Union list (EU Reg 872/2012) of authorised food flavourings substance with FL-no: 02.010. Following its evaluation by the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) in 2009 in flavouring group evaluation 54 (FGE.54Rev1), the Panel considered that benzyl alcohol was expected to be metabolised into innocuous products (EFSA CEF Panel, 2009). In 2012, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) prepared a scientific opinion on safety and efficacy of benzyl alcohols (including benzyl alcohol) when used as flavouring in all animal species (EFSA FEEDAP Panel, 2012).

European Medicines Agency (EMA) has registered the use of benzyl alcohol as an excipient in medical products (EMA, 2017).

Benzyl alcohol is registered under the REACH Regulation 1907/2006⁶ (ECHA, online).

Benzyl alcohol (PM Ref. 13150) is included in the Union list of authorised substances that may be intentionally used in the manufacture of plastic layers in plastic materials and articles (Annex I to Commission Regulation (EU) No 10/2011⁷). Furthermore, benzyl alcohol is permitted as an antioxidant in cosmetic products (European Commission database-CosIng⁸). Benzyl alcohol is included in the European Union Register⁹ of feed additives as a flavouring substance in feeding stuffs (Regulation (EC) No 1831/2003¹⁰).

2. Data and methodologies

2.1. Data

The Panel on Food Additives and Flavourings (FAF) was not provided with a newly submitted dossier. The Panel based its assessment on information submitted to EFSA following the public call for data,¹¹ information from previous evaluations and additional available literature up to 17 May 2019.

⁶ Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). OJ L 396, 30.12.2006.

⁷ Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. OJ L 12, 15.1.2011, p. 1–89.

⁸ Available online: <http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.simple>

⁹ Available online: http://ec.europa.eu/food/food/animalnutrition/feedadditives/comm_register_feed_additives_1831-03.pdf

¹⁰ Regulation (EC) No 1831/2003 of the European Parliament and the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, 29–43.

¹¹ Call for scientific data on food additives permitted in the EU and belonging to the functional classes of emulsifiers, stabilisers and gelling agents. Published: 22 November 2009. Available from: <http://www.efsa.europa.eu/en/dataclosed/call/ans091123>

Attempts were made at retrieving relevant original study reports on which previous evaluations or reviews were based; however, these were not always available to the Panel.

Food consumption data used to estimate the dietary exposure to benzyl alcohol (E 1519) were derived from the EFSA Comprehensive European Food Consumption Database (Comprehensive Database¹²).

The Mintel's Global New Products Database (GNPD) was checked to identify the uses of benzyl alcohol (E 1519) in food and beverage products and food supplements. The Mintel's GNPD is an online database that contains the compulsory ingredient information present on the label of numerous products.

2.2. Methodologies

This opinion was formulated following the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing guidance documents from the EFSA Scientific Committee.

The FAF Panel assessed the safety of benzyl alcohol (E 1519) as food additive in line with the principles laid down in Regulation (EU) 257/2010 and in the relevant guidance documents: Guidance on submission for food additive evaluations by the Scientific Committee on Food (SCF, 2001) and taking into consideration the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012).

When the test substance was administered in the feed or in the drinking water, but doses were not explicitly reported by the authors as mg/kg bw per day based on actual feed or water consumption, the daily intake was calculated by the Panel using the relevant default values as indicated in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012a,b,c) for studies in rodents or, in the case of other animal species, by JECFA (2000). In these cases the daily intake is expressed as equivalent.

Dietary exposure to benzyl alcohol (E 1519) from its use as a food additive was estimated combining food consumption data available within the EFSA Comprehensive European Food Consumption Database with the maximum levels according to Annex III to Regulation (EC) No 1333/2008.¹³ Different scenarios were used to calculate exposure (see Section 3.4.1). Uncertainties on the exposure assessment were identified and discussed.

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance

Benzyl alcohol (E 1519)

Benzyl alcohol (E 1519) has the chemical structure shown in Figure 1, the chemical formula C_7H_8O and a molecular weight of 108.14 g/mol. The substance is described as a colourless, clear liquid with a faint, aromatic odour and is characterised by the following physico-chemical properties: soluble in water, ethanol and ether; refractive index ($[n]_D^{20}$), 1.538–1.541; specific gravity (25°C/25°C), 1.042–1.047; distillation range, not less than 95% v/v distils between 202 and 208°C. Synonyms: phenylcarbinol; phenylmethyl alcohol; benzenemethanol; alfa-hydroxytoluene.

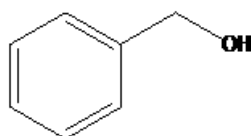


Figure 1: Structure of benzyl alcohol (E 1519)

¹² Available online: <http://www.efsa.europa.eu/en/food-consumption/comprehensive-database>

¹³ Commission Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16.

3.1.2. Specifications

The specifications for benzyl alcohol (E 1519) as defined in the Commission Regulation (EU) No 231/2012 and by JECFA (2009) are listed in Table 1.

Table 1: Specifications for benzyl alcohol (E 1519) according to Commission Regulation (EU) No 231/2012 and JECFA (2009)

	Commission Regulation (EU) No 231/2012	JECFA (2009)
Synonyms	Phenylcarbinol; phenylmethyl alcohol; benzenemethanol; alfa-hydroxytoluene	Phenylcarbinol; phenylmethyl alcohol; benzenemethanol; alfa-hydroxytoluene; INS No 1519
Definition	EINECS (EC) No: —	CAS No: 100-51-6
	Chemical names: benzyl alcohol; phenylmethanol	Chemical names: benzyl alcohol; phenylmethanol
	Chemical formula: C ₇ H ₈ O	Chemical formula: C ₇ H ₈ O
	—	Structural formula: see Figure 1
	Molecular weight (g/mol): 108.14	Formula weight (g/mol): 108.14
	Assay: not less than 98.0%	Assay: not less than 98.0%
Description	Colourless, clear liquid with a faint, aromatic odour	Colourless, clear liquid with a faint, aromatic odour
Functional uses	—	Flavouring agent, carrier
Identification	Solubility: soluble in water, ethanol and ether	Solubility: soluble in water, ethanol and ether
	Refractive index [n] _D ²⁰ : 1.538–1.541	Refractive index [n] _D ²⁰ : 1.538–1.541
	Specific gravity (25°C/25°C): 1.042–1.047	Specific gravity (25°C/25°C): 1.042–1.047
	Test for peroxides: passes test ^(c)	Test for peroxides: passes test ^{(a),(c)}
	Distillation range: not less than 95% v/v distils between 202 and 208°C	Distillation range: not less than 95% v/v distils between 202 and 208°C ^(a)
	—	Infrared absorption: passes test ^(b)
Purity	Acid value: not more than 0.5	Acid value: not more than 0.5
	Aldehydes: not more than 0.2 % v/v (as benzaldehyde)	Aldehydes: not more than 0.2 % v/v (as benzaldehyde)
	Lead: not more than 2 mg/kg	Lead: not more than 2 mg/kg
	—	Chlorinated organic compounds: passes test ^(c)

(a): In the JECFA data sheet, these specifications come under 'Purity'.

(b): A reference infrared spectrum is reported in the JECFA data sheet.

(c): The specific test is directly available from the JECFA data sheet.

As food additive, benzyl alcohol must have a purity equal to or greater than 98.0%, an acid value not higher than 0.5, a content of aldehydes and lead not greater than 0.2% v/v (as benzaldehyde) and 2 mg/kg, respectively, and a very low level of peroxides. In the regulation, no reference to the presence of chlorinated organic impurities is reported.

The Panel also noted that the EINECS (EC) identifier is missing in the Commission Regulation (EU) No 231/2012. The EC No 202-859-9 is allocated to benzyl alcohol (EC inventory, online) that corresponds to the CAS No 100-51-6 (SciFinder, software).

3.1.3. Manufacturing process

Large-scale production of benzyl alcohol is achieved by the reaction of sodium or potassium carbonate with benzyl chloride (Nair, 2001). Benzyl chloride is in general manufactured by the thermal or photochemical chlorination of toluene at 65–100°C (Seper, 2004), lower temperatures favouring the increase of ring-chlorinated by-products. As the chlorination reaction is sensitive to metallic impurities, the presence of the latter is avoided; the crude benzyl chloride is purged of dissolved hydrogen chloride, neutralised with alkali and distilled.

3.1.4. Methods of analysis in food

Methods to detect benzyl alcohol in food and beverages at the µg/L or µg/kg level are available. There is a vast number of scientific publications dealing with the detection of benzyl alcohol and other volatile organic compounds (VOCs) in various food matrices, wines, spirits and beverages (Antón-Díaz et al., 2016; Balcerek et al., 2017; Bellincontro et al., 2016; Cacho et al., 2013; Yang et al., 2012; Ganss et al., 2011; Rodríguez-Bencomo et al., 2011; Villanova and Martínez, 2007). The methods, often reaching limits of detection (LODs) in the µg/L or µg/kg range, make inter alia use of liquid–liquid extraction (LLE), solid-phase extraction (SPE), solid-phase micro-extraction (SPME), dynamic headspace (DHS), headspace solid-phase micro-extraction (HS-SPME), ultrasonic solvent extraction (USE), hydrodistillation (HD), CO₂-based supercritical fluid extraction (SFE) and micro simultaneous steam distillation-solvent extraction (MSDE) (Kuś and Jerković, 2018). The analysis is normally carried out by high-resolution gas chromatography equipped with a flame ionisation detector (GC-FID) or coupled to a mass spectrometer (GC-MS). An array of well-established tools is available to corroborate qualitative and/or quantitative results. These tools include the use of isotope-labelled or other internal standards (IS). The extraction solvents generally used are common volatile compounds (e.g. pentane, dichloromethane, diethyl ether, ethyl acetate or mixtures thereof). The utilisation of Freon 11 (trichlorofluoromethane) is also reported (Forcén et al., 1992).

Detection of benzyl alcohol frequently concerns its volatile unbound form only, although a quite large number of publications also deal with the assessment of the total amount of benzyl alcohol present as both unbound and glycoside- and/or phosphate-bound forms. The latter are subject to enzymatic hydrolysis (Garcia et al., 2011; Osorio et al., 2002; Ruiz-García et al., 2014; Vilanova et al., 2012) or chemical hydrolysis (Rodríguez-Bencomo et al., 2011) to obtain the free alcohol.

3.1.5. Stability of the substance, and reaction and fate in food

In the work of Sudareva and Chubarova (2006), aqueous solutions of benzyl alcohol – deprived of trace metal ions – were used to investigate the oxidative reaction



as function of: storage time (up to 189 days), in darkness or light; the initial concentration of the chemical; water purified with two different procedures; solutions saturated or not with argon. As the work was not designed to investigate the kinetics of benzyl alcohol reactions, no quantitative relationships to predict solution composition changes with time were provided.

Solutions contained from 0.005 to 2.09 mg benzyl alcohol/mL; the presence of benzyl alcohol, benzaldehyde and benzoic acid in these solutions was tested by liquid chromatography (LC) and spectrophotometry. The authors concluded that, at room temperature, benzyl alcohol underwent oxidation even when its aqueous solutions were stored in darkness and under careful conditions to prevent exposure to air oxygen, i.e. saturated with argon in sealed-off ampoules.

As reported in the preceding Section 3.1.4, benzyl alcohol can be found in food matrices, wines and beverages in the free form as well as in the bound form (glycoside- and/or phosphate-bound), which can represent quite a large fraction of the total amount of the benzyl alcohol present.

A moderate antioxidant activity was assigned to benzyl alcohol (Lee and Shibamoto, 2000, 2001a,b).

3.2. Authorised uses and use levels

Benzyl alcohol (E 1519) is authorised as a food additive in food flavourings according to Annex III, Part 4 to Regulation (EC) No 1333/2008¹⁴, as amended, with a maximum level of 100 mg/L in final food of liqueurs, aromatised wines, aromatised wine-based drinks and aromatised wine-products cocktails, and with a maximum level of 250 mg/kg in confectionery, including chocolate and fine bakery wares (from all sources in foodstuffs as consumed or as reconstituted according to instruction of the manufacturer).

Benzyl alcohol (E 1519) is not authorised to be added directly to food according to Annex II to Regulation (EC) No 1333/2008 on food additives.

¹⁴ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16.

According to Annex I of EU Regulation 1334/2008 (i.e. the EU Union List of flavouring substances Commission Implementing Regulation (EU) 872/2012), benzyl alcohol is authorised as a chemically defined flavourings substance (FL-no: 02.010) without restrictions.

3.3. Exposure data

3.3.1. Reported use levels or data on analytical levels of benzyl alcohol (E 1519)

Most food additives in the EU are authorised at a specific maximum permitted level (MPL). However, a food additive may be used at a lower level than the MPL. Therefore, information on actual use levels is required for performing a more realistic exposure assessment.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call¹⁵ for occurrence data (use level and/or analytical data) on benzyl alcohol (E 1519). In response to this public call, updated information on the actual levels of benzyl alcohol (E 1519) in foods was made available to EFSA by industry (levels resulting from carry over). No analytical data on the concentration of benzyl alcohol (E 1519) in foods were made available by the Member States.

Summarised data on reported use levels in foods provided by industry

Industry provided EFSA with 13 use levels of benzyl alcohol (E 1519) in foods belonging to four food categories. These levels were provided by FoodDrinkEurope (FDE) and Intersnack. These use levels referred to the use of benzyl alcohol (E 1519) in confectionery, including chocolate and fine bakery wares according to Annex III to Regulation (EC) No 1333/2008. One use level was excluded from further evaluation due to unclear description of the food product.

Appendix B provides an overview of the use levels of benzyl alcohol (E 1519) in foods as reported by industry.

3.3.2. Summarised data extracted from the Mintel's Global New Products Database

The Mintel's GNPD is an online database that monitors new introductions of packaged goods in the market worldwide. It contains information of over 2.5 million food and beverage products of which more than 900,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently it contains data from 25 out of its 28 Member States and Norway.¹⁶

For the purpose of this Scientific Opinion, Mintel's GNPD¹⁷ was used for checking the labelling of food and beverages products and food supplements for benzyl alcohol (E 1519) within the EU's food market as the database contains the compulsory ingredient information on the label.

According to Mintel's GNPD, benzyl alcohol (E 1519) was labelled on only one product belonging to the food subcategory of Mintel's GNPD 'Baking ingredients & mixes'.

The Panel noted that this information does not fully cover the actual use of benzyl alcohol (E 1519), because food additives authorised according to Annex III without a function as a food additive in the final product do not need to be labelled.¹⁸

3.3.3. Food consumption data used for exposure assessment

EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure

¹⁵ <https://www.efsa.europa.eu/en/data/call/170223>

¹⁶ Missing Cyprus, Luxembourg and Malta.

¹⁷ <http://www.gnpd.com/sinatra/home/> accessed on 17/5/2019.

¹⁸ Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. OJ L 304/18, 22.11.2011, p. 46.

Assessment' (EFSA, 2011a). Consumption surveys added in the Comprehensive Database in 2015 were also taken into account in this assessment.¹⁹

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible subjects' underreporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database includes the currently best available food consumption data across Europe.

Food consumption data from infants, toddlers, children, adolescents, adults and the elderly were used in the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 2).

Table 2: Population groups considered for the exposure estimates of benzyl alcohol (E 1519)

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From more than 12 weeks up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers ^(a)	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, UK
Children ^(b)	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK
The elderly ^(b)	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Netherlands, Sweden, UK

(a): The term 'toddlers' in the Comprehensive Database corresponds to 'young children' in Regulations (EC) No 1333/2008 and (EU) No 609/2013.

(b): The terms 'children' and 'the elderly' correspond to 'other children' and the merge of 'elderly' and 'very elderly' in Comprehensive Database, respectively.

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the food categorisation system (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D to perform an exposure assessment. In practise, FoodEx codes were matched to the FCS food categories.

Food categories considered in the exposure assessment of benzyl alcohol (E 1519)

As described in Section 3.2, benzyl alcohol (E 1519) is only authorised as a food additive in food flavourings without a function as a food additive in the final product according to Annex III to Regulation No 1333/2008. It is authorised in liqueurs, aromatised wines, aromatised wine-based drinks and aromatised wine-products cocktails, and in confectionery, including chocolate and fine bakery wares.

For the purpose of this assessment, relevant food categories of Annex II of Regulation (EC) No 1333/2008, part D were identified and assigned to the corresponding alcoholic-based beverages and confectionary food products as defined in Annex III.

The food categories in which the use of benzyl alcohol (E 1519) is authorised were selected from the nomenclature of the Comprehensive Database (FoodEx classification system), at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011b).

In the *regulatory maximum level exposure scenario*, the food categories taken into account were (in ascending order of the FCS codes):

- 05.1 Cocoa and Chocolate products as covered by Directive 2000/36/EC,
- 05.2 Other confectionery including breath freshening microsweets,

¹⁹ Available online: <http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm>

- 05.3 Chewing gum,
- 05.4 Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4,
- 07.2 Fine bakery wares,
- 14.2.6 Spirit drinks as defined in Reg (EC) No 110/2008, only liqueur,
- 14.2.7.1 Aromatised wines.

Some food categories or their restrictions/exceptions are not referenced in the Comprehensive Database and could therefore not be taken into account in the present estimate. This was the case for two food categories (Appendix C) and may have resulted in an underestimation of the exposure. The food categories that were not taken into account were:

- Aromatised wine-based drinks;
- Aromatised wine-product cocktails.

For the refined scenario, three additional food categories (FC 05.4 Decorations, coatings and fillings, FC 14.2.6 Spirit drinks, only liqueur and 14.2.7.1 Aromatised wines) were not taken into account because no concentration data were provided for these food categories to EFSA (Appendix C).

Overall, seven food categories were included in the *regulatory maximum level exposure scenario*, and four food categories were included in the refined scenarios (Appendix C).

3.4. Exposure estimates

3.4.1. Exposure to benzyl alcohol (E 1519) from its use as a food additive

The Panel estimated the chronic dietary exposure to benzyl alcohol (E 1519) for the following population groups: infants, toddlers, children, adolescents, adults and the elderly. Dietary exposure to benzyl alcohol (E 1519) was calculated by multiplying concentrations of benzyl alcohol (E 1519) per food category (Appendix B) with their respective consumption amount per kilogram body weight for each individual in the Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Dietary surveys with only 1 day per subject were excluded as they are considered as not adequate to assess repeated exposure.

The exposure was calculated for all individuals per survey and per population group, resulting in distributions of individual exposure per survey and population group (Table 2). Based on these distributions, the mean and 95th percentile of exposure were calculated per survey and per population group. The 95th percentile of exposure was only calculated for those population groups with a sufficiently large sample size (EFSA, 2011a). Therefore, in the present assessment, the 95th percentile of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain was not estimated.

The exposure assessment to benzyl alcohol (E 1519) was carried out by the FAF Panel based on two different sets of concentration data: (1) MPLs as set down in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*); and (2) reported use levels (defined as the *refined exposure assessment scenario*). These two scenarios are discussed in detail below.

Regulatory maximum level exposure assessment scenario

The *regulatory maximum level exposure assessment scenario* was based on the MPLs as set in Annex III to Regulation (EC) No 1333/2008.

The Panel considers the exposure estimates derived following this scenario as the most conservative since it is assumed that the population will be exposed to the food additive present in food at the MPL.

Refined exposure assessment scenario

The *refined exposure assessment scenario* is based on use levels reported by food industry. This exposure scenario can consider only food categories for which these data were available to the Panel.

Based on the available data set, the Panel calculated two refined exposure estimates for benzyl alcohol (E 1519) based on two model populations:

- The brand-loyal consumer scenario: It was assumed that a consumer is exposed long term to benzyl alcohol (E 1519) present at the maximum reported use level for one food category. This exposure estimate is calculated as follows:

- Combining consumption levels of the main contributing food category at the individual level with the maximum of the reported use levels.
- Combining consumption levels of the remaining food categories with the mean of the typical reported use levels for the remaining food categories.
- The non-brand-loyal consumer scenario: It was assumed that a consumer is exposed long term to benzyl alcohol (E 1519) present at the mean reported use levels in food. This exposure estimate is calculated using the mean of the typical reported use levels for all food categories.

Appendix B summarises the use levels of benzyl alcohol (E 1519) used in the regulatory maximum level exposure assessment scenario and refined exposure scenarios.

Dietary exposure to benzyl alcohol (E 1519)

Table 3 summarises the estimated exposure to benzyl alcohol (E 1519) from its use as a food additive in six population groups according to the different exposure scenarios. Detailed results per population group and survey are presented in Appendix C.

Table 3: Summary of dietary exposure to benzyl alcohol (E 1519) from its use as a food additive in the regulatory maximum level exposure assessment scenario and in the refined exposure assessment scenarios, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

	Infants (12 weeks– 11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Regulatory maximum level exposure assessment scenario						
• Mean	< 0.01–0.34	0.05–0.98	0.21–1.04	0.13–0.59	0.05–0.37	0.05–0.33
• 95th percentile	< 0.01–1.43	0.22–2.34	0.63–2.53	0.38–1.40	0.20–1.04	0.15–0.89
Refined exposure assessment scenario						
Brand-loyal scenario						
• Mean	< 0.01–0.03	0.01–0.27	0.05–0.26	0.04–0.18	0.01–0.05	< 0.01–0.03
• 95th percentile	< 0.01–0.12	0.05–0.81	0.21–0.67	0.15–0.47	0.05–0.20	0.02–0.11
Non-brand-loyal scenario						
• Mean	< 0.01–0.03	0.01–0.14	0.03–0.17	0.03–0.12	0.01–0.04	< 0.01–0.02
• 95th percentile	< 0.01–0.10	0.04–0.38	0.14–0.53	0.11–0.37	0.04–0.13	0.02–0.09

In the *regulatory maximum level exposure assessment scenario*, mean exposure to benzyl alcohol (E 1519) from its use as a food additive ranged from < 0.01 mg/kg bw per day in infants to 1.04 mg/kg bw per day in children. The 95th percentile of exposure to benzyl alcohol (E 1519) ranged from < 0.01 mg/kg bw per day in infants to 2.53 mg/kg bw per day in children.

In the *refined brand-loyal exposure scenario*, the mean exposure to benzyl alcohol (E 1519) from its use as a food additive ranged from < 0.01 mg/kg bw per day in infants and the elderly to 0.27 mg/kg bw per day in toddlers. The high exposure to benzyl alcohol (E 1519) ranged from < 0.01 mg/kg bw per day in infants to 0.81 mg/kg bw per day in toddlers. Corresponding estimates for the *non-brand-loyal scenario* were < 0.01 mg/kg bw per day in infants and the elderly and 0.17 mg/kg bw per day in children for the mean exposure and < 0.01 mg/kg bw per day in infants to 0.53 mg/kg bw per day in children for the 95th percentile of exposure.

Main food categories contributing to exposure to benzyl alcohol (E 1519)

In the *regulatory maximum level exposure assessment scenario*, the main contributing food category to the total mean exposure estimates for all population groups was fine bakery wares (see Appendix D for more details). The main contributing food category in the *brand-loyal* and *non-brand-loyal scenario* was cocoa and chocolate products for all population groups. (see Appendix D for more details).

Appendix D can be found in the online version of this output ('Supporting information' section): <https://doi.org/10.2903/j.efsa.2019.5876>

The Panel considered that the refined exposure assessment approach resulted in more realistic long-term exposure estimates compared to the *regulatory maximum level exposure assessment scenario*.

Benzyl alcohol (E 1519) is used as flavour enhancer and influences the organoleptic properties of the final food. Furthermore, the main contributing food category in the refined scenarios was cocoa and chocolate products, for which brand loyalty to a specific food product(s) can occur. For these reasons, the Panel considered the *brand-loyal scenario* as the most appropriate scenario for risk characterisation.

Uncertainty analysis

Uncertainties in the exposure assessment of benzyl alcohol (E 1519) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and summarised in Table 4.

Table 4: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction ^(a)
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/-
Methodology used to estimate high percentiles (95th) long-term (chronic) exposure based on data from food consumption surveys covering only a few days	+
Correspondence of reported use levels to the food items in the Comprehensive Database: uncertainties to which types of food the levels refer	+/-
Uncertainty in possible national differences in use levels of food categories	+/-
Use levels: • use levels considered applicable to all foods within the entire food category • unclear representativeness of foods on the EU market	+ +/-
Food categories used in the maximum regulatory exposure assessment scenario: exclusion of food categories due to missing FoodEx linkage (n = 2/9 total number of food categories)	-
Food categories used in the refined exposure assessment: Two food categories were excluded due to missing FoodEx linkage and three food categories were excluded due to missing use levels (n = 5/9 total number of food categories) The four remaining food categories out of all authorised food categories (n = 9), corresponded to 0.7–97% of the amount (g of foods by body weight) of food consumption documented in the Comprehensive Database	-
Regulatory maximum level exposure assessment scenario: – exposure calculations based on the MPL according to Annex III to Regulation (EC) No 1333/2008	+
Refined exposure assessment scenarios: – exposure calculations based on the maximum or mean levels (reported use from industry)	+/-

(a): +, uncertainty with potential to cause overestimation of exposure; -, uncertainty with potential to cause underestimation of exposure.

Overall the Panel considered that the uncertainties identified resulted in an overestimation of the exposure to benzyl alcohol (E 1519) as a food additive in European countries considered in the Comprehensive Database for the regulatory maximum level exposure scenario and for the refined exposure scenario. This was mainly due to the assumption that all foods within a relevant food category were assumed to contain the additive at the MPL or a level equal to the maximum or typical use level.

3.4.2. Other sources

Benzyl alcohol is also a naturally occurring substance, for example, wines, grapes, sour cherry, cider, mushrooms, chestnuts, almonds and clove at concentrations up to 3 mg/kg. In wine and tomato, benzyl alcohol is present either free or bound in the form of glycosides, and it can be released from the glycosides via a further fermentation or enzymatic activity (Diéguez et al., 2003; Marlatt et al., 2004; Ganss et al., 2011).

3.5. Biological and Toxicological data

3.5.1. Absorption, distribution, metabolism and excretion

The text below summarises common knowledge on the subject and was extracted from reports of JECFA (JECFA 1996, 2015) and the Scientific Committee on Food (SCF 2002) and the reviews by Nair et al. (2001), Adams et al. (2005), Belsito et al. (2012), Scognamiglio et al. (2012), Api et al. (2015), Gry et al. (2015).

In humans and various animal species, benzyl alcohol is metabolised to benzoic acid which reacts with glycine in the liver. The resulting hippuric acid is excreted quickly in urine and 75–100% of the dose is excreted within 6 h. Biotransformation of benzoic acid to hippuric acid follows saturable or Michaelis–Menten kinetics in humans (Kubota and Ishizaki, 1991) and the availability of glycine is the rate limiting step. When glycine is depleted, free benzoic acid maybe excreted unchanged or as the glucuronic acid conjugate. Even with saturation of hippuric acid formation, clearance of compounds in the benzyl group is relatively rapid in most laboratory animal species and in humans.

The octanol/water partition coefficient of benzyl alcohol ($\log[P_{ow}] = 1.1$) and the relatively high solubility in water (40 g/L at 20°C) indicate a low potential for bioaccumulation (Api et al., 2015; EFSA, 2012; OECD, 2001).

3.5.2. Acute toxicity

Acute oral toxicity (LD_{50}) reported for benzyl alcohol was 1,580 mg/kg bw in mice (Jenner et al., 1964 cited in Belsito et al. 2012), ranged from 1,230 to 3,100 mg/kg bw in rats (Graham and Kuizenga, 1945; Smyth et al., 1951; Jenner et al., 1964; Bar and Griepentrog, 1967; Koch et al., 1993; Nishimura et al., 1994) and was 1,040 mg/kg bw in rabbits (Graham and Kuizenga, 1945).

The Panel noted that benzyl alcohol was of low acute toxicity.

3.5.3. Short-term and subchronic toxicity

Mice

B6C3F1 mice (5/sex per group) received by gavage 0, 125, 250, 500, 1,000 and 2,000 mg benzyl alcohol/kg bw per day in corn oil for 5 day per week during 16 days (NTP, 1989). All mice from 2,000 mg/kg bw per day group and 1 male and 2 females from 1,000 mg/kg bw per day group died before the scheduled termination. Lethargy and rough hair coats were seen in males from 500 mg/kg bw per day group or higher and in females from the two highest dose groups. At necropsy, blood in urinary bladder was observed in two highest groups. There were no treatment-related microscopic findings in treated animals.

B6C3F1 mice (10/sex per group) received by gavage 0, 50, 100, 200, 400 or 800 mg/kg bw benzyl alcohol daily by gavage 5 days per week for a period of 13 weeks (NTP, 1989). Although the deaths occurred in all treatment groups, they were except one attributed by the authors of the study to the gavage error. High-dose females were lethargic during the first 2 weeks of the study. The terminal body weights of females from 400 and 800 mg/kg bw per day groups were 5 and 8% lower as compared to controls. The Panel considered the decrease in body weight as non-adverse because of the low magnitude of the change. There were no treatment-related microscopic findings at any of the doses.

Rats

F344 rats (5/sex per group) received by gavage 0, 125, 250, 500, 1,000 and 2,000 mg benzyl alcohol/kg bw per day in corn oil for 5 day per week during 16 days (NTP, 1989). All rats from 2,000 mg/kg bw per day group and two males and three females from 1,000 mg/kg bw per day group died before the scheduled termination. Lethargy was seen at two highest doses in animals of both sexes. Rough hair coats were observed at 500 and 1,000 mg/kg bw per day in males and at 250 and 500 mg/kg bw per day in females. The terminal body weights of males from 1,000 mg/kg bw per day groups was 18% lower as compared to controls. Rats from groups receiving doses of 1,000 or 2,000 mg/kg per day had blood around the mouth and nose, subcutaneous haemorrhages, and blood in the urinary and gastrointestinal tracts. There were no treatment-related microscopic findings at any of the doses.

F344 rats (10/sex per group) received by gavage 0, 50, 100, 200, 400 or 800 mg/kg bw benzyl alcohol 5 days per week for a period of 13 weeks (NTP, 1989). Eight males and two females at 800 mg/kg bw, one female at 400 mg/kg bw, one male at 200 mg/kg bw and one female in the control

group died prior to the scheduled termination. Some of these deaths were attributed by the authors to gavage error. Rats of each sex in the highest dose group manifested neurotoxicity in form of staggering, laboured breathing and lethargy. Furthermore, five of males from this group had blood around nose and mouth after 8 weeks of treatment. The body weight at the end of treatment was 7% and 5% lower in males and females from in the highest dose group as compared to controls. The Panel considered the decrease in body weight as non-adverse because of the low magnitude of the change. The treatment-related microscopic changes were recorded only in the highest dose group: necrosis of the dentate gyrus of the hippocampus (9/9 males and 7/7 females), skeletal muscle necrosis (5/10 males), thymic congestion, haemorrhage and atrophy (8/10 males).

Overall, oral short-term or subchronic treatment with benzyl alcohol resulted in neurotoxicity manifested as lethargy in mice (from 500 mg/kg per day) and lethargy or staggering (from 800 mg/kg bw per day) in rats. No clinical chemistry and haematology parameters were examined in these studies.

3.5.4. Genotoxicity

In vitro

A number of studies demonstrated that benzyl alcohol is not mutagenic in the bacterial reverse mutation assay with *Salmonella* Typhimurium TA92, TA94, TA98, TA100, TA1535, TA1537 and TA1538 with and without metabolic activation (NTP TR343, 1989; (Ishidate et al., 1984) (Rogan et al., 1986)). Equivocal results were obtained with the benzyl alcohol in the Rec DNA repair assay using *Bacillus subtilis* strains H17 and M45 (Oda et al., 1978; Kuroda et al., 1984; Yoo, 1986). In the SOS/umu test with *Salmonella* Typhimurium TA1535/pSK1002 benzyl alcohol did not induce positive response (Yasunaga et al., 2004).

Two studies of the *in vitro* mammalian cell gene mutation assay with mouse L5178Y/TK+/- lymphoma cells were performed with benzyl alcohol. In both studies, it was negative in the presence of metabolic activation, whereas without metabolic activation, an increase in mutant frequency was observed at 4,500 µg/mL with a relative total growth of 20% (NTP, 1989). The Panel noted that such increase was reported at a concentration above the maximum level recommended by the current OECD test guideline and in the presence of excessive cytotoxicity. Anyway, the mutation frequency did not exceed the Global Evaluation Factor indicated in OECD TG 490; therefore, the Panel considered the results of this study as negative.

In the two studies of the *in vitro* chromosomal aberrations assays, benzyl alcohol at high concentration (4,000 µg/mL) induced increase in chromosomal aberrations in Chinese hamster ovary cells in the presence but not in the absence of metabolic activation (NTP, 1989). A negative result of a chromosomal aberration assay was reported in Chinese hamster fibroblast cells exposed to up to 1 mg/mL benzyl alcohol without metabolic activation (Ishidate et al., 1984). The results of the induction of sister chromatid exchanges in CHO cells were equivocal (NTP, 1989). A more recent *in vitro* micronucleus assay that was performed according to OECD TG 487 with Chinese hamster lung cells and TK6 human lymphoblastoid cells gave negative results at the maximum test concentration of 10 mM (1,081 µg/mL) recommended in the guideline as the limit concentration (Fowler et al., 2012).

In rat hepatocytes, the induction of DNA damage by benzyl alcohol (1 and 10 mM) was determined with the alkaline elution assay. DNA damage was detected at 10 mM; however, it was associated with cytotoxicity and the author concluded that the result is negative (Storer et al., 1996). In the *in vitro* comet assay with isolated blood cells exposed to 1–50 mM benzyl alcohol induced statistically significant increase in DNA damage at 25 and 50 mM (Demir et al., 2010). However, the exposure conditions are not described and cytotoxicity was not determined; thus, the observed DNA damage might be due to the cytotoxicity; moreover, this test does not belong to the tests recommended by the SC for the assessment of genotoxicity (EFSA, 2011a,b).

In vivo

In a mouse micronucleus assay, benzyl alcohol was tested at doses 50, 100, 200 mg/kg administered by i.p. injection. The animals were sacrificed 24 h after the administration of the tested compound and the micronuclei were determined in bone marrow polychromatic erythrocytes. The test results were negative at all doses tested (Hayashi et al., 1988).

In summary, benzyl alcohol was tested with negative results in the base set of genotoxicity tests recommended by EFSA (Ames and *in vitro* micronucleus). Equivocal or weakly positive result were reported in some other *in vitro* tests, but only at high doses, above the current maximum recommended dose level (10 mM or 2,000 µg/mL, whichever is lower; OECD 2016). Negative results were obtained in

an *in vivo* micronucleus assay by i.p. administration. Overall, the Panel concluded that benzyl alcohol does not raise concern with respect to genotoxicity.

3.5.5. Chronic toxicity and carcinogenicity

No chronic toxicity studies were available.

Mice

In a 2-year carcinogenicity study, benzyl alcohol (purity 99%) was given by gavage to groups of 50 B6C3F1 mice of each sex, 8–9 weeks of age, at a dose of 0, 100, 200 mg/kg bw per day in corn oil on 5 days per week for 103 weeks (NTP, 1989). The mean body weights of treated and control mice were comparable throughout the study. The survival of control females was significantly lower than that of mice of the high-dose group after week 74, but no other differences in survival were observed. No significant treatment-related effects were noted at gross necropsy or histopathological examination. A dose-related negative trend was observed in the incidence of Harderian gland adenomas in male mice (8/50, in controls; 3/50, in low-dose group; 2/50, in high dose group). An increased incidence of adrenal cortex adenomas was noted in high-dose male mice (0/48; 0/44; 3/48), was within the historical control range and not considered compound related. The NOAEL in this study was 200 mg/kg bw per day, the highest dose tested.

Rats

Groups of 100 F344/N rats (50 each sex) were dosed by gavage with 0, 200 or 400 mg/kg bw per day benzyl alcohol in corn oil, 5 days per week for 103 weeks (NTP, 1989). Administration of benzyl alcohol did not affect survival in male rats, but reduced survival of female rats by half (70% of controls; 34% of low-dose females and 34% of high-dose females). Most of these accidental deaths were considered related to gavage errors. No effect on body weight gain was observed. No apparent compound-related non-neoplastic lesions were observed. A dose-related negative trend in incidence of anterior pituitary neoplasms was seen in female rats (vehicle control, 29/509; low dose, 17/47; high dose, 9/49). Epithelial hyperplasia of the forestomach was noted in 4/50 high-dose male rats, whereas it was not seen in controls or low-dose rats. The Panel noted that effect on forestomach is a local irritation due to high concentration when administered by gavage. The NOAEL was 400 mg/kg bw per day, the highest dose tested.

Overall, benzyl alcohol is not carcinogenic in mice and rats up to 200 and 400 mg/kg bw per day, respectively, the highest doses tested.

3.5.6. Reproductive and developmental toxicity

Reproductive toxicity studies

No studies available.

Developmental toxicity studies

Mice

Fifty female CD-1 mice were administered 550 mg/kg bw per day benzyl alcohol by gavage on days 6–15 of gestation. A control group of 50 mice received corn oil only. Body weight, clinical observations and mortality were recorded daily throughout treatment and up to 3 days postpartum. All parameters tested, including gestation index, average number of live pups/litter, postnatal survival and pup body weight, were statistically similar for the treated and control animals (York et al., 1986).

In a preliminary screening test, benzyl alcohol was administered by gavage to CD1 mice (50 females) at 750 mg/kg bw per day from gestational days 6–13 (Hardin et al., 1987; RIFM, 1986). Only 62% of the dams survived, and they showed a decrease in body weight. There were no changes in reproductive or gestation indices. Statistically significant reduction in litter weight, birth weight and weight gain of pups was also reported. It was reported in a review of this study that '*clinical signs of maternal toxicity were observed, including hunched posture, tremors, inactivity, prostration, hypothermia, ataxia, dyspnoea, swollen or cyanotic abdomen, and piloerection*' (York et al., 1986).

In summary, no reproductive toxicity studies were available for benzyl alcohol. The study design of the two developmental screening studies in mice was limited. In both studies, both only one dose of benzyl alcohol was tested. Benzyl alcohol was administered from gestational day (GD) 6–15 or GD 6–13 and dams were allowed to litter and necropsied on PND 3. No fetal pathology was performed. The

study of York et al. (1986) showed no effects at 550 mg/kg bw per day and in the study of Hardin et al. (1987), 38% mortality of the dams and effects on pup weight were observed at 750 mg/kg bw per day.

3.5.7. Hypersensitivity, allergenicity and food intolerance

Case reports of adverse reactions to benzyl alcohol that may point at hypersensitivity reactions after injection have been reported (Barbaud (1995), Shmunis (1984), Wilson et al. (1986), Turvey et al. (2004), Grant et al. (1982).

As reviewed by Api et al. (2015), based on the structure of the molecule benzyl alcohol is not predicted to be directly reactive to proteins, while in *in chemico* experimental studies, little to no reactivity to cysteine-based peptides has been reported. Benzyl alcohol has been reported to be both positive and negative in guinea pig test (GPMT) methods. Additionally, benzyl alcohol has been evaluated in the murine local lymph node assay (LLNA), and was reported to have an EC₃ value of > 50%. The dermal sensitisation potential of benzyl alcohol has also been evaluated in the Human Repeated Insult Patch Test (HRIPT) and the human maximisation test. Based on the available data, benzyl alcohol is considered to be a weak skin sensitiser.

Even if benzyl alcohol possesses some sensitising capacity, the intestinal immune system has a regulatory mechanism in place that induces oral tolerance to ingested low molecular compounds (Gutting et al., 2003; Wambre and Jeong, 2018). Low molecular weight compounds will therefore not readily induce hypersensitivity reactions after oral ingestion. In addition, no allergic reactions after ingestion of benzyl alcohol after ingestion have been reported in the population.

For this reason, whereas allergic reactions to benzyl alcohol after ingestion cannot be ruled out, the Panel considers the likelihood of such reactions to occur as low.

3.5.8. Other studies

The Panel is aware of cases of 'gasping syndrome' in pre-term new-born infants after intravenous administration of benzyl alcohol as an excipient of pharmaceutical product (add references). However, the use of this compound has been discontinued and this opinion does not consider this effect because benzyl alcohol (E 1519) is not authorised in food for infants below 16 weeks of age (<https://www.cdc.gov/mmwr/preview/mmwrhtml/00001109.htm>).

3.6. Discussion

Benzyl alcohol (E 1519) is authorised as a food additive in the EU in accordance with Annex III to Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012⁵.

In the EU, benzyl alcohol (E 1519) has been evaluated by the Scientific Committee for Food (SCF) in 2002 (SCF, 2002) who concluded that '*the metabolism of benzyl alcohol to benzoic acid by man is well established. In special studies, biochemical effects have been investigated. The toxicity has been studied extensively, including acute, short-term and long-term toxicity, carcinogenicity, genotoxicity and developmental toxicity*'.

Benzyl alcohol was evaluated by JECFA in 1980 (JECFA, 1980) and concluded a group ADI (for benzoic acid together with benzyl alcohol) of 0–5 mg/kg bw, subsequently agreed by the SCF in 2002.

In 2016, EFSA ANS Panel re-evaluated the safety of benzoic acid and benzoates (E210-231) and established a group ADI of 5 mg/kg bw per day (expressed as benzoic acid). The re-evaluation did not include but excluding benzyl-alcohol E 1519.

In humans and animal species benzyl alcohol is metabolised to benzoic acid which reacts with glycine in the liver and the resulting hippuric acid is excreted quickly in urine.

The Panel noted that benzyl alcohol was of low acute toxicity.

Oral short-term or subchronic studies with benzyl alcohol showed neurotoxicity manifested as lethargy in mice (from 500 mg/kg per day) and lethargy or staggering (from 800 mg/kg bw per day) in rats.

Benzyl alcohol was tested with negative results in the base set of genotoxicity tests and the Panel concluded that benzyl alcohol does not raise safety concern with respect to genotoxicity.

Benzyl alcohol is not carcinogenic in mice and rats up to 200 and 400 mg/kg bw per day, respectively, which were highest doses tested. The NOAEL in the carcinogenicity study in rats was 400 mg/kg bw per day.

The Panel noted that reproductive studies were not available and that two developmental screening studies in mice were of limited design.

Although some case reports of adverse reactions to benzyl alcohol after injection have been reported, the Panel noted that no allergic reactions after ingestion of benzyl alcohol have been reported in the population. For this reason, whereas allergic reactions to benzyl alcohol after ingestion cannot be ruled out, the Panel considers the likelihood of such reactions to occur as low.

Considering the available data set, the Panel established an ADI of 4 mg benzyl alcohol/kg bw per day based on a NOAEL of 400 mg/kg bw per day from the carcinogenicity study in rats, based on no effect in the highest dose tested.

The dietary exposure to benzyl alcohol (E 1519) from its use as a food additive, the exposure was calculated based on (1) MPLs set out in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*) and (2) the reported use levels (defined as the *refined exposure assessment scenario*). Dietary exposure through this latter scenario was assessed using reported use levels for food categories for which benzyl alcohol (E 1519) is authorised (Annex III to Regulation No 1333/2008).

Using the available data, the Panel calculated exposure estimates based on different assumptions as described in Section 3.4. The Panel considered that the refined exposure assessment approach resulted in more realistic long-term exposure estimates compared to the *regulatory maximum level exposure assessment scenario*. However, also the refined exposure estimates resulted in overestimates of the exposure to benzyl alcohol (E 1519) from its use as a food additive according to Annex III to Regulation (EC) No 1333/2008 (Section 3.4.1).

Because benzyl alcohol (E 1519) is authorised only according to Annex III, the information about its use from Mintel GNPD does not provide information about its actual use in food. Food additives authorised according to Annex III without a function as a food additive in the final product do not need to be labelled¹⁸.

The mean and high exposure estimates in the refined exposure scenarios were maximally 0.27 and 0.81 mg/kg bw per day in toddlers, respectively (Table 3). Considering the food categories for which the presence of benzyl alcohol (E 1519) can be due to carry over via its authorisation according to Annex III to Reg (EC) No 1333/2008, the exposure estimates to benzyl alcohol (E 1519) were below the ADI of 4 mg/kg bw per day in all population groups. The Panel noted that also the exposure in the *regulatory maximum level exposure assessment scenario* is well below the ADI in all population groups.

The Panel also noted that the refined exposure estimates are based on information provided on the reported level of use of food additive benzyl alcohol (E 1519). If actual practice changes, this refined estimate may no longer be representative and should be updated.

4. Conclusions

Based on the toxicological database available, the Panel established the ADI of 4 mg/kg bw per day for benzyl alcohol (E 1519) and concluded that the exposure to benzyl alcohol (E 1519) does not raise a safety concern at the reported uses and use levels.

5. Recommendations

The Panel recommend that:

- The European Commission considers introducing the maximum levels of chlorinated organic compounds as impurities in the specifications for purity of E 1519 in EU Regulation 231/2012.

Documentation provided to EFSA

- 1) FDE (FoodDrinkEurope), 2017. Data on use levels of benzyl alcohol (E 1519) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2017). Submitted to EFSA on 29 November 2017.
- 2) Intersnack, 2017. Data on use levels of benzyl alcohol (E 1519) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2017). Submitted to EFSA on 29 November 2017.

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Abbreviations

ADI	Acceptable Daily Intake
bw	Body Weight
CAS	Chemical Abstract Service
DHS	dynamic headspace
ECHA	European Chemicals Agency
EINECS	European Inventory of Existing Commercial chemical Substances
EMA	European Medicines Agency
F1	First generation pups
F2	Second generation pups
FAF	Food Additives and Flavourings
GC-FID	gas chromatography equipped with a flame ionization detector
GC-MS	gas chromatography coupled to a mass spectrometer
GD	Gestation Days
GNPD	Global New Products Database
HD	hydrodistillation
HRIPT	Human Repeated Insult Patch Test
HS-SPME	headspace solid-phase micro-extraction
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LLE	liquid–liquid extraction
LLNA	local lymph node assay
LOD	limit of detection
MPLs	Maximum Permitted Levels
MSDE	micro simultaneous steam distillation-solvent extraction
NOAEL	No Observable Adverse Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
P1	First generation adults
P2	Second generation adults
P3	Third generation adults
REACH	Registration, Evaluation, Authorisation and restriction of Chemicals
SCF	Scientific Committee for Food
SFE	supercritical fluid extraction
SPE	solid-phase extraction
SPME	solid-phase micro-extraction
TemaNord	Nordic Council of Ministers
USE	ultrasonic solvent extraction
VOCs	volatile organic compounds
WHO	World Health Organization

Appendix A – Summary of reported use levels (mg/kg) of benzyl-alcohol (E 1519) provided by industry

Appendix B – Concentration levels of benzyl-alcohol (E 1519) used in the exposure assessment scenarios (mg/kg or mL/kg as appropriate)

Appendix C – Summary of total estimated exposure of benzyl-alcohol (E 1519) from its use as a food additive for the regulatory maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day)

Appendix D – Main food categories contributing to exposure to benzyl-alcohol (E 1519) using the regulatory maximum level exposure assessment scenario and the refined exposure assessment scenarios (> 5% to the total mean exposure)

Appendices A–D can be found in the online version of this output ('Supporting information' section): <https://doi.org/10.2903/j.efsa.2019.5876>



**Ministry of Environment
and Food of Denmark**
Environmental
Protection

**Survey and risk
assessment of
chemical substances
in chemical products
used for "do-it-yourself"
projects in
the home – follow-up
project**

**Survey of chemical
substances in consumer
products**

Survey of chemical
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Preface

This report covers the survey and risk assessment of selected do-it-yourself (DIY) products conducted from April 2019 to November 2019. The project was carried out by the Danish Technological Institut with DHI as subcontractor on exposure scenarios and risk assessment.

The project was a follow-up study to “Survey and risk assessment of chemical substances in chemical products used for “do-it-yourself” projects in the home” Survey of chemical substances in consumer products No. 167, which in the current project is referred to as the “pre-project”.

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- Thomas Sørensen, Danish Technological Institute
- Poul Bo Larsen, DHI

The project has been followed by Nadine Heidi Brueckmann and Nellie Anne Martin from the Danish Environmental Protection Agency along with Sarah Yassine from the Chemical Inspection Service.

This project was funded by the Danish Environmental Protection Agency.

Summary and conclusion

Summary

The purpose of this project is to expand the existing knowledge obtained through mapping and analysis in the pre-project Survey and risk assessment of chemical substances in chemical products used for "do-it-yourself" projects in the home.

The project contains three parts: 1) Mapping of products and establishment of exposure scenarios, 2) Analysis of volatile chemical substances 3) Risk assessment.

The mapping is performed as a supplementary study to the pre-project, and new products relative to this have therefore been identified and chosen for analysis. A total of 86 new products are found, of which 20 are 2-component products, a product type than in general was deemed especially problematic in the pre-project.

The identified products are divided into the following categories: 1) Fillers 2) Primers/membranes 3) Adhesives 4) Paints and lacquers 5) Sealer products 6) Floor wax, polish and care products.

20 products have been chosen based on the mapping and these have been sampled by The Chemical Inspection Service. The analysis of volatile chemical substances in these products has been conducted by emissions testing in climate chambers after 5 hours, 3 days, and 28 days, respectively after application. The analysed products have been chosen based on their chemical content as well as their hazard classification, with the aim that all the aforementioned product categories are represented.

In the category of paints and lacquers eight products have been tested, which all emit high concentrations of volatile chemical substances. Six of these products have been selected for risk analysis, which shows that after 5 hours of degassing all samples exceeded the permitted risk characterization ratio for one or more chemicals (including chronically neurotoxic hydrocarbons, as well as multiple mucosal irritants including butanone oxime), and therefore have unacceptable exposure levels for the user. After 3 days none of the 2-component paints and lacquers exceed the tolerable exposure level, while this still is the case for 1-component paints and lacquers, for a range of eye and respiratory irritants (compounds that are strong irritants to eyes and upper respiratory system). For the latter product group, three out of four products displayed unacceptably high emissions of 2-ethylhexanoic acid, which is deemed to potentially be harmful to the unborn child.

Of the four tested fillers three products emit high concentrations of volatile chemical substances, which is also the case for the only sealer product that is tested regarding emissions. Only one filler is chosen for risk analysis, where degassing after 5 hours and 3 days, respectively is deemed unacceptably high for hydrocarbons especially, while this has reached an acceptable level after 28 days.

In the primers/membranes product group only one product is selected for testing. This product emits phenol, formaldehyde, and acetaldehyde, however it is chosen for risk assessment, as the concentration of emitted volatile compounds is relatively low and are therefore considered to not pose a significant exposure risk. In the category adhesives two products were tested that primarily also emitted phenol, formaldehyde, and acetaldehyde. Of these products only one adhesive product intended for wet room purposes was chosen for risk analysis. After 5

hours and 3 days, respectively this adhesive exhibits an exposure level above the tolerable for the mucosal irritant and preservative chemical 5-chloro-2-methyl-3(2H)-isothiazolone (CMIT).

Four products from the product group floor wax, polish and care products have been tested and two of these have further been selected for risk assessment. One oil-based product intended for wooden tables (product ID 72) exceeds acceptable tolerance levels after 5 hours for hydrocarbons (among others), but acceptable levels are achieved after 3 days. Product ID 85, which is a linseed oil-based product for wood care, exhibits limited emissions after 3 days, however after 28 days a range of aldehydes and organic acids are found, which are not previously observed for this product.

The recommendation of the project is to ensure thorough ventilation during and after all do-it-yourself projects in the home as well as taking the precaution of not using the room/rooms in question directly after renovation.

Conclusion

Mapping of products and establishment of exposure scenarios

Within the mapping of products, emphasis was on targeting sales locations and specific products that were not included in the pre-project. 31 companies selling products for private use were identified in total, where 12 of these have not previously been examined. Furthermore, the survey showed that consumers can buy do-it-yourself products in large retail stores, however, as these are usually promotional items and not part of the standard assortment, they were not examined further. It was not possible to get specific sales numbers for the products sold to private consumers, as this information is partially confidential and partially not known exactly. This is due to the fact that all identified sales locations sell to both private and professionals. However, it was assessed that the supply of products in the physical stores largely reflects the sales of these products, as the stores primarily have products on stock that are considered to have a high turnover. For all product groups it was possible to find eco-labelled products (M1, EU Ecolabel, the Swan etc.), making it possible for the consumer to choose an alternative to the traditionally used items. In the majority of DIY stores it was possible to get information from an employee, however it was not in all cases possible to get sufficiently qualified guidance. Safety Data Sheets (SDS) were only available in a few cases without actually purchasing a given item. It can therefore be concluded that it can be quite challenging for a consumer without previous knowledge within the area, to be sufficiently informed regarding safety, before purchasing a DIY product.

Analysis of volatile chemical substances

The products found through the mapping of the products, were analysed based on the available information. Based on this, 20 products were selected for further analysis according to the following criteria: 1) Hazard label on product 2) Ingredients with hazard classification 3) Each product group should be represented. The analysed products are shown below:

Fillers	4 products (ID 14, 26, 36, 71)
Primers/membranes	1 product (ID 56)
Adhesives	2 products (ID 60, 65)
Paints and lacquers	8 products consisting of 4 products 1-component (ID 4, 11, 29, 32) and 4 products 2-component (ID 2, 5, 20, 30)
Sealants	1 product (ID 88)
Floor wax, polish and care products	4 products (ID 72, 80, 83, 85)

Measurement of the volatile chemical substances from the products was performed by the same procedure as in the pre-project. The products were applied on glass plates in the recommended dosage according to the manufacturer's instructions. Emissions measurements was performed in small climate chambers of 113 litres at 23 °C and 50 % RH with an air change rate of 0.5 h⁻¹ corresponding to testing according to EN 16516: 2017. Three measurements

were performed for each sample, in order to assess the exposure during and right after application of the product (5 hours) after a short period (3 days) and after a longer period (28 days). The sampling of volatile organic compounds (VOC) was performed on Tenax and subsequently the substances were analysed by TDS-GC/MS. For the analysis a library of 67 reference substances was utilized. These chemicals were chosen based on the volatile substances found in the mapping of the products in addition to previous experience from analysis of similar construction products. Volatile aldehydes were collected on DNPH and analysed by HPLC for formaldehyde, acetaldehyde, propanal, acrolein and butanal.

2-ethylhexanoic acid was found in the emissions for three out of four 1-component paint products (product ID 4, 11 and 32). This substance is formed during drying and is derived from calcium bis (2-ethylhexanoate)¹, which is added to the paint to contribute to the deep drying of especially alkyd-based products. For all 1-component paints alkanes in the form of naphta or other substances were found.

In all the 2-component paints volatile substances were found in the emissions in high concentrations including 2-butoxy ethanol, 1-methoxy propan-2-ol, naphta, benzyl alcohol and a range of esters. In two products (product ID 2 and 5) CMIT was found, although CMIT was not declared in the SDS for product ID 5.

The tested sealer product (Product ID 88) emitted styrene in smaller quantities as well as 2-propenoic acid, 2-methyl-1,2 ethanediyl ester in very high concentrations. The filler products all emitted either styrene or phenol, while product ID 14 emitted naphta in very large quantities.

A single membrane product intended for wet room use (product ID 56) from product group primers/membranes was tested. Phenol, formaldehyde and acetaldehyde were found in the emissions of this product.

Two adhesive products were tested, which both mainly emitted phenol, formaldehyde and acetaldehyde, while one (product ID 60) also emitted CMIT, which was not declared in the SDS².

After 5 hours, four products from the product group floor wax, polish and care products (ID 72, 80, 83, 85) all emitted naphta, while 2-butanone oxime emitted in low concentration from product ID 85. Furthermore, various aldehydes and carboxylic acids were observed in the emissions for product ID 85 (raw linseed oil) after 28 days, although these were not detected after 5 hours nor 3 days.

CMIT and / or methyl isothiazolinone (MIT) emitted from six different products (Product ID 2, 5, 60, 65, 80 and 83). MIT was identified in the emissions from a floor polish (ID 83) for which no SDS was available.

For all the tested products the highest emissions were generally measured immediately after application i.e. after 5 hours. This is to be expected, as the products must cure after application, which happens through evaporation of solvent and/or by evaporation of new volatile chemical compounds, which are formed during the curing process. For some products, new

¹ New information became available during the final project phase via the complete list of ingredients acquired by the Chemical Inspection Service. Products with IDs 4 and 11 contain 2-ethyl hexanoic acid, however in concentrations below the limit of declaration.

² This substance was not mentioned on the SDS by the time analysis were performed. The manufacturer informed later on that the SDS was updated with information about the content of the substance.

emissions profiles (i.e. combination of volatile substances) were detected after 3 days and once again after 28 days, where the product finally had cured. The components subject to declaration in the SDS were known in advance for 13 of the 20 investigated products and overall there was a good correlation between the declared ingredients and the volatile chemical substances identified by analysis.

Risk assessment

Based on the results from the analysis of volatile compounds ten products were selected for risk assessment, as shown below:

Fillers	1 product (ID 14)
Adhesives	1 product (ID 60)
Paints and lacquers	6 products consisting of 4 products 1-component (ID 4, 11, 29, 32) and 2 products 2-component (ID 5, 30)
Floor wax, polish and care products	2 products (ID 72, 85)

Thus, risk assessment was not performed for the product groups primers/membranes or sealants. The selection of products for the risk assessment focused on the products that continued to emit dangerous substances after 3 and 28 days. There was a clear overrepresentation of paints and lacquers among the chosen products, as six of the ten selected products belong to this group.

The emissions of the products were evaluated in relation to the tolerable exposure values, the so-called DNEL-values (Derived No Effect Level). These values are either found within the literature or from expert assessments, while a few cases they are calculated from toxicological data.

The emission values, which can be directly related to exposure, are compared with the DNEL value and a Risk Characterization Ratio (RCR) is calculated:

$$\text{RCR (X)} = \text{Measured exposure (Substance X)} / \text{DNEL (Substance X)}$$

When the exposure exceeds the DNEL-value the RCR-value is over 1, which indicates increased risk in relation to safety defined by the DNEL-value.

In the event that several of the emitted substances have the same type of effect, e.g., mucous membrane irritation or neurotoxic effects, it is considered relevant to add the RCR values for these substances. Hereby, each of the single-acting substances is believed to contribute to the same type of effect. Thus, many small RCR values together may result in an overall risk that is unacceptable (i.e. a total RCR value above 1), although the values for the individual substances are below the tolerable exposure level.

The risk assessments are based on the use of one DIY product at a time. In the event that multiple products are utilized simultaneously, the accumulated emissions will be larger and thereby lead to increased risk potential.

All products from each product groups exceeded the acceptable exposure level ($\text{RCR} > 1$) for one or more substances in the emissions after 5 hours. Particularly 1- and 2-component paints and lacquers exhibited high emission levels. This is considered to be most critical for a number of eye and respiratory irritants, such as 2-butanone oxime and triethylamine. None of the 2-component paints and lacquers exceeded the RCR-values of 1 after 3 days, while this continued to be the case for some eye and respiratory irritants in 1-component paints and lacquers. Furthermore, it should be noted that for the 3-day measurement for three out of six products, unacceptably high emissions of 2-ethylhexanoic acid was detected, which is considered to potentially be harmful to the unborn child.

The tested adhesive for wet rooms (product ID 60) showed RCR-values of 18 and 21 after 5 hours and 3 days, respectively, for the mucosal irritant preservative CMIT. This had, however, dropped to an acceptable exposure level ($RCR = 0.08$) after 28 days.

Emissions after 5 hours and 3 days, respectively, are unacceptably high for the tested filler (product ID 14), especially with regards to hydrocarbons ($RCR \geq 238$), while after 28 days it has reached an acceptable level.

Two products from the product group floor wax, polish and care products have been selected for risk assessment. DNEL-values for product ID 72 (oil for wooden tabletops) exceed acceptable tolerance levels after 5 hours for 2-(2-ethoxyethoxyethanol) and C7-C13 hydrocarbons by a factor of 11 and 147, respectively, while acceptable levels are achieved after 3 days.

For product ID 85 (linseed oil for wood care) emissions of 2-butanone oxime and C7-C13 hydrocarbons exceeded DNEL-values by a factor of 6 and 3, respectively. This product exhibited limited emissions after 5 hours and 3 days, however, after 28 days a range of aldehydes and organic acids occur, indicating that these are degradation products from the linseed oil.

After 28 days emissions have generally dropped to much lower levels for all products, however for some substances tolerable exposure levels are still exceeded. This applies, for example, to the substance 2-ethylhexanoic acid from 1-component paints and lacquers, which is considered critical, as the substance is potentially harmful to unborn children. Furthermore, an unacceptably high emission of the mucosal irritant preservative CMIT was found for a 2-component paint after 28 days, which was not measured after neither 5 hours nor 3 days.

1. Introduction

1.1 Background for the survey

In "Survey and risk assessment of chemical substances in chemical products used for "do-it-yourself" projects in the home" henceforth referred to as the "pre-project", it was shown that there can be a health risk when working with a number of products for minor home improvement renovations, if the consumer does not use proper personal protective equipment. For some products, there is a risk in the case of direct skin contact, while for others the evaporation of respiratory irritants and carcinogenic substances occurs for up to 28 days after application.

The use of do-it-yourself products are not subject to the same stringent requirements as products for professional use, for example it is not required to submit information to the Product Register for products used by private consumers. Therefore, there is a risk that retailers unknowingly sell goods to consumers, which do not comply with current legislation for professional use. It is not mandatory to provide safety data sheets (SDS) for products intended for private use.

In the pre-project, the following product types were identified as posing a risk by emission: "Chemical wood" (two component), floor lacquer (acid-curing), solvent-based floor paint, water-based epoxy floor paint (two component), water based wet room paint, PU-sealing foam, white spirit (mineral turpentine) and floor waxes. These types of products are typically applied in liquid form and then cured. During the curing process, a chemical reaction occurs, whereby volatile organic compounds can be formed and emit.

1.2 Objective

The objective of this project is to:

- Expand the existing knowledge obtained through mapping and analysis in the pre-project
- Mapping the prevalence of previously studied product types including the amounts used by the average consumer
- Determine whether suitable alternatives are found for existing products
- Gain more knowledge about the origin of the volatile organic compounds that emit; Are they initially present in the products or are they formed during curing
- Analyse the emission of volatile organic compounds from several products within the previously investigated product groups

1.3 Delimitation

In this project products that have previously been investigated in "Survey and risk assessment of chemical substances in chemical products used for "do-it-yourself" projects in the home" will not be registered. Products that have eco-labels, with criteria regarding emissions of volatile compounds are not considered. This includes EU Ecolabel, the Nordic Swan Ecolabel, Blue Angel (Germany), M1 (Finland) and the French emission label. Additionally, products which are clearly labelled for outdoor use will not be included, while products that are intended for regular cleaning such as care products such as care oils/waxes for furniture and floors are likewise not included.

Furthermore, there will be only limited focus on grocery stores in this study, as they to some extent supply chemical products to the do-it-yourself market, especially cleaning products, but to some extent also paints and lacquers. These points of sale are mentioned in the project but have not been further investigated.

2. Mapping of products and establishment of exposure scenarios

2.1 Objective

This project seeks to:

- Map the prevalence of the above product types including the use of the general consumer
- Clarify whether there are suitable alternatives to the existing products and what their advantages and disadvantages are
- Gain more knowledge about the origin of the volatiles that emit gases; Are they present in the products or are they formed during curing
- Analyse the emissions of volatile substances from several products within the previously investigated product groups (e.g. epoxy products)

2.2 Mapping

The mapping is based on the pre-project and the methods below have been used.

- The products offered for private use have been investigated on the Internet
- Products available in physical stores have been detected during visits to the following retailers: Silvan, Bauhaus, Jem & Fix, Biltema, Beck & Jørgensen, Stark, Harald Nyborg and XL-byg. Product choices and options have also been discussed with the staff in the stores in the cases when it has been possible to obtain advice from competent employees.
- The store main offices have been contacted by telephone and through e-mail correspondences to obtain information on quantities of sold products. In addition, companies have been contacted to provide SDS, if these were not available online
- Contact has been made to the Confederation of Danish Industry and the Danish Chamber of Commerce, who do not have information on products sold exclusively for private use
- In the survey, special attention was paid to investigating 2-component products, as these were found to be particularly problematic in the pre-project and the application in professional use requires mandatory specialized training.
- In relation to identifying replacement products for 2-component acid curing varnishes, some 1-component acrylic, polyurethane and acrylic/polyurethane lacquers are found on the market, which claim: "The floor surface can be compared with the old 2-component floor surfaces"³. Typically, these products do not exhibit quite the same durable properties as the 2-component systems⁴, however they are included in the mapping of products on the market.

2.3 Scenarios

When assessing the use of do-it-yourself products, the specific project for which the product will be used must be defined. Do-it-yourself projects in the home relevant to this survey fall in to one of the following three categories.

1. General maintenance, where the primary purpose is to improve or maintain the appearance of a surface by washing and subsequent soap treatment, oiling or coating

³ Quote concerning the product from webpage of Maling.dk " FAXE GOLAK - EKSTREM STÆRK MAT"

⁴ Communication with professional painters

2. Extensive maintenance, where surfaces are cleaned thoroughly by removing wallpaper, paint or lacquer, and subsequently rebuilding the surface by spackling, glass flies/fabric setting, priming and finishing
3. New build or complete renovation where the surface is built from the beginning, which may be brick walls, concrete walls or light walls (plaster, wood or similar)

2.4 Do-it-yourself projects

2.4.1 Project for the scenario "Normal maintenance"

The primary goal is to improve or maintain the appearance of the surface.

Here the typical project operation will be:

- Cleaning
- Refinishing with soap, wood oil or paint

2.4.2 Project for the scenario "Extensive maintenance"

The project where the surfaces are cleaned thoroughly before rebuilding.

Here the typical project operation will be:

2.4.2.1 Renovation of walls

- Removal of wallpaper, paint or lacquer
- Spackling
- Grinding⁵
- Priming
- Mounting of fiberglass fabric or wallpaper with adhesives.
- Priming of fiberglass fabric
- Application of paint

2.4.2.2 Renovation of concrete floors

- Removal of paint or lacquer
- Spackling of holes and gaps with concrete filler
- Priming with acrylic/epoxy or floor levelling with epoxy filler
- Application of paint/lacquer

2.4.2.3 Renovation of woodwork

- Removal of paint or lacquer
- Spackling
- Grinding
- Priming
- Application of paint/lacquer

2.4.3 Project for the scenario "New build or complete renovation"

The project where everything is done from scratch.

Here the typical project operation will be:

- Spackling
- Grinding
- Priming
- Setting of tiles, glass flies/fabric or wallpaper with adhesives
- Priming of glass flies/fabric – grouting of tiles

⁵ Grinding is specifically mentioned, because harmful substances as well as dust may be released during this process and these may be inhaled by the user.

- Application of paint/lacquer
- Gluing of architraves, lists and panels
- Painting of architraves, lists and panels

2.5 Identified product types for do-it-yourself projects

The products for the projects can be divided into the following categories:

TABLE 1. Product types including descriptions

Product type	Description
Fillers	These products are available as either dry powder, which must be mixed with water, or as ready-to-use products. These products are available as 1 and 2 component products. These products are always used early on in a project, to level holes, gaps and other unevenness of the surface. These products are either porous like putties for walls or solid like wood fillers. These products are used as spot repair or as a whole surface pre-treatment for example on a full wall.
Primers/membranes	These products are available as ready-made liquid products and are used as pre-treatment. These products are mostly used on larger areas, like whole walls and floors, and are pre-treatment for gluing, painting and as moisture controlling treatment.
Adhesives (glues)	These products are available as either dry powder, which must be mixed with water or as ready-to-use products. These products are available as 1 and 2 component products. These products are used for setting of tiles, glass flies/fabric or wallpaper and gluing of architraves, lists and panels. The products are mainly used on large areas.
Paints and lacquers	These products are available as ready-made liquid products. These products are used as the aesthetic finishing of a project These products are mainly used on large areas
Sealants	These products are available as ready-made liquid products. These products are used for filling gaps and maintain the building envelope. The products are mainly used on smaller areas.
Floor wax, polish and care products	These products are available as ready-made liquid products. These products are used as the aesthetic finishing of projects on wood-based surfaces. These products are mainly used on large areas

2.6 Summary of survey results

The survey has identified a total of 31 companies selling products for private use, of which there are 12 new ones that were not covered by the pre-project, cf. Appendix 1.

In this project, the focus is primarily on products that were not covered by the pre-project. A total of 86 new products have been found, of which 20 are 2-component, cf. Appendix 2.

The products are categorised in the following groups:

- Fillers
- Primers/membranes
- Adhesives (glues)
- Paints and/or lacquers

- Sealer products
- Floor wax, polish and care products (including oils and waxes)

Based on the information found in the survey, it must be concluded that as a consumer it can in some cases be a challenge to get qualified advice in the shops regarding do-it-yourself products. This advice will depend on several factors, including the merchant's knowledge of the application area of the product and the seller's expectations of what the customer requires (ecolabelling, durability, price, etc.). These expectations are probably influenced by the customer's age, gender and appearance. The range of products will also depend on the geographical location in the country and whether the customer group for the current store is primarily rural or urban.

An online service called "Malerfagligt behandlingskatalog" (MBK)⁶ is used by Danish professionals and recommends appropriate procedures for a given project. It was observed that staff in the DIY stores can provide guidance on procedures in a similar way to MBK's guidance. An example of an MBK instruction for floor paint is given in Appendix 3 (only available in Danish).

Acrylic-based paints/lacquers can largely replace epoxy-based products but are generally not considered as strong as these. The acrylic based products are far less hazardous in relation to emissions than the epoxy products and often have the same properties. It was observed that the use of 2-component floor paints / lacquers (which includes epoxy-based products) is not directly recommended in the construction markets for floors in general, but they are recommended for floors needing high wear resistance, for example workshops and garages. In two construction markets, a curing agent for a floor lacquer (product ID 29 cf. appendix 2.1) could not be purchased, even though the packaging stated that this component must be added before use. The construction market stated that the product can be used without the addition of a curing agent and this was confirmed by telephone to the technical support department of the manufacturer. Curing agents are added in cases where the lacquered surfaces must be extremely durable, but the product dries within the same time as if the hardener is added.

The survey found 15 1-component fillers, which can be alternatives to the 9 found 2-component fillers. In most cases, 1-component products are easier to work with for the consumer, as the product is ready to use and therefore does not need to be prepared before use. However, if a corresponding 2-component product is significantly cheaper, this may also affect the consumer's choice. In all product categories, products with an eco-label (The Blue Label, EU Eco-label, The Nordic Swan etc.) were identified, giving the consumer an option to avoid problematic substances. Eco-labelled products are not included in the survey, as it is expected that these products will comply with their respective labelling requirements, which in most cases also include the emissions of problematic substances. In some cases, the durability and/or performance of these alternatives may be inferior to the traditional products, but a closer qualitative study of this would be very elaborate and was therefore not included in the project.

The products selected in the survey are available at the visited stores, which indicates that they are used by private customers. The DIY stores have no interest in having items on the shelves that will not be sold, as there is a limited shelf life of the products, which means that expired items must be discarded without profit. The distribution of the products in the stores thus reflects to some extent the demand from the consumers. This has been verified by physical visits to selected stores. A large assortment of a given product in several retail chains may indicate that this product is also used in large quantities by consumers. It has not been possi-

⁶ <https://www.teknologisk.dk/mbk>

ble to find exact figures for the sale of the different types of products, partly because most construction markets sell both to private and professional, and partly because this information is confidential.

2.7 Exposure scenarios

2.7.1 Background for exposure scenarios

The pre-project demonstrated a risk for the operator during application/use of all products except the water-based floor paint, if appropriate precautions (thorough ventilation) or personal protective equipment (respiratory protection and/or protective gloves) were not used. This risk was verified through measurements of the vapours in the inhalation zone, while working with the products. This was a general finding for all products containing organic solvents. It is unlikely that new measurements and assessments will contribute with new knowledge in relation to this compared to the pre-project. Therefore, it is also not expected that there will be changes in the recommendations for working with the specific products, as the user can protect himself/herself by using the proper protective equipment.

In the pre-project problematic substances were shown to emit to the indoor climate over a longer time period, which can be problematic. Therefore, this project focuses on measuring emissions in small climate chambers and the emissions of the chemical products for up to 28 days after use.

Furthermore, the exposure assessment and risk assessment in this project do not focus on skin contact with the products, but only on the emissions and inhalation of the vapours after the products are used.

2.7.2 Description of exposure scenarios

The do-it-yourself exposure scenarios focus on exposure to the emissions for the users of a renovated room in the first weeks after the renovation.

Relevant unit of measurement

Measurements are given in the concentration unit $\mu\text{g}/\text{m}^3$, since the concentration in the room can thus be directly compared with a tolerable exposure level stated in $\mu\text{g}/\text{m}^3$ (Derived No Effect Level, DNEL, see chapter 4 regarding exposure and risk assessment).

Target group and time of occupancy

The measurements from small climate chambers are used in the same way as in the pre-project to assess the exposure in a renovated room. The users of the room can include all age groups from infants to elderly/old people, and the exposure time can be set as a worst-case scenario to 24 hours per day in case the person is bedridden. However, for exposure in a bathroom, the daily exposure time of 1 hour is considered more appropriate.

Room size

Based on the measurements in the climate chamber, the exposure is assessed in a reference room, which is a room with a floor area of 12 m^2 with a volume of 30 m^3 , cf. the European standard EN 16516 for measuring the emission of VOC from construction products to the indoor environment. Thus, the area of the treated surfaces of the test pieces can be scaled down relative to the size of the test chamber so that the results can be used directly as exposure levels for user occupancy in a standard room of 30 m^3 .

The emission rate and the measured concentration in the climate chamber will also depend on the following factors:

Amount of used product and applied area:

As in the pre-project, climate chamber test specimens will be prepared according to current test standards and recommendations for the current product type. The surface area and film thickness of liquid products such as coatings and fillers are important for the measured concentration.

As mentioned above, the area of the treated surfaces of the test specimens is scaled down to the size of the test chamber at 113 L, so that the results can be used directly as exposure levels for user occupancy in a standard room of 30 m³.

Measuring Time:

As in the pre-project, the concentration of emissions is measured after 5 hours, 3 days, and 28 days in order to assess the emissions during the first weeks after the renovation work and to assess any changes in the emissions from the product.

Air exchange:

Air exchange in the chamber in relation to the material load is adjusted to correspond to an air exchange of 0.5 times per hour in the reference room, since this is the building regulations minimum ventilation requirement in housing (BR18).

In connection with risk assessment of the listed products in Table 5, it is not considered necessary to change the assumptions in relation to the corresponding risk assessments carried out in the pre-project.

3. Analysis of volatile chemical compounds

3.1 Objective

The objective is to determine the emissions of volatile chemical compounds from selected do-it-yourself (DIY) products. These results will form the basis of the subsequent health and risk assessments.

3.2 Selection of products for analysis

From the list of products found in the survey, 20 products were selected for analysis based on the following criteria:

- Hazard labelling of the product (CLP), if available
- Ingredient substances with hazard classification, if SDS is available⁷
- The 6 different product types must be represented

Products for testing have been obtained by the Chemical Inspection Service of the Danish Environmental Protection Agency, which in addition to participation on this project, inspects the CLP marking of the products. The list of products selected for laboratory analysis is highlighted with green in the product overview in appendix 2.1.

Based on available information from the SDS of the products, a list of declared ingredient substances with hazard classification has been prepared as shown in Appendix 4.1. Not all products from the survey (Appendix 2.1) are included in this list, as some of the found products were subsequently considered to be outside the scope of the defined DIY scenarios.

3.3 Analysis programme

There are numerous methods for measuring the emission of volatiles from building materials and liquid products. Since emissions from liquid products such as adhesives and coatings (lacquers, paints etc.) are very high in the initial period after application, most product test methods use a curing time of 3 days under controlled climatic conditions before the sample is introduced into the climate chamber with air samples taken after 3 and 28 days, respectively (GEV-Testing method, EN 16402). This means that the first emissions are in fact measured 6 days after application of the product. The measurements in the current project focus on exposure immediately during and just after application (5 hours), after a short time (3 days) and long-term (28 days).

Although it is a technical challenge to measure the initial high concentrations of volatiles, it was decided to use the same methodology as in the pre-project. It was considered to introduce a smaller material surface in the climate chambers than the standard room to reduce the concentration of VOC in the gas phase, thus avoiding over-saturation of sampling media and subsequent analyses. Emission of volatiles from material surfaces is an equilibrium between gas phase and substrate in a so-called boundary layer (Salthammer and Uhde, 2009). If the amount of material is reduced and thereby the VOC in the chamber, the equilibrium will shift towards the gas phase and thus not give a correct emission profile. It was therefore assessed that measurements performed under the same test conditions as in the pre-project, would give the most accurate results.

⁷ As it is only required that SDS must be provided for products intended for professional use, it has not been possible to obtain SDS for all purchased products.

Measurements of volatile chemical substances from the products were therefore carried out similarly to the pre-project in small climate chambers of 113 litres at 23°C and 50% RH at an air change of 0.5 h⁻¹ according to EN 16516: 2017. EN 16516: 2017 is a specification of the ISO 16000 standard test methods for emission testing of dangerous substances from construction products to the indoor environment. Thus, the test conditions reflect a typical indoor climate in dwellings, where a standard room has been defined with loading factors for different types of construction products i.e. floors, walls, ceilings, sealants etc.

3.3.1 Preparation of samples for chamber testing

Products were applied in the recommended amount according to the product-specific instructions. Representative sampling is performed after thorough mixing of the product. The product coverage (m²/L) and density as stated by the manufacturer are used for calculation of application amounts, cf. Appendix 2.1. The product is applied in an even layer with a brush or roller according to the manufacturer's instructions on to a clean glass plate with an area corresponding to the loading factor for chamber test cf. EN 16516. For some of the products, including sealants and oils, a recommended amount is not specified, so instructions given in ISO 16000-11 and GEV-Testing method was followed. Fillers and sealants were applied to a 3 mm deep mould and smoothed with a trowel. An overview of the tested products is shown in Table 2.

TABLE 2. Tested do-it-yourself products (c = number of components)

ID	Product type	C	Application/scenario	Surface	Load (m ² /m ³)	Amount (g/m ²)
60	Adhesive	1C	Mounting of fiberglass fabric	Ceiling + Wall	1.4	222
65	Adhesive	1C	Mounting of fiberglass fabric	Ceiling + Wall	1.4	254
2	Paint & lacquer	2C	Painting of floor	Floor	0.4	108
4	Paint & lacquer	1C	Painting of ceiling and walls	Ceiling + Wall	1.4	274
5	Paint & lacquer	2C	Painting of floor	Floor	0.4	253
11	Paint & lacquer	1C	Painting of wall	Wall	1.0	110
20	Paint & lacquer	2C	Painting of floor	Floor	0.4	301
29	Paint & lacquer	1C	Lacquering of floor	Floor	0.4	109
30	Paint & lacquer	2C	Painting of floor	Floor	0.4	152
32	Paint & lacquer	1C	Painting of floor	Floor	0.4	146
56	Membrane	1C	Bathroom waterproof coating membrane	Floor + Wall	1.4	997
72	Floor wax, polish & care product	1C	Oil for polish & care of wood	Tabletop	0.1	66
80	Floor wax, polish & care product	1C	Oil for polish & care of wood and stone floors	Floor	0.4	50

83	Floor wax, polish & care product	1C	Oil for polish & care of wood and stone floors	Floor	0.4	50
85	Floor wax, polish & care product	1C	Linseed oil for polish of wood floors	Floor	0.4	60
14	Filler	1C	Wood surface repair and levelling	Very small surfaces	0.007	-
26	Filler	2C	Wood surface repair and levelling	Very small surfaces	0.007	-
36	Filler	1C	Wood surface repair and levelling	Very small surfaces	0.007	-
71	Filler	1C	Wood surface repair and levelling	Very small surfaces	0.007	-
88	Sealant	2C	Repair of concrete/cracked concrete	Very small surfaces	0.007	-

3.3.2 Chemical analyses of the air samples

Air samples of volatile compounds were collected on suitable sampling media; VOCs on Tenax and VVOC C1-C4 aldehydes on DNPH, according to the pre-project set-up shown in Table 3 using the calibration list of the chemical substances given in Appendix 5. Detection limits for measured concentrations depend on the amount of air collected on the media.

TABLE 3. Sampled air volumes from climate chamber

Air sampling media	Method	5 hours	3 days	28 days
Tenax (VOC)	ISO 16000-6	0.05 L – 2 L	1 L, 3 L	3 L, 5 L
DNPH (C1-C4 aldehydes)	ISO 16000-3	30 L	30 L	30 L*

*Air sampling after 28 days was carried out if aldehydes > 10 µg/m³ after 3 days

Determination of VOCs in air by TDS-GC / MS

The collected VOCs on Tenax were thermally desorbed, separated by gas chromatography (GC) and detected by mass spectrometry (MS) on a Gerstel-Agilent system with a 60 m DB-5 column mounted with a 10 m precolumn (DB-5ms) according to ISO 16000-6. The identity of each VOC was verified by searching on Wiley W9N11 with a minimum match of the MS spectrum of 80%. The retention times were verified by pure reference substances according to availability. VOCs were quantified by calibration with pure reference substances, and otherwise as toluene equivalents cf. EN 16516 at concentrations 5 µg/m³ and above. The analysis included VOC reference substances usually found construction products emissions (including DIY products) and purchased reference substances for the identified volatiles found in the survey. The calibrated linear range for 67 substances is shown in Appendix 5.1, where the measuring range of air concentrations is calculated for the smallest volume of air (0.05 L) and the largest volume of air (5 L). The measurement uncertainty is not determined for all individual substances. The expanded analytical measurement uncertainty of the validated method is 30%.

Determination of carbonyls in air (VVOC C1-C4 aldehydes) by HPLC-UV

The aldehydes were collected on 2,4-dinitrophenylhydrazine (DNPH) filter by derivatization. The collected carbonyls are eluted with acetonitrile and analysed by HPLC, identified by retention time and UV spectrum, and quantified using calibrated reference substances: Formaldehyde, acetaldehyde, propanal, butanal and acrolein. The results are reported with one decimal

place down to 1.0 µg/m³ which is lower than the EN 16516 reporting limit of 5 µg/m³. Expanded analytical measurement accuracy is 15%. The limit of detection is given in appendix 5.2.

3.4 Results

The results for all the measured volatiles in the 20 products are given in appendix 6.

Since the emission of VOC is very high at the beginning of the chamber test, the capacity of the sampling medium (Tenax) has been exceeded for several samples upon sampling after 5 hours. In the GC-MS analysis, some values are so high that the concentrations are outside the calibrated range. It cannot be ruled out that the air samples, in addition to the identified substances, also contain other substances at low concentrations which are "overshadowed" by the very high background concentrations. Thus, the measured concentrations may be underestimated and subject to greater analytical uncertainty than usual.

Substance names are in English and the chemical nomenclature is variable depending on the library search on MS spectra for VOC analyses. However, in all cases the substances can be identified by their unique CAS no.

The hydrocarbons listed in SDS for some of the products are of technical grade with different chain lengths and degree of branching depending on the composition, cracking process and distillation of the crude oil. It is therefore difficult to determine the exact hydrocarbon composition of the individual raw materials with the GC/MS VOC analysis used.

An overview of identified volatiles that have a hazard classification according to the risk of inhalation and skin contact, cf. the pre-project⁸ is given in the following tables (4-8). Substances that are expected to be present in the product and mentioned in SDS are marked in gray. When the substance is not detected within the linear range, "ND" is indicated for the given measurement. Where a substance or group of substances is measured in concentrations that exceed the linear range, a greater than sign ">" is added to the concentration.

The lower limit for reporting measured concentration in air is 5 µg/m³ with two significant digits according to EN 16516, but C1-C4 aldehydes and carcinogenic VOC including CMIT are reported down to 1 µg/m³ air. Only aldehydes are reported with one decimal place and "N" indicates cases where C1-C4 aldehydes have not been measured after 28 days.

TABLE 4. Volatiles measured in 1-component paints and lacquers

Substance	CAS	5 hours (µg/m ³)	3 days (µg/m ³)	28 days (µg/m ³)
<i>ID 4: 1C Paint – painting of ceiling and wall:</i>				
Formaldehyde	50-00-0	ND	22	7.8
Acetaldehyde	75-07-0	ND	230	18
Acrolein	107-02-8	ND	39	ND
Acetic acid	64-19-7	ND	230	38
Propanoic acid	79-09-4	ND	200	37
Butanoic acid	107-92-6	ND	97	31
Hexanal	66-25-1	ND	ND	230
2-Butanone, oxime	96-29-7	≥220000	ND	1

⁸ Acute tox 1+2+3, Skin Corr 1A+1B+1C, Skin sens 1A+1B, Resp sens 1A+1B, STOT RE 1+2, STOT SE 1+2, Carc 1A+1B+2, Repr 1A+1B, Muta 1A+1B

Substance	CAS	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Hexanoic acid	142-62-1	ND	3200	600
2-Ethylhexanoic acid	149-57-5	ND	3200	410
Alkaner, iso-, < 2% aromater C7-C13	927-285-2	≥1100000	≥110000	ND
ID 11: 1C Paint - painting of ceiling and wall:				
Formaldehyde	50-00-0	ND	16	6.5
Acetaldehyde	75-07-0	ND	111	20
Acrolein	107-02-8	ND	22	ND
Acetic acid	64-19-7	ND	250	21
Propanoic acid	79-09-4	ND	250	34
2-Butanone, oxime	96-29-7	≥58000	ND	ND
Butanoic acid	107-92-6	ND	55	ND
Phenol	108-95-2	ND	49	3
Hexanoic acid	142-62-1	ND	3400	340
Di(propylene glycol) methyl ether, mixture of isomers (A)	34590-94-8	≥3900	76	ND
2-Ethylhexanoic acid	149-57-5	ND	3500	200
Naphtha C7-C13	64742-82-1/64742-48-9	>2600000	4200	81
ID 29: 1C Lacquer – lacquering of floors:				
Formaldehyde	50-00-0	ND	ND	2.0
Acetaldehyde	75-07-0	16	2.2	1.6
Diethylamine	109-97-7	2300	ND	ND
Triethylamine	121-44-8	≥19000	140	9
Oxiraner	68609-97-2	<100	430	ND
Propylene glycol butyl ether	5131-66-8	300	ND	ND
Di(propylene glycol) methyl ether, mixture of isomers (A)	34590-94-8	≥190000	100	<5
1-Ethyl-2-pyrrolidinone	2687-91-4	ND	ND	1
Naphtha C7-C13	64742-82-1/64742-48-9	370	780	ND
ID 32: 1C Paint – painting of floors:				
Formaldehyde	50-00-0	2.6	7.1	2.9
Acetaldehyde	75-07-0	2.0	30	5.4
Acetic acid	64-19-7	ND	36	ND
1-Methoxypropan-2-ol	107-98-2	650	ND	ND
Propanoic acid	79-09-4	ND	77	ND
Butanoic acid	107-92-6	ND	31	22
2-Butanone, oxime	96-29-7	≥60000	ND	ND
Hexanal	66-25-1	ND	600	44
Phenol	108-95-2	ND	16	3
Hexanoic acid	142-62-1	ND	790	240
Di(propylene glycol) methyl ether, mixture of isomers (A)	34590-94-8	ND	16	ND
2-Ethylhexanoic acid	149-57-5	ND	600	50

Substance	CAS	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Naphtha C7-C13	64742-82-1/64742-48-9	≥1600000	1900	84

TABLE 5. Volatiles measured in 2-component paints and lacquers

Substance	CAS	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
ID 2: 2C Paint – painting of floors:				
Formaldehyde	50-00-0	10	24	4.9
Acetaldehyde	75-07-0	3.6	1.8	6.6
1-Methoxypropan-2-ol	107-98-2	160	ND	ND
Triethylamine	121-44-8	44	ND	ND
1,2-Ethanediol #	107-21-1	2300	45	ND
Butanoic acid	107-92-6	ND	ND	21
Oxiranes (Dodecyl and tetradecyl glycidyl ethers) #	68609-97-2	200	290	<5
2-Butoxy ethanol	111-76-2	6300	7	ND
Hexanoic acid	142-62-1	ND	ND	13
Di(propylene glycol) methyl ether, mixture of isomers (A)	34590-94-8	5600	ND	ND
Benzyl alcohol	100-51-6	220	29	ND
2-Ethylhexanoic acid	149-57-5	ND	ND	6
5-Chloro-2-methyl-1,2-thiazol-3(2H)-on (CMIT)	26172-55-4	17	16	ND
Naphtha C7-C13 #	64742-82-1/64742-48-9	420	6	<5
ID 5: 2C Paint – painting of floors:				
Formaldehyde	50-00-0	ND	2.4	N
Acetaldehyde	75-07-0	11	2.6	N
2-Propanol	67-63-0	360	<5	ND
1-Methoxypropan-2-ol	107-98-2	130	ND	ND
1,4-Dioxane	123-91-1	460	ND	ND
Ethylbenzene	100-41-4	7	ND	ND
Styrene	100-42-5	2	ND	ND
2-Butoxy ethanol	111-76-2	≥23000	230	12
Hexanoic acid	142-62-1	ND	ND	14
Phenol	108-95-2	ND	1	ND
Benzyl alcohol	100-51-6	67	10	<5
2-Ethylhexanoic acid	149-57-5	ND	ND	7
5-Chloro-2-methyl-3(2H)-isothiazolone (CMIT)	26172-55-4	ND	ND	7
ID 20: 2C Paint – painting of floors:				
Formaldehyde	50-00-0	ND	1.1	N
Acetaldehyde	75-07-0	22	1.6	N
1-Methoxypropan-2-ol	107-98-2	7100	ND	ND
Acetone oxime	127-06-0	170	ND	ND
1,4-Dioxane	123-91-1	7	ND	ND

Substance	CAS	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Toluene	108-88-3	4	ND	ND
2-Butoxy ethanol	111-76-2	12	ND	ND
Benzyl alcohol	100-51-6	610	18	ND
Naphtha C7-C13 (3) #	64742-82-1/64742-48-9	1100	<5	<5
ID 30: 2C Paint - painting of floors:				
Formaldehyde	50-00-0	ND	1.0	N
Acetaldehyde	75-07-0	13	1.4	N
Hexane	110-54-3	210	ND	ND
Acetic acid	64-19-7	4100	ND	ND
Benzene	71-43-2	180	ND	ND
1-Methoxypropan-2-ol	107-98-2	≥42000	49	8
Propanoic acid	79-09-4	900	ND	ND
1,4-Dioxane	123-91-1	320	ND	ND
Toluene	108-88-3	130	ND	ND
2-Butanone, oxime	96-29-7	9700	ND	ND
Ethylbenzene	100-41-4	230	ND	ND
Phenol	108-95-2	110	ND	ND
Benzyl alcohol	100-51-6	≥14000	200	29
2-Ethylhexanoic acid	149-57-5	100	ND	ND
Butyldiglycol	112-34-5	430	17	ND
Dibutyl phthalate #	84-74-2	200	ND	ND

TABLE 6. Volatiles measured in fillers and sealants

Substance	CAS	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
ID 14: 1C Filler –Wood surface repair & leveling				
Formaldehyde	50-00-0	1.5	2.0	N
Acetaldehyde	75-07-0	35	18	N
Propanoic acid	79-09-4	ND	ND	20
Butanoic acid	107-92-6	ND	ND	22
Hexanal	66-25-1	ND	53	18
Hexanoic acid	142-62-1	ND	ND	33
Phenol	108-95-2	160	15	3
2-Ethylhexanoic acid	149-57-5	ND	110	18
Naphtha C7-C13	64742-82-1/64742-48-9	>340000	12000	220
ID 26: 2C Filler –Wood surface repair & leveling				
Formaldehyde	50-00-0	4.2	1.1	N
Acetaldehyde	75-07-0	4.9	1.5	N
Styrene	100-42-5	380	6	ND
2-Butoxy ethanol	111-76-2	32	5	ND

Substance	CAS	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Hexanoic acid	142-62-1	ND	ND	22
Phenol	108-95-2	ND	5	ND
Styrene oxid	96-09-3	16	ND	ND
2-Ethylhexanoic acid	149-57-5	ND	ND	10
1-Ethyl-2-pyrrolidinone	2687-91-4	12	2	ND
Naphtha C7-C13	64742-82-1/64742-48-9	32	ND	ND
ID 36: 1C Filler –Wood surface repair & leveling				
Formaldehyde	50-00-0	1.7	1.2	N
Acetaldehyde	75-07-0	1.6	1.2	N
Styrene	100-42-5	4	4	ND
2-Butoxy ethanol	111-76-2	8	ND	ND
Benzyl alcohol	100-51-6	<5	ND	ND
ID 71: 1C Filler –Wood surface repair & leveling				
Formaldehyde	50-00-0	7.2	2.1	N
Acetaldehyde	75-07-0	2.9	1.8	N
Hexanoic acid	142-62-1	ND	ND	23
Phenol	108-95-2	110	12	3
2-Ethylhexanoic acid	149-57-5	ND	ND	10
Naphtha C7-C13	64742-82-1/64742-48-9	670	19	ND
ID 88: 2C Sealant – Repair of concrete				
Formaldehyde	50-00-0	9.2	1.7	N
Acetaldehyde	75-07-0	28	2.4	N
1,2-Ethanediol	107-21-1	64	ND	ND
Styrene	100-42-5	7	2	ND
2-Butoxy ethanol	111-76-2	10	ND	ND
2-Propanoic acid, 2-methyl-, 1,2-ethanediyl ester #	97-90-5	5800	360	<5

TABLE 7. Volatiles in floor wax, polish and care products

Substance	CAS	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
ID 72: Floor wax, polish & care product – Oil for polish and care of wood				
Formaldehyde	50-00-0	2.8	6.6	N
Acetaldehyde	75-07-0	15	6.9	N
Butanoic acid	107-92-6	ND	ND	21
Hexanoic acid	142-62-1	ND	360	25
Phenol	108-95-2	53	11	3
Di(propylene glycol) methyl ether, mixture of isomers (A)	34590-94-8	290	9	ND
Ethanol, 2-(2-ethoxyethoxy)- #	111-90-0	3700	ND	ND

Substance	CAS	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
2-Ethylhexanoic acid	149-57-5	ND	55	10
Naphtha C7-C13	64742-82-1/ 64742-48-9	≥210000	310	ND
ID 80: Floor wax, polish & care product – Oil for polish & care of wood and stone floors				
Formaldehyde	50-00-0	ND	1.8	N
Acetaldehyde	75-07-0	4.8	1.3	N
1,4-Dioxane	123-91-1	70	ND	ND
1,2-Ethanediol	107-21-1	19	ND	ND
2-Butoxy ethanol	111-76-2	1900	<5	ND
Phenol	108-95-2	ND	<5	4
2-Methyl-isothiazolin-3-one (MIT)	2682-20-4	16	ND	ND
Butyldiglycol	112-34-5	310	9	ND
Naphtha C7-C13	64742-82- 1/64742-48-9	330	23	<5
ID 83: Floor wax, polish & care product – Oil for polish & care of wood and stone floors				
Formaldehyde	50-00-0	ND	1.9	N
Acetaldehyde	75-07-0	2.5	1.4	N
1,4-Dioxane	123-91-1	68	ND	ND
2-Butoxy ethanol	111-76-2	460	ND	ND
Benzyl alcohol	100-51-6	830	6	ND
2-Methyl-isothiazolin-3-one (MIT)	2682-20-4	ND	10	ND
Naphtha C7-C13	64742-82- 1/64742-48-9	450	93	<5
ID 85: Floor wax, polish & care product – Linseed-oil for polish of wood floors				
Formaldehyde	50-00-0	ND	1.9	12
Acetaldehyde	75-07-0	ND	1.6	30
Propanal	123-38-6	5.9	2.0	257
Acetic acid	64-19-7	ND	ND	120
Propanoic acid	79-09-4	ND	ND	420
Hexanal	66-25-1	<5	ND	670
Hexanoic acid	142-62-1	ND	ND	260
2-Butanone, oxime	96-29-7	92	ND	ND
Naphtha C7-C13	64742-82- 1/64742-48-9	4500	48	<5

TABLE 8. Volatile chemicals measured in adhesives (for walls) and membranes (water-based)

Substance	CAS	5 hours (µg/m ³)	3 days (µg/m ³)	28 days (µg/m ³)
ID 56: Membrane - Bathroom waterproof coating membrane				
Formaldehyde	50-00-0	70	5.7	N
Acetaldehyde	75-07-0	10	1.6	N
Phenol	108-95-2	110	11	2
ID 60: Adhesive - Mounting of fiberglass fabric				
Formaldehyde	50-00-0	120	1.4	N
Acetaldehyde	75-07-0	11	1.3	N
Acetic acid	64-19-7	ND	1000	31
Propanoic acid	79-09-4	ND	22	ND
Butanoic acid	107-92-6	ND	15	13
Phenol	108-95-2	100	5	3
2-Methyl-1,2-thiazol-3(2H)-on (MIT)	2682-20-4	ND	9	<5
5-Chloro-2-methyl-3(2H)-isothiazolone (CMIT)	26172-55-4	18	21	<5
ID 65: Adhesive - Mounting of fiberglass fabric				
Formaldehyde	50-00-0	15	5.0	N
Acetaldehyde	75-07-0	107	1.5	N
Acetic acid	64-19-7	ND	64	43
Propanoic acid	79-09-4	ND	ND	20
Butanoic acid	107-92-6	ND	ND	13
Styrene	100-42-5	ND	ND	1
Phenol	108-95-2	10	8	2
2-Methyl-isothiazolin-3-one (MIT)	2682-20-4	ND	<5	14
5-Chloro-2-methyl-1,2-thiazol-3(2H)-on (CMIT)	26172-55-4	ND	13	ND

3.5 Discussion of the results

Information on the ingredients was available for 13 of the 20 tested products. In general, there was a good correlation between the declared ingredients and the results of the performed analyses.

More volatiles have been found in the emissions from the tested products than those listed in the corresponding SDS. There are several reasons for this:

- Cf. CLP rules ingredient substances are declared only when above a certain concentration depending on CLP hazard classification, i.e. a substance measured in the emissions may well be derived from the product, however it is present at such low concentrations that it need not be declared as an ingredient
- The identified substances are formed during curing of the product by chemical reactions in the liquid phase, including polymerisation
- Gas phase reactions occur between the volatiles, including rearrangement and cleavage into smaller molecules
- Gas phase reactions occur with oxygen, whereby radicals are formed and cleavage into smaller molecules happens

In general, it is observed that the concentrations of declared ingredients concentration are very high immediately after application of the product and decrease over time. After 5 hours, the

identified volatiles are primarily solvents and other volatile constituents in the products, while semi-volatile substances and the degradation products formed are identified in the emissions after 3 and 28 days.

Solvents are applied to dissolve typical ingredients such as pigments, polymers, reactive monomers and other additives that are included in the production of liquid DIY products. It is expected and necessary that solvents evaporate during curing to form the final product.

Substances that are not initially present are presumed to be formed during the curing process and in gas-phase reactions in the chamber. As a rule, these substances are not present in the products and therefore cannot be expected to be declared as product ingredients.

3.5.1 Paints and lacquers 1-component

The organic acid 2-Ethylhexanoic acid is present in 3 of 4 of the products after 3 days of testing, however it is not observed after 5 hours in any of the products. It is released during the curing process and is derived from the salt Calcium bis (2-ethylhexanoate) which is a declared ingredient in product ID 11. Calcium bis (2-ethylhexanoate) is a so-called siccative, which is a catalyst for the deep drying of alkyd-based products. Similarly, in the same 3 products there are findings of "2-butanone oxime", which is also a declared ingredient. 2-Butanone oxime is only measured after 5 hours, as it is a volatile constituent intended to prevent skin formation of the product during in-can storage.

Naphta is found in 3 paints and in high concentrations in 2 paints (above the upper quantification limit). In the fourth product, high concentrations of alkanes (above upper quantification limit) were found, however these were declared ingredients.

3.5.2 Paints and lacquers 2-component

All 4 products emit high amounts of volatiles such as 2-butoxy ethanol, 1-methoxy propane-2-ol and several ethers. There were also findings of naphta, benzyl alcohol, acetic acid, etc. In 2 products (product ID 2 and 5) CMIT was found, and in the SDS for product ID 5 CMIT was not declared. In SDS for Product ID 2, CMIT and MIT are declared in a 3:1 ratio, i.e. content of CMIT is 3 times higher than MIT, so it is possible that MIT has been present in the emissions below the analytical detection limit.

3.5.3 Fillers and sealants

Styrene is found in a high concentration in product ID 26 (380 µg/m³) and in a lower concentration in product ID 36 and 88 (4 µg/m³ and 7 µg/m³, respectively). Naphta was found in very high concentration in product ID 14 (340,000 µg/m³), however this was declared in SDS. Phenol was found in the emissions from 3 of the products ID 14, 26, and 71.

3.5.4 Floor wax, polish and care products

Naphta was found in all 4 products (ID 72, 80, 83, 85). 2-Butoxyethanol was found in 2 products (ID 83, 80). Benzyl alcohol was found in one product (ID 83). After 5 hours, a low concentration of 2-butanone oxime is emitted from product ID 85. Many carboxylic acids (acetic acid, propanoic acid, hexanoic acid) and aldehydes (pentanal, hexanal, heptanal, octanal) are measured after 28 days from product ID 85, although they could not be detected after 5 hours and 3 days. Thus, an atypical emission profile is seen for product ID 85 compared to the other tested products. This is due to the fact that product ID 85 is based on crude linseed oil, which is degraded over time by oxidation of double bonds in the unsaturated fatty acids, thereby forming aldehydes and acids, which have also been identified in previous studies of linseed oil (Clausen et al., 2008).

3.5.5 Adhesives and membranes

Phenol, formaldehyde and acetaldehyde emit from all 3 products (ID 56, 60, 65). Phenol reacts with aldehydes (typically formaldehyde) in conventional adhesive systems, which is consistent with the observations of the tested products.

4. Risk assessment

4.1 Objective

Based on the results from the emissions analysis a selection of relevant products will be made for further risk assessment. The subsequent risk assessment will then be carried out in the same way as in the pre-project for the assessment of VOC emissions after 5 hours, 3 days and 28 days.

4.2 Selection of products for risk assessment

When reviewing the analysis results, it was considered most appropriate to focus the risk assessment on the products that continued to emit over 3 and 28 days. For these products, there is potentially the worst risk of exposure to persons/family members who subsequently reside in the renovated room/rooms.

In collaboration with the Danish Environmental Protection Agency, the project group selected 10 products for detailed risk assessment (see Table 9), as these products all emitted volatiles for up to 28 days. Products were selected which as broadly as possible represented the 20 tested products. The selection also included a wide range of different degassing volatiles.

TABLE 9. Products selected for risk assessment

Fillers	1 product (ID 14)
Adhesives	1 product (ID 60)
Paints and lacquers	6 products consisting of 4 products 1-component (ID 4, 11, 29, 32) and 2 products 2-component (ID 5, 30)
Floor wax, polish and care products	2 products (ID 72, 85)

4.3 Risk assessment of substances in the emissions, tolerable exposure levels

In the pre-project a risk assessment has been carried out for a number of the substances also found in this project. Therefore, the hazard assessments and tolerable exposure levels from the pre-project are used in this project. The tolerable exposure levels are specified as DNEL (Derived No Effect Level) values, as this is the terminology used in the REACH chemical control (REACH 2006). The DNEL values given in this project are aimed at the general population, i.e. consumers in general.

For substances, where no DNEL values are specified in the pre-project, a so-called Lowest Concentration of Interest (LCI) values are being utilized to protect consumers from emissions from building materials. These values (which were also widely used in the pre-project) are derived according to the same principles as DNEL values under REACH. Thus, the EU Commission has prepared a report describing how LCI values are derived based on the same assessment methods used in REACH in connection with the derivation of DNEL values to the general population i.e. consumers (ECA 2013).

Table 10 lists the relevant DNEL values that will be utilised for risk assessment in this project based on values from the previous do-it-yourself project (the pre-project) as well as reviewing the last updated EU list of LCI values (Agreed EU-LCI values, 2018).

In Table 10 the chemical substances measured by emission from the selected products are listed with corresponding DNEL values. The hazard classification of the substances regarding human health hazards is also indicated here. For the hazard classification the EU harmonized classification is indicated. If this is not available, the hazard classification of the substance

used in its REACH registration is used. The column "Critical effect" indicates the effect of the substance that is considered to occur at the lowest exposure level and which thus is considered as the basis for the protective DNEL value.

TABLE 10. Volatile substances with hazard classification and DNEL values in air

Substance	CAS	Classification health	DNEL (air)	Critical effect
Hydrocarbons			mg/m ³	
Benzene	071-43-2	Carc. 1A Muta. 1B Asp. Tox. 1 STOT RE 1 Skin Irrit. 2 Eye Irrit. 2	0.600 ^a 0.019 ^b	Neurotoxic Carcinogenic
Hexane	110-54-3	Repr. 2 Asp. Tox. 1 STOT SE 3 STOT RE 2 Skin Irrit. 2	0.700 ^a	Neurotoxic
Ethylbenzene	100-41-4	Acute Tox. 4 Asp. Tox. 1 STOT RE 2	0.200 ^a	Neurotoxic
Styrene	100-42-5	Repr. 2 Acute Tox. 4 STOT RE 1 Skin Irrit. 2 Eye Irrit. 2	0.175 ^a	Neurotoxic
Toluene	108-88-3	Repr. 2 Asp. Tox. 1 STOT SE 3 STOT RE 2 Skin Irrit. 2	0.725 ^a	Neurotoxic
Alkanes, iso-, < 2% aromatics C7-C13	927-285-2	Asp Tox. 1	1.425 ^a	Neurotoxic
Naphtha C7-C13	64742-82-1/ 64742-48-9	Asp. Tox. 1	1.425 ^a	Neurotoxic
Aldehydes				
Acetaldehyde	75-07-0	Carc. 2 STOT SE 3 Eye Irrit. 2	1.200 ^c	Eye and respiratory tract irritation
Acrolein	107-02-8	Acute Tox. 1 Acute Tox. 2 Acute Tox. 3 Skin Corr. 1B	0.007 ^d	Mucous membrane irritation
Formaldehyde	50-00-0	Carc. 1B Muta. 2 Acute Tox. 3 Skin Corr. 1B Skin Sens. 1		Eye and respiratory tract irritation

Substance	CAS	Classification health	DNEL (air)	Critical effect
Hexanal	66-25-1	Skin Irrit. 2 Eye Irrit. 2	0.900 ^c	Mucous membrane irritation
Organic acids				
2-Ethylhexanoic acid	149-57-5	Repr. 2	0.150 ^c	Effects on fetal development
Acetic acid	064-19-7	Skin Corr. 1A	1.200 ^c	Mucous membrane irritation
Butanoic acid	107-92-6	Skin Corr. 1B	1.800 ^c	Mucous membrane irritation
Hexanoic acid	142-62-1	Skin Corr. 1B	2.100 ^c	Mucous membrane irritation
Propanoic acid	79-09-4	Skin Corr. 1B	1.500 ^c	Mucous membrane irritation
Alcohols/ ethers /epoxides/ esters				
1-Methoxypropan-2-ol	107-98-2	STOT SE 3	7.900 ^c	No value available
1,4-Dioxane	123-91-1	Carc. 2, STOT SE 3 Eye Irrit. 2	0.400 ^c	Mucous membrane irritation
2-Butoxy ethanol	111-76-2	Acute tox. 4 Skin Irrit. 2 Eye Irrit. 2	1.600 ^c	Effects on liver and blood
2-Propanol	67-63-0	Eye Irrit. 2 STOT SE 3	26 000 ^b	Neurotoxic
Benzyl alcohol	100-51-6	Acute Tox. 4	0.440 ^c	No value available
Butyldiglycol	112-34-5	Eye Irrit. 2	0.670 ^c	No value available
Di(propylene glycol) methyl ether, mixture of isomers (A) Dipropylene glycol monomethyl ether	34590-94-8	No classification	3.100 ^c	No value available
Dibutyl phthalate	84-74-2	Repr. 1B	0.023 ^b	Effects on fetal development
Ethanol, 2-(2-ethoxyethoxy)-	111-90-0	Ingen klass.	0.350 ^c	Most likely mucous membrane irritation
Oxiranes	68609-97-2	Skin Irrit. 2 Skin Sens. 1	0.020 ^e	Mucous membrane irritation (value for allylglycidylether, CAS 106-92-3)
Phenol	108-95-2	Muta. 2, Acute Tox. 3 STOT RE 2 Skin Corr. 1B	0.070 ^c	Respiratory effects
Propylene glycol butyl ether	5131-66-8	Skin Irrit. 2 Eye Irrit. 2	0.900 ^b	Effects on eyes

Substance	CAS	Classification health	DNEL (air)	Critical effect
Nitrogen-containing substances				
1-Ethyl-2-pyrrolidinone	2687-91-4	Repr. 1B, Eye Dam. 1	0.400 ^c	Mucous membrane irritation
2-Butanone, oxime	96-29-7	Carc. 2 Acute Tox. 4 Eye Dam. 1 Skin Sens. 1 (Carc. 1B Acute Tox. 4 Acute Tox. 3 STOT SE 3 STOT SE 1 STOT RE 2 Skin Irrit. 2 Eye Dam. 1 Skin Sens. 1) ^f	0.015 ^c	Irritation of upper respiratory tract
2-Methyl-isothiazolin-3-one	2682-20-4	Acute Tox. 3 Skin Corr. 1B Skin Sens. 1A Eye Dam. 1 STOT SE 3	0.100 ^c	Most likely mucous membrane irritation
5-Chloro-2-methyl-3(2H)-isothiazolone (CMIT)	26172-55-4	Acute Tox. 3 Skin Corr. 1B Skin Sens. 1 Eye Dam. 1 STOT SE 3	0.001 ^c	Mucous membrane irritation
Diethylamine	109-97-7	Acute Tox. 4 Skin Corr. 1A	As triethylamine 0.060 ^c	Eye and respiratory tract irritation
Triethylamine	121-44-8	Acute Tox. 4 Skin Corr. 1A	0.060 ^c	Eye and respiratory tract irritation

a) Environmental Protection Agency (2016a). Survey and risk assessment of toluene and other neurotoxic substances in children's rooms. Survey of Chemical Substances in Consumer Products # 145. These values pay particular attention to children's increased sensitivity to the influence of the central nervous system.

(b) Derived from this report, see Appendix 7

c) EU-LCI Values. Agreed EU-LCI values (July 2018) https://ec.europa.eu/growth/sectors/construction/eu-lci/values_en

d) Danish Environmental Protection Agency (2016b) Survey and risk assessment of chemical substances in rugs for children. Survey of Chemical Substances in Consumer Products No. 147.

e) Environmental Protection Agency (2018). Survey and risk assessment of chemical substances in chemical products used for "do-it-yourself" projects in the home. Survey of Chemical Substances in Consumer Products No. 167.

f) New harmonized classification under discussion in the European Chemicals Agency (personal information from the Environmental Protection Agency, November 2019).

For the substances that are reported to cause mucosal irritation and effects on the eyes and respiratory tract, the values will typically target acute damage/irritation due to the direct irritant effects of the substances, where even short-term exposure to many of these substances may cause acute irritation. Next, in the context of a risk assessment, it will be relevant to add the

contributions of these substances, since the substances can be considered to have similar and thus additive effects.

For the substances listed as neurotoxic, the values, on the other hand, are aimed at protecting from long-term exposure and protection from chronic damage to the central nervous system, and here short-term exceeding of the values is less critical, as the acute toxicity level is significantly higher than the values protecting against chronic effects. For substances that affect the central nervous system, it is also considered relevant to add the contributions in connection with the risk assessment.

With regards to carcinogens, short-term exceeding of the DNEL value, as for the chronic neurotoxic substances, is considered less critical, as it is the overall long-term exposure that is important for increased risk of cancer.

For substances harmful to the unborn child (developmental toxicants), on the other hand, short-term exceeding of the DNEL value must also be considered critical, since the effects on fetal development can be manifested by even short-term exposure in particularly sensitive periods during pregnancy.

4.4 Exposure Assessment

In this project, the focus has been solely on inhalation of the emitting chemical substances. Thus, skin exposure to the substances will not be assessed in terms of direct skin contact with the treated surfaces. As stated in section 2.7, the emission tests in the climate chambers are carried out so that the measured values represent the concentrations that would have been obtained if the product had been used in a standard room in a home. Therefore, the measured values can be used directly as exposure values in connection with a risk assessment.

Continuous exposure of 24 hours/day is used as a realistic worst-case exposure scenario, since such a scenario may be relevant for bedridden individuals or people who spend most of the day in the room. However, a modified scenario is used for one product intended for wet room purposes (Section 4.6.5.1.), where the exposure time is set to 1 hour per day.

4.5 Risk assessment

4.5.1 Method

The chosen methodology for risk assessment corresponds to the method used in the pre-project. The risk assessment covers all age groups, which should therefore be taken into account since some age groups e.g. very young children can be more sensitive than adults. This applies, for example, to effects on the central nervous system, where the DNEL values for the chronic neurotoxic substances are reduced 4 times compared to DNEL values for adults (see Danish Environmental Protection Agency 2016b).

4.5.1.1 Calculation of risk characterization ratio (RCR)

The risk assessment in this project is based on the guidelines used in connection with the REACH chemical regulation (ECHA, 2012).

Risk assessment is done by comparing the exposure of a given substance to the substance's DNEL value and calculating the risk characterization ratio (RCR) of the substance, where RCR is calculated as:

$$RCR (X) = \text{Calculated or measured exposure (Substance X)} / \text{DNEL (Substance X)}$$

If the calculated or measured exposure to a given substance exceeds the DNEL value and the RCR thus becomes greater than 1, the exposure in that scenario is considered to present an unacceptable risk.

When exposed to multiple similar-acting substances in the exposure scenario, it may be relevant to assess the overall risk of a given effect. This can be done by summing the individual RCR values for the similar-acting substances:

$$RCR (sum) = RCR (1) + RCR (2) + \dots RCR (n)$$

This approach may be particularly relevant in this project for those substances that are considered to affect the central nervous system or those substances that cause irritation of mucous membranes (eye and respiratory tract irritation). If the RCR (sum) exceeds 1, the total exposure of the substances with similar effect is considered to result in an unacceptable risk

4.6 Risk assessment for selected products

In the following, a risk assessment is carried out for each product. Subsequently, a more general assessment of the overall results is made and a discussion of which substances, if any, can be considered particularly critical.

4.6.1 1-component paints and lacquers

4.6.1.1 Assessment of product 4

The emissions from product 4 (see Table 11) is largely dominated by the substances 2-butanone oxime and C7-C13 hydrocarbons (white spirit with low aromatic content), which are the two substances listed as ingredients in the product (see Appendix 4).

After 5 hours, exposure levels above 14667 times the DNEL for 2-butanone oxime and almost 800 times above the DNEL for hydrocarbons are observed. Thus, it is believed that there will be considerable discomfort in the form of mucosal irritation from butanone oxime at this level. However, for hydrocarbons, the exposure level is still considered to be below acute toxic levels regarding central nervous system effects.

After 3 days, emissions of 2-butanone oxime are no longer seen, but emission of acrolein, which is a potent respiratory and eye irritant, is seen. The level of acrolein is 5.6 times the DNEL value. Similarly, emissions of 2-ethylhexanoic acid is now seen, where the level is 21 times above the DNEL value protecting against developmental toxicity. For C7-C13 hydrocarbons, the level has dropped to 1/10 compared to the 5-hour measurement, but the level is still significantly above the DNEL value (77 times) protecting against chronic neurotoxicity in relation to long term exposure to the substance. The relatively delayed/ slow emission of the hydrocarbons is probably due to the fact that it is a low aromatic white spirit with a high content of C12-13 in the product.

After 28 days, the levels of 2-butanoxime and C7-C13 hydrocarbons are below the DNEL, while the level of 2-ethylhexanoic acid remains almost a factor of 3 above the DNEL.

As stated in the preliminary project, it is expected that high exposure levels of volatiles may occur during the first hours and days at levels exceeding the DNEL if no specific precautions such as additional ventilation are taken in the first days after use. It is worth noting that there is a delayed emission of 2-ethylhexanoic acid and that an unacceptably high emission level may be maintained by this substance for more than 28 days.

TABLE 11. Risk assessment of product 4 (1-component paint)

Product 4									
Substance	CAS no	Room concentration			DNEL (µg/m³)	RCR			Critical effect
		5 hours	3 days	28 days		5 hours	3 days	28 days	
		(µg/m³)	(µg/m³)	(µg/m³)		(µg/m³)	(µg/m³)	(µg/m³)	
Formaldehyde	50-00-0	ND	22	7.8	100	-	0.22	0.08	Mucous membrane irritation
Acetaldehyde	75-07-0	ND	230	18	1200	-	0.20	0.02	Mucous membrane irritation
Acrolein	107-02-8	ND	39	ND	7	-	5.6	-	Mucous membrane irritation
Hexanal	66-25-1	ND	ND	230	900	-	-	0.26	Mucous membrane irritation
Acetic acid	64-19-7	ND	230	38	1200	-	0.20	0.03	Mucous membrane irritation
Propanoic acid	79-09-4	ND	200	37	1500	-	0.13	0.02	Mucous membrane irritation
Butanoic acid	107-92-6	ND	97	31	1800	-	0.05	0.02	Mucous membrane irritation
Hexanoic acid	142-62-1	ND	3200	600	2100	-	1.5	0.29	Mucous membrane irritation
2-Butanone, oxime	96-29-7	≥220000	ND	1	15	≥14667	-	0.01	Mucous membrane irritation
Cumulated RCR for mucous membrane irritation						≥14667	8.0	0.73	Mucous membrane irritation
2-Ethylhexanoic acid	149-57-5	ND	3200	410	150	-	21	2.7	Effects on fetal development
Alkaner, iso-, < 2% aromatics C7-C13	927-285-2	≥1100000	110000	ND	1425	≥772	77	-	Neurotoxic

4.6.1.2 Assessment of product 11

For product 11 emission after 5 hours is dominated by 2-butanone oxime and C7-C8 hydrocarbons (SDS ingredients, see Appendix 4), with the levels of these substances exceeding their respective DNELs by 3867 and 1825 times, respectively (see Table 12). Thus, it is assumed that there is considerable mucosal irritation from butanone oxime at this level. For hydrocarbons, exposure levels are estimated to be close to acute toxic levels with an impact on the central nervous system. Furthermore, di (propylene glycol) methyl ether is emitting at a concentration exceeding 1.3 times the DNEL value.

After 3 days emission of 2-butanone oxime no longer occurs and C7-C8 hydrocarbons have reached a level that merely exceeds the DNEL by a factor of 2.9. However, emission of acrolein and 2-ethylhexanoic acid is now seen, and the levels here exceed the DNEL values by a factor of 2.3 and 23, respectively, giving a calculated, cumulative RCR value of 6.1 for mucosal irritation.

After 28 days only the emission of 2-ethylhexanoic acid exceeds the DNEL value, hereby reaching an RCR value of 1.3. It is worth noting that there is a delayed emission of 2-ethylhexanoic acid and that an unacceptably high emission level may be maintained by this substance for more than 28 days. Emission of 2-ethylhexanoic acid probably derives from the compound calcium bis (2-ethylhexanoate) in the product, which according to the safety data sheet is present in up to 1%. (see Appendix 4).

TABLE 12. Risk assessment of product 11 (1-component paint)

Product 11									
Substance	CAS no	Room concentration			DNEL (µg/m³)	RCR			Critical effect
		5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)		5 hours	3 days	28 days	
Formaldehyde	50-00-0	ND	16	6.5	100	-	0.16	0.07	Mucous membrane irritation
Acetaldehyde	75-07-0	ND	111	20	1200	-	0.09	0.02	Mucous membrane irritation
Acrolein	107-02-8	ND	22	ND	7	-	3.1	-	Mucous membrane irritation
Acetic acid	64-19-7	ND	250	21	1200	-	0.21	0.02	Mucous membrane irritation
Propanoic acid	79-09-4	ND	250	34	1500	-	0.17	0.02	Mucous membrane irritation
Butanoic acid	107-92-6	ND	55	ND	1800	-	0.03	-	Mucous membrane irritation
Hexanoic acid	142-62-1	ND	3400	340	2100	-	1.6	0.16	Mucous membrane irritation
2-Butanone, oxime	96-29-7	≥58000	ND	ND	15	≥3867	-	-	Mucous membrane irritation
Phenol	108-95-2	ND	49	3	70	-	0.7	0.04	Respiratory effects
Cumulated RCR for mucous membrane irritation						≥3867	6.1	0.33	Mucous membrane irritation
Di(propylene glycol) methyl ether, mixture of isomers (A)	34590-94-8	≥3900	76	ND	3100	≥1.3	0.02	-	No value available
2-Ethylhexanoic acid	149-57-5	ND	3500	200	150	-	23	1.3	Effects on fetal development
Naphtha C7-C13	64742-82-1/ 64742-48-9	≥260000 0	4200	81	1425	≥1825	2.9	0.06	Neurotoxic

4.6.1.3 Assessment of product 29

Dipropylene glycol methyl ether is the only declared chemical substance in the product SDS (see Appendix 4). After 5 hours, the emission is dominated by diethylamine, triethylamine and dipropylene glycol methyl ether, for which the DNEL values are exceeded by 38, 317 and 61

times, respectively (see table 13). The amines must be considered to cause both malodour and mucosal irritation. The source of diethylamine and triethylamine is unknown.

After 3 days, the level of amines has dropped significantly, but triethylamine still exceeds the DNEL by a factor of 3. The level of dipropylene glycol methyl ether has dropped to a toxicologically insignificant level. Oxiranes, which are highly mucosal irritants, are now emitting at a level exceeding the DNEL value 22 times. The source of the oxiranes is unknown.

After 28 days, none of the emitted substances exceed the DNEL levels. It is noted that the identified critical substances from this product were not disclosed in SDS (Appendix 4).

TABLE 13. Risk assessment of product 29 (1-component lacquer)

Product 29									
Substance	CAS no	Room concentration			DNEL	RCR			Critical effect
		5 hours	3 days	28 days	(µg/m³)	5 hours	3 days	28 days	
		(µg/m³)	(µg/m³)	(µg/m³)					
Formaldehyde	50-00-0	ND	ND	2.0	100	-	-	0.02	Mucous membrane irritation
Acetaldehyde	75-07-0	16	2.2	1.6	1200	0.01	0.002	0.001	Mucous membrane irritation
Diethylamine	109-97-7	2300	ND	ND	60	38	-	-	Mucous membrane irritation
Triethylamine	121-44-8	≥19000	140	9	60	≥317	2.3	0.15	Mucous membrane irritation
Oxiranes	68609-97-2	<100	430	ND	20	<5	22	-	Mucous membrane irritation
1-Ethyl-2-pyrrolidinone	2687-91-4	ND	ND	1	400	-	-	0.003	Mucous membrane irritation
Propylene glycol butyl ether	5131-66-8	300	ND	ND	900	0.33	-	-	Mucous membrane irritation
Cumulated RCR for mucous membrane irritation						≥360	24	0.17	Mucous membrane irritation
Di(propylene glycol) methyl ether, mixture of isomers (A)	34590-94-8	≥190000	100	<5	3100	≥61	0.03	0.002	No value available
Naphtha C7-C13	64742-82-1/ 64742-48-9	370	780	ND	1425	0.26	0.55	-	Neurotoxic

4.6.1.4 Assessment of product 32

After 5 hours, considerable emission of 2-butanone oxime and naphtha C7-C13 (white spirit), both of which are indicated, occur (Appendix 4). The DNEL values are exceeded by over 4000 and 1123 times, respectively (see table 14).

After 3 days the emission of 2-butanone oxime has ceased, while naphtha C7-C13 is still present, but at a significantly lower level just exceeding the DNEL value. Furthermore, emission of 2-ethylhexanoic acid is now approximately 4 times above the DNEL (probably due to the co-

balt bis (2-ethylhexanoate) content). The cumulative RCR value for mucous membrane irritation is exceeded due to the presence of a number of aldehydes and organic acids, the latter possibly being generated from the decomposition products of fatty acids in the product.

After 28 days, emission has decreased to levels significantly below the DNELs.

In the initial phase, very high emissions of 2-butanone oxime and white spirit is observed, which however decreases rapidly. 2-Ethylhexanoic acid is considered to be the substance that exceeds the DNEL value for most days, although this has reached an acceptable level after 28 days.

TABLE 14. Risk assessment of product 32 (1-component floor paint)

Product 32									
Substance	CAS no	Room concentration			DNEL (µg/m³)	RCR			Critical effect
		5 hours	3 days	28 days		5 hours	3 days	28 days	
		(µg/m³)	(µg/m³)	(µg/m³)					
Formaldehyde	50-00-0	2.6	7.1	2.9	100	0.03	0.07	0.03	Mucous membrane irritation
Acetaldehyde	75-07-0	2.0	30	5.4	1200	0.002	0.03	0.005	Mucous membrane irritation
Acetic acid	64-19-7	ND	36	ND	1200	-	0.03	-	Mucous membrane irritation
Propanoic acid	79-09-4	ND	77	ND	1500	-	0.05	-	Mucous membrane irritation
Butanoic acid	107-92-6	ND	31	22	1800	-	0.02	0.01	Mucous membrane irritation
2-Butanone, oxime	96-29-7	≥60000	ND	ND	15	≥4000	-	-	Mucous membrane irritation
Hexanal	66-25-1	ND	600	44	900	-	0.67	0.05	Mucous membrane irritation
Phenol	108-95-2	ND	16	3	70	-	0.23	0.04	Respiratory effects
Hexanoic acid	142-62-1	ND	790	240	2100	-	0.38	0.11	Mucous membrane irritation
Cumulated RCR for mucous membrane irritation						≥4000	1.48	0.25	Mucous membrane irritation
Di(propylene glycol) methyl ether, mixture of isomers (A)	34590-94-8	ND	16	ND	3100	-	0.005	-	No value available
1-Methoxypropan-2-ol	107-98-2	650	ND	ND	7900	-	0.08	-	No value available
2-Ethylhexanoic acid	149-57-5	ND	600	50	150	-	4.0	0.33	Effects on fetal development
Naphtha C7-C13	64742-82-1 / 64742-48-9	≥1600000	1900	84	1425	≥1123	1.3	0.06	Neurotoxic

4.6.2 2-component paints and lacquers

4.6.2.1 Assessment of product 5

After 5 hours, the DNEL values for 1,4-dioxane and 2-butoxyethanol are exceeded by 1.2 and 14 times, respectively (see table 15). 2-butoxyethanol, but not 1,4-dioxane is listed as an ingredient, cf. Appendix 4. After 3 days, none of the emitted substances exceed their respective DNEL values.

On the contrary CMIT, which is a potent mucosal irritant, is found after 28 days and as the DNEL value is very low (1 µg/m³), it is exceeded by a factor of 7. It is remarkable that the substance appears as a delayed emission after 28 days. The substance is not listed as an ingredient (Appendix 4).

TABLE 15. Risk assessment of product 5 (2-component paint)

Product 5									
Substance	CAS no	Room concentration			DNEL	RCR			Critical effect
		5 hours	3 days	28 days		5 hours	3 days	28 days	
		(µg/m ³)	(µg/m ³)	(µg/m ³)	(µg/m ³)				
Formaldehyde	50-00-0	ND	2.4	N	100	-	0.02	-	Mucous membrane irritation
Acetaldehyde	75-07-0	11	2.6	N	1200	0.009	0.002	-	Mucous membrane irritation
2-Propanol	67-63-0	360	<5	ND	26000	0.01	-	-	Mucous membrane irritation
Phenol	108-95-2	ND	1	ND	70	-	0.01	-	Respiratory effects
1,4-Dioxane	123-91-1	460	ND	ND	400	1.2	-	-	Mucous membrane irritation
Hexanoic acid	142-62-1	ND	ND	14	2100	-	-	0.007	Mucous membrane irritation
5-Chloro-2-methyl-3(2H)-isothiazolone (CMIT)	26172-55-4	ND	ND	7	1	-	-	7.0	Mucous membrane irritation
Cumulated RCR for mucous membrane irritation						1.2	0.03	7.0	Mucous membrane irritation
1-Methoxypropan-2-ol	107-98-2	130	ND	ND	7900	0.02	-	-	No value available
Toluene	108-88-3	ND	ND	ND	725		-	-	Neurotoxic
Ethylbenzene	100-41-4	7	ND	ND	200	0.035	-	-	Neurotoxic
Styrene	100-42-5	2	ND	ND	175	0.01	-	-	Neurotoxic
2-Butoxy ethanol	111-76-2	≥23000	230	12	1600	≥14	0.14	0.008	Effects on liver and blood
Benzyl alcohol	100-51-6	67	10	<5	440	0.15	0.02	-	No value available
2-Ethylhexanoic acid	149-57-5	ND	ND	7	150	-	-	0.05	Effects on fetal development

4.6.2.2 Assessment of product 30

This product results in the most complex emission of all the investigated products. After 5 hours, an emission concentration of 2-butanone oxime is seen, exceeding the DNEL value by 647 times (see table 16). Other respiratory irritants such as acetic acid and phenol also exceed their DNEL values with RCR values of 3.4 and 1.6, respectively. The cumulated exposure to neurotoxic hydrocarbons reaches a total RCR value of 2, while benzene as a carcinogen exceeds the DNEL value 9.5 times. In addition, the DNEL value are exceeded for benzyl

alcohol (32 times), 1-methoxypropan-2-ol (5.3 times) and dibutyl phthalate (8.7 times). No information regarding the composition was available for the product.

After 3 days and 28 days, emission is very limited and all levels are significantly below the relevant DNEL values.

TABLE 16. Risk assessment of product 30 (2-component floor paint)

Product 30									
Substance	CAS no	Room concentration			DNEL (µg/m³)	RCR			Critical effect
		5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)		5 hours	3 days	28 days	
Formaldehyde	50-00-0	ND	1.0	N	100	-	0.01	-	Mucous membrane irritation
Acetaldehyde	75-07-0	13	1.4	N	1200	0.01	0.001	-	Mucous membrane irritation
Acetic acid	64-19-7	4100	ND	ND	1200	3.4	-	-	Mucous membrane irritation
Propanoic acid	79-09-4	900	ND	ND	1500	0.60	-	-	Mucous membrane irritation
1,4-Dioxane	123-91-1	320	ND	ND	400	0.80	-	-	Mucous membrane irritation
2-Butanone, oxime	96-29-7	9700	ND	ND	15	647	-	-	Mucous membrane irritation
Phenol	108-95-2	110	ND	ND	70	1.6	-	-	Respiratory effects
Cumulated RCR for mucous membrane irritation						653	0.01		Mucous membrane irritation
Benzene	71-43-2	180	ND	ND	600	0.30	-	-	Neurotoxic
Hexane	110-54-3	210	ND	ND	700	0.30	-	-	Neurotoxic
Toluene	108-88-3	130	ND	ND	725	0.18	-	-	Neurotoxic
Ethylbenzene	100-41-4	230	ND	ND	200	1.2	-	-	Neurotoxic
Cumulated RCR for neurotoksicitet						2.0			Neurotoxic
Benzene	71-43-2	180	ND	ND	19	9.5	-	-	Carcinogenic
Benzyl alcohol	100-51-6	≥14000	200	29	440	≥32	0.45	0.07	No value available
1-Methoxypropan-2-ol	107-98-2	≥42000	49	8	7900	≥5.3	0.006	0.001	No value available
Butyldiglycol	112-34-5	430	17	ND	670	0.64	0.03	-	No value available
Dibutyl phthalate	84-74-2	200	ND	ND	23	8.7	-	-	Effects on fetal development
2-Ethylhexanoic acid	149-57-5	100	ND	ND	150	0.67	-	-	Effects on fetal development

4.6.3 Fillers

4.6.3.1 Assessment of product 14

After 5 hours, the DNEL value for C7-C13 hydrocarbons (white spirit) is exceeded 238 times (see table 17). Additionally, an excess of the substance phenol (RCR: 2.3) is observed, although this is not declared as a constituent of the product (Appendix 4).

After 3 days, the levels of C7-C13 hydrocarbons are still above the DNEL value (RCR: 8.4), and initial emission of 2-ethylhexanoic acid is observed here probably due to the content of zirconium 2-ethylhexanoate in the product. However, the DNEL value is not exceeded for the substance. No volatiles exceed the DNEL value after 28 days.

TABLE 17. Risk assessment of product 14 (Filler)

Product 14									
Substance	CAS no	Room concentration			DNEL	RCR			Critical effect
		5 hours	3 days	28 days		5 hours	3 days	28 days	
		(µg/m ³)	(µg/m ³)	(µg/m ³)	(µg/m ³)				
Formaldehyde	50-00-0	1.5	2.0	N	100	0.02	0.02	-	Mucous membrane irritation
Acetaldehyde	75-07-0	35	18	N	1200	0.03	0.02	-	Mucous membrane irritation
Propanoic acid	79-09-4	ND	ND	20	1500	-	-	0.01	Mucous membrane irritation
Butanoic acid	107-92-6	ND	ND	22	1800	-	-	0.01	Mucous membrane irritation
Hexanal	66-25-1	ND	53	18	900	-	0.06	0.02	Mucous membrane irritation
Hexanoic acid	142-62-1	ND	ND	33	2100	-	-	0.02	Mucous membrane irritation
Phenol	108-95-2	160	15	3	70	2.3	0.21	0.04	Respiratory effects
Cumulated RCR for mucous membrane irritation						2.4	0.31	0.10	Mucous membrane irritation
2-Ethylhexanoic acid	149-57-5	ND	110	18	150	-	0.73	0.12	Effects on fetal development
Naphtha C7-C13	64742-82-1/ 64742-48-9	≥340000	12000	220	1425	≥238	8.4	0.15	Neurotoxic

4.6.4 Floor wax, polish and care products

4.6.4.1 Assessment of product 72

After 5 hours, the DNEL value has been exceeded by a factor 11 for 2- (2-ethoxyethoxy)-ethanol, while the DNEL value for C7-C14 hydrocarbons has been exceeded by 147 times (see table 18). Emission decreases rapidly and there are no substances exceeding the DNEL value after either 3 or 28 days. There are no data regarding ingredients of the product, cf. Appendix 4.

TABLE 18. Risk assessment of product 72 (Oil for care of tabletops)

Product 72									
Substance	CAS no	Room concentration			DNEL (µg/m³)	RCR			Critical effect
		5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)		5 hours	3 days	28 days	
Formaldehyde	50-00-0	2.8	6.6	N	100	0.03	0.07	-	Mucous membrane irritation
Acetaldehyde	75-07-0	15	6.9	N	1200	0.01	0.006	-	Mucous membrane irritation
Butanoic acid	107-92-6	ND	ND	21	1800	-	-	0.01	Mucous membrane irritation
Hexanoic acid	142-62-1	ND	360	25	2100	-	0.17	0.01	Mucous membrane irritation
Phenol	108-95-2	53	11	3	70	0.76	0.16	0.04	Respiratory effects
Ethanol, 2-(2-ethoxyethoxy)-	111-90-0	3700	ND	ND	350	11	-	-	Mucous membrane irritation
Cumulated RCR for mucous membrane irritation						12	0.41	0.06	Mucous membrane irritation
2-Ethylhexanoic acid	149-57-5	ND	55	10	150	-	0.37	0.07	Effects on fetal development
Naphtha C7-C13	64742-82-1/ 64742-48-9	≥210000	310	ND	1425	≥147	0.22	-	Neurotoxic
Di(propylene glycol) methyl ether, mixture of isomers (A)	34590-94-8	290	9	ND	3100	0.09	0.003	-	No value available

TABLE 19. Risk assessment of product 85 (Linseed oil for wood care)

Product 85									
Substance	CAS no	Room concentration			DNEL (µg/m³)	RCR			Critical effect
		5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)		5 hours	3 days	28 days	
Formaldehyde	50-00-0	ND	1.9	12	100		0.019	0.12	Mucous membrane irritation
Acetaldehyde	75-07-0	ND	1.6	30	1200		0.001	0.025	Mucous membrane irritation
Acetic acid	64-19-7	ND	ND	120	1200			0.10	Mucous membrane irritation
Propanoic acid	79-09-4	ND	ND	420	1500			0.28	Mucous membrane irritation
Hexanal	66-25-1	<5	ND	670	900			0.74	Mucous membrane irritation
Hexanoic acid	142-62-1	ND	ND	260	2100			0.12	Mucous membrane irritation
2-Butanone, oxime	96-29-7	92	ND	ND	15	6.1			Mucous membrane irritation
Cumulated RCR for mucous membrane irritation						6.1	0.020	1.3	Mucous membrane irritation
Naphtha C7-C13	64742-82-1/ 64742-48-9	4500	48	<5	1425	3.2	0.0007		Neurotoxic

4.6.4.2 Assessment of product 85

After 5 hours, emission of 2-butanone oxime exceeds DNEL by a factor of 6.1 and emission of C7-C13 hydrocarbons (white spirit) exceeds the DNEL value by a factor of 3.2 (see table 19). No significant emission is measured after 3 days. After 28 days, a number of aldehydes and organic acids not previously measured are released, suggesting that the degradation products of the linseed oil, which makes up the main content of the product. When the RCR values for these irritants are added up, the tolerable level of mucosal irritants is exceeded after 28 days. It is not known how long the emission of these substances will take place.

TABLE 20. Risk assessment of product 60 (Wet room adhesive)

Product 60									
Substance	CAS no	Room concentration			DNEL	RCR			Critical effect
		5 hours	3 days	28 days		5 hours	3 days	28 days	
		(µg/m³)	(µg/m³)	(µg/m³)	(µg/m³)				
Formaldehyde	50-00-0	120	1.4	N	100	1.2	0.01	-	Mucous membrane irritation
Acetaldehyde	75-07-0	11	1.3	N	1200	0.009	0.001	-	Mucous membrane irritation
Acetic acid	64-19-7	ND	1000	31	1200	-	0.83	0.03	Mucous membrane irritation
Propanoic acid	79-09-4	ND	22	ND	1500	-	0.03	-	Mucous membrane irritation
Butanoic acid	107-92-6	ND	15	13	1800	-	0.008	0.007	Mucous membrane irritation
Phenol	000108-95-2	100	5	3	70	1.4	0.07	0.04	Respiratory effects
2-Methyl-1,2-thiazol-3(2H)-one (MIT)	2682-20-4	ND	9	<5	100	-	0.09	-	Mucous membrane irritation
5-Chloro-2-methyl-3(2H)-isothiazolone (CMIT)	026172-55-4	18	21	<5	1	18	21	-	Mucous membrane irritation
Cumulated RCR for mucous membrane irritation						20.6	22	0.08	Mucous membrane irritation

4.6.5 Adhesive for walls and membranes (water-based)

4.6.5.1 Assessment of product 60

After 5 hours, emissions are observed over the DNEL levels of phenol (RCR: 1.4) and CMIT (RCR: 18) (see table 20). The latter is listed as an ingredient in the product.

While the emission of phenol is decreased after 3 days, a slight increase is seen for CMIT resulting in an RCR value of 21. After 28 days, only emissions at very low levels persist and a total RCR of 0.08 can be calculated for the cumulated emission with respect to mucosal irritants.

It should be noted that the exposure duration in a wet room (bathroom or laundry room) is estimated to be significantly shorter (approx. 1 hour) than the exposure time in other rooms (living room or bed room), thus, 1 hour of exposure to phenol (RCR of 1.4) after 5 hours is not considered critical. However, the relatively high RCR values for CMIT of 18 and 21 after 5 hours

and 3 days, respectively, is considered critical due to the risk of irritation during the stay in the wet room.

4.7 Overview of the emission results

4.7.1 Emission patterns over time

Table 21 below lists the chemical gases emitted from the investigated products at levels that exceed the DNEL value for the measurement series.

TABLE 21 Critical substances and emission patterns

1-component paints and lacquers (product 4, 11, 29, 32)						
Substance	CAS no	DNEL	5 hours	3 days	28 days	Critical effect
		(µg/m ³)	RCR	RCR	RCR	
Acrolein	107-02-8	7		3.1 – 5.6		Mucous membrane irritation
Hexanoic acid	142-62-1	1800		1.5 – 1.6		Mucous membrane irritation
2-Butanone, oxime	96-29-7	15	≥3867 - ≥14667			Mucous membrane irritation
Diethylamine	109-97-7	60	38			Mucous membrane irritation
Triethylamine	121-44-8	60	317	2.3		Mucous membrane irritation
Oxiranes	68609-97-2	20	<5	22		Mucous membrane irritation
2-Ethylhexanoic acid	149-57-5	150		4-23	1.3 – 2.7	Effects on fetal development
Di(propylene glycol) methyl ether, mixture of isomers (A)	34590-94-8	3100	1.3 - 61			No value available
Naphtha C7-C13 Alkanes, iso-, < 2% aromatics C7-C13	64742-82-1/ 64742-48-9 927-285-2	1425	772-1825	1.3-77		Neurotoxic

2-component paints and lacquers (product 5, 30)						
Acetic acid	64-19-7	1200	3.4			Mucous membrane irritation
1,4-Dioxane	123-91-1	400	1.2			Mucous membrane irritation
2-Butanone, oxime	96-29-7	15	647			Mucous membrane irritation
2-Butoxy ethanol	111-76-2	1600	14			Mucous membrane irritation
5-Chloro-2-methyl-3(2H)-isothiazolone (CMIT)	26172-55-4	1		7.0		Mucous membrane irritation
Phenol	108-95-2	70	1.6			Respiratory effects
Ethylbenzene	100-41-4	230	1.2			Neurotoxic
Benzene	71-43-2	19	9.5			Carcinogenic
Benzyl alcohol	100-51-6	440	32			No value available
1-Methoxypropan-2-ol	107-98-2	7900	5.3			No value available
Dibutyl phthalate	84-74-2	23	8.7			Effects on fetal development
Fillers (product 14)						
Phenol	108-95-2	70	2.3			Respiratory effects
Naphtha C7-C13	64742-82-1/ 64742-48-9	1425	238	8.4		Neurotoxic
Floor wax, polish and care products (product 72, 85)						
Substance	CAS no	DNEL (µg/m³)	5 hours RCR	3 days RCR	28 days RCR	Critical effects
Ethanol, 2-(2-ethoxyethoxy)-	111-90-0	350	11			Mucous membrane irritation
Naphtha C7-C13	64742-82-1/ 64742-48-9	1425	3.2 - 147			Neurotoxic
2-Butanone, oxime	96-29-7	15	6.1			Mucous membrane irritation
Adhesive (product 60)						
Formaldehyde	50-00-0	100	1.2			Mucous membrane irritation
Phenol	108-95-2	70	1.4			Respiratory effects
5-Chloro-2-methyl-3(2H)-isothiazolone (CMIT)	026172-55-4	1	18	21		Mucous membrane irritation

4.7.1.1 Emission after 5 hours

After 5 hours, 23 substances have been measured that exceed the DNEL value. This is most pronounced for 2-butanone oxime from 1-component paints and varnishes exceeding the DNEL by more than 14667 times. As the substance is a potent mucosal irritant, pronounced irritation of eyes and respiratory tract is expected for people are staying in the room in question. Similarly, C7-C13 hydrocarbons (white spirit) from 1-component paints and varnishes and from filler and sealant exceed DNEL up to 1825 times. As this DNEL value is based on the development of chronic neurotoxicity after many years of exposure, this temporary exceedance is considered to be less critical than for 2-butanone oxime, where the effect is acute.

To avoid irritation from these very high concentrations of volatiles after 5 hours, persons should not use the room and instead wait a few days before putting it into service and/or ensuring a very thorough ventilation.

It should be noted that there is increased focus on 2-butanone, as it is currently being discussed in the Risk Assessment Committee of the European Chemicals Agency, whether the substance is proposed to be upgraded from Carc 2 to Carc 1B and further to be subjected to classification as STOT SE 1 and STOT RE 1 due to the harmful effects of the substance on the nasal mucosa in short-term as well as by prolonged/repeated exposure.

4.7.1.2 Emission after 3 days

The total emission has decreased significantly after 3 days, with 7 substances exceeding their DNEL value. A number of these substances are substances that have not been measured after 5 hours e.g. acrolein, hexanoic acid, oxiranes, 2-ethylhexanoic acid. The substances measured after 3 days considered to be most problematic are acrolein, oxiranes and CMIT, all of which are potent respiratory and eye irritants. Furthermore, the emission of 2-ethylhexanoic acid is also considered critical due to the potential effects on fetal development. Thus, when using the rooms after 3 days, very thorough ventilation is necessary to reduce the exposure to acceptable levels.

4.7.1.3 Emission after 28 days

After 28 days, the emissions from most products have dropped to very low values below the critical levels. An exception is one 1-component paint/lacquer, which continues to exceed the DNEL value for 2-ethylhexanoic acid and another exception is a filler, where the emission of CMIT causes the DNEL value to be exceeded. Finally, a linseed oil product, which had no significant emissions at 5 hours, emits irritants such as aldehydes and organic acids after 28 days, which results in a cumulated exposure above the tolerable exposure level.

Furthermore, it is seen that the content of the preservative CMIT can cause long-term emissions at levels that exceed the DNEL value of this substance. Products containing metal salts of 2-ethylhexanoic acid as a desiccant may also give rise to a delayed and prolonged emission, which may cause the DNEL values to be exceeded. Similarly, delayed emission also applies to the linseed oil product, which can emit mucosal irritant conversion products several weeks after application.

However, after 28 days, the exceedances of the DNEL values are relatively limited and critical levels can therefore be countered by undertaking thorough venting.

4.8 Conclusion of risk analysis

Significant emissions, which far exceed the tolerable exposure level after 5 hours have been demonstrated from the investigated products. Emissions of potent eye and upper airways irritants like 2-butanone oxime and triethylamine were evaluated to be the most critical compounds.

After 3 days, significant reductions in the total emissions of volatile substances from products was found. However, the tolerable exposure levels are exceeded for eye and respiratory irritants. These substances with delayed emission may either be degradation products or less volatile substances than those measured after 5 hours. Furthermore, after 3 days unacceptably high emissions of 2-ethylhexanoic acid was found, a substance that may be potentially harmful to the developing fetus.

After 28 days, the overall emissions generally decrease to very low levels, however for some substances the tolerable exposure levels are still exceeded. This applies, for example, to the substance 2-ethylhexanoic acid, which is considered critical since the substance as mentioned before, is considered potentially harmful to the developing fetus. Additionally, after 28 days unacceptably high emissions of the biocidal preservative CMIT, critical for mucous membrane irritation. After 28 days, linseed oil display unacceptably high emissions of aldehydes and organic acids that may cause mucous membrane irritation of the eyes and respiratory tract.

The risk assessments are based on the use of one product at a time. In case of simultaneous use of more products, the total emissions will increase, thus leading to a higher potential risk. It is assumed the general population is aware of the occurrence of high emissions on the day of working with DIY products and therefore users do not stay in the room right after use. Therefore, the delayed evaporation after 3 and 28 days is considered to be more critical, as it is easy to envision that a renovated room will be in use after 1-3 days. However, the results of this project show that for some DIY products, a longer “quarantine” period before use of the room may be relevant or significantly increased ventilation must be provided to avoid harmful levels of volatile compounds.

5. Outlook

In the pre-project, 19 companies selling DIY products were identified, while a total of 57 DIY products were found. In this project, an additional 12 companies and a total of 86 products were identified. The survey showed that DIY products can be purchased many other places than in conventional DIY stores. When purchasing DIY products in a supermarket for example, it is assumed that assistance is minimal and that purchasing is predominantly based on spontaneous decisions. As a consumer it is difficult to know, whether a product is safe to use indoors, when there is a lack of relevant safety information in combination with lack of suitable guidance at the time of purchase.

The emissions analysis in the pre-project was primarily focused on the declared and/or expected ingredients in the products. Experience from the pre-project as well as analysis of other similar materials showed a need for further investigation regarding several other substances. The analyses must be performed using defined reference standards and therefore a limited number of substances can be determined. Therefore, it is not surprising that more dangerous substances have been identified and determined in the current project than in the pre-project. Furthermore, in the present project, increased attention was paid to identifying substances potentially formed during curing of the DIY product, which could therefore not be expected to be found in the list of declared ingredients.

In the pre-project tests for human exposure were performed in a large chamber while applying the products. These analyses did not give new information compared to the measurements after 5 hours in the small chambers. It has therefore been possible to focus in this project on testing a wider range of products within the given financial framework. The analysis program of the project has focused on the volatiles emitting over time to assess the risk related to the products in the short, medium and long term. The measurements performed after 5 hours give an overview of the substances the performing DIY person is exposed to, while the analysis after 3 and 28 days, respectively, give an indication of the exposure levels the rest of the household is subject to in the short and long term, when the renovated room/rooms in question are in taken into use after renovation.

Thorough and regular ventilation of the renovated room/rooms significantly reduces the exposure to harmful volatile substances. This is especially important for humans, who are more vulnerable to dangerous substances such as pregnant women and young children. Thus, it is recommended to ensure thorough ventilation during all DIY projects in the home and as well as taking the precaution of not using the room/rooms directly after renovation. The general recommendation for all product groups is that consumers should choose a product with an indoor climate label, which adhere to established criteria in relation emissions of dangerous volatiles and thus also set limits for exposure to harmful substances. Limit values for emissions are set in several existing labelling schemes such as AgBB, Blue Label, GEV Emicode, Danish Indoor Climate Label, M1 and Blauer Engel.

Liquid products may have different eco-labels with requirements regarding specific ingredients (including carcinogens and allergenic substances) and total VOC content. Under the CLP Regulation Safety Data Sheets are only required to include state hazard labelled ingredients for a product, but this does not take into consideration the formation of reaction products and volatiles during curing of a given product. In general, there are no legal requirements for testing of or criteria for product emissions to the indoor environment, and therefore consumers also have no way of knowing, whether products are safe to apply indoors or whether they are safe to use indoors in the long term. Therefore, the recommendation to consumers is to use products that

have documentation of testing and adhere to recommended limit values for hazardous substances.

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Appendix 1. List of companies

Appendix 1.1 In ” Survey and risk assessment of chemical substances in chemical products used for "do-it-yourself" projects in the home ”*
product information was obtained from the following companies
with retail stores of DIY products

Company name	Type of company
Alfix	Producer
Bauhaus A/S	DIY store
Beck & Jørgensen A/S	Producer
Beckmann A/S	Paint shop/Producer
Borup Kemi I/S	Producer
Casco - Sika Danmark A/S	Producer
DanaLim A/S	Producer
Deco Farver ApS	Paint shop
Flügger A/S	Paint shop/Producer
Harald Nyborg A/S	DIY store
Jem & Fix A/S	DIY store
Junckers Industrier A/S	Producer
LavprisVVS	DIY store
Malgodt.dk	Paint shop
Sadolin - Akzo Nobel	Producer
Saint-Gobain Weber A/S	Producer
Scandinova A/S	Wholesaler
XL-Byg	DIY store
Æ´Gulvsliver	DIY store (Floor planning)

*) Survey and risk assessment of chemical substances in chemical products used for "do-it-yourself" projects in the home. Survey of Chemical Substances in Consumer Products No. 167, Environmental Protection Agency 2018.

Appendix 1.2 Product information gathered from identified DIY companies, stores and manufacturers of building materials for private use

Company name	Type of company
Bilka (Salling Group A/S)	Retail/groceries
Biltema	Retail/DYI store
Coop Danmark (amba)	Retail/groceries
Damgaard	Retail/DYI store
Davidsen (DAVIDSENS TØMMERHANDEL A/S)	Retail/DYI store, lumber
Esbjerg Farve- & Lakfabrik A/S	Producer
Idé Møbler (IDdesign A/S)	Retail/Furniture
JYSK A/S	Retail/DYI store
Maling.dk	Retail/paints and varnishes
Røverkøb	Retail/paints and varnishes
SILVAN A/S (Aurelius Group)	DYI store
thansen (T. Hansen Gruppen A/S)	Retail/DYI store

Appendix 2. Product overview

Appendix 2.1 Product overview with product ID.

Products selected for testing are indicated with green background

ID	1/2c	Product type (application)	Project	Amount	Drying time	Number of treatments	SDS	Density	MAL-code*	CLP hazard label*	VOC content (g/l)
1		Sealant	Sealing				Yes	1.3 g/cm ³ (20°C)	1-5	-	No
2	A	Paint & lacquer	Painting of floor				Yes	~1.050 g/cm ³	00-5	Health hazard, Environmental hazard	5
2	B	Paint & lacquer	Painting of floor				Yes	1.290 g/cm ³	No	Corrosion	5
3	A	Paint & lacquer	Lacquering of floor				Yes	~1.050 g/cm ³	No	-	5
3	B	Paint & lacquer	Lacquering of floor				Yes	~1.050 g/cm ³	No	-	55
4		Paint & lacquer	Painting	4-6 m ² /liter	4-8 hours	1-2	Yes	No	1-1	-	<350 g/l
5	A	Paint & lacquer	Painting	150-200 g/m ²	24-96 hours	3	Yes	1.1 g/cm ³	00-1	Corrosion, Health hazard	No
5	B	Paint & lacquer	Painting	150-200 g/m ²	24-96 hours	3	Yes	1.52 g/cm ³	0-5	Health hazard	No
6		Sealant	Sealing	-	-	-	Yes	≤ 1.3 g/cm ³ (PMDI)	3-3	-	No
7		Sealant	Sealing	-	-	-	Yes	≤ 1.3 g/cm ³ (PDMI)	3-3	-	No
8		Sealant	Sealing	-	-	-	Yes	1.2 g/cm ³	3-3	-	No
9		Sealant	Sealing	-	-	-	Yes	1.2 g/cm ³	3-1	-	No

ID	1/2c	Product type (application)	Project	Amount	Drying time	Number of treatments	SDS	Density	MAL-code*	CLP hazard label*	VOC content (g/l)
10		Paint & lacquer	Painting of floor	-	-	2-3	Yes	1.18 g/cm ³	2-1	-	<500 g/l
11		Paint & lacquer	Painting	-	-	-	Yes	1.1 g/cm ³	2-1	Health hazard, Flammable	<500 g/l
12		Paint & lacquer	Lacquering of floor	-	-	-	Yes	-	2-1	-	<400 g/l
13	A	Filler	Sealing	-	-	-	No	-	-	-	-
13	B	Filler	Sealing				No	-	-	-	-
14		Filler	Sealing	-	2-3 hours	-	Yes	-	-	Serious health hazard, Flammable	-
15		Filler	Sealing	1-2 mm layer	8-10 hours	-	No	-	-	-	-
16		Sealant	Sealing	-	-	-	No	-	-	-	-
17		Filler	Sealing				No	-	-	-	-
18		Filler	Sealing	-	-	-	No	-	-	-	-
19		Filler	Sealing	1 cm layer	2 hours	-	No	-	-	-	-
20	A	Paint & lacquer	Painting	6 m ² /liter	9 hours (surface dry)	2	No	-	00-6	Corrosion, Serious health hazard, Health hazard	-
20	B	Paint & lacquer	Painting				No	-	00-5	Health hazard, Environmental hazard	-
21	A	Paint & lacquer	Painting of floor	8 m ² /kg	24 hours (surface dry)	2	No	-	-	-	-
21	B	Paint & lacquer	Painting of floor				No	-	-	-	-
22	A	Filler	Sealing	-	15 min	-	No	-	-	-	-
22	B	Filler	Sealing				No	-	-	-	-
23	A	Filler	Sealing	-	15 min	-	No	-	-	-	-
23	B	Filler	Sealing				No	-	-	-	-
24	A	Filler	Sealing	-	15 min	-	No	-	-	-	-

ID	1/2c	Product type (application)	Project	Amount	Drying time	Number of treatments	SDS	Density	MAL-code*	CLP hazard label*	VOC content (g/l)
24	B	Filler	Sealing				No	-	-	-	-
25		Sealant	Sealing	-	12 min	-	No	<25 kg/kvm	-	-	-
26	A	Filler	Sealing	-	10 min	-	Yes	-	-	Flammable, Serious health hazard, Health hazard	-
26	B	Filler	Sealing				Yes	-	-	Flammable, Health hazard	-
27	A	Filler	Sealing	-	10 min	-	No	-	-	-	-
27	B	Filler	Sealing				No	-	-	-	-
28		Sealant	Sealing	10-30 liter	-	-	No	-	-	-	-
29	A	Paint & lacquer	Lacquering of floor	-	-	-	Yes	-	-	-	-
29	B	Paint & lacquer	Lacquering of floor	-	-	-	Yes	-	-	-	-
30	A	Paint & lacquer	Painting of floor	-	-	-	No	-	-	Health hazard, Corrosion	-
30	B	Paint & lacquer	Painting of floor	-	-	-	No	-	-	Health hazard	-
31		Paint & lacquer	Painting of floor	8 m²/0.9 l (8.8 m²/l)	-	-	No	-	-	-	-
32		Paint & lacquer	Painting of floor	-	-	-	No	-	-	Flammable, Health hazard	-
33		Paint & lacquer	Lacquering of floor	-	2 min	-	No	-	-	-	-
34		Filler	Sealing	-	-	-	No	-	-	-	-
35		Filler	Sealing	-	-	-	No	-	-	-	-
36	A	Filler	Sealing	-	20 min	-	Yes	-	-	-	-
36	B	Filler	Sealing				Yes	-	-	-	-
38		Filler	Sealing	-	10 min	-	No	-	-	-	-
39		Filler	Sealing				No	-	-	-	-

ID	1/2c	Product type (application)	Project	Amount	Drying time	Number of treatments	SDS	Density	MAL-code*	CLP hazard label*	VOC content (g/l)
40		Filler	Sealing	-	-	-	No	-	-	-	-
41		Filler	Sealing	max 5 mm	1.5 hours	-	No	-	-	-	-
42		Filler	Sealing	-	-	-	No	-	-	-	-
43		Filler	Sealing	-	-		No	-	-	-	-
44		Filler	Sealing	-	-		No	-	-	-	-
45	A	Filler	Sealing	-	10 min	-	Yes	1.79 g/cm ³	5-6	-	105 g/l
45	B	Filler	Sealing				Yes	1.1 g/cm ³	0-4	-	0 g/l
46		Filler	Sealing	-	-		No	-	-	-	-
47		Tile adhesive	Gluing	-	-		No	-	-	-	-
48		Tile adhesive	Gluing	-	-		No	-	-	-	-
49		Tile adhesive	Gluing	-	-		No	-	-	-	-
50		Tile adhesive	Gluing	-	-		No	-	-	-	-
51		Tile adhesive	Gluing	-	-		No	-	-	-	-
52		Filler	Sealing	-	-		No	-	-	-	-
53		Tile adhesive	Gluing	-	min. 2 hours		No	-	-	-	-
54		Tile adhesive	Gluing	-	min. 12 hours		No	-	-	-	-
55		Tile adhesive	Gluing	-	24-48 hours		No	-	-	-	-
56		Membrane	Sealing	-	1. layer: 1-2 2-3 hours; 2. layer: 12 hours		Yes	-	-	-	-

ID	1/2c	Product type (application)	Project	Amount	Drying time	Number of treatments	SDS	Density	MAL-code*	CLP hazard label*	VOC content (g/l)
57		Membrane	Sealing	-	1. layer: 1-2 hours; 2. layer 8-16 hours	2	No	-	00-1	-	-
58	A	Adhesive	Gluing	-	-	-	No	-	-	-	-
58	B	Adhesive	Gluing	-	-	-	No	-	-	-	-
59		Adhesive	Gluing	-	-	-	No	-	-	-	-
60		Adhesive	Gluing	-	-	-	Yes	-	-	-	-
61		Adhesive	Gluing	-	-	-	No	-	-	-	-
62		Adhesive	Gluing	-	-	-	No	-	-	-	-
63		Adhesive	Gluing	-	-	-	No	-	-	-	-
64		Adhesive	Gluing	-	-	-	No	-	-	-	-
65		Adhesive	Gluing	-	-	-	Yes	-	-	-	-
66	A	Adhesive	Gluing	-	-	-	No	-	-	-	-
66	B	Adhesive	Gluing	-	-	-	No	-	-	-	-
67		Adhesive	Gluing	-	-	-	No	-	-	-	-
68		Adhesive	Gluing	-	-	-	No	-	-	-	-
69		Floor wax, polish and care products (wax)	Waxing	-	-	-	Yes	0.79 g/cm ³	2-1	-	-
70		Sealant	Sealing	-	10-14 min	-	Yes	1.3 g/cm ³	1-5	-	-
71		Filler	Sealing	-	-	-	Yes	1.5 ± 0.05 kg/dm ³	-	-	-
72		Floor wax, polish and care products (oil)	Polish and care of wood	-	-	-	No	-	-	-	-
73		Sealant	Sealing	-	-	-	No	-	-	-	-
74		Floor wax, polish and care	Polish and care	-	-	-	No	-	-	-	-

ID	1/2c	Product type (application)	Project	Amount	Drying time	Number of treatments	SDS	Density	MAL-code*	CLP hazard label*	VOC content (g/l)
		care products									
75		Floor wax, polish and care products (wax)	Polish and care	-	-	-	No	-	-	-	-
76		Floor wax, polish and care products	Polish and care	-	-	-	No	-	-	-	-
77		Floor wax, polish and care products (wax)	Polish and care	-	-	-	No	-	-	-	-
78		Floor wax, polish and care products (wax)	Polish and care	-	-	-	No	-	-	-	-
79		Floor wax, polish and care products (wax)	Polish and care	-	-	-	No	-	-	-	-
80		Floor wax, polish and care products (wax)	Polish and care	-	-	-	No	-	-	-	-
81		Floor wax, polish and care products	Polish and care	-	-	-	No	-	-	-	-
82		Floor wax, polish and care products	Polish and care	-	-	-	No	-	-	-	-

ID	1/2c	Product type (application)	Project	Amount	Drying time	Number of treatments	SDS	Density	MAL-code*	CLP hazard label*	VOC content (g/l)
83		Floor wax, polish and care products (wax)	Polish and care		-	-	No	-	00-3	-	-
84		Sealant	Sealing		-	-	No	-	-	-	-
85		Floor wax, polish and care products	Polish and care of wooden floors	Apply to surface and remove excess	-	-	No	-	-	-	-
87 [±]		Adhesive	Gluings		-	-	Yes	1.71	-	Health hazard	3.6
88 [¥]		Sealant	Repair and levelling		-	-	Yes	1.52-1.68 g/ml	-	Health hazard	-

Product-ID numbers 37 and 86 were not applied. In order to avoid errors in data, the original ID-numbering has been kept.

*MAL-codes and CLP classification is stated for all products, depending on availability of information. For all the tested products (marked with green background) the MAL-codes and CLP classification was stated on the packaging and therefore added in the table.

[±]The product was received from the Danish Chemical Inspection due to packaging issues. The product was considered outside the scope of the current project, since it is a specialty product.

[¥]The product was received from the Danish Chemical Inspection. The product is from a store that is not included in this project.

Appendix 3. MBK

MBK is a Danish database for application of coating products and the following instructions are only available in Danish.

Appendix 3.1 Anvisning V 6453C



Malerfagligt Behandlings-Katalog

Gulve > Vedligehold > Træ, brædder/parket

Vedligehold V 6453C

Byggemateriale:	Træ, brædder/parket
Funktionsklasse:	Æstetiske og middel funktionelle krav II
Nuværende behandling:	Plastgulvlak
Tilstand og behov:	C: Almindeligt vedligehold
Forventet udfald:	Ensartet, lukket og glat flade
Slutbehandling:	Plastgulvlak
Kodenummer:	00-1



Behandlingsanvisning

Malebehandling

- vask
- afrensning af evt. sæberester, plejemidler o.lign.
- let slibning
- kitning
- 2 gange plastgulvlak, glans: _____

Vedhæftning: Tapeprøve, kl. 0-1

Forudsætninger

Overfladen er:
stort set intakt (slidt, enkelte små huller/revner),
fastsiddende og bæredygtig,
uden væsentlig afsmitning,
tilsmudset.

C-anvisningen kan give tilfredsstillende funktion/udseende.

Vejl. prøvemethoder: Klima-Afsmitning-Tapeprøve

Egenskaber

Behandling til trægulve i tørre rum med let gangtrafik, fx bolig.
C-behandling anviser det rettidige vedligehold.
Plastgulvlak tørrer op til klar film, blank til halvmat. Har tilfredsstillende slidstyrke, men begrænset ridsefasthed og vandbestandighed. Rengørigheden er god, men skal udføres skånsomt og vandspild skal fjernes straks.
NB: Undersøg forlidelighed mellem nuværende beh. og ny lak.



Malerfagligt Behandlings-Katalog

Gulve > Nybehandling > Træ, brædder/parket

Nybehandling V 6452

Byggemateriale:	Træ, brædder/parket
Funktionsklasse:	Æstetiske og middel funktionelle krav II
Forventet udfald:	Ensartet, lukket og glat flade
Slutbehandling:	Plastgulvlak
Kodenummer:	00-1



Behandlingsanvisning

Malebehandling

grundning med plastgulvlak

2 gange plastgulvlak, glans: _____

Vedhæftning: Tapeprøve, kl. 0

Forudsætninger

Trægulvet skal være fastgjort fordækt eller med udfyldte huller. Gulvat skal være rent, uden ujævnhed fra overfladeopfugtning og uden misfarvninger som følge af biologisk angreb, samt glatte (uden skader fra maskinbearbejdning og meget synlige kutterslag). Samlinger og stød skal være tætte og i plan, uden slibespor. Barkrings- og rådknaster, knasthuller og harpikslommer må ikke forekomme. Revner max. bredde 0,5 mm og længde max. 10% af emnet. Fugtighed 8 ± 2 vægt%.

Vejl. prøvemethoder: Klima-Træfugtighed

Egenskaber

Behandling til trægulva i tørre rum med let gangtrafik, fx bolig.

Plastgulvlak tørrer op til klar film, blank til halvmat. Har tilfredsstillende slidstyrke, men begrænset ridsefasthed og vandbestandighed. Rengørigheden er god, men skal udføres skånsomt og vandspild skal fjernes straks.

Plastgulvlak er let at genbehandle.

Appendix 4. Dangerous substances in do-it-yourself products

Appendix 4.1 Dangerous substances in do-it-yourself products according to their respective safety data sheets (SDS).

Products selected for testing are indicated with green background

ID	C	Product type	Substance	Type	CAS no.	EINECS no.	Classification cf. SDS	Conc.(%)
1		Sealant	Dimethylether	VVOC	115-10-6	204-065-8	H220, H280, Flam. Gas 1, Press. Gas (*),	<10
1		Sealant	Isobutane	VVOC	75-28-5	200-857-2	H220, H280, Flam. Gas 1, Press. Gas (*),	<15
1		Sealant	Butane	VVOC	106-97-8	203-448-7	H220, H280, Flam. Gas 1, Press. Gas (*),	<15
1		Sealant	Dimethylether	VVOC	115-10-6	204-065-8	H220, H280, Flam. Gas 1, Press. Gas (*),	<10
1		Sealant	Chlorinated paraffins C14-17	N	85535-85-9	287-477-0	H362, H400, H410, Aquatic Acute 1, Aquatic Chronic 1, Lact.,	<30
1		Sealant	Diphenylmethandiisocyanate, isomers and homologues	(VOC)	9016-87-9	618-498-9	H315, H317, H319, H332, H334, H335, H351, H373, Acute tox. 4, Carc. 2, Eye Irrit. 2, Resp. Sens. 1, Skin Irrit. 2, Skin Sens. 1, STOT RE 2, STOT SE 3,	20-60
2	A	Paint & lacquer	Alcohols, C8-22, ethoxylated	(VOC)	69013-19-0	-	H302, H315, H318, H400, Acute tox. 4, Aquatic Acute 1, Eye D am. 1, Skin Irrit. 2,	<0.5
2	A	Paint & lacquer	1,2-ethandiol	VOC	107-21-1	203-473-3	H302, Acute tox. 4,	1
2	A	Paint & lacquer	5-chloro-2-methyl-2H-isothiazol-3-on [EU no. 247-500-7], mixture (3:1) with 2-methyl-2H-isothiazol-3-one [EU no. 220-239-6]	VOC	55965-84-9	247-500-7	H301+H311, H314, H317, H331, H400, H410, Acute tox. 3, Aquatic Acute 1, Aquatic Chronic 1, Skin Corr. 1B, Skin Sens. 1,	0.001
2	B	Paint & lacquer	5-chloro-2-methyl-2H-isothiazol-3-on [EU no. 247-500-7], mixture (3:1) with 2-methyl-2H-isothiazol-3-one [EU no. 220-239-6]	VOC	55965-84-9	247-500-7	H301+H311, H314, H317, H331, H400, H410, Acute tox. 3, Aquatic Acute 1, Aquatic Chronic 1, Skin Corr. 1B, Skin Sens. 1,	~0.001
2	A	Paint & lacquer	oxiran, mono[(C12-14-alkyloxy)methyl]derivatives; (C12C14) alkylglycidylether	N	68609-97-2	271-846-8	H315, H317, Skin Irrit. 2, Skin Sens. 1,	5-10
2	A	Paint & lacquer	Bisphenol F reaction product	N	9003-36-5	500-006-8	H315, H317, H319, H411, Aquatic Chronic 2, Eye Irrit. 2, Skin Irrit. 2, Skin Sens. 1,	10-20

ID	C	Product type	Substance	Type	CAS no.	EINECS no.	Classification cf. SDS	Conc.(%)
2	A	Paint & lacquer	Reaction product: bisphenol-A-diglycidylether, homologues with molecular weight ≤ 700	N	25068-38-6	500-033-5	H315, H317, H319, H411, Aquatic Chronic 2, Eye Irrit. 2, Skin Irrit. 2, Skin Sens. 1,	30-60
2	B	Paint & lacquer	Epoxy resin/polyamine adduct	N	238080-05-2	607-272-5	H302, H318, Acute tox. 4, Eye Dam. 1,	10-20
3	A	Paint & lacquer	Alcohols, C8-22, ethoxylated	(VOC)	69013-19-0	-	H302, H315, H318, H400, Acute tox. 4, Aquatic Acute 1, Eye Dam. 1, Skin Irrit. 2,	<0.5
3	B	Paint & lacquer	Poly(oxy(methyl-1,2-ethanediyl)), *alpha*-(2-aminomethylethyl)-*omega*-(2-aminomethylethoxy)-	N	9046-10-0	-	H314, H318, H411, Aquatic Chronic 2, Eye Dam. 1, Skin Corr. 1C,	<10
3	A	Paint & lacquer	Bronopol (INN)	(VOC)	52-51-7	200-143-0	H302, H312, H315, H318, H335, H400, Acute tox. 4, Aquatic Acute 1, Eye Dam. 1, Skin Irrit. 2, STOT SE 3,	0.025
3	B	Paint & lacquer	Benzylalcohol	VOC	100-51-6	202-859-9	H302, H332, Acute tox. 4,	1-5
3	A	Paint & lacquer	1,2-ethandiol	VOC	107-21-1	203-473-3	H302, Acute tox. 4,	1
3	B	Paint & lacquer	1-methoxy-2-propanol	VOC	107-98-2	203-539-1	H226, H336, Flam. Liq. 3, STOT SE 3,	<5
3	A	Paint & lacquer	5-chloro-2-methyl-2H-isothiazol-3-on [EU no. 247-500-7], mixture (3:1) with 2-methyl-2H-isothiazol-3-one [EU no. 220-239-6]	VOC	55965-84-9	247-500-7	H301+H311, H314, H317, H331, H400, H410, Acute tox. 3, Aquatic Acute 1, Aquatic Chronic 1, Skin Corr. 1B, Skin Sens. 1,	0.0005
3	A	Paint & lacquer	oxiran, mono[(C12-14-alkyloxy)methyl]derivate; (C12C14) alkylglycidylether	N	68609-97-2	271-846-8	H315, H317, Skin Irrit. 2, Skin Sens. 1,	5-10
3	A	Paint & lacquer	Bisphenol F reaction product	N	9003-36-5	500-006-8	H315, H317, H319, H411, Aquatic Chronic 2, Eye Irrit. 2, Skin Irrit. 2, Skin Sens. 1,	10-20
3	A	Paint & lacquer	bisphenol-A-diglycidylether, homologues with molecular weight ≤ 700	N	25068-38-6	500-033-5	H315, H317, H319, H411, Aquatic Chronic 2, Eye Irrit. 2, Skin Irrit. 2, Skin Sens. 1,	30-60
4		Paint & lacquer	2-butanone oxime	VOC	96-29-7	202-496-6	Carc. 3, Xn, R21,R40,R41,R43,H312, H317, H318, H351, Acute tox. 4, Carc. 2, Eye Dam. 1, Skin Sens. 1,	0.1-1
4		Paint & lacquer	ALKANES, C11-C15-ISO-	VOC	-	927-285-2	Xn, R65,R66, H304, Asp. tox. 1,	10-30
5	B	Paint & lacquer	Polyoxypropylenediamine (Reaction products of di-, tri-, and tetra-propoxylated Propane-1,2-diol with ammonia)	N	-	-	Skin Corr. 1C, Eye Dam. 1, Acquat. Chronic 3, H314, H318, H412	>1

ID	C	Product type	Substance	Type	CAS no.	EINECS no.	Classification cf. SDS	Conc.(%)
5	B	Paint & lacquer	propan-2-ol	VOC	67-63-0	200-661-7	H225, H319, H336	1-3
5	A	Paint & lacquer	1-methoxypropan-2-ol	VOC	107-98-2	203-539-1	H226, H336	5-10
5	B	Paint & lacquer	2,2'-iminodi(ethylamine)	(VOC)	111-40-0	203-865-4	Acute Tox. 1, Acute Tox. 4, STOT SE 3, Skin Corr. 1B, Eye Dam. 1, Skin Sens. 1, H302, H312, H314, H317, H318, H330, H335	<1
5	B	Paint & lacquer	2-butoxyethanol	VOC	111-76-2	203-905-0	H302, H312, H315, H319, H332	1-3
5	B	Paint & lacquer	adduct of aliphatic polyamine (Bis A epoxy adduct with 3-aminomethyl-3,5,5-trimethyl-cyclohexylamine)	N	2855-13-2	220-666-8	H302, H312, H314, H317, H412	5-10
5	A	Paint & lacquer	Bisphenol-A- (epichlorhydrin); epoxy resin	N	25068-38-6	500-033-5	H315, H317, H319	60-80
5	A	Paint & lacquer	Water	NN	7732-18-5			25-40
5	B	Paint & lacquer	Water	NN	7732-18-5			25-40
6		Sealant	Propane	VVOC	74-98-6	200-827-9	Flam. Gas 1, Press.Gas; H220, H280	<15
6		Sealant	Isobutane	VVOC	75-28-5	200-857-2	Flam. Gas 1, Press.Gas; H220, H280	<15
6		Sealant	Butane	VVOC	106-97-8	203-448-7	Flam. Gas 1, Press.Gas; H220, H280	<15
6		Sealant	Dimethylether	VVOC	115-10-6	204-065-8	Flam. Gas 1, Press.Gas; H220, H280	<10
6		Sealant	Tris(2-chloro-1-methylethyl)phosphate	VOC	13674-84-5	237-158-7	Acute Tox. 4; H302	<25
6		Sealant	Alkanes, C14-17-, chloro	N	85535-85-9	287-477-0	Lact., Aquatic Acute 1, Aquatic Chronic 1; H362, H400, H410, EUH066	<20
6		Sealant	Diphenylmethandiisocyanat, isomers and homologues	(VOC)	9016-87-9		Acute Tox. 4, Skin Irrit. 2, Eye Irrit. 2, Resp. Sens. 1, Skin Sens. 1, Carc. 2, STOT SE 3, STOT RE 2; H332, H373, H315, H319, H334, H317, H351, H335	30-60
7		Sealant	Propane	VVOC	74-98-6	200-827-9	Flam. Gas 1, Press.Gas; H220, H280	<15
7		Sealant	Isobutane	VVOC	75-28-5	200-857-2	Flam. Gas 1, Press.Gas; H220, H280	<15
7		Sealant	Butane	VVOC	106-97-8	203-448-7	Flam. Gas 1, Press.Gas; H220, H280	<15
7		Sealant	Dimethylether	VVOC	115-10-6	204-065-8	Flam. Gas 1, Press.Gas; H220, H280	<10

ID	C	Product type	Substance	Type	CAS no.	EINECS no.	Classification cf. SDS	Conc.(%)
7		Sealant	Tris(2-chloro-1-methylethyl) phosphate	VOC	13674-84-5	237-158-7	Acute Tox. 4; H302	<25
7		Sealant	Alkanes, C14-17-, chloro	N	85535-85-9	287-477-0	Lact., Aquatic Acute 1, Aquatic Chronic 1; H362, H400, H410, EUH066 – M=1	<20
7		Sealant	Diphenylmethandiisocyanate, isomers and homologues	(VOC)	9016-87-9		Acute Tox. 4, Skin Irrit. 2, Eye Irrit. 2, Resp. Sens. 1, Skin Sens. 1, Carc. 2, STOT SE 3, STOT RE 2; H332, H373, H315, H319, H334, H317, H351, H335	30-60
8		Sealant	Propane	VVOC	74-98-6	200-827-9	Flam. Gas 1, Press.Gas; H220, H280	<15
8		Sealant	Isobutane	VVOC	75-28-5	200-857-2	Flam. Gas 1, Press.Gas; H220, H280	<15
8		Sealant	Butane	VVOC	106-97-8	203-448-7	Flam. Gas 1, Press.Gas; H220, H280	<15
8		Sealant	Dimethylether	VVOC	115-10-6	204-065-8	Flam. Gas 1, Press.Gas; H220, H280	<8
8		Sealant	Alkanes, C14-17-, chloro	N	85535-85-9	287-477-0	Lact.;H362, Aquatic Acute 1;H400, Aquatic Chronic 1;H410 – M = 100, EUH066	<20
8		Sealant	Diphenylmethandiisocyanate, isomers and homologues	(VOC)	9016-87-9		Skin Irrit. 2;H315, Skin Sens. 1;H317, Eye Irrit. 2;H319, Acute Tox. 4;H332, Resp. Sens. 1;H334, STOT SE 3;H335, Carc. 2;H351, Lact. ;H362, STOT RE 2;H373	38-55
9		Sealant	Liquid Propane	VVOC	74-98-6	200-827-9	Comp. Gas, Flam. Gas 1 H220, H280	<15
9		Sealant	Isobutane	VVOC	75-28-5	200-857-2	Comp. Gas, Flam. Gas 1 H220, H280	<15
9		Sealant	Butane	VVOC	106-97-8	203-448-7	Comp. Gas, Flam. Gas 1 H220, H280	<15
9		Sealant	Dimethylether	VVOC	115-10-6	204-065-8	Comp. Gas, Flam. Gas 1 H220, H280	<10
9		Sealant	Alkanes, C14-17-, chloro	N	85535-85-9	287-477-0	Lact., Aquatic Chronic 1 H362, H410, EUH066 (M-chronic = 1)	<25
9		Sealant	Diphenylmethane Diisocyanate, isomers and homologues	(VOC)	9016-87-9	618-498-9	Acute Tox. 4, STOT RE 2, STOT SE 3, Skin Irrit. 2, Eye Irrit. 2, Resp. Sens. 1, Skin Sens. 1, Carc. 2 H315, H317, H319, H332, H334, H335, H351, H373	30-60
10		Paint & lacquer	2-butanone oxime	VOC	96-29-7	202-496-6	Acute Tox. 4 H312, Eye Dam. 1 H318, Skin Sens. 1 H317, Carc. 2 H351	≤0.30
10		Paint & lacquer	calciumbis(2-ethylhexanoate)	N	136-51-6	205-249-0	Eye Dam. 1 H318, Repr. 2 H361fd	<1
10		Paint & lacquer	cobaltbis(2-ethylhexanoate)	N	136-52-7	205-250-6	Eye Irrit. 2 H319, Skin Sens. 1 H317, Repr. 2, H361f	≤0.30
10		Paint & lacquer	2-ethylhexanoic acid, zirconiumsalt	N	22464-99-9	245-018-1	Repr. 2, H361fd	≤1

ID	C	Product type	Substance	Type	CAS no.	EINECS no.	Classification cf. SDS	Conc.(%)
10		Paint & lacquer	Naphtha (petroleum), hydrotreated heavy <0,1% benzene	VOC	64742-48-9	265-150-3	Flam. Liq. 3 H226, Asp. Tox. 1 H304	≥25 - ≤50
11		Paint & lacquer	2-butanone oxime	VOC	96-29-7	202-496-6	H312, H317, H318, H351, Acute tox. 4, Carc. 2, Eye Dam. 1, Skin Sens. 1,	<1
11		Paint & lacquer	Calcium bis(2-ethylhexanoate)	N	136-51-6	205-249-0	H318, H361d, Eye Dam. 1, Repr. 2,	<1
11		Paint & lacquer	N-Formylmorpholine	VOC	4394-85-8	224-518-3	H317, Skin Sens. 1,	0,5
11		Paint & lacquer	Trizinkbis(orthophosphate)	N	7779-90-0	231-944-3	H400, H410, Aquatic Acute 1, Aquatic Chronic 1,	<1
11		Paint & lacquer	5-chloro-2-methyl-2H-isothiazol-3-on [EU no. 247-500-7], mixture (3:1) with 2-methyl-2H-isothiazol-3-one [EU no. 220-239-6]	VOC	55965-84-9	247-500-7	H301+H311, H314, H317, H331, H400, H410, Acute tox. 3, Aquatic Acute 1, Aquatic Chronic 1, Skin Corr. 1B, Skin Sens. 1,	<0.001; 5
11		Paint & lacquer	Hydrocarbons, C9-C11, n-alkanes, isoalkanes, cyclics, <2% aromatics	VOC	-	919-857-5	H226, H304, H336, Asp. tox. 1, Flam. Liq. 3, STOT SE 3,	30-60
12		Paint & lacquer	2-butanone oxime	VOC	96-29-7	202-496-6	H312, H317, H318, H351, Acute tox. 4, Carc. 2, Eye Dam. 1, Skin Sens. 1,	<1
12		Paint & lacquer	Naphtha (petroleum), hydrotreated heavy <0,1% benzene	VOC	64742-48-9	265-150-3	EUH 066, H226, H304, Asp. tox. 1, Flam. Liq. 3,	30-60
14		Filler	Naphtha (petroleum), hydrotreated heavy <0,1% benzene	(VOC)	64742-48-9	265-150-3	Flam. Liq. 3, Asp. Tox. 1, STOT SE 3	2.5 - <5
14		Filler	Titandioxide	N	13463-67-7	236-675-5		1 - <2.5
14		Filler	2-ethylhexanoic acid, zirconiumsalt	N	22464-99-9	245-018-1	Acute Tox. 4, Repr. 2	0.25 - <1
14		Filler	Hydrocarbons, C10-C13, n-alkanes, isoalkanes, cyclics, aromatics (2-25%)	VOC	64742-82-1	919-164-8	STOT RE 1, Asp. Tox. 1, Aquatic Chronic 3	5 - <10
14		Filler	Hydrocarbons, C9-C11, n-alkanes, isoalkanes, cyclics, <2% aromatics	VOC	64742-48-9	919-857-5	Flam. Liq. 3, STOT SE 3, Asp. Tox. 1	2.5 - <5
20		Paint & lacquer	SDS missing					
26		Filler	Styrene	VOC	100-42-5	202-851-5	Flam. Liq. 3 H226 Acute Tox. 4 H332 Asp. Tox. 1 H304 Eye Irrit. 2 H319 Skin Irrit. 2 H315 STOT RE 1; Inhalation H372 Repr. 2 H361d Aquatic Chronic 3 H412 STOT SE 3 H335	10-20

ID	C	Product type	Substance	Type	CAS no.	EINECS no.	Classification cf. SDS	Conc.(%)
29		Paint & lacquer	1-butoxypropan-2-ol	VOC	5131-66-8	225-878-4	Flam. Liq. 3, Skin Irrit. 2, Eye Irrit. 2, H226, H315, H319	1-3
29		Paint & lacquer	(2-methoxymethylethoxy)propanol	VOC	34590-94-8	252-104-2	N/A	1-3
29		Paint & lacquer	Pentanoic acid, 5-(dimethylamino)-2-methyl-5-oxo, methyl ester	(VOC)	1174627-68-9	N/A	Eye Irrit. 2 H319	<1
30		Paint & lacquer	SDS missing					
32		Paint & lacquer	2-Butanone oxime	VOC	96-29-7	202-496-6	Acute Tox. 4, H312, Eye Dam. 1, H318, Skin Sens. 1, H317, Carc. 2, H351	≥0.3 - <1
32		Paint & lacquer	1-Methoxy-2-propanol	VOC	107-98-2	203-539-1	Flam. Liq. 3, H226, STOT SE 3, H336	≥1 - <3
32		Paint & lacquer	cobaltbis(2-ethylhexanoate)	N	136-52-7	205-250-6	Eye Irrit. 2, H319, Skin Sens. 1, H317, Repr. 2, H361fd, (Suspected of damaging fertility or the unborn child), Aquatic Acute 1, H400, Aquatic Chronic 3, H412	≥0.1 - <0.3
32		Paint & lacquer	Xylene	VOC	1330-20-7	215-535-7	Flam. Liq. 3, H226, Acute Tox. 4, H312, Acute Tox. 4, H332, Skin Irrit. 2, H315, Eye Irrit. 2, H319, STOT SE 3, H335, STOT RE 2, H373 (oral), Asp. Tox. 1, H304	≥3 - <5
32		Paint & lacquer	2-ethylhexanoic acid, zirconiumsalt	N	22464-99-9	245-018-1	Repr. 2, H361fd, (Suspected of damaging fertility or the unborn child)	≥0.1 - <0.3
32		Paint & lacquer	Naphtha (petroleum), hydrotreated heavy <0,1% benzene	VOC	64742-48-9	265-150-3	Flam. Liq. 3, H226, STOT SE 3, H336, Asp. Tox. 1, H304, EUH066	≥25 - <50
32		Paint & lacquer	Fatty acids, tall-oil, compds. with oleylamine	N	N/A	288-315-1	Eye Dam. 1, H318, Skin Sens. 1A, H317	<0.1
36		Filler	SDS: Dangerous substances: not relevant					
45	B	Filler	BENZOYLPEROXIDE	N	94-36-0	202-327-6	Org. Perox. B H241 Eye Irrit. 2 H319 Skin Sens. 1 H317 Aquatic Acute 1 H400 Aquatic Chronic 1 H410 M-factor (Very toxic to aquatic life with long lasting effects): 10 Mfactor (chronic toxicity to aquatic life) 10	45-52
45	A	Filler	Styrene	VOC	100-42-5	202-851-5	Flam. Liq. 3 H226 Acute Tox. 4 H332 Asp. Tox. 1 H304 Eye Irrit. 2 H319 Skin Irrit. 2 H315 STOT RE 1; Inhalation H372 Repr. 2 H361d Aquatic Chronic 3 H412 STOT SE 3 H335	1.5 - <20
56		Membrane	SDS: Does not contain any substances eligible for CLP hazard labelling.					
60		Adhesive	1,2-benzisothiazol-3(2H)-one	N	2634-33-5	220-120-9	Acute Tox. 4, Skin Irrit. 2, Eye Dam. 1, Skin Sens. 1, Aquatic Acute 1, H302, H315, H317, H318, H400	<0.05%

ID	C	Product type	Substance	Type	CAS no.	EINECS no.	Classification cf. SDS	Conc.(%)
60		Adhesive	5-chloro-2-methyl-2H-isothiazol-3-one, mixture (3:1) with 2-methyl-2H-isothiazol-3-one	VOC	55965-84-9	N/A	Acute Tox. 3, Skin. Corr. 1B, Skin Sens. 1, Aquatic Acute 1, Aquatic Chronic 1, H301, H311, H314, H317, H331, H400, H410 (M-acute = 10)	<0.0015
65		Adhesive	1,2-benzisothiazol-3(2H)-one	N	2634-33-5	220-120-9	Acute Tox. 4, Skin Irrit. 2, Skin Sens. 1, Eye Dam. 1, Acute Tox. 2, Aquatic Acute 1, Aquatic Chronic 2, H302, H315, H317, H318, H330, H400, H411 (M-acute = 1)	<0.01
65		Adhesive	5-chloro-2-methyl-4-isothiazolin-3-one [EC no.247- 500-7] / 2-Methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1)	VOC	55965-84-9	N/A	Acute Tox. 3, Acute Tox. 3, Skin Corr. 1B, Skin Sens. 1, Eye Dam. 1, Acute Tox. 3, Aquatic Acute 1, Aquatic Chronic 1, H301, H311, H314, H317, H318, H331, H400, H410 (M-acute = 100) (M-chronic = 10)	
69		Floor wax, polish & care product	Paraffin wax	N	8002-74-2	232-315-6	-	5-10
69		Floor wax, polish & care product	Isododecane	VOC	31807-55-3	250-816-8	Flam.Liq 3, Asp. Tox. 1; H226, H304	50-100
69		Floor wax, polish & care product	Distillates (petroleum), hydrotreated light	VOC	64742-47-8	265-149-8	Asp. Tox. 1; H304	10-25
70		Sealant	Propane	VVOC	74-98-6	200-827-9	F+, R12, H220, H280, Flam. Gas 1, Press. Gas (*),	<15
70		Sealant	Isobutane	VVOC	75-28-5	200-857-2	F+, R12, H220, H280, Flam. Gas 1, Press. Gas (*),	<15
70		Sealant	Butane (content ≥ 0.1 % butadiene (203-450-8))	VVOC	106-97-8	203-448-7	F+, R12, H220, H280, Flam. Gas 1, Press. Gas (*),	<15
70		Sealant	Dimethylether	VVOC	115-10-6	204-065-8	F+, R12, H220, H280, Flam. Gas 1, Press. Gas (*),	<10
70		Sealant	Tris(2-chloro-1-methylethyl) phosphate	VOC	13674-84-5	237-158-7	Xn, R22, H302, Acute tox. 4,	<25
70		Sealant	Chlorinated paraffines C14-17	N	85535-85-9	287-477-0	N, R50/53, R64, R66, EUH 066, H362, H400, H410, Aquatic Acute 1, Aquatic Chronic 1, Lact.,	<20
70		Sealant	Polymethylenpolyphenylisocyanate	(VOC)	9016-87-9	618-498-9	Cancer 3, Xn, R20, R36/37/38, R40, R42/43, R48/20, EUH 204, H315, H317, H319, H332, H334, H335, H351, H373, Acute tox. 4, Carc. 2, Eye Irrit. 2, Resp. Sens. 1, Skin Irrit. 2, Skin Sens. 1, STOT RE 2, STOT SE 3,	30-60
71		Filler	SDS: Does not contain any substances eligible for CLP hazard labelling. Does not contain substances harmful to the environment.					

ID	C	Product type	Substance	Type	CAS no.	EINECS no.	Classification cf. SDS	Conc.(%)
72		Floor wax, polish & care product	SDS missing					
76		Floor wax, polish & care product	SDS missing					
80		Floor wax, polish & care product	Ammonia		1336-21-6	215-647-6	Skin Corr. 1B; H314, Aquatic Acute 1; H400	< 1
83		Floor wax, polish & care product	SDS missing					
85		Floor wax, polish & care product	Linseed oil	(VOC)	8001-26-1	232-278-6		100
88		Sealant	Ethylendimethacrylate	VOC	97-90-5	202-617-2	STOT SE 3: H335 – Skin Sens. 1: H317	10 - <15
88		Sealant	Methacrylic acid, monoester with propan-1,2-diol	(VOC)	27813-02-1	248-666-3	Eye Irrit. 2: H319 – Skin Sens. 1: H317	1 - <10
88		Sealant	Glycerine	(VOC)	56-81-5	200-289		1 - <20
88		Sealant	Dibenzoylperoxide	N	94-36-0	202-327-6	Org. Perox. B: H241 – Eye Irrit. 2: H319 – Skin Sens. 1: H317	1-<2.5
88		Sealant	1,1'-(p-tolylimino)dipropan-2-ol	(VOC)	38668-48-3	254-075-1	Acute Tox. 2: H300 – Eye Dam. 1: H318 – Aquatic Chronic 3: H412	0.1 - <1

Explanation:

C: Component A or B for 2 component products

VVOC: Very volatile organic compound with low boiling point < 60°C, may require specialized analysis

VOC: Volatile organic compound with low boiling point > 60°C, measured by ISO 16000-6/EN 16516

(VOC): Instable, decomposes in air/by GC-analysis (ISO 16000-6/EN 16516), may possibly be measured specialized analysis

N: Not a volatile compound or VOC, degradation- or reaction products may be monitored as VOC

NN: Inorganic volatile substance

Appendix 5. Analysis methods

Appendix 5.1 Calibrated range for quantification of 67 volatile organic compounds (VOC) by Tenax analysis standard method ISO 16000-6

CAS no	Substance	Tenax (ng)	Tenax (ng)	Calibrated 0.05 L air (µg/m ³)		Calibrated 5 L air (µg/m ³)	
		Min.	Max.	Min.	Max.	Min.	Max.
000064-17-5	Ethanol	10	1000	200	20000	2	200
000067-64-1	Acetone	5	1000	100	20000	1	200
000067-63-0	2-Propanol	5	1000	100	20000	1	200
000071-23-8	1-Propanol	5	1000	100	20000	1	200
000078-93-3	2-Butanone	5	1000	100	20000	1	200
000109-97-7	Ethanamine, N-ethyl-	50	400	1000	8000	10	80
000110-54-3	Hexane	5	1000	100	20000	1	200
000064-19-7	Acetic acid	96	398	1920	7960	19	80
000078-83-1	Isobutyl Alcohol	5	194	100	3880	1	39
000071-43-2	Benzene	5	500	100	10000	1	100
000071-36-3	1-Butanol	5	1000	100	20000	1	200
000107-98-2	2-Propanol, 1-methoxy-	5	194	100	3880	1	39
000127-06-0	Acetone oxime	5	400	100	8000	1	80
000121-44-8	Triethylamine	5	400	100	8000	1	80
000142-82-5	Heptane	5	1000	100	20000	1	200
000110-62-3	Pentanal	5	333	100	6664	1	67
000123-91-1	1,4-Dioxane	5	333	100	6666	1	67
000079-09-4	Propanoic acid	108	733	2160	14660	22	147
000108-10-1	2-pentanone-4-methyl	5	300	100	6000	1	60
000057-55-6	1,2-Propandiol	50	300	1000	6000	10	60
000108-88-3	Toluene (TIC)	5	300	100	6000	1	60
000108-88-3	Toluene	5	500	100	10000	1	100
000079-31-2	Propanoic acid, 2-methyl-	15	870	300	17400	3	174
000107-92-6	Butanoic acid	147	871	2940	17420	29	174
000111-65-9	Octane	5	1000	100	20000	1	200
000066-25-1	Hexanal	5	333	100	6666	1	67
000123-86-4	Butylacetat	5	300	100	6000	1	60
000096-29-7	2-Butanone, oxime	5	400	100	8000	1	80
000100-41-4	Ethylbenzene	5	400	100	8000	1	80
179601-23-1	P+M-xylene	5	1000	100	20000	1	200
000109-52-4	Pentanoic acid	86	1010	1720	20200	17	202
000100-42-5	Styrene	5	500	100	10000	1	100
000095-47-6	O-Xylene	5	1000	100	20000	1	200

CAS no	Substance	Tenax (ng)	Tenax (ng)	Calibrated 0.05 L air (µg/m ³)		Calibrated 5 L air (µg/m ³)	
		Min.	Max.	Min.	Max.	Min.	Max.
000108-94-1	Cyclohexanone	5	400	100	8000	1	80
000111-84-2	Nonane	5	1000	100	20000	1	200
000111-76-2	Ethanol, 2-butoxy-	5	194	100	3880	1	39
007785-26-4	S-.alpha.-Pinene	5	1000	100	20000	1	200
005131-66-8	2-Propanol, 1-butoxy-	5	194	100	3880	1	39
000620-14-4	3-ethyltoluene	5	1000	100	20000	1	200
000108-67-8	Benzene, 1,3,5-trimethyl-	5	400	100	8000	1	80
000108-95-2	Phenol	5	300	100	6000	1	60
000142-62-1	Hexanoic acid	49	1149	980	22980	10	230
000611-14-3	2-ethyltoluene	5	400	100	8000	1	80
018172-67-3	b-pinene	5	750	100	15000	1	150
034590-94-8	top a Di(propylene glycol) methyl ether,	5	194	100	3880	1	39
000095-63-6	1,2,4-trimethyl-benzene	5	400	100	8000	1	80
000124-18-5	Decane	5	400	100	8000	1	80
000526-73-8	Benzene, 1,2,3-trimethyl-	5	400	100	8000	1	80
005989-27-5	Limonene	5	1000	100	20000	1	200
000100-51-6	Benzyl alcohol	5	194	100	3880	1	39
000111-14-8	Heptanoic acid	55	1300	1100	26000	11	260
000096-09-3	Styrene oxide	5	333	100	6667	1	67
001120-21-4	Undecane	5	1000	100	20000	1	200
000124-19-6	Nonanal	5	1000	100	20000	1	200
002687-91-4	1-Ethyl-2-pyrrolidinone	5	450	100	9000	1	90
000149-57-5	2-Ethylhexanoic acid	17	664	340	13280	3	133
002682-20-4	2-Methyl-isothiazolin-3-one	13	400	260	8000	3	80
000112-34-5	Butyldiglycol	10	333	200	6667	2	67
000112-40-3	Dodecane	5	750	100	15000	1	150
000112-31-2	Decanal	5	1000	100	20000	1	200
000103-11-7	2-Ethylhexyl-acrylate	5	300	100	6000	1	60
026172-55-4	5-Chloro-2-methyl-3(2H)-isothiazolone	5	450	100	9000	1	90
000629-50-5	Tridecane	5	750	100	15000	1	150
000629-59-4	Tetradecane	5	750	100	15000	1	150
000629-62-9	Pentadecane	5	500	100	10000	1	100
002425-79-8	Butandiol diglycidylether	100	333	2000	6667	20	67
000544-76-3	Hexadecane	5	400	100	8000	1	80

Appendix 5.2 Detection limits (LOD) of C1-C4 aldehydes (VVOC) by DNPH-analysis and standard method ISO 16000-3

CAS no	Substance	LOD (µg)	LOD 30 L air sample (µg/m ³)
50-00-0	Formaldehyde	0.03	1.0
75-07-0	Acetaldehyde	0.03	1.0
123-38-6	Propanal	0.05	1.7
123-72-8	Butanal	0.03	1.0
107-02-8	Acrolein	0.03	1.0

Appendix 6. Results

Appendix 6.1 ID 2 – Floor paint 2-component (Paint & lacquer)

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0	10	24	4.9
Acetaldehyde	75-07-0	3.6	1.8	6.6
Propanal	123-38-6	15		
Acetone	000067-64-1	8		7
1-Butanol	000071-36-3	17	<5	
1-Methoxypropan-2-ol	000107-98-2	160		
Triethylamine	000121-44-8	44		
1,2-Ethanediol #	000107-21-1	2300	45	
Butanoic acid	000107-92-6			21
Hexanal	000066-25-1			<5
Oxiranes (Dodecyl and tetradecyl glycidyl ethers) #	068609-97-2	200	290	<5
Pentanoic acid	000109-52-4			20
2-Butoxy ethanol	000111-76-2	6300	7	
alpha-Pinene	007785-26-4		<5	
Hexanoic acid	000142-62-1			13
Benzaldehyde #	000100-52-7			<5
Di(propylene glycol) methyl ether, mixture of isomers (A)	034590-94-8	5600		
1-Hexanol, 2-ethyl- #	000104-76-7			<5
Benzyl alcohol	000100-51-6	220	29	
Heptanoic acid	000111-14-8			17
Acetophenone #	000098-86-2			<5
2-Ethylhexanoic acid	000149-57-5			6
Nonanal	000124-19-6	5	<5	
Decanal	000112-31-2	7	6	<5
5-Chloro-2-methyl-1,2-thiazol-3(2H)-one (CMIT)	26172-55-4	17	16	
Tetradecane	000629-59-4	41	<5	
Unknown alcohol? #			290	
Pentadecane	000629-62-9	<5		
Hexadecane	000544-76-3	12		
Oxirane, [(dodecyloxy)methyl]- #	002461-18-9			51
Naphtha C7-C13 #	64742-82-1/64742-48-9	420	6	<5
Sum of unidentified VVOC	Rt < C6	6		
Sum of unidentified VOC	Rt C6-C16	1400	38	46
Sum of unidentified SVOC	Rt >C16	12	37	15
Sum of all measured VVOC*	Rt < C6	14	<5	7

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Sum of all measured VOC*	Rt C6-C16	16743	727	123
Sum of all measured SVOC*	Rt >C16	12	37	66
TVOC (Toluene equivalents)		7000	640	28
TSVOC (Toluene equivalents)		200	300	59

Appendix 6.2 ID 4 Paint 1-component (Paint & lacquer)

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0		22	7.8
Acetaldehyde	75-07-0		230	18
Propanal	123-38-6		131	4.6
Butanal	123-72-8		103	7.2
Acrolein	107-02-8		39	
Ethanol	000064-17-5	50	31	
Acetone	000067-64-1	78	74	
2-Butanone	000078-93-3	1400	44	
Acetic acid	000064-19-7		230	38
1-Butanol	000071-36-3		34	
1-Methoxypropan-2-ol	000107-98-2	770		
Pentanal	000110-62-3		960	32
Propanoic acid	000079-09-4		200	37
1-Pentanol #	000071-41-0		290	
Butanoic acid	000107-92-6		97	31
Hexanal	000066-25-1			230
Cyclotrisiloxane, hexamethyl- #	000541-05-9	<25	47	8
2-Butanone, oxime	000096-29-7	≥220000		1
1-Hexanol #	000111-27-3		64	
Pentanoic acid	000109-52-4		560	100
Heptanal #	000111-71-7		180	17
Oxirane, pentyl- #	005063-65-0		170	
Benzaldehyde #	000100-52-7		44	9
Di(propylene glycol) methyl ether, mixture of isomers (A)	034590-94-8	3700	270	
Hexanoic acid	000142-62-1		3200	600
Octanal	000124-13-0		81	52
Acetophenone #	000098-86-2	<25		
Heptanoic acid	000111-14-8		68	160
Undecane	001120-21-4			<5
Nonanal	000124-19-6	<25		78
2-Ethylhexanoic acid	000149-57-5		3200	410
Decane #	000124-18-5			7
Octanoic acid #	000124-07-2			23

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Decanal	000112-31-2	<25		18
Nonanoic acid #	000112-05-0			10
Tetradecane	000629-59-4	<25	38	
Pentadecane	000629-62-9	<25	11	
Hexadecane	000544-76-3	<25	<5	
Alkaner, iso-, < 2% aromater C7-C13	927-285-2	≥1100000	≥110000	
Sum of unidentified VVOC	Rt < C6	84	160	
Sum of unidentified VOC	Rt C6-C16	≥15000	≥9200	260
Sum of unidentified SVOC	Rt >C16			12
Sum of all measured VVOC*	Rt < C6	1612	260	
Sum of all measured VOC*	Rt C6-C16	≥1339470	≥128944	2121
Sum of all measured SVOC*	Rt >C16	<5	<5	12
TVOC (Toluene equivalents)		≥1200000	≥240000	1200
TSVOC (Toluene equivalents)		<5	<5	7

Appendix 6.3 ID 5 Floor lacquer 2-component (Paint & lacquer)

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0		2.4	N
Acetaldehyde	75-07-0	11	2.6	N
Propanal	123-38-6			N
Butanal	123-72-8	1.4		N
Acetone	000067-64-1	40		7
2-Propanol	000067-63-0	360	<5	
2-Butanone	000078-93-3	<5		
1-Butanol	000071-36-3	250	<5	
1-Methoxypropan-2-ol	000107-98-2	130		
Heptane	000142-82-5		54	
1,4-Dioxane	000123-91-1	460		
1,2-Propandiol	000057-55-6	≥9400	570	22
Ethylbenzene	000100-41-4	7		
m,p-Xylene	179601-23-1	18		
Styrene	000100-42-5	2		
o-Xylene	000095-47-6	8		
Nonane	000111-84-2	81	<5	<5
2-Butoxy ethanol #	000111-76-2	≥23000	230	12
Hexanoic acid	000142-62-1			14
Phenol	000108-95-2		1	
Propylene Carbonate #	000108-32-7	4700	360	
Decane	000124-18-5	4200	42	<5
1-Hexanol, 2-ethyl- #	000104-76-7	1200		
Benzyl alcohol	000100-51-6	67	10	<5

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Heptanoic acid	000111-14-8			18
Undecane	001120-21-4	12000	150	11
2-Ethylhexanoic acid	000149-57-5			7
Dodecane	000112-40-3	11000	120	9
5-Chloro-2-methyl-3(2H)-isothiazolone (CMIT)	026172-55-4			7
Tridecane	000629-50-5	4900	70	<5
Tetradecane	000629-59-4	48	<5	
Oxirane, [(dodecyloxy)methyl]- #	002461-18-9		86	<5
Sum of unidentified VVOC	Rt < C6	38		
Sum of unidentified VOC	Rt C6-C16	550	80	8
Sum of unidentified SVOC	Rt >C16			
Sum of all measured VVOC*	Rt < C6	438	<5	7
Sum of all measured VOC*	Rt C6-C16	≥72021	1687	108
Sum of all measured SVOC*	Rt >C16	<5	86	<5
TVOC (Toluene equivalents)		≥52000	1000	54
TSVOC (Toluene equivalents)		<5	86	<5

Appendix 6.4 ID 11 Paint 1-component (Paint & lacquer)

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0		16	6.5
Acetaldehyde	75-07-0		111	20
Propanal	123-38-6	4.7	284	9.4
Butanal	123-72-8		39	4.4
Acrolein	107-02-8		22	
Ethanol	000064-17-5	<200	36	
Acetone	000067-64-1	150	31	5
2-Butanone	000078-93-3	350	23	
Acetic acid	000064-19-7		250	21
1-Butanol	000071-36-3	<100		<5
Pentanal	000110-62-3		490	19
Propanoic acid	000079-09-4		250	34
1-Pentanol #	000071-41-0		97	
2-Butanone, oxime	000096-29-7	≥58000		
Butanoic acid	000107-92-6		55	
Octane	000111-65-9		38	
Hexanal	000066-25-1		5300	250
Butylacetat	000123-86-4		19	
Cyclotrisiloxane, hexamethyl- #	000541-05-9			<5
Oxiraner	068609-97-2	<100	<5	<5
Butylacetat	000123-86-4	570		
Pentanoic acid	000109-52-4		370	39

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Cyclohexanone	000108-94-1		24	
Benzaldehyde #	000100-52-7			<5
Phenol	000108-95-2		49	3
Hexanoic acid	000142-62-1		3400	
2-ethyltoluene	000611-14-3		<5	
beta-pinene	018172-67-3	180		
Hexanoic acid	000142-62-1			340
Di(propylene glycol) methyl ether, mixture of isomers (A)	034590-94-8	≥3900	76	
Decane	000124-18-5			<5
Octanal	000124-13-0		59	14
1-Hexanol, 2-ethyl- #	000104-76-7			<5
Heptanoic acid	000111-14-8		50	48
Undecane	001120-21-4			5
Nonanal	000124-19-6		48	29
2-Ethylhexanoic acid	000149-57-5		3500	200
Dodecane	000112-40-3			10
Decanal	000112-31-2			6
2-Ethylhexyl-acrylate	000103-11-7		11	
Cyclohexasiloxane, dodecamethyl- #	000540-97-6			<5
Tetradecane	000629-59-4	100	73	
Pentadecane	000629-62-9	<100	15	
Hexadecane	000544-76-3	<100	<5	
Naphtha C7-C13	064742/064742-48-9	≥2600000	4200	81
Sum of other aliphatic hydrocarbons VOC	Rt C6-C16		160	
Sum of unidentified VVOC	Rt < C6		100	5
Sum of unidentified VOC	Rt C6-C16	1900	2700	200
Sum of unidentified SVOC	Rt >C16		90	
Sum of all measured VVOC*	Rt < C6	500	190	10
Sum of all measured VOC*	Rt C6-C16	≥2664650	21234	1299
Sum of all measured SVOC*	Rt >C16	<5	90	<5
TVOC (Toluene equivalents)		≥2600000	12000	780
TSVOC (Toluene equivalents)		<5	76	<5

Appendix 6.5 ID 14 Filler (Fillers)

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0	1.5	2.0	N
Acetaldehyde	75-07-0	35	18	N
Propanal	123-38-6	1.9	2.6	N
Butanal	123-72-8	1.2	1.5	N

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Acetone	000067-64-1	150	12	<5
1-Methoxypropan-2-ol	000107-98-2		180	
1-Butanol	000071-36-3	170	17	<5
1-Methoxypropan-2-ol	000107-98-2	3100		
Propanoic acid	000079-09-4			20
2-pentanone-4-methyl	000108-10-1	<100		
1,2-Propandiol	000057-55-6	2700	680	51
1-Pentanol #	000071-41-0			9
Butanoic acid	000107-92-6			22
Hexanal	000066-25-1		53	18
Oxiraner	068609-97-2	<100	<5	<5
1-Pentanol #	000071-41-0			<5
Pentanoic acid	000109-52-4			22
3-Heptanone #	000106-35-4			<5
Heptanal #	000111-71-7			<5
Benzaldehyde #	000100-52-7			7
Hexanoic acid	000142-62-1			33
Phenol	000108-95-2	160	15	3
Di(propylene glycol) methyl ether, mixture of isomers (A)	034590-94-8	510	56	<5
Octanal #	000124-13-0			<5
Heptanoic acid	000111-14-8			28
2-Ethylhexanoic acid	000149-57-5		110	18
Decanal	000112-31-2			<5
Cyclohexasiloxane, dodecamethyl- #	000540-97-6			<5
Tetradecane	000629-59-4	<100	<5	
1,1'-Biphenyl #	000092-52-4	<100	<5	
Pentadecane	000629-62-9		<5	
Hexadecane	000544-76-3	<100		
Naphtha C7-C13	64742-82-1/64742-48-9	≥340000	12000	220
Sum of unidentified VVOC	Rt < C6		7	
Sum of unidentified VOC	Rt C6-C16	30000	1700	7
Sum of unidentified SVOC	Rt >C16			
Sum of all measured VVOC*	Rt < C6	150	19	<5
Sum of all measured VOC*	Rt C6-C16	≥376640	14811	458
Sum of all measured SVOC*	Rt >C16	<5	<5	<5
TVOC (Toluene equivalents)		≥370000	13000	260
TSVOC (Toluene equivalents)		<5	<5	<5

Appendix 6.6 ID 20 Epoxy-based floor paint 2-component (Paint & lacquer)

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0		1.1	N
Acetaldehyde	75-07-0	22	1.6	N
Propanal	123-38-6	84		N
Butanal	123-72-8	75		N
Ethanol	000064-17-5	230		
Acetone	000067-64-1	150	10	
2-Butanone #	000078-93-3	5		
1-Butanol	000071-36-3	130	<5	
1-Methoxypropan-2-ol	000107-98-2	≥7100		
Acetone oxime	000127-06-0	170		
1,4-Dioxane	000123-91-1	7		
2-pentanone-4-methyl	000108-10-1	7		
1,2-Propandiol	000057-55-6	75		
Toluene	000108-88-3	4		
Cyclohexanone	000108-94-1	<5		
2-Butoxy ethanol	000111-76-2	12		
Benzaldehyde #	000100-52-7			
Di(propylene glycol) methyl ether, mixture of isomers (A)	034590-94-8			
Benzyl alcohol	000100-51-6	610	18	
Nonanal	000124-19-6	<5		<5
Decanal	000112-31-2	6	<5	
Tetradecane	000629-59-4	110	<5	
Pentadecane	000629-62-9	<5		
Hexadecane	000544-76-3	16	<5	
Oxirane, [(dodecyloxy)methyl]-	002461-18-9	33	59	
Naphtha C7-C13 (3) #	64742-82-1/64742-48-9	1100	<5	<5
Sum of unidentified VVOC	Rt < C6	38		5
Sum of unidentified VOC	Rt C6-C16	720	45	
Sum of unidentified SVOC	Rt >C16	39	18	
Sum of all measured VVOC*	Rt < C6	423	10	5
Sum of all measured VOC*	Rt C6-C16	≥10100	122	<5
Sum of all measured SVOC*	Rt >C16	39	18	<5
TVOC (Toluene equivalents)		≥6200	34	<5
TSVOC (Toluene equivalents)		72	76	<5

Appendix 6.7 ID 26 Chemical wood (Filler)

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0	4.2	1.1	N

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Acetaldehyde	75-07-0	4.9	1.5	N
Acetone	000067-64-1		6	12
2-Propanol	000067-63-0		<5	
1-Methoxypropan-2-ol	000107-98-2	44		
Hexanal	000066-25-1			<5
Styrene	000100-42-5	380	6	
O-xylene	000095-47-6		<5	
Heptanal #	000111-71-7		<5	
2-Butoxy ethanol	000111-76-2	32	5	
alpha-Pinene	007785-26-4	5		
3-ethyltoluene	000620-14-4	<5		
Hexanoic acid	000142-62-1			22
Phenol	000108-95-2		5	
1,2,4-trimethyl-benzene	000095-63-6		<5	
Benzyl alcohol	000100-51-6	<5	<5	
Heptanoic acid	000111-14-8			28
Styrene oxide	000096-09-3	16		
2-Ethylhexanoic acid	000149-57-5			10
Nonanal	000124-19-6		11	<5
1-Ethyl-2-pyrrolidinone	002687-91-4	12	2	
Naphtha C7-C13 #	64742-82-1/64742-48-9	32		
Sum of unidentified VVOC	Rt < C6	9	9	
Sum of unidentified VOC	Rt C6-C16	130	25	
Sum of unidentified SVOC	Rt >C16			
Sum of all measured VVOC*	Rt < C6	9	15	12
Sum of all measured VOC*	Rt C6-C16	651	54	60
Sum of all measured SVOC*	Rt >C16	<5	<5	<5
TVOC (Toluene equivalents)		830	18	<5
TSVOC (Toluene equivalents)		<5	<5	<5

Appendix 6.8 ID 29 Floor lacquer (Paint & lacquer)

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0			2.0
Acetaldehyde	75-07-0	16	2.2	1.6
Propanal	123-38-6	343	32	3.6
Butanal	123-72-8	7.6		
Acetone	000067-64-1	840		<5
1-Propanol	000071-23-8		<5	
Diethylamine	000109-97-7	2300		
2-Butanone	000078-93-3		<5	
1-Butanol	000071-36-3			<5

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Disiloxane, hexamethyl- #	000107-46-0	120		
Triethylamine	000121-44-8	≥19000	140	9
Heptane	000142-82-5			<5
2-pentanone-4-methyl	000108-10-1	<100		
Cyclotrisiloxane, hexamethyl- #	000541-05-9	230		<5
Naphtha C7-C13	064742/064742-48-9	370	780	
Oxiraner	068609-97-2	<100	430	
Trisiloxane, octamethyl- #	000107-51-7	810		
Cyclohexanone	000108-94-1		21	
2-Butoxy ethanol	000111-76-2	140		
Propylene glycol butyl ether	005131-66-8	300		
Benzaldehyde #	000100-52-7	<100		<5
Cyclotetrasiloxane, octamethyl- #	000556-67-2	130		
Phenol	000108-95-2		12	2
Di(propylene glycol) methyl ether, mixture of isomers (A)	034590-94-8	≥190000	100	<5
1-Propanol, 2-(2-methoxy-1-methylethoxy)- #	055956-21-3	1700		
1-Propanol, 2-(2-methoxypropoxy)- #	013588-28-8	2800		
Tetrasiloxane, decamethyl- #	000141-62-8	1200		
Acetophenone #	000098-86-2			<5
Nonanal	000124-19-6	<100	6	<5
1-Ethyl-2-pyrrolidinone	002687-91-4			1
Cyclopentasiloxane, decamethyl- #	000541-02-6	150	<5	
Decanal	000112-31-2	110	<5	<5
Unknown ether (002396-61-4?) #		16000	2200	9
Unknown ether (002396-61-4?) #		18000	2400	15
Cyclohexasiloxane, dodecamethyl- #	000540-97-6	<100	<5	
Tetradecane	000629-59-4	<100		
Pentadecane	000629-62-9	<100		
Butylated Hydroxytoluene #	000128-37-0	<100		
Hexadecane	000544-76-3	<100		
Sum of unidentified VOC	Rt C6-C16	1800	560	59
Sum of unidentified SVOC	Rt >C16	110	5	5
Sum of all measured VVOC*	Rt < C6	3140	<5	<5
Sum of all measured VOC*	Rt C6-C16	≥252860	6649	95
Sum of all measured SVOC*	Rt >C16	110	5	5
TVOC (Toluene equivalents)		≥150000	5700	74
TSVOC (Toluene equivalents)		110	5	5

Appendix 6.9 ID 30 Epoxy-based floor paint 2-component (Paint & lacquer)

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0		1.0	N
Acetaldehyde	75-07-0	13	1.4	N
Propanal	123-38-6	5.3		N
Butanal	123-72-8	3.6		N
Ethanol	000064-17-5	230		
Acetone	000067-64-1	300	6	8
2-Propanol	000067-63-0	<100		
2-Butanone	000078-93-3	200		
Hexane	000110-54-3	210		
Acetic acid	000064-19-7	4100		
Benzene (Carc.)	000071-43-2	180		
1-Butanol	000071-36-3	170	<5	
1-Methoxypropan-2-ol	000107-98-2	≥42000	49	8
Heptane	000142-82-5	810		
Propanoic acid	000079-09-4	900		
1,4-Dioxane	000123-91-1	320		
2-pentanone-4-methyl	000108-10-1	<100		
1,2-Propandiol	000057-55-6	4300		
Toluene	000108-88-3	130		
2-Butanone, oxime	000096-29-7	≥9700		
Butylacetat	000123-86-4	280		
Ethylbenzene	000100-41-4	230		
m,p-Xylene	179601-23-1	520		
Styrene	000100-42-5	<100		
O-xylene	000095-47-6	290		
Nonane	000111-84-2	<100		<5
2-Butoxy ethanol	000111-76-2	220	<5	
alpha-Pinene	007785-26-4	<100	<5	
3-ethyltoluene	000620-14-4	<100		
Benzene, 1,3,5-trimethyl-	000108-67-8	<100		
Phenol	000108-95-2	110		4
Di(propylene glycol) methyl ether, mixture of isomers (A)	034590-94-8	≥13000		
1,2,4-Trimethyl-benzene	000095-63-6	<100	<5	
Benzene, 1,2,3-trimethyl-	000526-73-8	<100		
Limonene	005989-27-5		<5	
Benzyl alcohol	000100-51-6	≥14000	200	29
Undecane #	001120-21-4	<100		
Nonanal	000124-19-6		8	<5
2-Ethylhexanoic acid	000149-57-5	100		
Butyldiglycol	000112-34-5	430	17	
Dodecane	000112-40-3	<100		

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Decanal	000112-31-2	<100		<5
Dibutyl phthalate #	000084-74-2	200		
Sum of unidentified VVOC	Rt < C6		7	
Sum of unidentified VOC	Rt C6-C16	850	94	2
Sum of unidentified SVOC	Rt >C16			
Sum of all measured VVOC*	Rt < C6	730	13	8
Sum of all measured VOC*	Rt C6-C16	≥92850	368	43
Sum of all measured SVOC*	Rt >C16	200	<5	<5
TVOC (Toluene equivalents)		≥41000	140	18
TSVOC (Toluene equivalents)		200	<5	<5

Appendix 6.10

ID 32 Floor paint 1-component (Paint & lacquer)

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0	2.6	7.1	2.9
Acetaldehyde	75-07-0	2.0	30	5.4
Propanal	123-38-6	7.4	31	2.8
Butanal	123-72-8		12	3.7
Ethanol	000064-17-5	<200	28	
Acetone	000067-64-1	140	10	<5
2-Butanone	000078-93-3	530	23	
Acetic acid	000064-19-7		36	
1-Butanol	000071-36-3	420	<5	<5
1-Methoxypropan-2-ol	000107-98-2	650		
Pentanal	000110-62-3		59	7
Propanoic acid	000079-09-4		77	
2-pentanone-4-methyl	000108-10-1	<100		
1-Pentanol	000071-41-0		16	
Butanoic acid	000107-92-6		31	22
2-Butanone, oxime	000096-29-7	≥60000		
Octane	000111-65-9			8
Hexanal	000066-25-1		600	44
Cyclotrisiloxane, hexamethyl- #	000541-05-9	170	5	<5
Butylacetat	000123-86-4	210		
Pentanoic acid	000109-52-4		120	40
2-Heptanone #	000110-43-0			<5
Cyclohexanone	000108-94-1			<5
Heptanal #	000111-71-7			<5
Benzaldehyde #	000100-52-7		20	<5
Phenol	000108-95-2		16	3
Hexanoic acid	000142-62-1		790	240

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Di(propylene glycol) methyl ether, mixture of isomers (A)	034590-94-8		16	
Octanal	000124-13-0		32	8
Heptanoic acid	000111-14-8		46	49
Acetophenone #	000098-86-2			<5
Nonanal	000124-19-6		32	11
2-Ethylhexanoic acid	000149-57-5		600	50
Hexanoic acid, 3,5,5-trimethyl- #	003302-10-1		270	
Octanoic acid #	000124-07-2			18
Decanal	000112-31-2			<5
Tridecane #	000629-50-5			<5
Nonanoic acid #	000112-05-0			6
Tetradecane	000629-59-4	100	8	<5
Pentadecane	000629-62-9	<100	<5	<5
Hexadecane	000544-76-3	<100	<5	
Naphtha C7-C13	064742/064742-48-9	≥1600000	1900	84
Sum of unidentified VVOC	Rt < C6		63	7
Sum of unidentified VOC	Rt C6-C16		150	96
Sum of unidentified SVOC	Rt >C16		43	
Sum of all measured VVOC*	Rt < C6	780	124	7
Sum of all measured VOC*	Rt C6-C16	≥1661550	4824	686
Sum of all measured SVOC*	Rt >C16	<5	43	<5
TVOC (Toluene equivalents)		≥1600000	3100	400
TSVOC (Toluene equivalents)		<5	57	<5

Appendix 6.11

ID 36 Filler (Filler)

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0	1.7	1.2	N
Acetaldehyde	75-07-0	1.6	1.2	N
Acetone	000067-64-1	13	<5	<5
2-Propanol	000067-63-0	<5		
1-Butanol	000071-36-3	<5		
1-Methoxypropan-2-ol	000107-98-2	43		
Ethylbenzene	000100-41-4	<5	<5	
Styrene	000100-42-5	4	4	
Nonane	000111-84-2	5	<5	<5
2-Butoxy ethanol	000111-76-2	8		
3-ethyltoluene	000620-14-4	<5		
Benzene, 1,3,5-trimethyl-	000108-67-8	<5		
2-ethyltoluene	000611-14-3	<5		
1,2,4-trimethyl-benzene	000095-63-6	<5		

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Benzene, 1,2,3-trimethyl-	000526-73-8	<5		
Benzyl alcohol	000100-51-6	<5		
Undecane	001120-21-4	<5		<5
Nonanal	000124-19-6	8	<5	<5
Sum of unidentified VVOC	Rt < C6		9	
Sum of unidentified VOC	Rt C6-C16		1	6
Sum of unidentified SVOC	Rt >C16			
Sum of all measured VVOC*	Rt < C6	13	9	<5
Sum of all measured VOC*	Rt C6-C16	68	5	6
Sum of all measured SVOC*	Rt >C16	<5	<5	<5
TVOC (Toluene equivalents)		16	<5	6
TSVOC (Toluene equivalents)		14	<5	<5

Appendix 6.12

ID 56 Waterproofing coating (Primer/membrane)

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0	70	5.7	N
Acetaldehyde	75-07-0	10	1.6	N
Propanal	123-38-6	-	-	N
Butanal	123-72-8	2.0	-	N
Acetone	000067-64-1		<5	
2-Propanol	000067-63-0	860		
2-Methyl-1-propanol (isobutyl alcohol)	000078-83-1	100		
1,2-Propandiol #	000057-55-6		10	
alpha-Pinene	007785-26-4	<5		
Benzaldehyde #	000100-52-7	100	<5	<5
Cyclotetrasiloxane, octamethyl- #	000556-67-2		<5	
Phenol	000108-95-2	110	11	2
Ethanone, 1-phenyl- #	000098-86-2			<5
Nonanal	000124-19-6	<5		
Cyclopentasiloxane, decamethyl- #	000541-02-6			<5
Decanal	000112-31-2		<5	<5
Cyclohexasiloxane, dodecamethyl- #	000540-97-6	<5		
Sum of unidentified VVOC	Rt < C6	7		
Sum of unidentified VOC	Rt C6-C16	390	52	
Sum of unidentified SVOC	Rt >C16			
Sum of all measured VVOC*	Rt < C6	867	<5	<5
Sum of all measured VOC*	Rt C6-C16	700	73	2
Sum of all measured SVOC*	Rt >C16	<5	<5	<5
TVOC (Toluene equivalents)		1500	230	24
TSVOC (Toluene equivalents)		<5	<5	<5

Appendix 6.13

ID 60 Adhesive for wet room (bathroom)

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0	120	1.4	N
Acetaldehyde	75-07-0	11	1.3	N
Propanal	123-38-6	3.9		N
Butanal	123-72-8	2.3		N
Acetone	000067-64-1	48		
2-Methyl-1-propanol (isobutyl alcohol)	000078-83-1	<5		
Acetic acid	000064-19-7		1000	31
1-Butanol	000071-36-3	650	<5	<5
Heptane	000142-82-5		12	<5
Propanoic acid	000079-09-4		22	
1,2-Propandiol #	000057-55-6		44	
Butanoic acid	000107-92-6		15	13
Butylacetat	000123-86-4	170		
Benzaldehyde #	000100-52-7	130		<5
Cyclotetrasiloxane, octamethyl- #	000556-67-2	<5		<5
Phenol	000108-95-2	100	5	3
Di(propylene glycol) methyl ether, mixture of isomers (A)	034590-94-8		<5	
Benzyl alcohol	000100-51-6		<5	
Acetophenone #	000098-86-2			<5
Nonanal	000124-19-6	10	<5	<5
Cyclopentasiloxane, decamethyl- #	000541-02-6	<5		
2-Methyl-1,2-thiazol-3(2H)-one (MIT)	2682-20-4		9	<5
Decanal	000112-31-2	7	<5	
5-Chloro-2-methyl-3(2H)-isothiazolone (CMIT)	026172-55-4	18	21	1
Sum of unidentified VVOC	Rt < C6	260		
Sum of unidentified VOC	Rt C6-C16	610		
Sum of unidentified SVOC	Rt >C16			
Sum of all measured VVOC*	Rt < C6	48	<5	<5
Sum of all measured VOC*	Rt C6-C16	1085	1128	47
Sum of all measured SVOC*	Rt >C16	<5	<5	<5
TVOC (Toluene equivalents)		1400	78	<5
TSVOC (Toluene equivalents)		<5	<5	<5

Appendix 6.14

ID 65 Wall adhesive

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0	15	5.0	N
Acetaldehyde	75-07-0	107	1.5	N

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Propanal	123-38-6	-	-	N
Butanal	123-72-8	1.4	-	N
Acetone	000067-64-1		<5	<5
2-Propanol	000067-63-0	<5		
Hexane	000110-54-3		6	
Acetic acid	000064-19-7		64	43
1-Butanol	000071-36-3	32	<5	<5
Propanoic acid	000079-09-4			20
Heptane	000142-82-5		10	
1,2-Propandiol #	000057-55-6		82	
Butanoic acid	000107-92-6			13
Cyclotrisiloxane, hexamethyl- #	000541-05-9		<5	
Styrene	000100-42-5			1
Cyclohexanone	000108-94-1			
Benzaldehyde #	000100-52-7	8	<5	<5
Cyclotetrasiloxane, octamethyl- #	000556-67-2			1
Phenol	000108-95-2	10	8	2
Acetophenone #	000098-86-2	10		<5
Nonanal	000124-19-6	8	5	<5
2-Methyl-isothiazolin-3-one	002682-20-4		<5	14
Decanal	000112-31-2	8		<5
5-Chloro-2-methyl-1,2-thiazol-3(2H)-one (CMIT)	26172-55-4		13	
Sum of unidentified VVOC	Rt < C6	10		
Sum of unidentified VOC	Rt C6-C16	87	7	12
Sum of unidentified SVOC	Rt >C16			
Sum of all measured VVOC*	Rt < C6	10	<5	<5
Sum of all measured VOC*	Rt C6-C16	163	195	106
Sum of all measured SVOC*	Rt >C16	<5	<5	<5
TVOC (Toluene equivalents)		600	7	12
TSVOC (Toluene equivalents)		<5	<5	<5

Appendix 6.15

ID 71 Wood repair (Filler)

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0	7.2	2.1	N
Acetaldehyde	75-07-0	2.9	1.8	N
Propanal	123-38-6	3.2		N
Butanal	123-72-8	4.1		N
Acetone	000067-64-1	<100		<5
2-Propanol	000067-63-0	<100		
1-Butanol	000071-36-3	150		<5
Heptane	000142-82-5		11	

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Hexanal	000066-25-1			<5
Cyclotrisiloxane, hexamethyl- #	000541-05-9	<100	16	
Butylacetat	000123-86-4	<5		
Propylene glycol butyl ether	005131-66-8	<5		
Hexanoic acid	000142-62-1			23
Benzaldehyde #	000100-52-7	<100	<5	<5
Cyclotetrasiloxane, octamethyl- #	000556-67-2	<100	1	
Phenol	000108-95-2	110	12	3
Acetophenone #	000098-86-2	<100		<5
2-Ethylhexanoic acid	000149-57-5			10
Cyclopentasiloxane, decamethyl- #	000541-02-6	<100		
Decanal	000112-31-2	<100		<5
Unknown ether #	(055956-25-7 el 029911-28-2)	1400	210	67
Unknown ether #	(055956-25-7 el 029911-28-2)	1700	270	89
Cyclohexasiloxane, dodecamethyl- #	000540-97-6	<100		
Pentadecane	000629-62-9	<100		
Hexadecane	000544-76-3	<100		
Naphtha C7-C13	64742-82-1/64742-48-9	670	19	
Sum of unidentified VVOC	Rt < C6			
Sum of unidentified VOC	Rt C6-C16		13	
Sum of unidentified SVOC	Rt >C16			
Sum of all measured VVOC*	Rt < C6	<5	<5	<5
Sum of all measured VOC*	Rt C6-C16	4030	552	192
Sum of all measured SVOC*	Rt >C16	<5	<5	<5
TVOC (Toluene equivalents)		3700	520	160
TSVOC (Toluene equivalents)		<5	<5	<5

Appendix 6.16

ID 72 Oil (Floor wax, polish and care products)

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0	2.8	6.6	N
Acetaldehyde	75-07-0	15	6.9	N
Propanal	123-38-6	71	6.2	N
Butanal	123-72-8	13	4.2	N
Acetone	000067-64-1	110	<5	<5
2-Propanol	000067-63-0	<25		
1-Butanol	000071-36-3		<5	<5
Butanoic acid	000107-92-6			21
Hexanal	000066-25-1	340	120	7
Pentanoic acid	000109-52-4		19	21

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Cyclohexanone	000108-94-1	680		<5
Benzaldehyde #	000100-52-7	58	<5	6
Hexanoic acid	000142-62-1			25
Cyclotetrasiloxane, octamethyl- #	000556-67-2	94		
Hexanoic acid	000142-62-1		360	
Phenol	000108-95-2	53	11	3
Di(propylene glycol) methyl ether, mixture of isomers (A)	034590-94-8	290	9	
Ethanol, 2-(2-ethoxyethoxy)- #	000111-90-0	3700		
Octanal	000124-13-0		7	
Heptanoic acid	000111-14-8		12	
Acetophenone #	000098-86-2			<5
Nonanal	000124-19-6	<25		<5
2-Ethylhexanoic acid	000149-57-5		55	10
Decanal	000112-31-2	320		
Tetradecane	000629-59-4	2200		<5
Pentadecane #	000629-62-9	<25	8	
Hexadecane	000544-76-3	<25		
Naphtha C7-C13	64742-82-1/64742-48-9	≥210000	310	
Sum of other aliphatic hydrocarbons VOC	Rt C6-C16	4800	490	
Sum of unidentified VVOC	Rt < C6	39		
Sum of unidentified VOC	Rt C6-C16	3900	82	
Sum of unidentified SVOC	Rt >C16			
Sum of all measured VVOC*	Rt < C6	149	<5	21
Sum of all measured VOC*	Rt C6-C16	≥226435	1483	72
Sum of all measured SVOC*	Rt >C16	<5	<5	<5
TVOC (Toluene equivalents)		≥220000	1000	6
TSVOC (Toluene equivalents)		<5	<5	<5

Appendix 6.17

ID 80 Oil for polish & care of wood and stone floors

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0		1.8	N
Acetaldehyde	75-07-0	4.8	1.3	N
Propanal	123-38-6		1.3	N
Acetone	000067-64-1	8	<5	<5
2-Propanol	000067-63-0	<5	<5	
1-Butanol	000071-36-3	17		<5
1,4-Dioxane	000123-91-1	70		
2-pentanone-4-methyl	000108-10-1		<5	
1,2-Ethanediol #	000107-21-1	19		
2-Butoxy ethanol	000111-76-2	1900	<5	

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
alpha-Pinene	007785-26-4	<5	<5	
Benzaldehyde #	000100-52-7		<5	<5
Phenol	000108-95-2		<5	4
1,2,4-trimethyl-benzene	000095-63-6		<5	
Ethanol, 2-(2-ethoxyethoxy)- #	000111-90-0	≥18000	97	
Limonene	005989-27-5	<5		
Nonanal	000124-19-6	9	7	
2-Methyl-isothiazolin-3-one	002682-20-4	16		
Butyldiglycol	000112-34-5	310	9	
Decanal	000112-31-2	9	8	
Pentadecane	000629-62-9		<5	
Naphtha C7-C13 #	64742-82-1/64742-48-9	330	23	<5
Sum of unidentified VVOC	Rt < C6			
Sum of unidentified VOC	Rt C6-C16	340		
Sum of unidentified SVOC	Rt >C16			
Sum of all measured VVOC*	Rt < C6	8		
Sum of all measured VOC*	Rt C6-C16	≥21020	144	4
Sum of all measured SVOC*	Rt >C16			
TVOC (Toluene equivalents)		≥20000	110	
TSVOC (Toluene equivalents)		<5	<5	<5

Appendix 6.18

ID 83 Oil for polish & care of wood and stone floors

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0		1.9	N
Acetaldehyde	75-07-0	2.5	1.4	N
Propanal	123-38-6	4.7		N
Acetone	000067-64-1	21	7	<5
2-Propanol	000067-63-0	<5		
1-Butanol	000071-36-3	23	<5	<5
1,4-Dioxane	000123-91-1	68		
Ethanol, 2-ethoxy- #	000110-80-5	27		
2-pentanone-4-methyl	000108-10-1	<5	<5	
Propanoic acid, 2-methyl-	000079-31-2	1800		
Hexanal	000066-25-1	<5		
2-Butoxy ethanol	000111-76-2	460		
alpha-Pinene	007785-26-4	<5		
Ethanol, 2-(2-ethoxyethoxy)- #	000111-90-0	≥17000	300	
Limonene	005989-27-5	<5		
Benzyl alcohol	000100-51-6	830	6	
Nonanal	000124-19-6	7		
1,3-Pentanediol, 2,2,4-trimethyl- #	000144-19-4		110	
2-Methyl-isothiazolin-3-one	002682-20-4		10	

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Butyldiglycol	000112-34-5	35		
Decanal	000112-31-2	9		
Texanol A+B #	025265-77-4	5300	910	39
Tetradecane	000629-59-4	<5		
Pentadecane	000629-62-9	24	<5	
Tributyl phosphate #	000126-73-8	60		
Pentan-1,3-dioldiisobutyrate, 2,2,4-trimethyl- #	006846-50-0		5	
Unknown (TXIB?) / Butandioldiglycidylether #	002425-79-8	45		
Naphtha C7-C13 #	64742-82-1/64742-48-9	450	93	<5
Sum of unidentified VVOC	Rt < C6			
Sum of unidentified VOC	Rt C6-C16	620	48	
Sum of unidentified SVOC	Rt >C16			
Sum of all measured VVOC*	Rt < C6	21	7	<5
Sum of all measured VOC*	Rt C6-C16	≥26758	1482	39
Sum of all measured SVOC*	Rt >C16	<5	<5	<5
TVOC (Toluene equivalents)		≥26000	1300	39
TSVOC (Toluene equivalents)		<5	<5	<5

Appendix 6.19

ID 85 Linseed oil for polish of wood floors

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0		1.9	12
Acetaldehyde	75-07-0		1.6	30
Propanal	123-38-6	5.9	2.0	257
Butanal	123-72-8			2.8
Acetone	000067-64-1	12		
2-Propanol	000067-63-0		<5	
Acetic acid	000064-19-7			120
1-Butanol	000071-36-3	7		
Acetone oxime	000127-06-0			<5
Pentanal	000110-62-3			44
Propanoic acid	000079-09-4			420
2-pentanone-4-methyl	000108-10-1	<5		
2-Butanone, oxime	000096-29-7	92		
Butanoic acid	000107-92-6			16
Hexanal	000066-25-1	<5		670
Pentanoic acid	000109-52-4			19
Heptanal #	000111-71-7			<5
alpha-Pinene	007785-26-4	<5		
beta-pinene	018172-67-3	<5		
Hexanoic acid #	000142-62-1			260
Octanal	000124-13-0			17

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Limonene	005989-27-5	5		
Heptanoic acid	000111-14-8			12
Undecane	001120-21-4			<5
Nonanal	000124-19-6		<5	23
Decanal	000112-31-2			<5
Tetradecane	000629-59-4	<5		
Naphtha C7-C13 (3) #	64742-82-1/64742-48-9	4500	48	<5
Sum of unidentified VVOC	Rt < C6	13		40
Sum of unidentified VOC	Rt C6-C16	590	34	370
Sum of unidentified SVOC	Rt >C16			
Sum of all measured VVOC*	Rt < C6	25	<5	40
Sum of all measured VOC*	Rt C6-C16	5194	82	1971
Sum of all measured SVOC*	Rt >C16	<5	<5	<5
TVOC (Toluene equivalents)		5100	67	810
TSVOC (Toluene equivalents)		<5	<5	<5

Appendix 6.20

ID 88 Sealant (repair of concrete)

Substance	CAS no.	5 hours (µg/m³)	3 days (µg/m³)	28 days (µg/m³)
Formaldehyde	50-00-0	9.2	1.7	N
Acetaldehyde	75-07-0	28	2.4	N
Propanal	123-38-6	2.3		N
Acetone	000067-64-1	120	6	15
2-Propanol	000067-63-0		<5	
1-Methoxypropan-2-ol	000107-98-2	39		
1,2-Ethandiol #	000107-21-1	64		
1,2-Propandiol	000057-55-6	94		
Toluene	000108-88-3	<5	<5	
Octane	000111-65-9		<5	<5
Ethylbenzene	000100-41-4	<5		
Styrene	000100-42-5	7	2	
O-xylene	000095-47-6		<5	
Nonane	000111-84-2		5	<5
2-Butoxy ethanol	000111-76-2	10		
1,2,4-trimethyl-benzene	000095-63-6	<100		
Decane	000124-18-5	<5	<5	
Octanal	000124-13-0			<5
Unknown (2-Hydroxyethyl methacrylate?) #	000868-77-9	100	<5	
Unknown (2-Hydroxyethyl methacrylate?) #	000868-77-9	4200	41	
Unknown (acrylate?) #		2100	21	
Undecane	001120-21-4	<5		
Nonanal	000124-19-6		8	<5

Substance	CAS no.	5 hours (µg/m ³)	3 days (µg/m ³)	28 days (µg/m ³)
2-Propenoic acid, 2-methyl-, 1,2-ethanediyl ester #	000097-90-5	5800	360	<5
Dimethyl phthalate #	000131-11-3		60	<5
Pentan-1,3-dioldiisobutyrate, 2,2,4-trimethyl- #	006846-50-0	130	98	<5
Diethyleneglycol dimethacrylate #	002358-84-1			<5
Hexadecane	000544-76-3	<100		
Sum of unidentified VVOC	Rt < C6	11	10	
Sum of unidentified VOC	Rt C6-C16	1300	29	6
Sum of unidentified SVOC	Rt >C16			
Sum of all measured VVOC*	Rt < C6	131	16	15
Sum of all measured VOC*	Rt C6-C16	13844	624	6
Sum of all measured SVOC*	Rt >C16	<5	<5	<5
TVOC (Toluene equivalents)		13000	550	6
TSVOC (Toluene equivalents)		<5	100	<5

General explanation to the tables of results:

* Sum of all calibrated substances plus toluene equivalents of identified and unidentified substances

Substance quantified using toluene calibration curve

N: Measurement not carried out

≥X: Substance measured at concentration above the calibrated range, larger or equal to X µg/m³

<X: Substance identified and measured at concentration below the calibrated linear range, larger or equal to X µg/m³ at collected volume of air

Whenever a substance has not been detected, there is no entry (number or symbol) and the cell is left blank at a given measuring point.

Appendix 7. Calculation of DNEL values

Appendix 7.1 Benzene

Hitherto as well as in the pre-project benzene has been regarded as a carcinogenic substance with no lower limit for the carcinogenic effect. Therefore, a very low threshold value of 0.00017 mg/m³ has been defined as a tolerable exposure level. In 2018 the Committee for Risk Assessment at the European Chemicals Agency reassessed benzene and ascertained that sufficient data is available to define a threshold value for the detrimental effect on DNA and thus on the carcinogenic effect of benzene (ECHA 2018).

Based on this data the committee adopted an 8-hour threshold value of 0.16 mg/m³ in the working environment. Converting this value from worker exposure to exposure to the general population requires a conversion factor of 2 due to higher susceptibility of the more diverse general population. Furthermore, the value must be converted from 8 hours per week for a worker to 24 hours per day 7 days per week for DIY consumers. This gives the following DNEL value for the general population:

$$DNEL_{inhalation\ general\ population} = \frac{DNEL_{inh\ worker}}{2} \times \frac{8\ hours}{24\ hours} \times \frac{5\ days}{7\ days}$$
$$DNEL_{inhalation\ general\ population} = \frac{0,16\ mg/m^3}{2} \times \frac{8\ hours}{24\ hours} \times \frac{5\ days}{7\ days} = 0.019\ mg/m^3$$

ECHA (2018). Opinion on scientific evaluation of occupational exposure limits for Benzene. Committee for Risk Assessment, RAC. ECHA/RAC/ O-000000-1412-86-187/F. Adopted 9 March 2018.

Appendix 7.2 Dibutylphthalate

The Committee for Risk Assessment of the European Chemicals Agency assessed dibutyl phthalate together with a number of other phthalates and concluded a DNEL value of 0.0067 mg/kg/d for dibutylphthalate calculated as an internal dose after absorption into the body (ECHA 2017). Further, an absorption of 100% by inhalation was assumed. If an adult (70 kg) inhales 20 m³ per day, the following DNEL value in air can be calculated:

$$DNEL_{inh} = \frac{DNEL_{inh} \times 70\ kg}{20\ m^3/day} = \frac{0.0067\ \frac{mg}{kg}/day \times 70\ kg}{20\ m^3/day} = 0.023\ mg/m^3$$

ECHA (2017). Opinion on an Annex XV dossier proposing restrictions on FOUR PHTHALATES (DEHP, BBP, DBP, DIBP). Committee for Risk Assessment (RAC), Committee for Socio-economic Analysis (SEAC). RES-O-0000001412-86-140/F. Adopted 10 March 2017

Appendix 7.3 Propylene glycol butyl ether

No LCI value has been found for this substance. Data from the REACH registration of the substance indicate that signs of eye irritation may occur in rats at concentration levels of 300 and 600 ppm (1620 and 3240 mg/m³) when the animals were exposed for 6 hours/day for 9 days. In other studies, opaque cornea was observed in one animal at 700 ppm (Data from REACH registration of the substance).

Data is not sufficient to determine a NOAEL value (No Observed Effect Level), which is needed to establish a DNEL value. Instead these somewhat limited data may be used to estimate a LOAEL (Lowest Observed Adverse Effect Level) value of 1620 mg/m³.

$$DNEL = LOAEL / (AF I \times AF II \times AF III \times AF IV) = 0.9 \text{ mg/m}^3$$

$$DNEL = 1620 \text{ mg/m}^3 \times 6\text{hours}/24\text{hours} / (2.5 \times 10 \times 3 \times 6) = 0.9 \text{ mg/m}^3$$

AF I: In the extrapolation from rat to human a factor of 2.5 is used for local eye irritation.

AF II: A factor of 10 is used to take varying susceptibility among individuals in the human population into account

AF III: A factor of 3 is used to extrapolate from LOAEL to a no-effect-level

AF IV: A factor of 6 is used due to the short duration of the study (9 days)

Appendix 7.4 2-propanol

An acceptable exposure level of 31.25 ppm (77 mg/m³) for an exposure period of 8 hours was determined in connection with approval of this substance as an active biocide (ECHA 2015). This level was based on effect on the central nervous system, which is considered to be the critical effect of this compound. The calculated value is further supposed to protect against eye and respiratory tract irritation. Based on this, a DNEL value of 26 mg/m³ is calculated, since a factor 8/24 is used to estimate the tolerable level for continuous exposure for 24 hours:

$$DNEL = 77 \text{ mg/m}^3 \times 8\text{hours}/24\text{hours} = 26 \text{ mg/m}^3$$

ECHA (2015). Propan-2-ol. Evaluation of active substances under Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products- Assessment Report Product-type 1 (Human hygiene biocidal products)

**Survey and risk assessment of chemical sub-stances in chemical products
used for "do-I yourself" projects in the home – follow-up project**

The project contains three parts: 1) Mapping of products and establishment of exposure scenarios, 2) Analysis of volatile chemical substances 3) Risk assessment.

20 products have been chosen based on the mapping and these have been sampled by The Chemical Inspection Service. The analysis of volatile chemical substances in these products has been conducted by emissions testing in climate chambers after 5 hours, 3 days, and 28 days, respectively after application. The analysed products have been chosen based on their chemical content as well as their hazard classification, with the aim that all the aforementioned product categories are represented.

After 28 days emissions have generally dropped to much lower levels for all products, however for some substances tolerable exposure levels are still exceeded. This applies, for example, to the substance 2-ethylhexanoic acid from 1-component paints and lacquers, which is considered critical, as the substance is potentially harmful to unborn children. Furthermore, an unacceptably high emission of the mucosal irritant preservative CMIT was found for a 2-component paint after 28 days, which was not measured after neither 5 hours nor 3 days.



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Committee for Risk Assessment
RAC

Opinion
proposing harmonised classification and labelling
at EU level of

Benzyl alcohol

EC Number: 202-859-9

CAS Number: 100-51-6

CLH-O-0000007024-83-01/F

Adopted
16 September 2021

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: Benzyl alcohol

EC Number: 202-859-9

CAS Number: 100-51-6

The proposal was submitted by Germany and received by RAC on 29 September 2020.

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at <http://echa.europa.eu/harmonised-classification-and-labelling-consultation/> on 19 October 2020. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by 18 December 2020.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: Lea Stine Tobiassen

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on 16 September 2021 by consensus.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	603-057-00-5	Benzyl alcohol	202-859-9	100-51-6	Acute Tox. 4* Acute Tox. 4*	H302 H332	GHS07 Wng	H302 H332			
Dossier submitters proposal	603-057-00-5	Benzyl alcohol	202-859-9	100-51-6	Add Eye Irrit. 2 Skin Sens. 1B Modify Acute Tox. 4 Remove Acute Tox. 4	Add H319 H317 Modify H302 Remove H332	Retain GHS07 Wng	Add H319 H317 Modify H302 Remove H332		Add Oral: ATE = 1570 mg/kg bw	
RAC opinion	603-057-00-5	Benzyl alcohol	202-859-9	100-51-6	Add Eye Irrit. 2 Skin Sens. 1B Modify Acute Tox. 4 Remove Acute Tox. 4	Add H319 H317 Modify H302 Remove H332	Retain GHS07 Wng	Add H319 H317 Modify H302 Remove H332	-	Add Oral: ATE = 1200 mg/kg bw	
Resulting Annex VI entry if agreed by COM	603-057-00-5	Benzyl alcohol	202-859-9	100-51-6	Acute Tox. 4 Eye Irrit. 2 Skin Sens. 1B	H302 H319 H317	GHS07 Wng	H302 H319 H317		Oral: ATE = 1200 mg/kg bw	

GROUND S FOR ADOPTION OF THE OPINION

RAC general comment

Benzyl alcohol is used as a solvent for inks, paints and lacquers. It has biocidal use (preservative) in a wide range of products (e.g. cosmetics, detergents, food additives) and is used as an odorant in e.g. cosmetics. The substance is also used in pharmaceutical products including treatment of head lice. Benzyl alcohol is a high tonnage chemical ($\geq 10\,000$ tons per annum) under REACH.

The substance was classified under Directive 67/548/EC, preceding the CLP regulation. However, the data and reasoning for the classification at that time are not available. A re-evaluation for the use as food additive (E1519) was published in 2019¹. The substance is currently under scrutiny as a new biocidal active substance (PT6) under the BPR; however, no information is yet publicly available.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter’s proposal

Acute oral toxicity

Eleven studies on the acute oral toxicity of benzyl alcohol in rats, mice, rabbits and guinea pigs are presented by the dossier submitter. The studies were conducted between 1918 and 1980. All reports lack study details in varying degree. The LD₅₀s reported across all studies range from 1040 mg/kg bw (in the mouse and rabbit from 1918 and 1945, respectively) to 3100 mg/kg bw in one rat study (from 1951). One study in Wistar rats (males only) from 1978, and one in Sprague-Dawley rats (both sexes) from 1980 have been conducted according to OECD TG 401 or similar protocols. The DS proposed to use the LD₅₀ from these two studies, i.e. 1620 mg/kg bw and 1570 mg/kg bw as the basis for confirming the existing classification as Acute Oral Toxicity category 4, H302. An ATE for acute oral toxicity of 1570 mg/kg bw is proposed.

Acute dermal toxicity

Only a few, older reports from 1945, 1973 and 1974 with very limited information on study designs were available to the DS. The LD₅₀ values are reported to be over 2000 mg/kg bw in rabbits, less than 5.0 ml/kg bw (≈ 5225 mg/kg bw) in guinea pigs and 2940 mg/kg bw in cats. Based on these data, the dossier submitter proposed not to classify for acute dermal toxicity.

¹ EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Engel K-H, Fowler P, Fürst P, Gürtler R, Gundert-Remy U, Husøy T, Mennes W, Moldeus P, Oskarsson A, Shah R, Waalkens-Berendsen I, Wölfle D, Boon P, Crebelli R, Di Domenico A, Filipic M, Mortensen A, Van Loveren H, Woutersen R, Gergelova P, Giarola A, Lodi F and Frutos Fernandez MJ, 2019. Scientific Opinion on the re-evaluation of benzyl alcohol (E 1519) as food additive. EFSA Journal 2019;17(10):5876, 25 pp. <https://doi.org/10.2903/j.efsa.2019.5876>

Acute inhalation toxicity

Two OECD TG 403 compliant unpublished studies¹ in rats from 1990 and 1993, conducted with aerosols were available using nose-only application. The maximum achievable concentrations 4.18 mg/L and 5.4 mg/L did not cause any mortalities in the studies. Only transient clinical signs (unkempt fur and slower breathing) were reported in the first study, and no effects were reported in the second study. The LC₅₀ values in both studies is concluded to be above 5 mg/L.

Two older studies using rats exposed to vapour were also quoted in the dossier. However, details on study conduct are scarce. The study from 1949 (Carpenter et al. 1949), reported an LC₅₀ for 4h exposure to be 8.8 mg/L (2000 ppm). The DS notes that the concentrations given are nominal only, and that no analytical measurements were performed. In the other of the older studies (Smyth et al. 1951), 3 out of 6 rats died following 8 hours exposure to 1000 ppm (4.4 mg/L) benzyl alcohol. An LC₅₀ of 5.5 mg/L for a 4 hour exposure was extrapolated. A handbook citation (Clayton 1982) referring to the latter study reports that testing of a saturated vapour concentration of 200 ppm (>0.9 mg/L) did not lead to any mortalities after 2 hrs exposure, while one third of the animals died (LC₃₃) following 4 hour exposure, and 8 hours led to the death of all animals. However, the relation to the original report quoted above is very unclear.

The DS notes that the MAK-Kommission² (Hartwig 2017) calculated a saturated vapour concentration of 567 mg/m³ indicating that there is an equilibrium between vapour and aerosol at 0.5-0.6 mg/L.

Having regard to the uncertainties related to the vapour data, the DS considers that the classification for acute toxicity by inhalation should be based on the two OECD TG 403 compliant studies from 1990 and 1993, conducted with aerosols, leading to a conclusion not to classify benzyl alcohol for acute inhalation toxicity.

Comments received during consultation

Acute oral toxicity

Comments were received from 2 MSCAs, both agreeing to the proposed classification for Acute Oral Toxicity in category 4. One of the commenting MSCAs stressed the uncertainty related to the scarce reporting of the studies used to derive the classification, including lack of information on the purity of the tested substance. The other MSCA commenter added that, due to the low level of details in the reports, using the generic ATE of 500 mg/kg bw may be considered in preference to the proposed ATE of 1570 mg/kg bw.

The DS responded that the ATE of 1570 mg/kg was proposed due to the similarity of the results in the two most reliable studies.

Acute dermal toxicity:

One MSCA expressed support for the proposal not to classify for this endpoint.

Another MSCA commented on the low quality of studies on acute dermal toxicity, and the lack of studies in rats or mouse. The MSCA noted that the guinea pig data do not exclude an LD₅₀

¹ Robust study summary available in REACH registration dossier. However, a full study report unavailable

² MAK: Maximale Arbeitsplatzkonzentrationen – German national occupational limit values.

below 2000 mg/kg bw. Finally, the MSCA proposed to stress the insufficiency of the database in the reasoning for no classification.

The DS responded that all the currently available data were included in the present proposal of no classification.

Acute inhalation toxicity

One MSCA refers to the CLP guidance for differentiation between aerosols/mists and vapours, offering a calculation of the LC₅₀ level of 3 mg/L above which the values for aerosols should be used to decide on classification of benzyl alcohol. However, the low data quality could raise a doubt when deleting an existing classification.

Another MSCA notes that the results reported from the aerosols would not lead to classification. However, due the poor database, and having regard to the current classification in category 4, the MSCA proposed to point to the inconclusiveness of the data in the justification for no classification.

Assessment and comparison with the classification criteria

Acute oral toxicity

All available results on acute oral toxicity show LD₅₀ values between 1040 and 3100 mg/kg bw, across the species tested. RAC agrees to disregard LD₅₀ values from studies where no details at all are available.

The DS based the proposal on two acute oral toxicity studies from 1978 and 1980 similar to OECD TG (no information on the purity of the test substance available), reporting LD₅₀ values of 1620 mg/kg bw and 1570 mg/kg bw, respectively. RAC considers that the information on the study in rats by Jenner et al. (1964) included in the CLH report should also be considered. Although performed prior to OECD and GLP, using a lower number of animals and without information on the purity of the substance, the study is considered to be reported in sufficient details in the article for it to be suitable for classification purposes. The study reported acute oral toxicity testing of a number of chemicals in rats, guinea pigs and/or mice. With respect to benzyl alcohol, the study used 10 Osborne-Mendel rats fasted for 18 hours prior to treatment. The animals were treated by intubation with the undiluted substance. The LD₅₀ value confidence limits are given as 1130 to 1330 mg/kg bw. The animals were observed for 14 days reporting clinical signs as depression and coma within 10-15 min, excitability for 3-4 days, and mortalities occurring from 4 hours up to 3 days. The reported LD₅₀ from the rat study is 1230 mg/kg bw, calculated by the method of Litchfield and Wilcoxon (1949).

The guidance on classification recommends to use the most sensitive result from a relevant study. Although the age and reporting of acute oral studies brings some uncertainties to their evaluation, and the use of the generic ATE of 500 mg/kg bw could be considered, RAC considers that there are data available that point to an LD₅₀ between 1000 and 2000 mg/kg bw.

The DS proposes to classify on the basis of LD₅₀ values of 1620 mg/kg bw and 1570 mg/kg bw. RAC notes that the study by Jenner et al.(1964) appears to be reliable, and would include this study in the evaluation of acute oral toxicity. The LD₅₀ value of 1230 mg/kg bw from this study, although slightly lower than from the two studies proposed by the DS, also indicates classification as Acute Tox. cat 4.

RAC agrees with the DS to base the classification on the most reliable experimental data and recommends classification as Acute Tox. 4; H302 for benzyl alcohol.

RAC further proposes to use the LC₅₀ value from the rat study by Jenner et Al. (1964) and set a rounded ATE of 1200 mg/kg bw for acute oral toxicity of benzyl alcohol.

Acute dermal toxicity

The DS evaluated that the dermal LD₅₀ values were over 2000 mg/kg bw, and that the criteria for acute dermal toxicity classification were therefore not met. No classification is proposed for this endpoint.

RAC considers that the available data, although from elder studies and scarcely reported are sufficient to conclude on no classification for acute dermal toxicity.

Acute inhalation toxicity

The reported LC₅₀ values from studies conducted with vapours appear to be within the criteria for classification of vapours in category 3; H331 (LC₅₀ between 2.0 and 10.0 mg/L).

However, there are uncertainties on the conduct and results from these studies. Also, because benzyl alcohol is of low volatility (vp is 0.12hPa at 25°C), the aerosols would be predominant in concentrations above the saturated concentration (calculated as 0.57 mg/L at 25°C by the MAK-commission). The DS therefore proposes to disregard these studies in the classification of benzyl alcohol for acute inhalation toxicity.

The two studies available using aerosols in nose-only application are considered to have LC₅₀ over 5 mg/L. As the criteria for classifying in category 4 (dusts and mist: 1.0 mg/L < ATE ≤ 5.0mg/L) are not met, the DS proposes not to classify benzyl alcohol for acute inhalation toxicity.

RAC notes that the CLP guidance recommends classification based on the mists (aerosols) for substances for which LC₅₀ values are above the Saturated Vapour Concentration (SVC): "*An LC₅₀ well below the SVC will be considered for classification according to the criteria for vapours; whereas an LC₅₀ close to or above the SVC will be considered for classification according to the criteria for mists.*" At 20°C, the SVC would be slightly lower than 0.57 mg/L at 25°C as calculated by the MAK commission (i.e. ≈ 0.31 mg/L, when applying the equation proposed in the CLP guidance $SVC = 0.0412 \times MW \times vp$).

RAC agrees that the classification should be based on the data for aerosols, as both available studies conducted according to OECD test guidelines are regarded as valid and reliable based on the summaries available in the REACH registration dossier.

RAC considers that a new evaluation of the classification of benzyl alcohol for acute inhalation toxicity is justified, as the substance would occur as an aerosol at the concentrations of concern, and as the data on aerosols may not have been available when the substance was first classified under DSD.

As the LC₅₀ values from the studies on aerosols are higher than 5 mg/L, RAC concurs with the DS that no classification for acute inhalation toxicity is warranted. In conclusion, RAC recommends to remove the existing classification for acute inhalation toxicity on benzyl alcohol.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS presented two unpublished OECD TG 405 compliant studies in rabbits from 1990 and 1998 (only summaries available). Both studies used 3 animals that were treated with 100µl benzyl alcohol of 99.99% purity. Observation periods were 21 and 18 days, respectively. Another unpublished eye irritation study conducted in 1979 with only 2 rabbits and an observation period of only 7 days was also available. This study also used 100µl benzyl alcohol (no information on the purity of the substance).

The scores from both studies led to an overall categorisation as an eye irritant. All effects were reversible, at the latest by day 21. The study from 1979 also led to an overall conclusion of moderate eye irritancy from benzyl alcohol. The results from three older studies were also included in the proposal, one reporting benzyl alcohol to be highly irritating to the rabbit eye, whilst 2 other testing very low concentrations showed no irritancy. However, these results were considered of low relevance due to poor reporting.

Based on the result from the OECD 405 compliant studies, the DS considered that benzyl alcohol should be classified as Eye Irritant, category 2; H319.

Comments received during consultation

Two MSCAs supported DS proposal for classification as Eye Irritant, category 2; H319.

The scores from the two OECD TG compliant studies are tabled below. For comparison, the results from the 2 animals in the 1979 study are also included. However, as this study is terminated at 7 days, the irreversibility of the effect on the cornea by the end of the study is not considered relevant for the evaluation. Also, it is noted that the scores are similar or less severe than in the two OECD TG studies.

Year of study report	Mean scores (no animals / scores of individual animal)			
	Corneal opacity	Iritis	Conjunctival redness	Conjunctival oedema (chemosis)
1990- study	1 (3 / score 2)	0.1 (1/mean score 0.3; 2/ score 0)	2 (3 / score 2)	0.8 (1/ score 3; 2/mean score 0.7)
	Time to full reversibility 21 days	Time to full reversibility 48 hrs	Time to full reversibility 21 days	Time to full reversibility 7 days
1998-study	2 (3 / score 2)	1 (3 / score 1)	2.4 (1 / score 3; 1 / mean score 2.3; 1 / score 2)	2.2 (1/score 3; 1/score 2; 1/mean score 1.7)
	Time to full reversibility 18 days	Time to full reversibility 11 days	Time to full reversibility 11 days	Time to full reversibility 7 days
1979-study	2 / score 1 Time to full reversibility: > 7 days	2/score 1-2 Time to full reversibility: 7 hrs	2/score 0-2 Time to full reversibility: 7 days	1/score 0-1; 1/score 0-2 Time to full reversibility: 7 days

Assessment and comparison with the classification criteria

For classification in category 2, one or more of the following scores should be fulfilled in at least 2 of 3 tested animals: corneal opacity ≥ 1 ; iritis ≥ 1 ; conjunctival redness ≥ 2 and/or conjunctival oedema ≥ 2 .

Both studies following the OECD TG protocol fulfil the criteria for category 2 (see scores in bold in the table above). RAC agrees with the DS that benzyl alcohol should be classified as Eye Irritant in category 2; H319.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Benzyl alcohol has a broad pattern of use in consumer and professional applications, and a large number of animal studies and human data investigating the skin sensitisation potential of benzyl alcohol as well as some *in vitro* data are presented in the classification proposal.

Benzyl alcohol is regulated in annex III (substances subject to restrictions due to its sensitising potential) of the Cosmetics Regulation and should be declared on the label of leave-on cosmetic products from 0.001%, and in rinse-off products from 0.001%. The DS further notes that the substances biocidal use as a preservative in ready-for-use is regulated at 1%.

Human data

There is a large data base on benzyl alcohol including results from experimental data in human volunteers (Human Repeat Insult Patch Tests, HRIPT, Human maximisation tests, HMT) and from patch tests in consecutive dermatitis patients from hospitals medical clinics, and case studies.

Table 1: Experimental data in humans:

Study description	Test substance, concentration	Number of volunteers	Results	Reference *
Human repeat insult patch test (HRIPT) 9 inductions under occlusion for 24 hrs over 3 weeks with 0.3 ml Benzyl alcohol 10-14 day rest period, then challenge patch for 24hrs (unexposed site) Reactions scored at 24, 48, 72 and/or 96hrs after application	20 % Benzyl alcohol in diethyl phthalate:EtOH (3:1) Hill Top chamber: 23622 μ g/cm ²	56	Induction: oedematous skin reactions in 5 subjects <i>Challenge:</i> Oedema: 2 / 56 (3.6 %) score 2+ 3 / 56 (5.4 %) score 1+ Remaining subjects +/- (transient reaction) <i>Re-challenge:</i> Oedema: 1 / 56 (1.8 %) score 2 1 / 56 (1.8 %) score 1+ 2 / 56 (3.6 %) score +/-	(RIFM, 2002) Only short summary available
	15 % Benzyl alcohol in diethyl phthalate:EtOH (3:1) Hill Top chamber: 17717 μ g/cm ²	46	Induction: oedema in 5/46 subjects <i>Challenge:</i> Oedema: 4 / 46 (8.7 %) score 2+ 1 / 46 (2.2 %) score 1+ 1 / 46 (2.2 %) score +/- (transient reaction)	(RIFM, 2003) Only short summary available

	7.5 % Benzyl alcohol in diethyl phthalate:EtOH (3:1) Hill Top chamber: 8858µg/cm ²	110	Induction: Severe irritation in 1 subject <i>Challenge:</i> Oedema: 1/110 (0.9%) score 2+ (same subject as above - persistent at 96 h after challenge) 2 / 110 (1.8 %) score not reported <i>Re-challenge:</i> 1 / 3 positive at challenge sensitised (after occlusive and semi-occlusive patches)	(RIFM, 2004b) Only short summary available
	5 % Benzyl alcohol in diethyl phthalate:EtOH (3:1) Hill Top chamber: 5906µg/cm ²	101	Induction: Oedema: 2 subjects - also at new sites. Transient reaction in 1 subject. <i>Challenge:</i> Oedema: 1 / 101 (1 %) score 3+ 1 / 101 (1 %) score 1+ Same subjects affected as at induction. Effects indicative of pre-sensitisation	(RIFM, 2005b)
	3 % Benzyl alcohol in diethyl phthalate:EtOH (3:1) Hill Top chamber: 3 543 µg/cm ²	107	Negative, no skin reactions	(RIFM, 2004a)
Human maximisation test (HMT) according to (Kligman, 1966) Patches on volar forearms under occlusion 5 alternate-day 48h periods 24 h pretreatment with 5% aqueous sodium lauryl sulphate (SLS) under occlusion. 10-14 day rest period 48h challenge Reading at 0, 48 and 72hrs upon removal	10 % Benzyl alcohol in petrolatum Hill Top chamber: 6 900 µg/cm ²	25	Negative	(RIFM, 1970)

Five unpublished reports (summaries available) from HRIPTs with doses ranging from 3543 µg/cm² to 23622 µg/cm² using 3 to 20% Benzyl alcohol for induction and challenge were reported. Scoring of skin reactions was performed, scores >1 being regarded as a positive sensitisation reaction. Three of the studies including 47-110 subjects resulted in sensitisation

in 2.7%-11% of subjects occurring from doses above 8858 µg/cm² or 7.5% benzyl alcohol. In a fourth HRPIT using 5% benzyl alcohol, pre-sensitisation was suspected in 2 subjects reacting already in the induction phase, and the results are ambiguous. The individual studies are reliable with restrictions, with scarce information given in the available summaries. Also, some studies use a relatively low number of subjects.

One HMT in 25 volunteers from 1970 for 10% Benzyl alcohol available as a summary was negative. The low number of subjects and low concentration of benzyl alcohol reduces the robustness of the result.

Table 2: Human patch tests studies

Study description	Test substance concentration	Number of patients	Results	Reference
Human patch test Retrospective analysis of data on patch testing of preservatives contained in the standard series and special series collected by the IVDK 1996–2009.	1 % Benzyl alcohol in petrolatum Purity not specified	79770	258/79770 subjects with positive reactions (0.28 %) 64 men (0.18 %), 194 women (0.34 %)	(Schnuch et al., 2011a)
Human patch test Data on all patients patch tested in the departments of the Information Network of Departments of Dermatology between 2005 and 2008. Diagnostic procedure follows international guidelines.	1 % Benzyl alcohol Purity and vehicle not specified	23257	51/23257 subjects with positive reactions (0.22 %)	(Uter et al., 2010)
Human patch test Frequency of sensitisation to preservatives analysed on the basis of data from the IVDK (2006–2009).	Benzyl alcohol Purity, vehicle and test concentrations not specified	17740	31 subjects with positive reactions (0.17 %)	(Schnuch et al., 2011b)
Human patch test Analysis of data on the frequency of sensitisation to selected antimicrobials in all patients with current or previous atopic eczema compared with patients without past or current atopic eczema, patch test data collected by Departments of Dermatology participating in the IVDK (1995-1999). (patient groups standardised for age and sex, patients with current leg ulcer/stasis dermatitis were excluded)	1 % Benzyl alcohol in petrolatum Purity not specified	14722 (non-atopic) 5183 (atopic)	44 / 14722 non-atopic patients with positive reactions (0.3 %) 15/5183 atopic patients with positive reactions (0.28 %)	(Jappe et al., 2003)
Human patch test Retrospective study on patients with suspected allergic contact dermatitis tested with a preservative series, data collected between 1990 and	1 % Benzyl alcohol in petrolatum Purity not specified	11373	46/11373 subjects with positive reactions (0.4 %)	(Schnuch et al., 1998)

Study description	Test substance concentration	Number of patients	Results	Reference
1994 from 24 departments participating in the German Information Network of Departments of Dermatology (IVDK).				
Human patch test Retrospective multicentre survey of patch test reactions to standard, cosmetic and hairdressing series collected by 7 Finnish dermatological clinics representing the Finnish Contact Dermatitis Group (comparing results from 1995-1996 and 2000-2002).	Benzyl alcohol Purity, vehicle and test concentrations not specified	6125 (2000-2002) 4922 (1995-1996)	2000-2002: No allergic reactions 1995-1996: 1/4922 subject with positive reaction (0.02 %)	(Hasan et al., 2005)
Human patch test Study on patients tested with Belgian Contact Patch-test series.	Benzyl alcohol Purity, vehicle and test concentrations not specified	5202 (with known contact dermatitis)	48 subjects with positive reactions (0.9 %)	(Broeckx et al., 1987)
Human patch test Retrospective study of patch testing results, aggregated from four patch test clinics in three centres in Melbourne and Sydney (1993–2006). Data were collected for a minimum of five years from each centre.	1 % Benzyl alcohol Purity not specified	4552	18/4552 subjects with positive reactions (0.4 %)	(Chow et al., 2013)
Human patch test Study on the frequency of sensitisation to fragrances to be labelled according to current European regulation. 25 fragrances were successively patch-tested additionally to the standard series in a total of 21 325 unselected patients; the number of patients tested with each of the fragrances ranged from 1658 to 4238 in 2003-2004.	1 % Benzyl alcohol Purity and vehicle not specified	2 166	7/2166 subjects with positive reactions (0.3 %): 3 subjects 1+, 3 subjects 2+, 1 subject 3+) 12 irritant or doubtful reactions Sensitisation to Benzyl alcohol associated with leg dermatitis (29 %).	(Schnuch et al., 2007)
Human patch test Retrospective study on data from all eczema patients routinely tested 2011-2012 with the fragrance series and the European baseline series at St John's Institute of Dermatology at St Thomas' Hospital, London. Co-reactions also reported.	10 % Benzyl alcohol in petrolatum Purity not specified	1 951	4 subjects with positive reactions (0.21 %) Co-reactions with any fragrance marker 3/4 (75%) of reactions to fragrance series substance Co-reactions with fragrance mix I (FM I) 1/4 (25%)	(Mann et al., 2014)

Study description	Test substance concentration	Number of patients	Results	Reference
			of reactions to ingredient. Co-reactions with fragrance mix II (FM II) 2/4 (50%) of positive reactions to ingredient.	
Human patch test Patients were tested for their reaction to three different fragrance mixes (FM I, FM II, and "further fragrances"). Patients tested positive to a mix were tested with the individual components. The frequency of sensitisation in the study population was extrapolated from the frequency of reactions to the single compound.	Fragrances mix and Benzyl alcohol Composition of fragrances mix, purity of test substance(s), vehicle and test concentrations not specified	1 870	0.7 % of the patients sensitised to the fragrances mix tested positive for Benzyl alcohol. This corresponded with a frequency of 0.16 % when extrapolated to all 1 870 patients.	(Schnuch et al., 2015)
Human patch test Retrospective study based on data from the Department of Dermato-Allergology, Copenhagen University Hospital Gentofte. Eczema patients were patch tested (2008- 2010) with the 26 fragrance ingredients, including Benzyl alcohol. All eczema patients suspected of having contact allergy were tested consecutively according to international guidelines.	1 % Benzyl alcohol Purity and vehicle not specified	1 508	2/1508 subjects with positive reaction (0.1 %) In addition: - 3 subjects with doubtful reaction - 1 subject with irritant reaction	(Heisterberg et al., 2011)
Human patch test Prospective study of cosmetic adverse reactions by eleven dermatologists (1977-1980) using standard screening, perfume or vehicle-preservative series of the North American Contact Dermatitis Group	Benzyl alcohol Purity, vehicle and test concentrations not specified	487	2 subjects with positive reactions (0.4 %)	(Eiermann et al., 1982)
Human patch test (short report) Patients with clinical suspicion of cosmetic contact dermatitis patch tested at Contact Dermatitis Clinic of Rabin Medical Center in Israel from 1997-2000. European standard series and cosmetic series used.	Benzyl alcohol Purity, vehicle and test concentrations not specified	244	5 subjects with positive reactions (2.0 %)	(Trattner et al., 2002)
Human patch test Frequency of cosmetics as causal factors of allergic contact dermatitis are reported and the cosmetic allergens identified during the	Benzyl alcohol Purity, vehicle and test concentrations not specified	147	1 subject with positive reaction (0.68 %)	(Goossens, 2016)

Study description	Test substance concentration	Number of patients	Results	Reference
previous six years are discussed (2010–2015). The data were retrieved from and evaluated with a patient database developed in-house.				
Human patch test Prospective study of 93 consecutive patients suspected of having allergic contact dermatitis tested 2005-2006 with the European standard series and cosmetic series at the Dermatology Department, Baskent University Faculty of Medicine, Ankara, Turkey.	10 % Benzyl alcohol Purity and vehicle not specified	93	1 subject with positive reaction (1.1 %)	(Ada and Seckin, 2010)
Human patch test 4-year retrospective study of selected patients tested with a fragrance series (2004-2008). Patients selected were either positive to the Spanish baseline series (54 patients) or there was clinical suspicion (32 patients).	1 % Benzyl alcohol in petrolatum Purity not specified	86	2/86 subjects with positive reactions (2.3 %)	(Cuesta et al., 2010)
Human patch test Study on 35 consecutive patients tested with chemical compounds recommended by North American Contact Dermatitis Group. In cases with positive reactions chemical compounds were re-applied at day 7 and read again at day 9.	Benzyl alcohol Purity, vehicle and test concentrations not specified	35	2 subjects with positive reactions on day 2 and at re-testing (6 %)	(Mitchell, 1977)

The largest study included 79 770 consecutive dermatitis patients from 1996 to 2009 analysed retrospectively (Schnuch et al. 2011a), in which a concentration of 1% benzyl alcohol was used for patch testing. The authors of that study conclude that benzyl alcohol is a rare contact allergen (combined incidence of 0.28%) and report a higher incidence in women (0.34 %) compared to men (0.18 %).

Two human patch testing studies of dermatitis patients (table 2) with 10% Benzyl alcohol resulted in sensitisation rates of 0.21 % and 1.1 %, respectively, whilst patch testing of benzyl alcohol at a concentration of 1% led to sensitisation rates ranging from 0.1 % to 2.3 %. There are also a number of studies not specifying the concentration of benzyl alcohol used, resulting in sensitisation rates in the same range (up to 2% positives).

The highest incidence of sensitisation reported stems from a study based on 35 patients (6 %) (Mitchell 1977). The lowest incidence reported was of no positive reactions amongst 6 125 patients between 2000 and 2002, and 0.02 % positive reactions amongst 4 922 between 1995 and 1996 (Hasan et al. 2005).

Overall, patch test studies including more than 100 patients show sensitisation rates between 0.1 and 1%.

Furthermore, 38 patch test studies as summarised by the DS from a review article (Scognamiglio et al. 2012) are included in the table below. Details of these studies are lacking.

Table 3: Human diagnostic patch test data

Concentration of benzyl alcohol	Incidence	Original references
20% in petrolatum	5 % from 1971-74; 4 % from 1975-77, 1 % from 1978-80 in cosmetic dermatitis patients Number of patients not reported	(Nakayama et al., 1984)
10% in petrolatum	0/501 (0%)	(De Groot et al., 1986)
10% in petrolatum	2/394 (0.5 %)	(Mid-Japan Contact Dermatitis Research Group, 1984) (Ueda, 1994)
5% in petrolatum	1/394 (0.3 %)	
1% in petrolatum	0/394 (0 %)	
10% (vehicle not reported)	3/182 (1.6 %)	(Malten et al., 1984)
5% in petrolatum	1/2261 (0.04 %) from 1978-79 0/1934 (0 %) from 1979-80	(Mitchell et al., 1982)
5% in petrolatum	3/991 (0.3 %)	(Dickel et al., 2001)
5% in petrolatum	3/669 (0.4 %)	(Katoh et al., 1995)
5% in petrolatum	0/667 (0 %)	(van Joost et al., 1984)
5% in petrolatum	6/661 (0.9 %)	(Itoh et al., 1988)
5% in petrolatum	9/585 (1.5 %)	(Itoh et al., 1986)
5% in petrolatum	3/425 (0.71 %)	(Nagareda et al., 1992)
5% in petrolatum	1/479 (0.2 %)	(Nagareda, 1996)
5% in petrolatum	1/398 (0.3 %)	(Sugai, 1996)
5% in petrolatum	2/200 (1 %)	(Nethercott, 1982)
5% in petrolatum	0/241 (0 %)	(Ferguson and Sharma, 1984)
5% in petrolatum	8/102 (7.8 %)	(Hausen, 2001)
5% in petrolatum or 10% in alcohol	19/95 (20%)	(Hjorth, 1961)
5% (vehicle not reported)	0/3037 (0 %)	(Angelini et al., 1985)
5% (vehicle not reported)	13/1206 (1.1 %)	(Sugai, 1982)
5% (vehicle not reported)	0/574 (0 %)	(Hirose et al., 1987)
5% (vehicle not reported)	1/457 (0.2 %)	(Addo et al., 1982)
5% (vehicle not reported)	8/427 (1.9 %)	(Nishimura et al., 1984)
5% (vehicle not reported)	2/242 (1.7 %)	(Van Joost et al., 1985)
5% (vehicle not reported)	6/220 (2.7 %)	(Ishihara et al., 1979)
5% (vehicle not reported)	0/178 (0 %)	(Hirano and Yoshikawa, 1982)
5% (vehicle not reported)	3/167 (1.8 %)	(Larsen et al., 1996)
5% (vehicle not reported)	0/145 (0 %)	(Suzuki et al., 1997)
5% (vehicle not reported)	1/84 (1.1%)	(Takase et al., 1984)

Concentration of benzyl alcohol	Incidence	Original references
5% (vehicle not reported)	1/81 (1.2%)	(Haba et al., 1993)
5% (vehicle not reported)	3/78 (3.8%)	(Ishihara et al., 1979)
2% (vehicle not reported)	2/78 (2.6%)	
1% (vehicle not reported)	2/78 (2.6%)	
1% in petrolatum	7/2166 (0.3%)	(Schnuch et al., 2007)
1% in petrolatum	1/1082 (0.1%)	(Geier et al., 2003)
1% in petrolatum	1/320 (0.3%)	(van Oosten et al., 2009)
1% (vehicle not reported)	0/3115 (0%)	(Cooper and Shaw, 2000)
1% (vehicle not reported)	0/436 (0%)	(Penchalaiah et al., 2000)
1% (vehicle not reported)	0/422 (0%)	(An et al., 2005)
1% (vehicle not reported)	1/390 (0.3%)	(Torgerson et al., 2007)
0.2% (vehicle not reported)	18/614 (2.9%)	(Fuji et al., 1972)

The results of diagnostic patch tests with benzyl alcohol summarised in table 3 above showed incidences of sensitisation in 0 to 20% of the patients. However, the DS notes that the robustness of studies including less than 100 individuals may be questioned. When disregarding those studies sensitisation occurs in frequencies of 0-7.8%, with 12 negative studies, 14 studies with less than 1% of the patients reacting and 9 studies with more than 1% reactions.

The DS also reported 19 case reports from consecutive dermatitis patients reacting to benzyl alcohol. Most patients reacted strongly to patch testing with benzyl alcohol, but also mild and negative results were reported.

Table 4: Case reports of sensitisation

Study description	Results	References
30 year-old facial dermatitis patient Patch testing with Benzyl alcohol (no test concentration reported) produced	Macular erythema	Case reports cited by (Johnson et al., 2017)
38 year-old eczema patient 1% aqueous Benzyl alcohol Prick test and intradermal injection	Negative prick test result Positive (++) intradermal injection test result. (injection test were negative in 10 healthy controls)	
39 year-old female with pruritic erythema of foot 5 % Benzyl alcohol in petrolatum Patch test and Repeated open application test	Weak (+) reaction in patch test Strong positive reaction in repeated open application test	
67 year-old male with leg dermatitis Occlusive patch test with 1% benzyl alcohol in petrolatum Prick test with 0.9% benzyl alcohol in saline	Positive (++) occlusive patch test reaction. Negative prick test reaction at 0.5 hours reading, but marked induration and proximal spread over arm at days 3 to 8	

Study description	Results	References
53 year-old with stasis dermatitis Patch test with 1% Benzyl alcohol in petrolatum	Redness and swelling at 1 hour after patch application, wheal 1 day later, and mild urticaria at day 5	
16 year-old female with possible anaphylactic reaction after injection with B12 preparation containing 0.9 % Benzyl alcohol Prick test and intradermal test with benzyl alcohol preparation (concentration not reported)	Negative in prick test, Positive in intradermal test	
57 year-old female with pruritic dermatitis Patch testing with Benzyl alcohol (concentration not reported)	Allergic contact dermatitis after patch testing	
40 year-old female with dermatitis Patch test using 9.5% benzyl alcohol in petrolatum	Positive patch test reaction (+++)	
65 year-old female with eyelid dermatitis Patch testing with benzyl alcohol (concentration not stated)	Macular erythema	
30 year-old female with eyelid dermatitis Patch testing with benzyl alcohol (concentration not stated)	Positive (+) patch test reaction	
46 year-old man with atopic excema Patch testing with 5% Benzyl alcohol in petrolatum	Positive (+ +) reaction at day 2 and day 3	(Corazza et al., 1996)
43 year-old patient with recurrent right leg ulceration Patch testing with 0.1% Benzyl alcohol in aqueous solution	Strong positive (+ + +) reaction at day 1 and day 3	(Jager et al., 1995)
63 year-old woman Patch testing with 5% Benzyl alcohol (vehicle not reported)	Positive (+ +) reaction	(Li and Gow, 1995)
37 year-old woman with acute excema Patch testing with 1% Benzyl alcohol in petrolatum	Strong positive (+ + +) reaction at day 2 and 4	(Aguirre et al., 1994)
50 year-old man Patch testing with 5% Benzyl alcohol in petrolatum	Strong positive (+ + +) reaction after 48 and 96 hours	(Wurbach et al., 1993)
28 year-old metal grinder with patchy rash Patch testing with 1% Benzyl alcohol in petrolatum	Positive (+ +) reaction at day 2 and 3	(Mitchell and Beck, 1988)
41 year-old Japanese women Patch testing with 5% Benzyl alcohol (vehicle not reported)	Positive reaction (+ +) after 48 and 72 hours in patch test Negative in open patch test	(Shoji, 1983)
80 year-old man Patch testing with 5% Benzyl alcohol in petrolatum	Positive reaction at days 2 (+) and 4 (++)	(Kleyn et al., 2004)
36 year-old female and 43-year old male with contact dermatitis Patch testing with 1% Benzyl alcohol in petrolatum Scratch, intradermal and subcutaneous injections of 1% Benzyl alcohol in saline solution	Strong positive reaction in patch test. Negative in intradermal and subcutaneous injections	(Fisher, 1975)

DS conclusion on human data

A large database from human studies including HRIPTs, one HMT, and several patch test studies from dermatological hospitals and case reports was available on benzyl alcohol.

The HRIPTs available on benzyl alcohol are considered to be valid, although the reporting is lacking some information. Three of the studies including 47-110 subjects resulted in sensitisation in 2.7%-11% of subjects occurring from increasing doses (0-11%) benzyl alcohol from 7.5% (8858 µg/cm²). The HMT showed no sensitisation from exposure to 10% benzyl alcohol in 25 volunteers. Due to the low number of subjects tested and that only one dose was applied, the reliability of the results is uncertain.

Multicenter patch testing studies of collective of dermatitis patients show sensitisation rates from 0.1 to 2.3% when tested with benzyl alcohol from in concentrations 1 to 10%. Short reports from further patch test studies performed with 0.2-20% benzyl alcohol show sensitisation rates from 0 up to 20%, with studies with more than 100 patients generally showing sensitisation of <1%, perceived as a low to moderate sensitisation rate according to the Guidance (the ECHA Guidance on the Application of the CLP criteria, 2017).

The information on exposure to benzyl alcohol from both experimental and clinical studies is however scarce, and no reliable estimate of the level exposure is possible. The DS points especially to the ubiquitous presence of Benzyl alcohol in a broad range of cosmetic products that may be applied several times per day and result in a prolonged or repeated exposure over years. Applying the criteria for scoring exposure set in the Guidance, table 3.3, the DS concludes that exposure to benzyl alcohol is relatively high.

Animal data

The animal studies presented include results from one LLNA test and a number of guinea pig tests, three being maximisation tests, and others including older test protocols such as the Draize test, the open and closed epicutaneous test (OET and CET), and one Freund's complete adjuvant (FCA) test. The studies are only available as short summaries of unpublished reports, and one is in Japanese.

The LLNA test from 2005, conducted according to OECD TG 409 with 99.8% pure benzyl alcohol in diethyl phthalate:ethanol (3:1), using 4 animals and 5 treatment groups up from 2.5 to 50% w/v was negative. The stimulation indices (SI) were 0.5-1.2% and EC₃ >50%.

In a GPMT conducted in a protocol similar to OECD TG 406 and deemed reliable with restrictions, 5% intradermal and 25% epicutaneous induction did not result in sensitisation (Klecak et al. 1977).

Another article reported a GMPT using 10% benzyl alcohol for induction and challenge to be positive (Ishihara et al. 1986), whilst the same main author had earlier reported a GMPT to be negative (Ishihara et al. 1981). However, it was not possible to assess the reliability of these results.

Kashima and coworkers reported benzyl alcohol to be a weak sensitiser in a non-standard "modified cumulative contact enhancement test" using 30% for induction and 10% benzyl alcohol for challenge (Kashima et al. 1993). The DS considered the report reliable with restrictions based on the level of detail available.

A negative modified Draize sensitisation test conducted prior to OECD TG regarded to be reliable with restrictions was reported to have used the challenge concentration of 0.25 and 10% intradermally and epidermally, respectively (Sharp 1978).

The paper by Klecak et al. (1977) also reported a FCA test, an OET, and a Draize test stated to be a conducted according to OECD TG 406 comparable protocols. The FCA test, in which undiluted

benzyl alcohol for induction was used, gave a positive result with a “non-irritating challenge dose”. The OET using several dilutions up to undiluted benzyl alcohol for induction also resulted in sensitisation after challenge with a “non-irritating challenge dose”, whilst the Draize test using 5 doses of 0.1% at induction over 10 days for induction and 0.1% at challenge was negative (Klecak 1977). The information from the studies in this article is considered reliable with restrictions.

Two later papers from Klecak (1979, 1985) reported a negative result from an OET using 10% benzyl alcohol, but reliability could not be attributed due to lack of detail.

Hausen et al. (1992) reported benzyl alcohol (component of Balsam of Peru) to be a moderate sensitiser in a FCA test and a weak sensitiser in a modified FCA test. However, very few study details were given and reliability therefore not assessed.

Overall, the animal studies are of variable quality, their reporting is poor, and the results are contradictory. One out of three GPMTs and two out of three OETs were positive, the FCA tests showed benzyl alcohol to be a weak to moderate sensitiser whilst the Draize tests were negative. The LLNA test was negative, however it is noted that the maximum concentration tested was 50%, and no justification not using a higher concentration was offered in the summary available.

The DS concluded that the animal data indicate that benzyl alcohol is a weak sensitiser in animal studies of variable quality.

Other data

Summaries from reports from *in vitro* tests relating to key events an adverse outcome pathway (AOP) leading to skin sensitisation were available on benzyl alcohol.

Table 5: Summary table of *in vitro* / *in chemico* data

Key event Type of test	Result	Remarks from study author or DS	Reference
Key event 1: Peptide/ protein binding Test: Direct peptide reactivity assay (DPRA) <i>in chemico</i>	Negative	Metabolic activation of benzyl alcohol may be necessary, according to author.	(Urbisch et al., 2015)
Key event 2: Keratinocyte response ARE-Nrf2 luciferase assay Test 1: KeratinoSens™ (<i>in vitro</i>)	Negative	The DS notes that the different results may be due to differences in sensitivity in detecting a weak sensitising potential	
Key event 2: Keratinocyte response ARE-Nrf2 luciferase assay Test 2: LuSens (<i>in vitro</i>)	Positive		
Key event 3: Monocytic/ Dendritic cell response Test: Human cell line activation test (h-CLAT (<i>in vitro</i>))	Positive		

Based on the results and remarks included in the table above the DS regards that the *in vitro/in chemico* data point towards a sensitising potential of benzyl alcohol.

In conclusion, regarding all the data in a weight of evidence assessment, from the HRIPT studies on Benzyl alcohol, from dermatitis patients, animal data, and indications o sensitisting potential

from *in vitro/in chemico* assays, the results all point to a weak to moderate skin sensitising potential of benzyl alcohol. A classification as Skin Sens. in category 1B is proposed by the DS.

Comments received during consultation

A total of 10 organisations or individuals filed detailed comments, often including public and confidential comments in the public consultation.

Two MSCAs supported the proposed classification based on weight of evidence.

Six Industrial organisations, including one industrial expert group commented that the quality of the database was not satisfactory. Several organisations questioned whether the status of the patched tested individuals, pointing to the possibility of the patients being selected rather than unselected. The commenters also commented on the low to insignificant sensitising potential of benzyl alcohol. Considering classification in category 1B to overestimate the hazard from benzyl alcohol.

The academic organisation IVDK provided additional information on patch test results from several dermatology departments performed from 2010 to 2019 with benzyl alcohol. The largest resulted in incidences of positive results of 146/70867 (0.21%) and 99/54062 (0.18%). They provided analyses to demonstrate that a number of patch tested patients had previous sensitisation history or some reactions were irritative rather than sensitisation response to benzyl alcohol. Comparison with other sensitizer led IVDK to conclude that benzyl alcohol is a very rare skin sensitizer not meriting classification.

One individual commented on the animal and *in vitro* data, stating that only OECD TG studies should be used for classification and considered the outcome of the *in vitro* tests negative

The DS stressed that the CLP criteria rules that a weight of evidence approach using all available and suitable animal, *in vitro* and human data should be performed in order to reach a conclusion on classification. The DS further explained that the human data were evaluated in accordance with CLP criteria and supplemented by the Guidance, *inter alia* in the evaluation of frequency of effect and exposure.

Assessment and comparison with the classification criteria

Human data

The numerous human data available for benzyl alcohol include experimental and clinical studies. RAC notes that conducting experimental studies in humans such as HRIPT and HMT, as underlined in the CLP regulation, is not allowed for ethical reasons. Information from existing studies may however be considered for classification purposes, and therefore criteria for the use of such data are also included in the classification criteria.

RAC notes that specific information on the exposure levels of the patients tested are often missing in clinical data from diagnostic patch tests, as it is also the case for benzyl alcohol. However, in view of the broad use pattern of benzyl alcohol and especially its use in cosmetics, exposure is expected to occur repeatedly and over longer periods of time. The Guidance (ECHA 2017) includes a scoring system to evaluate the size of exposure qualitatively in table 3.4 applied below:

Table: Level of exposure

Exposure data	Relatively low exposure	Relatively high exposure	Benzyl Alcohol
Concentration/ dose criteria	<1.0% or <500µg/cm ² (score 0)	≥1.0% or ≥500µg/cm ² (score 2)	Unknown (score 1 ¹)
Repeated exposure	< once daily (score 1)	≥ once daily (score 2)	≥ once daily (score 2)
Number of exposures	< 100 exposures (score 0)	≥100 exposures (score 2)	≥100 exposures (score 2)
Total score	1-3	4-6	5

¹An intermediate score of 1 is attributed as the exposure concentration is not reported in the available studies.

The resulting total score achieved for benzyl alcohol of 5 is regarded as reflecting a high exposure. RAC notes that IFRA use recommendations for different product groups in rinse-off and leave-on cosmetics indicate that concentrations over 1% might occur (IFRA standards 2020) indicating that the overall score in the estimation of the level of exposure to benzyl alcohol could be higher than 5.

The criteria relating to diagnostic patch tests data further distinguishes between “a *relatively high and substantial incidence of reactions...*” and “a *relatively low but substantial incidence of reactions...*” (category 1A and sub-category 1B). Table 3.2 on Frequency of occurrence of skin sensitisation in the Guidance (ECHA 2017) considers test data in unselected, consecutive dermatitis patients resulting in incidences above ≥ 1.0% to be of high frequency, and incidences <1.0% of low/moderate frequency. The results of patch testing studies reported in table 1. Experimental data in humans testing consecutive, unselected patients and using standard series fall under category of high frequency.

If the dermatitis patients are selected, and the testing aimed, the limit between high and low/moderate frequency is 2.0%. Due to the scarce information available for the studies reported in table 3 on human diagnostic patch test data, it cannot be excluded that the some of the studies included selected patients, and the information is thus difficult to assess under this part of the criteria.

Animal data

Only few details are available from the animal study summaries on benzyl alcohol. One LLNA study from 2005 conducted according to OECD TG 429 and GLP for which a robust study summary is available can be considered as reliable with restrictions, although there appears to be no justification available for the lower concentration used in relation to the OECD TG 429 requirements. The reporting of the majority of adjuvant and non-adjuvant studies in guinea pigs carried too few details to enable evaluation of their reliability. The results are often only listed as positive or negative, and application of sub-categorisation criteria is not possible as the condition of induction are not reported. Overall, the animal data are insufficient for classification on their own. However, the results are included in the table below on the weight of evidence assessment for the classification of benzyl alcohol.

Other data

There are as yet no specific criteria in CLP on the use of *in vitro/in chemico* data for classification for skin sensitization. The CLP regulation and the Guidance recommends to include the information from *in vitro* methods in a weight of evidence approach with other data. OECD TGs have in the recent years included *in vitro* methods describing key event steps relating to an Adverse Outcome Pathway (AOP) approach for skin sensitisation, developed by the OECD in 2016

including three key events to investigated in *in vitro* tests. These tests are referred in the Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7a: endpoints specific guidance (2017). Most recently, a defined approach for skin sensitisation (DASS) was adopted by the OECD WNT (May 2021) including a refined Integrated Testing Strategy (ITS) v2 (OECD 2021a, 2021b). The data from key events tests related to skin sensitisation performed on benzyl alcohol are therefore also included in the table below on the weight of evidence approach for the classification of benzyl alcohol. RAC notes that benzyl alcohol is one of the substances included in the OECD database (OECD 2021b) supporting the new defined approach on skin sensitisation adopted by OECD (OECD 2021a). Application of the OECD ITS v2. approach (Integrated Testing Strategy including use of OECD toolbox for *in silico* data scoring) gives total score of 2 (1 from OECD Toolbox and 1 from the positive hCLT) which is considered to warrant sub-categorisation as Skin Sens. 1B.

Conclusion

The table below includes all the available human data from volunteer studies (HRIPT and HMT), diagnostic patch test in consecutive patients, diagnostic patch tests where information on the history of the patients is unavailable, and human patch test cases. With respect to animal data available in the dossier, the table includes the LLNA, 3 GMPTs, 3 FCA tests, 3 OETs, one closed epicutaneous test, one delayed contact hypersensitivity test, and 3 Draize tests available in the classification report. Finally, the table also includes the 4 available *in vitro/in chemico* tests. A short mention on reliability of the studies and RACs evaluation on the contribution to classification from the individual results in order to facilitate the weight of evidence based conclusion on the classification of benzyl alcohol are included.

Table : Weight of evidence approach for the classification of benzyl alcohol

Study type	Result /Reliability	CLP criteria, and detail from the Guidance on the Application of classification criteria	RAC evaluation on classification outcome of each individual study ^a
Human data			
HRIPT	3 positive results at 8 858 µg/cm ² and above in studies including >100 volunteers Reliable with restrictions	Sub-category 1A: (a) positive responses at ≤ 500 µg/cm ² (HRIPT, HMT-induction threshold) (Annex I: 3.4.2.2.2.1.) Sub-category 1B: (a) positive responses at > 500 µg/cm ² (HRIPT, HMT-induction threshold); (Annex I: 3.4.2.2.2.2)	Skin Sens. 1B
HMT	Negative Uncertainty due to low number of volunteers. Not reliable	Sub-category 1A: (a) positive responses at ≤ 500 µg/cm ² (HRIPT, HMT-induction threshold) (Annex I: 3.4.2.2.2.1.) Sub-category 1B: (a) positive responses at > 500 µg/cm ² (HRIPT, HMT-induction threshold); (Annex I: 3.4.2.2.2.2)	Not considered suitable for classification in this case
Diagnostic patch tests in consecutive dermatitis patients	Overall sensitisation rate of <1% Low/moderate frequency, Relatively high exposure	Skin Sens. 1: Relatively high frequency (≥ 1.0%*) and "relatively high exposure" or Relatively low/moderate frequency (< 1.0%*) and "relatively low exposure"	Skin Sens. 1B

Study type	Result /Reliability	CLP criteria, and detail from the Guidance on the Application of classification criteria	RAC evaluation on classification outcome of each individual study ^a
	Reliable with restrictions	Skin Sens. 1A: Relatively high frequency ($\geq 1.0\%$ *) and "relatively low exposure" Skin Sens. 1B: Relatively low/moderate frequency ($< 1.0\%$ *) and "relatively high exposure"	
Diagnostic patch tests Conditions of testing unclear	Sensitisation rates 0-7 %. Most studies with >100 patients <2% Reliable with restrictions	Skin Sens. 1: Relatively high frequency ($\geq 1.0\%$ * or $\geq 2.0\%$ **) and "relatively high exposure" or Relatively low/moderate frequency ($< 1.0\%$ * or $< 2.0\%$ **) and "relatively low exposure" Skin Sens. 1A: Relatively high frequency ($\geq 1.0\%$ * or $\geq 2.0\%$ **) and "relatively low exposure" Skin Sens. 1B: Relatively low/moderate frequency ($< 1.0\%$ * or $< 2.0\%$ **) and "relatively high exposure"	Skin Sens. 1B
Case reports	< 100 cases Relatively high exposure estimated above (score 5) Reliable with restrictions	Skin Sens. 1: Relatively high frequency (Number of published cases ≥ 100) and "relatively high exposure" or Relatively low frequency (number of published cases < 100) and "relatively low exposure" Skin Sens. 1A: Relatively high frequency (Number of published cases ≥ 100) and "relatively low exposure" Skin Sens. 1B: relatively low frequency (Number of published cases < 100) and "relatively high exposure"	Skin Sens. 1B
Animal data			
LLNA test	EC3 > 50 % Maximum used concentration. Uncertainty as justification on choice of dose levels unavailable Reliable with restrictions	Skin Sens. 1A: EC3 $\leq 2\%$ Skin Sens. 1B: EC3 > 2 %	No classification
Guinea Pig Maximisation Test	1 positive reliability unknown		(Skin sensitiser)
	1 negative reliability unknown		(No classification)

Study type	Result /Reliability	CLP criteria, and detail from the Guidance on the Application of classification criteria	RAC evaluation on classification outcome of each individual study ^a
	1 negative reliable with restrictions		No classification
Freund's Complete Adjuvant Test	1 positive reliable with restrictions		Skin sensitiser
	1 positive reliability unknown		(Skin sensitiser)
	1 weakly positive reliability unknown		(Skin sensitiser/ no classification)
Open Epicutaneous Test	2 positive reliability unknown		(Skin sensitiser)
	1 positive reliable with restrictions,		Skin sensitiser
	1 negative reliability unknown		(No classification)
Closed epicutaneous test	negative reliability unknown		(No classification)
Delayed contact hypersensitivity test	positive reliable with restrictions		Weak sensitiser
Draize Guinea Pig Sensitisation Test	1 negative reliable with restrictions		No classification
	1 negative reliable with restrictions		No classification
	1 negative reliability unknown		(No classification)
		<i>In vitro</i> data ¹	
Key event 1 protein binding: Direct peptide reactivity assay	Negative Reliable without restrictions	Skin sensitiser when 2 out of 3 key events confirmed in test	Inconclusive result: 1 or 2 out of 3 key events are positive
Key event 2: ARE-Nrf2 luciferase assay Keratinocyte response	Negative Reliable without restrictions		
Key event 2: ARE-Nrf2 luciferase assay LuSens (in vitro) Keratinocyte response	Positive Reliable without restrictions		
Key event 3: h-CLAT (in vitro)	Positive Reliable without restrictions		

^a Text in bold refers to a reliable study (with or without restrictions), text in brackets to study with unknown reliability, which are not considered in the WoE. The WoE gives preference to reliable results.

* Limit applies to unselected dermatitis patients

** Limit applies to selected dermatitis patients

¹ One *in vitro* test cannot stand alone, but overall build up to an AOP on skin sensitisation.

The data base on benzyl alcohol is very large, albeit of varying reliability and suitability for classification purposes. In the weight of evidence assessment above, the human data from HRIPTs and from diagnostic patch tests and case reports are regarded as suitable for classification, and all point to benzyl alcohol being a low-moderately potent skin sensitiser.

The most reliable animal data is the LLNA although there is uncertainty whether the maximum dose of 50% was sufficiently high to detect an apparently weak sensitiser as benzyl alcohol. The rest of the animal results are contradictory, and the reliability and suitability for classification is low, as details of exposure are unavailable.

Finally, data included in the AOP (OECD 2016a, referred in IR&CSA 2017) do not point to a clear conclusion, as there is both a positive and a negative result for one of the three key events of this AOP, but indicate activity that can lead to sensitisation.

In conclusion RAC agrees with the DS that the weight of evidence assessment including all human, animal, *in silico* and *in vitro* data available on benzyl alcohol, with the extensive human data from HRIPT and diagnostic patch test data as the most important evidence, leads to the conclusion that benzyl alcohol should be classified as Skin Sensitiser, subcategory 1B; H317 - May cause an allergic skin reaction.

RAC further agrees with the DS that attribution of SCL is not warranted.

Additional references

IFRA standards (2020) amendment 49 – benzyl alcohol

(https://ifrafragrance.org/standards/IFRA_STD_008.pdf)

OECD (2021a): OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects - Guideline No. 497: Defined Approaches on Skin Sensitisation. Organisation for Economic Co-operation and Development. https://www.oecd-ilibrary.org/environment/guideline-no-497-defined-approaches-on-skin-sensitisation_b92879a4-en

OECD (2021b). Supporting document to the OECD Guideline 497 on Defined Approaches for Skin Sensitisation- Series on Testing and Assessment No. 336. Organisation for Economic Co-operation and Development. ENV/CBC/MONO(2021)11. [https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/CBC/MONO\(2021\)11&docLanguage=En](https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/CBC/MONO(2021)11&docLanguage=En)

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).

SAFETY DATA SHEET

Creation Date 24-Dec-2009

Revision Date 23-Jun-2022

Revision Number 3

1. Identification

Product Name Benzyl alcohol

Cat No. : L03292

CAS No 100-51-6
Synonyms Phenylmethanol

Recommended Use Laboratory chemicals.
Uses advised against Food, drug, pesticide or biocidal product use.

Details of the supplier of the safety data sheet

Company

Alfa Aesar
Thermo Fisher Scientific Chemicals, Inc.
30 Bond Street
Ward Hill, MA 01835-8099
Tel: 800-343-0660
Fax: 800-322-4757
Email: tech@alfa.com
www.alfa.com

Emergency Telephone Number

During normal business hours (Monday-Friday, 8am-7pm EST), call (800) 343-0660.
After normal business hours, call Carechem 24 at (866) 928-0789.

2. Hazard(s) identification

Classification

This chemical is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Acute oral toxicity	Category 4
Acute Inhalation Toxicity - Vapors	Category 4
Serious Eye Damage/Eye Irritation	Category 2

Label Elements

Signal Word

Warning

Hazard Statements

Causes serious eye irritation
Harmful if swallowed or if inhaled

**Precautionary Statements****Prevention**

Wash face, hands and any exposed skin thoroughly after handling
Do not eat, drink or smoke when using this product
Use only outdoors or in a well-ventilated area
Wear eye/face protection
Do not breathe dust/fume/gas/mist/vapors/spray

Response

Get medical attention/advice if you feel unwell

Inhalation

IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
Call a POISON CENTER or doctor/physician if you feel unwell

Eyes

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
If eye irritation persists: Get medical advice/attention

Ingestion

IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell
Rinse mouth

Disposal

Dispose of contents/container to an approved waste disposal plant

Hazards not otherwise classified (HNOC)

None identified

3. Composition/Information on Ingredients

Component	CAS No	Weight %
Benzyl alcohol	100-51-6	>95

4. First-aid measures

General Advice	If symptoms persist, call a physician.
Eye Contact	Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Get medical attention.
Skin Contact	Wash off immediately with plenty of water for at least 15 minutes. If skin irritation persists, call a physician.
Inhalation	Remove to fresh air. If not breathing, give artificial respiration. Get medical attention if symptoms occur.
Ingestion	Clean mouth with water and drink afterwards plenty of water.
Most important symptoms and effects	Difficulty in breathing. Symptoms of overexposure may be headache, dizziness, tiredness, nausea and vomiting
Notes to Physician	Treat symptomatically

5. Fire-fighting measures

Suitable Extinguishing Media Water spray, carbon dioxide (CO₂), dry chemical, alcohol-resistant foam.

Unsuitable Extinguishing Media No information available

Flash Point 96 °C / 204.8 °F

Method - No information available

Autoignition Temperature 435 °C / 815 °F

Explosion Limits

Upper 13 vol %

Lower 1.3 vol %

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. Containers may explode when heated. Keep product and empty container away from heat and sources of ignition.

Hazardous Combustion Products

Carbon monoxide (CO). Carbon dioxide (CO₂).

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
2

Flammability
1

Instability
1

Physical hazards
N/A

6. Accidental release measures

Personal Precautions Use personal protective equipment as required. Ensure adequate ventilation.

Environmental Precautions Should not be released into the environment. Do not flush into surface water or sanitary sewer system.

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/face protection. Do not get in eyes, on skin, or on clothing. Ensure adequate ventilation. Avoid ingestion and inhalation.

Storage. Keep containers tightly closed in a dry, cool and well-ventilated place. Keep away from heat, sparks and flame. Store under an inert atmosphere. Incompatible Materials. Strong oxidizing agents.

8. Exposure controls / personal protection

Exposure Guidelines This product does not contain any hazardous materials with occupational exposure limit established by the region specific regulatory bodies.

Engineering Measures Use only under a chemical fume hood. Ensure that eyewash stations and safety showers are close to the workstation location.

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	Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.
Hygiene Measures	Handle in accordance with good industrial hygiene and safety practice.

9. Physical and chemical properties

Physical State	Liquid
Appearance	Light yellow
Odor	aromatic
Odor Threshold	No information available
pH	No information available
Melting Point/Range	-15 °C / 5 °F
Boiling Point/Range	205 °C / 401 °F
Flash Point	96 °C / 204.8 °F
Evaporation Rate	No information available
Flammability (solid,gas)	Not applicable
Flammability or explosive limits	
Upper	13 vol %
Lower	1.3 vol %
Vapor Pressure	0.13 mbar @ 20 °C
Vapor Density	3.72 (Air = 1.0)
Specific Gravity	1.044
Solubility	Soluble in water
Partition coefficient; n-octanol/water	No data available
Autoignition Temperature	435 °C / 815 °F
Decomposition Temperature	No information available
Viscosity	6.6 mPa.s at 20 °C
Molecular Formula	C7 H8 O
Molecular Weight	108.14

10. Stability and reactivity

Reactive Hazard	None known, based on information available
Stability	Air sensitive. Hygroscopic.
Conditions to Avoid	Excess heat. Exposure to moist air or water. Exposure to air.
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
Component Information

Component	LD50 Oral	LD50 Dermal	LC50 Inhalation
Benzyl alcohol	LD50 = 1230 mg/kg (Rat)	LD50 = 2 g/kg (Rabbit)	LC50 > 4178 mg/m ³ (Rat) 4 h

Toxicologically Synergistic Products No information available

Delayed and immediate effects as well as chronic effects from short and long-term exposure
Irritation Irritating to eyes

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
Benzyl alcohol	100-51-6	Not listed	Not listed	Not listed	Not listed	Not listed

Mutagenic Effects Not mutagenic in AMES Test

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 Symptoms of overexposure may be headache, dizziness, tiredness, nausea and vomiting

Endocrine Disruptor Information No information available

Other Adverse Effects The toxicological properties have not been fully investigated.

12. Ecological information

Ecotoxicity

Contains no substances known to be hazardous to the environment or that are not degradable in waste water treatment plants.

Component	Freshwater Algae	Freshwater Fish	Microtox	Water Flea
Benzyl alcohol	Not listed	LC50: = 460 mg/L, 96h static (Pimephales promelas) LC50: = 10 mg/L, 96h static (Lepomis macrochirus)	Not listed	EC50: = 23 mg/L, 48h (water flea)

Persistence and Degradability Soluble in water Persistence is unlikely based on information available.

Bioaccumulation/ Accumulation No information available.

Mobility . Will likely be mobile in the environment due to its water solubility.

Component	log Pow
Benzyl alcohol	1.05

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

<u>DOT</u>	Not regulated
<u>TDG</u>	Not regulated
<u>IATA</u>	Not regulated
<u>IMDG/IMO</u>	Not regulated

15. Regulatory information

United States of America Inventory

Component	CAS No	TSCA	TSCA Inventory notification - Active-Inactive	TSCA - EPA Regulatory Flags
Benzyl alcohol	100-51-6	X	ACTIVE	-

Legend:

TSCA US EPA (TSCA) - Toxic Substances Control Act, (40 CFR Part 710)

X - Listed

'-' - Not Listed

TSCA - Per 40 CFR 751, Regulation of Certain Chemical Substances & Mixtures, Under TSCA Section 6(h) (PBT) Not applicable

TSCA 12(b) - Notices of Export Not applicable

International Inventories

Canada (DSL/NDSL), Europe (EINECS/ELINCS/NLP), Philippines (PICCS), Japan (ENCS), Japan (ISHL), Australia (AICS), China (IECSC), Korea (KECL).

Component	CAS No	DSL	NDSL	EINECS	PICCS	ENCS	ISHL	AICS	IECSC	KECL
Benzyl alcohol	100-51-6	X	-	202-859-9	X	X	X	X	X	KE-02570

KECL - NIER number or KE number (<http://ncis.nier.go.kr/en/main.do>)

U.S. Federal Regulations

SARA 313 Not applicable

SARA 311/312 Hazard Categories See section 2 for more information

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

Component	Massachusetts	New Jersey	Pennsylvania	Illinois	Rhode Island
Benzyl alcohol	X	-	X	-	-

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

Authorisation/Restrictions according to EU REACH Not applicable

Component	CAS No	REACH (1907/2006) - Annex XIV - Substances Subject to Authorization	REACH (1907/2006) - Annex XVII - Restrictions on Certain Dangerous Substances	REACH Regulation (EC 1907/2006) article 59 - Candidate List of Substances of Very High Concern (SVHC)
Benzyl alcohol	100-51-6	-	-	-

Safety, health and environmental regulations/legislation specific for the substance or mixture

Component	CAS No	OECD HPV	Persistent Organic Pollutant	Ozone Depletion Potential	Restriction of Hazardous Substances (RoHS)
Benzyl alcohol	100-51-6	Listed	Not applicable	Not applicable	Not applicable

Component	CAS No	Seveso III Directive (2012/18/EC) - Qualifying Quantities for Major Accident Notification	Seveso III Directive (2012/18/EC) - Qualifying Quantities for Safety Report Requirements	Rotterdam Convention (PIC)	Basel Convention (Hazardous Waste)
Benzyl alcohol	100-51-6	Not applicable	Not applicable	Not applicable	Not applicable

16. Other information

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End of SDS

EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

Fifty-seventh report of the
Joint FAO/WHO Expert Committee on
Food Additives



The World Health Organization was established in 1948 as a specialized agency of the United Nations serving as the directing and coordinating authority for international health matters and public health. One of WHO's constitutional functions is to provide objective and reliable information and advice in the field of human health, a responsibility that it fulfils in part through its extensive programme of publications.

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EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

Fifty-seventh report of the
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Food Additives



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Joint FAO/WHO Expert Committee on Food Additives

Rome, 5–14 June 2001

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Monographs containing summaries of relevant data and toxicological evaluations are available from WHO under the title:

Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 48, 2002.

Specifications are issued separately by FAO under the title:

Compendium of food additive specifications, addendum 9. FAO Food and Nutrition Paper, No. 52, Add. 9, 2001.

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

The preparatory work for toxicological evaluations of food additives and contaminants by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is actively supported by certain of the Member States that contribute to the work of the International Programme on Chemical Safety (IPCS).

The International Programme on Chemical Safety (IPCS) is a joint venture of the United Nations Environment Programme, the International Labour Organization and the World Health Organization. One of the main objectives of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment.

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives met in Rome from 5 to 14 June 2001. The meeting was opened by Mr W. Clay, Chief, Nutrition Programmes Service, Food and Nutrition Division, FAO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Mr Clay reminded the Committee that one of its tasks was to provide scientific advice to Member States of the two organizations with respect to food regulations and control. He noted that dioxins and some related compounds were to be discussed by the Committee for the first time, almost 25 years after the accident in Seveso, Italy, in which large quantities of dioxins had been released into the environment. That event had raised awareness and concern both in the general population and among regulators, leading to a greater demand for global assessment, management and communication of risks relating to environmental contamination and food. The Committee's deliberations on the topic would therefore be important and should help to improve communication between those responsible for risk assessment and risk management. Mr Clay informed the Committee that its activities would be part of a wider effort by FAO and WHO to improve food safety. The two organizations were planning to establish a Global Forum for Food Safety Regulators, in order to promote the exchange of information about ways of dealing with issues of potential importance to public health and international food trade among those responsible for regulating food safety.

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955 (1), there have been fifty-six previous meetings of the Committee (Annex 1). The present meeting was convened on the basis of a recommendation made at the fifty-fifth meeting (Annex 1, reference 149).

The tasks before the Committee were:

- to elaborate further principles for evaluating the safety of food additives and contaminants (section 2);
- to undertake toxicological evaluations of certain food additives, flavouring agents and contaminants (sections 3–5 and Annex 2);
- to review and prepare specifications for selected food additives and flavouring agents (sections 3 and 4 and Annex 2).

2.1 **Modification of the agenda**

Annatto extracts were scheduled for evaluation at a future meeting, when the results of toxicological studies that were being performed would become available to the Committee for consideration. Amyloglucosidase from *Aspergillus oryzae*, var. had been included in the call for data erroneously.

Sodium ethyl *p*-hydroxybenzoate, sodium propyl *p*-hydroxybenzoate, sodium methyl *p*-hydroxybenzoate, calcium sulfite, sodium formate, calcium formate, synthetic γ -tocopherol, synthetic δ -tocopherol, calcium tartrate, sorbitan trioleate, dipotassium diphosphate and dimagnesium diphosphate have been removed from the draft Codex General Standard for Food Additives and were referred to the Committee for evaluation. There was no indication, however, that any of these substances are used as food additives, and consequently little information was provided that would permit the establishment of Acceptable Daily Intakes (ADIs) or the preparation of specifications. Phenyl salicylate was removed from the agenda because no data were available.

2.2 **Principles governing the toxicological evaluation of compounds on the agenda**

In making recommendations on the safety of food additives and contaminants, the Committee took into consideration the principles established and contained in Environmental Health Criteria, No. 70, *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference 76), as well as the principles elaborated subsequently at a number of its meetings (Annex 1, references 77, 83, 88, 94, 101, 107, 116, 122, 131, 137, 143 and 149), including the present one. Environmental Health Criteria, No. 70 (Annex 1, reference 76) contains the most important observations, comments and recommendations made, up to the time of its publication, by the Committee and associated bodies in their reports on the safety assessment of food additives and contaminants. At its present meeting, the Committee noted that the publication included recommendations that are still appropriate and indicated potential problems associated with those that are no longer valid in the light of technological changes.

2.3 **Principles for the safety assessment of chemicals in food**

The Committee was informed that FAO and WHO are intending to update and consolidate principles and methods for the safety assessment of chemicals in food, including food additives, contaminants, and residues of veterinary drugs and pesticides. The project was

initiated on the basis of a recommendation of the Conference on International Food Trade Beyond 2000 that was held in Melbourne, Australia, in October 1999 (2), and in view of the scientific advances, changes in procedures and the increasing complexity of assessments of chemicals in food that have occurred since the publication of Environmental Health Criteria, No. 70 (Annex 1, reference 76) and Environmental Health Criteria, No. 104, *Principles for the toxicological assessment of pesticide residues in food* (3). The project would include consideration of all those aspects of the assessment of chemicals in food that are addressed by the Committee and by the Joint FAO/WHO Meeting on Pesticide Residues.

The Committee recognized the importance of this initiative and recommended that it be undertaken as soon as possible.

2.4 Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents

The Committee questioned whether some of the substances included in the lists of flavouring agents that it had been asked to evaluate at its present meeting were in fact used as flavouring agents. The Committee noted that some of the substances were used extensively in food processing as solvents, emulsifiers or preservatives.

The Committee stressed that the Procedure for the Safety Evaluation of Flavouring Agents is intended for application to flavouring agents used to impart flavour to foods and not to other uses of these substances or to other chemicals that may be used in flavouring formulations. Consequently, the Committee was unable to finalize the evaluations of certain substances listed on the agenda,¹ pending confirmation of their use and intake as flavouring agents.

A clear definition of “flavouring agent” has not been elaborated by the Committee. Although Environmental Health Criteria, No. 70 (Annex 1, reference 76) provides some guidance, the Committee recommended that this issue be addressed at a future meeting.

2.5 α,β -Unsaturated carbonyl compounds and aldehydes

The α,β -unsaturated carbonyl group is a reactive moiety that represents a potential structural alert for toxicity. Five flavouring agents containing such a group were considered by the Committee at its forty-ninth meeting (Annex 1, reference 131), but their evaluation was postponed, pending consideration of other α,β -unsaturated carbonyl compounds. The safety of these five agents was reconsidered by

¹ See sections 4.1.3–4.1.5.

the Committee at its fifty-fifth meeting (Annex 1, reference 149), when it also evaluated furfural, cinnamaldehyde, structural analogues of cinnamaldehyde, pulegone and esters of the corresponding alcohols, which are predicted to be metabolized by formation of α,β -unsaturated carbonyls. The available data on the toxicity of these compounds in experimental animals showed a number of adverse effects at high doses, and no-observed-effect-levels (NOELs) for these effects were identified. The presence of protective processes in cells, such as conjugation with glutathione, provides adequate capacity for detoxification at the low doses associated with the use of such compounds as flavouring agents. In consequence, the Committee concluded that the presence of an α,β -unsaturated carbonyl group in a flavouring agent, or its formation during metabolism, would not preclude assessment of that substance by the Procedure for the Safety Evaluation of Flavouring Agents. This conclusion was supported by data on the toxicokinetics of 4-phenyl-3-buten-2-one (No. 820), which was considered by the Committee at its present meeting. This α,β -unsaturated carbonyl compound undergoes complete first-pass metabolism in rats and mice after oral administration and is rapidly eliminated (with a half-life of 20 min in rats and 10 min in mice) after intravenous administration. A number of other α,β -unsaturated carbonyl compounds were also evaluated by the Committee at its present meeting (Nos 821, 826 and 829), as were several compounds predicted to be metabolized to an α,β -unsaturated carbonyl compound (Nos 819, 944, 946 and 948).

Aldehyde groups are also chemically reactive and can bind to soluble proteins and protein components of membranes. Several aldehydes were evaluated previously by the Committee, and the potential genotoxicity of furfural was considered in detail at the fifty-first meeting (Annex 1, reference 137). Furfural was reported to be genotoxic in three of 16 assays for reverse mutation in *Salmonella typhimurium* and in one of three assays for *rec* gene mutation in *Bacillus subtilis*. A few chromosomal aberrations were seen in Chinese hamster ovary cells in culture when furfural was added at relatively high concentrations (Annex 1, reference 138). Sister chromatid exchanges and forward mutations were induced in mouse lymphoma cells. The Committee concluded that the weak activity seen in vitro in some tests for genotoxicity might be explained by the reactivity of the aldehyde group. Various metabolic processes (i.e. oxidation, conjugation and condensation) effectively eliminate the reactive aldehyde functional group, when the metabolic pathways are not saturated by high, non-physiological doses. The flavouring agents evaluated at the present meeting included a number of aldehydes (Nos 22, 865, 866,

868, 869, 877–879, 889–893, 896–898 and 937) and compounds that are predicted to be metabolized to aldehydes, such as acetals (Nos 837–840, 867, 940–949 and 954). Metabolism of these flavouring agents is predicted to result in gradual formation of aldehydes, which undergo extensive biotransformation, resulting in only low concentrations of the aldehydes per se. The results of tests for reverse mutation in bacteria were positive for pyruvaldehyde (No. 937), but consistently negative for Nos 22, 80, 95, 98, 867, 868, 877–879, 889, 893, 896, 897 and 953; the results of assays for *rec* gene mutation in *B. subtilis* were negative (Nos 878, 889 and 893) or equivocal (Nos 22 and 896). Chromosomal aberrations were reported in vitro in some studies with Nos 22, 878, 889, 893, 896 and 937, but not with No. 80. Similarly, sister chromatid exchanges were reported in some studies with Nos 22, 80, 878, 889 and 937, but not with Nos 868, 893 and 897. Mutations were reported in mouse lymphoma cells exposed to some aldehydes (Nos 80, 877, 878 and 893), but not other aldehydes or acetate (Nos 867, 889 and 896). The results of studies in vivo did not indicate genotoxicity after oral administration in a variety of test systems: in *Drosophila melanogaster* (with Nos 22, 80, 879 and 893), in assays for micronucleus formation in mice (with Nos 879, 889 and 893) and in assays for dominant lethal mutations in mice (No. 896). Sister chromatid exchange was induced in mice and hamsters by intraperitoneal injection of acetaldehyde (No. 80), and weakly positive results were obtained in several tests in vivo with pyruvaldehyde (No. 937) at very high doses (>200 mg/kg of body weight). Pyruvaldehyde is a natural component of some foods, and the amount ingested due to its use as a flavouring agent would be much less than the estimated intake from natural sources. The Committee concluded that metabolic processes such as oxidation and conjugation effectively eliminate reactive aldehyde functional groups from such substances when they are consumed in the amounts that would arise from their use as flavouring agents.

2.6 Minimum assay values for flavouring agents

At its fifty-third meeting, the Committee developed criteria for establishing specifications for flavouring agents (Annex 1, reference 143). The Committee noted that these criteria — chemical formula and relative molecular mass, identity test and the minimum amount that can be determined (minimum assay value) — constitute the core information required to establish acceptable specifications. At its present meeting, the Committee considered that a minimum assay value of 95% for an individual flavouring agent would apply to both the flavouring agent itself and to the agent plus its known secondary components. The minimum assay values of about 90% of the flavouring agents evaluated to date meet or exceed 95%, and the Committee received

information about the nature of the secondary components of the others. The Committee noted that 95% is not a fixed value for the acceptability of specifications for flavouring agents and that flexibility can be used in establishing an acceptable level of secondary components, taking into account the probable levels of intake and other considerations.

Many of the secondary components are structurally related to the named flavouring agents. They typically comprise small amounts of starting materials, isomers and other flavouring agents. As these secondary components share many of the properties of the named flavouring agents, and in some cases are metabolites, their safety would not be expected to present a concern or can be evaluated from appropriate data on metabolism and toxicity.

The Committee noted that, in applying the Procedure for the Safety Evaluation of Flavouring Agents, information on secondary components included in the specifications should be considered with data on intake and on the potential toxicity of the flavouring agent and its structural analogues. The Committee therefore recommended that data on specifications be submitted before or at the same time as all other information necessary for evaluating the safety of a flavouring agent.

2.7 Requests for data relating to intake assessments

The Committee recognized that it is unnecessary to request data for assessing intake for all the substances on its agenda, as it had done recently, and developed criteria for determining when such information would be needed. These are described below. In general, calls for data should specify the information required for each substance on the agenda, as the data required for evaluating food additives are different from those required for evaluating contaminants.

2.7.1 Food additives

Data for assessing intake should be requested in the case of food additives that are being evaluated for the first time or are being re-evaluated, except for food additives:

- for which only specifications are to be considered;
- for which the Committee has recently deferred an evaluation, pending the provision of a specific toxicological study or information on specifications, provided it has evaluated intake of the additive during the preceding 3–5 years.

Information on proposed maximum levels should be provided in the call for data for food additives included in the draft Codex General

Standard for Food Additives, so that national assessments of intake based on such maximum levels, national maximum levels and/or actual levels of use can be submitted. The Committee has formulated data sheets for submission of national data, which are included in the guidelines for the preparation of working papers on the intake of food additives.

2.7.2 Contaminants

For contaminants, an intake assessment is required in all cases. The call for data should request data on:

- the occurrence and concentrations of the contaminant (both individual and summary data) from all available sources, preferably submitted on data sheets, with information on sampling and analytical techniques, data quality and reliability, reporting conventions and appropriate processing factors;
- national intake of the contaminant derived from national surveys of food consumption and concentrations.

2.8 Principles governing the establishment and revision of specifications

2.8.1 Inclusion of raw materials and manufacturing methods in specifications

Principles for the safety assessment of food additives and contaminants in food (Annex 1, reference 76) states that “to establish the chemical identities of additives, it is necessary to know the nature of the raw materials, methods of manufacture and impurities. This information is used to assess the completeness of analytical data on the composition of additives, and to assess the similarity of materials used in biological testing with those commercially produced.”

As there are increasing volumes of food additives in international trade, specifications must include brief descriptions of the raw materials and methods of manufacture used, excluding proprietary details, in order to provide a full account of the product being evaluated. If this information is not available, the Committee cannot know whether the products being evaluated were produced from materials or methods that are different from those in the specifications; consequently, impurities might have arisen that were not considered during the toxicological evaluation. The level of detail of the descriptions should be similar to that in specifications elaborated by the Committee for additives made by fermentation or from plant materials.

2.8.2 **General specifications and considerations for enzyme preparations used in food processing**

The Committee has, on many occasions, addressed issues related to specifications for enzyme preparations used in food processing. The general specifications currently in use for enzymes were first elaborated by the Committee at its twenty-sixth meeting (Annex 1, reference 59). Several revisions have been made, including the following:

- an addendum to address issues arising from use of enzymes from genetically modified microorganisms (Annex 1, references 94, 96, 137 and 139);
- addition of an appendix to describe the method for determining antimicrobial activity (Annex 1, reference 58);
- an amendment to address inclusion of microbial strain numbers in the specifications for enzyme preparations (Annex 1, reference 139);
- addition of the general requirement that source microorganisms be non-pathogenic and non-toxicogenic (Annex 1, reference 145).

At its fifty-fifth meeting (Annex 1, reference 149), the Committee reiterated its view, expressed at its fifty-third meeting (Annex 1, reference 145), that Annex 1 (General specifications for enzyme preparations used in food processing) of the *Compendium of food additive specifications* (Annex 1, reference 96) required updating in the light of technological developments and to ensure consistency and coherence with the appendices, including Appendix B (General considerations and specifications for enzymes from genetically manipulated microorganisms).

At its present meeting, the Committee noted that the revised general specifications require that all new enzyme preparations undergo a general safety assessment. Many of the requirements previously outlined for enzyme preparations from genetically modified microorganisms are appropriate for all preparations, regardless of source, and the Committee revised the general specifications to reflect those requirements. For enzymes from genetically modified sources, information is now required on the microbial strain used as the source organism and the genetic material introduced into and remaining in the final microbial strain used in production.

At its present meeting, the Committee noted that the list of mycotoxins contained in the existing general specifications was not relevant to all food enzyme preparations from fungal sources. It agreed that an attempt to list all known mycotoxins of potential concern was impractical and unwarranted. The Committee further agreed that enzyme preparations derived from fungal sources be evaluated for those mycotoxins that are known to be synthesized by strains of the species or related species used in the production of the enzyme preparation.

With regard to limits on heavy metals, the Committee agreed that the specifications for lead contained in the existing general specifications should be lowered from 10mg/kg to 5mg/kg. The Committee recognized that arsenic is not a concern in enzyme preparations, and therefore deleted the limit for this metal. Moreover, as there is no traceable source of cadmium or mercury in enzyme preparations, the Committee saw no need to establish limits for those metals. Such changes are consistent with the Committee's current policy on heavy metals (Annex 1, reference 145).

In considering microbiological contamination of enzyme preparations, the Committee agreed that the existing microbiological criteria (for *Salmonella* spp., *Escherichia coli* and total coliforms) and the requirement that use of preparations should not increase the total microbial count in treated food above the threshold considered to be acceptable for the respective foods are sufficient to ensure microbial safety. The criteria and the requirement were thus retained. The Committee noted that the specification for a total viable count of 5×10^4 organisms per gram included in the existing general specifications did not provide an indication of the safety of an enzyme preparation. It was therefore deleted.

In considering allergenic potential, the Committee emphasized that, when the source organism of an enzyme preparation is a genetically modified microorganism, the necessity for evaluating the allergenic potential of the gene products encoded by the inserted DNA should be assessed. The Committee agreed that, when the DNA sequence of an enzyme from a genetically modified production microorganism is comparable to that coding for an enzyme already known to have a history of safe use in food, there would be no need to assess the allergenic potential of that enzyme further.

Finally, the Committee recognized that the revised specifications include many criteria for safety evaluation that would be more appropriately listed elsewhere. The Committee strongly recommended that *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference 76) be revised to include the safety assessment of enzymes intended for use in food and that such guidelines should subsequently be removed from the general specifications.

3. **Specific food additives (other than flavouring agents)**

The Committee evaluated two food additives for the first time and re-evaluated a number of others. Information on the safety evaluations

and on specifications is summarized in Annex 2. Details of further toxicological studies and other information required for certain substances are given in Annex 3.

3.1 **Safety evaluations**

3.1.1 **Emulsifiers**

3.1.1.1 *Diacetyltartaric and fatty acid esters of glycerol*

Diacetyltartaric and fatty acid esters of glycerol were reviewed by the Committee at its tenth and seventeenth meetings (Annex 1, references 13 and 32). At its seventeenth meeting, the Committee allocated an ADI of 0–50 mg/kg of body weight on the basis of the results of biochemical and metabolic studies and feeding tests in animals. At the same meeting, the Committee also reviewed mixed tartaric, acetic and fatty acid esters of glycerol and allocated an ADI “not limited”, with the provision that the total intake of tartaric acid from food additives should not exceed 30 mg/kg of body weight per day.

Specifications established by the Committee at its fifty-first meeting (Annex 1, reference 137) covered both the above-mentioned products under the name “diacetyltartaric and fatty acid esters of glycerol”, as the Committee was aware that the two products could not be distinguished analytically. At that meeting, the Committee recommended that the material defined in the specifications be evaluated toxicologically. At its present meeting, the Committee considered the data that were available previously as well as newly submitted information.

The diacetyltartaric and fatty acid esters of glycerol consist of mixed glycerol esters of mono- and diacetyltartaric acid and fatty acids of food fats. They can be manufactured either by the interaction of diacetyltartaric anhydride and mono- and diglycerides of fatty acids in the presence of acetic acid, or by the interaction of acetic anhydride and mono- and diglycerides of fatty acids in the presence of tartaric acid.

Owing to inter- and intramolecular exchange of acyl groups, the two methods of production result in essentially the same components, the distribution of which depends on the relative proportions of the basic raw materials, on temperature and on reaction time. Diacetyltartaric and fatty acid esters of glycerol may contain small amounts of free glycerol, free fatty acids and free tartaric and acetic acids. They may be further specified as to the acid value, total tartaric acid content, free acetic acid content, saponification value, iodine value, free fatty acid content and the solidification point of the free fatty acids.

The draft Codex General Standard for Food Additives includes use of diacetyltartaric and fatty acid esters of glycerol as an emulsifier, sequestrant and stabilizing agent in a wide range of foods at a maximum concentration of 10 g/kg.

Biological data. Biochemical studies suggest that diacetyltartaric and fatty acid esters of glycerol are hydrolysed in the gastrointestinal tract to yield mono- and diglycerides and acetylated tartaric acid. As mono- and diglycerides are natural dietary constituents, they would be subjected to natural digestion and absorption processes. Diacetyltartaric acid is not a natural constituent of the diet, and there is evidence that it may be further hydrolysed to yield acetic and tartaric acids. When labelled diacetyltartaric and fatty acid esters of glycerol were administered to rats, only about one-third of a ^{14}C label on tartaric acid was absorbed; slightly more was excreted in expired air than in urine.

The studies reviewed previously indicated little toxicity after administration of a single oral dose of diacetyltartaric and fatty acid esters of glycerol. Three studies of the potential long-term toxicity of this product when given in the diet to small numbers of rats showed no adverse effects of dietary concentrations of up to 200 g/kg on mortality rate, physical appearance, body weight, food consumption, reproduction or the histological appearance of the main organs. Dogs also showed no adverse effects when fed diets containing concentrations of up to 200 g/kg for more than 2 years.

The information reviewed for the first time at the present meeting consisted of a 2-week study of palatability, a long-term study of toxicity and carcinogenicity, a two-generation study of reproductive toxicity and a study of developmental toxicity, all conducted in rats, plus two studies of genotoxicity, for point mutations in bacteria and for clastogenicity in isolated human lymphocytes. In addition, a 6-month study was conducted in male rats to elucidate some of the effects seen in the long-term study of toxicity and carcinogenicity.

In the short- and long-term studies in rats, diacetyltartaric and fatty acid esters of glycerol at a concentration of 100 g/kg of diet caused a transient occurrence of soft stools, particularly in males. Food consumption was frequently depressed at this concentration, most consistently during the first weeks of treatment. The palatability of the diet was not improved by incorporating diacetyltartaric and fatty acid esters of glycerol into breadcrumbs or by volatilizing the fatty acids in the substance by freeze-drying the diet before administration. Body-weight gain tended to be reduced, but this effect was not observed consistently in the short-term study. In the long-term study, consumption of a diet containing diacetyltartaric and fatty acid esters of glycerol at 100 g/kg was associated with decreased body weight. This effect was transient in male rats, but body weights more than 10% lower than those of controls persisted in females into the second year of the study. Supplementing the diet with additional protein,

magnesium, pyridoxine (vitamin B₆) and cyanocobalamin (vitamin B₁₂) reduced the decrease in weight observed with the compound at 100 g/kg of diet in the 6-month study.

Administration of diacetyltartaric and fatty acid esters of glycerol was associated with a decrease in the proportion of lymphocytes and an increase in the proportion of neutrophils in the total leukocyte count during the 6-month study and during the first year of the long-term study. However, these effects were transient and dependent on the type of diet.

In the 6-month study, inclusion of diacetyltartaric and fatty acid esters of glycerol at a concentration of 60 or 100 g/kg of diet was associated with an increase in the urinary excretion of calcium. In the long-term study, differences in the weights of the adrenal glands, kidneys and spleen were observed after 1 year of treatment but were no longer evident after 2 years. Males fed a diet containing 100 g/kg showed an increase in both the incidence and the severity of mineralization in the kidney papilla and pelvis after 1 and 2 years of treatment. Administration at 100 g/kg of diet for 2 years resulted in an increased prevalence of microscopic abscesses in the kidneys of males and an increased severity of nephrocalcinosis in females.

After 2 years of treatment, a dose-related increase in the incidence of adrenal medullary adenomas was seen in males, affecting 4/50, 6/50, 11/50 and 15/50 (statistically significant) animals at 0, 30, 60 and 100 g/kg of diet, respectively, and 1/50 and 4/50 females at 0 and 100 g/kg of diet, respectively. Focal medullary hyperplasia was observed in 3/50, 10/48 (statistically significant), 15/50 (statistically significant) and 15/50 (statistically significant) males at 0, 30, 60 and 100 g/kg of diet, respectively, and in 0/50 and 9/50 (statistically significant) females at 0 and 100 g/kg of diet, respectively.

Statistically significantly higher incidences of haemangioma and haemorrhage in the mesenteric lymph nodes were observed in males fed diets containing 100 g/kg of diacetyltartaric and fatty acid esters of glycerol, while the incidence of sinus histiocytosis of the mesenteric lymph nodes was statistically significantly increased in all treated males.

Myocardial fibrosis was observed more frequently in males at the highest dietary concentration (13/50) than in the control group (3/50). Females at the highest concentration had higher incidences of endometrial hyperplasia (7/50 vs 0/50) and cystic endometrial hyperplasia (14/50 vs 7/50) than controls at the end of the study. Histopathological examinations were carried out on the hearts of only

some of the males and on the uteri of only some of the females at the two lower doses.

In a two-generation study of reproductive toxicity, parental males of the F₀ generation ate less of the diet containing 100 g/kg of diacetyltartaric and fatty acid esters of glycerol and gained less weight during the pre-mating period. Although F₀ females at this dietary concentration also ate less food during the first few weeks of the study, their body-weight gains were not affected. The body weights, body-weight gains and food consumption of the F₁ adults were unchanged. The survival of the F₁ and F₂ litters was not affected by treatment. The weight gains during lactation of the F₁ generation litters of dams given diacetyltartaric and fatty acid esters of glycerol at concentrations of 60 or 100 g/kg of diet and of the F₂ generation litters of dams at 100 g/kg of diet were significantly reduced. The reproductive organs were not assessed histologically. The NOEL for reproductive toxicity was 30 g/kg of diet, equivalent to 1500 g/kg of body weight per day.

Evaluation. High dietary concentrations of diacetyltartaric and fatty acid esters of glycerol were associated with decreased body weights in adult rats and their offspring, but it could not be ascertained from the available data whether these decreases were secondary to or independent of decreased food consumption.

In the 2-year study in rats, the groups treated with diacetyltartaric and fatty acid esters of glycerol were apparently compared with controls fed diets containing monoglyceride. In order to assess whether some of the adverse effects were treatment-related, it would be necessary to compare the effects in treated groups with those in both untreated and monoglyceride-treated control groups, and to compare the control groups with one another. In the absence of additional data on the incidence of myocardial fibrosis and adrenal medullary hyperplasia in animals at the lowest and intermediate doses, no NOEL could be identified in the long-term study. The previous ADI of 0–50 mg/kg of body weight was made temporary until 2003, pending submission of all the necessary additional information.

The specifications for diacetyltartaric and fatty acid esters of glycerol were revised. As specifications no longer exist for tartaric, acetic and fatty acid esters of glycerol, mixed, the previous ADI is no longer applicable and was withdrawn.

A toxicological monograph was prepared, incorporating information from the earlier monographs and summaries of the studies reviewed for the first time at the present meeting.

3.1.1.2 *Quillaia extracts*

Quillaia extracts (synonyms: bois de Panama, Panama bark extracts, quillai extracts, Quillay bark extracts, soapbark extracts) are obtained by aqueous extraction of the milled inner bark or wood of pruned stems and branches of *Quillaja saponaria* Molina (family Rosaceae), which is a large evergreen with shiny, leathery leaves and a thick bark, native to China and several South American countries, principally Bolivia, Chile and Peru. The term “quillaia” refers to the dried inner bark of the tree.

Unpurified extracts contain over 60 triterpenoid saponins, consisting predominantly of glycosides of quillaic acid. Polyphenols and tannins are major components. Some simple sugars and calcium oxalate are also present. The saponin concentration of freshly prepared, unpurified extracts is 190–200 g/kg of solids (about 20%). The extracts are treated with “stabilizing agents” such as egg albumin and polyvinylpyrrolidone and then filtered through diatomaceous earth. The stabilizing agents remove substances that would probably precipitate during storage, such as protein–polyphenol complexes. After filtration, the liquid is concentrated, and the concentrate may be sold as such (solids constituting about 550 g/l) or be spray-dried and sold as a powder containing carriers such as lactose and maltodextrin. The unpurified extracts are used in food applications, primarily for their foaming properties.

Semi-purified powdered extracts are produced by subjecting unpurified extracts to ultra-filtration or affinity chromatography to remove most solids other than saponins, such as polyphenols. These semi-purified extracts have higher saponin concentrations (750–800 g/kg of solids; about 80%) and better emulsifying properties than unpurified extracts.

Highly purified extracts are produced for use as adjuvants in the production of animal and human vaccines and not for food use. These products generally contain more than 90% saponins.

In previous evaluations, the Committee considered data on unpurified quillaia extracts. Quillaia extracts were reviewed toxicologically by the Committee at its twenty-sixth meeting (Annex 1, reference 59). The available toxicological data included adequate lifetime studies in mice and rats, from which a NOEL was identified. However, in the absence of data, no specifications were prepared, and, hence, no ADI could be allocated. At its twenty-ninth meeting (Annex 1, reference 70), the Committee prepared new tentative specifications and established an ADI of 0–5 mg/kg of body weight. The present evaluation was conducted in response to a request by the Codex Committee on Food Additives and Contaminants at its Thirty-

second Session (4) that the Expert Committee re-evaluate all relevant information on the toxicity and, in particular, the intake of quillaia extracts. No new data were submitted to the Committee at its present meeting. The Committee evaluated published reports on quillaia extracts or specific saponins that provided information relevant to a toxicological assessment of quillaia extracts.

Biological data. Quillaia extracts are mixtures of biologically active compounds that include saponins, tannins, polyphenols and calcium oxalate. The saponins present in quillaia extracts have a variety of biological activities: they are haemolytic, cytotoxic, enhance immune reactions, cause mucosal irritation and inflammation and are anti-hypercholesterolaemic. The biological activities and the potency of individual saponins vary widely and depend primarily on the route of administration.

Studies of acute toxicity showed that quillaia extracts are less toxic when administered orally than when administered subcutaneously or intravenously. Fractions isolated from *Q. saponaria* differed widely in acute toxicity as well as in adjuvant activity and cholesterol-binding capacity. QS-18, the major saponin of quillaia extracts, was more acutely toxic to mice than two other saponins that were isolated and was more toxic than the extract itself when administered intradermally.

In a 90-day study, rats were fed diets containing 40g/kg quillaia extract (equivalent to 2000mg/kg of body weight per day). The specifications of the preparation conformed to the Emulsifiers and Stabilisers in Food Regulations 1975 of the United Kingdom, but information on the actual composition of the material tested was not available. The animals showed decreased body-weight gain, decreased weight of the liver relative to body weight and increased stomach weight, with no treatment-related histological changes. The NOEL was 6 g/kg of diet, equivalent to 400mg/kg of body weight per day.

In a more recent 90-day study, rats were given quillaia saponins in deionized water by gavage at a dose of 1200mg/kg of body weight. Severe and lethal toxic effects were observed during the study. In the surviving animals, the weights of several organs were increased, and several haematological and clinical parameters were changed. Histopathological examination showed inflammatory changes in the forestomach, larynx, trachea and lungs.

Minor changes in body-weight gain and in the relative weights of some organs were reported in lifetime studies in mice and rats given

quillaia extracts (with specifications conforming to the Emulsifiers and Stabilisers in Food Regulations 1975 of the United Kingdom), at dietary concentrations of up to 30 g/kg for mice and 15 g/kg for rats. No compound-related histopathological changes were reported. The NOELs for quillaia extracts in the diet were 5 g/kg (equivalent to 700 mg/kg of body weight per day) for mice and 10 g/kg (equivalent to 500 mg/kg of body weight per day) for rats.

The Committee noted that the differences in toxicity observed in the 90-day studies in rats treated orally, outlined above, might have been due to differences in the concentrations and types of saponins present in the test material and/or differences in the method of administration, i.e. in the diet and by gavage in water.

Evaluation. The existing specifications for quillaia extracts were revised in order to clarify the differences between unpurified and semi-purified extracts. As additional information on composition was determined to be necessary, the specifications were designated as tentative. Once the requested information has been received, the Committee will consider whether separate specifications for unpurified and semi-purified extracts are required.

The Committee decided that the previously established ADI of 0–5 mg/kg of body weight for unpurified extracts should be made temporary and extended it until 2003, pending clarification of the specifications for quillaia extracts; further studies of toxicity with specified quillaia products similar to the product consumed by humans may be required. The Committee emphasized that the temporary ADI is not applicable to the semi-purified extract or to any other product derived from *Q. saponaria* or from other species of *Quillaia*.

Assessment of intake. Quillaia extracts can be used as foaming agents in soft drinks and cocktail mixes and as emulsifiers in foods such as baked goods, sweets, frozen dairy products, gelatine and puddings. Their major food use is in soft drinks such as ginger beer, root beer and cream soda.

Calculations based on the temporary ADI of 0–5 mg/kg of body weight and the assumption that quillaia extracts are used in soft drinks at a concentration of 500 mg/kg indicated that a person weighing 60 kg could drink up to 600 g/day of a soft drink before exceeding the ADI, while a child weighing 15 kg could drink only 150 g/day of a soft drink before exceeding the ADI. Data on food consumption submitted to the Committee indicated that consumers in Australia who are at the 95th percentile of the distribution of consumption of soft drinks that are likely to contain the additive and children aged 1.5–4 years in the United Kingdom who consume soft drinks at the

97.5th percentile could exceed these amounts. However, these calculations may have overestimated long-term consumption because the data were derived from short-term surveys.

Estimates of intake based on consumption of soft drinks likely to contain this food additive and the levels of use of quillaia extracts permitted in the draft Codex General Standard for Food Additives were submitted by Australia and the USA. Estimates of the mean intake in the United Kingdom were also available, which were based on consumption of all water-based flavoured drinks and are therefore conservative. In Australia, the mean intakes were 3 mg/kg of body weight per day (60% of the ADI) for consumers of drinks containing the additive at the level permitted in the draft Codex General Standard for Food Additives (500 mg/kg) and 7.2 mg/kg of body weight per day (145% of the ADI) for persons at the 95th percentile of consumption. In the USA, the estimated mean intakes of quillaia extracts were 1.5 mg/kg of body weight per day (30% of the ADI) for consumers of drinks containing the additive at the level permitted in the draft Codex General Standard for Food Additives and 2.7 mg/kg of body weight per day (54% of the ADI) for consumers at the 90th percentile.

Estimates of intake based only on consumption of soft drinks likely to contain the food additive and national levels of use were submitted by the USA. The maximum level of use of quillaia extracts by manufacturers in the USA is 100 mg/kg. The estimated mean intake by consumers was 0.3 mg/kg of body weight per day (6% of the ADI), and that by consumers at the 90th percentile was 0.54 mg/kg of body weight per day (11% of the ADI). Data from the United Kingdom, based on a level of use by manufacturers of 95 mg/kg, indicated that children who consume soft drinks at the 97.5th percentile level would consume quillaia extracts at 5.2 mg/kg of body weight per day (105% of the ADI), but this value may be an overestimate of intake as it is based on consumption of all water-based flavoured drinks.

Use at a maximum level of 95–100 mg/day (that reported by the manufacturers), as in the United Kingdom and the USA, appeared to be adequate for the technological functioning of quillaia extracts as foaming agents in soft drinks and did not appear to result in intakes that exceed the ADI. Young children are a possible exception, but, as the results of a short-term nutritional survey were used, the frequency or duration of their potential excursion above the ADI could not be determined.

The Committee recommended that the Codex Committee on Food Additives and Contaminants review the use of quillaia extracts at

500mg/kg proposed in the draft Codex General Standard for Food Additives.

A toxicological monograph was prepared.

3.1.2 **Enzyme preparation**

3.1.2.1 *Invertase from *Saccharomyces cerevisiae**

Invertase from *Saccharomyces cerevisiae*, or “bakers’ yeast”, hydrolyses sucrose to a mixture of glucose and fructose (invert sugar). This substance was reviewed by the Committee at its fifteenth meeting (Annex 1, reference 26) as one of the active principles of carbohydrase from *Saccharomyces* species. It is produced by controlled, submerged fermentation of a pure culture of *S. cerevisiae*. At the end of fermentation, the yeast cells are collected, washed and subjected to autolysis. The lysate is centrifuged and/or filtered to remove cell debris. The resulting enzyme preparation may be dried or ultra-filtered to a desired enzyme concentration. Liquid ultra-filtered products can be treated further with activated charcoal to remove colour and then filtered under sterile conditions. The invertase product is standardized with food-grade diluents.

At its fifteenth meeting (Annex 1, reference 26), the Committee concluded that enzymes derived from microorganisms that are traditionally accepted as constituents of foods or are normally used in the preparation of foods should themselves be regarded as foods. Invertase from *S. cerevisiae* was evaluated at the present meeting because it was being considered for inclusion in the draft Codex General Standard for Food Additives.

Invertase is fundamental to the manufacture of soft-filled chocolates and liquid-centre confectionery, there being no additive that fulfils the same technological function. In the filling for chocolates, invertase is used at a concentration of 1 g/kg of sucrose, resulting in a concentration of 0.6 g/kg (600 mg/kg) in the finished product.

The intake of invertase predicted by the Scientific Committee on Food of the Commission of the European Union was 15 mg/day, assuming consumption of 25 g of filled chocolates per day out of a total of 50 g of chocolate of all types and a concentration of invertase of 600 mg/kg of chocolate.

The potential intake of invertase from its use in chocolates was also predicted for the Australian population from individual dietary records obtained in a survey in 1995. Assuming consumption of 600 mg/kg of filled chocolate, the mean invertase intake by consumers was 20 mg/day (33 g/day of filled chocolate), and that of persons at the 95th percentile of consumption was 61 mg/day (100 g/day of filled choco-

late). These estimates were based on 24-h recalls of food consumption, which tend to result in overestimates of consumption on a long-term basis. The intake of invertase by young children and adolescents was similar to that of adults but would be relatively higher than that of the general population if expressed per kilogram of body weight.

No biological data were available. *S. cerevisiae* has a well-established history of use in fermented foods, including bread, alcoholic beverages, some milk products and cocoa. In line with the general principles outlined in *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference 76), invertase from *S. cerevisiae* that meets the specifications developed at the present meeting was considered to be acceptable, as *S. cerevisiae* is commonly used in the preparation of food. Its use should be limited by good manufacturing practice.

A toxicological monograph was not prepared. New specifications were prepared.

3.1.3 **Food colours**

3.1.3.1 *β*-Carotene from *Blakeslea trispora*

The Committee did not undertake a general re-evaluation of β -carotene for use as a colouring agent but focused its assessment on the production and analytical characteristics of β -carotene produced from *Blakeslea trispora*.

β -Carotene is obtained from *B. trispora* by co-fermentation of the two sexual types of the fungus in specific proportions. Both types are stable in cultures maintained under conditions consistent with good manufacturing practice. These source organisms are neither pathogenic nor toxinogenic. The compound is isolated from the fungal biomass by solvent extraction and crystallized. The main articles of commerce are suspensions in food-grade vegetable or plant oil and water-dispersible powders, which are easy to use and improve stability, as carotenes readily undergo oxidation.

As in synthetic β -carotene, the colouring principle of β -carotene from *B. trispora* consists predominantly of the all-*trans* isomer of β -carotene. The content of total colouring matter is not less than 96% (expressed as β -carotene). β -Carotene from *B. trispora* may also contain other carotenoids, of which γ -carotene accounts for the major part, at concentrations up to 3%. These molecules occur naturally in carotenoid-containing vegetables.

The Committee considered that the source organisms, the production process and the composition of β -carotene from *B. trispora* do not

raise specific concerns and that the material should be considered toxicologically equivalent to chemically synthesized β -carotene, for which an ADI of 0–5 mg/kg of body weight was established by the Committee at its eighteenth meeting (Annex 1, reference 35). This opinion was given further credence by the negative results obtained in two tests for genotoxicity (mutagenicity and chromosomal aberration) considered at the present meeting. Therefore, the Committee established a group ADI of 0–5 mg/kg of body weight for synthetic β -carotene and β -carotene derived from *B. trispora*. The ADI relates strictly to use of β -carotene as a food colouring agent and not to its use as a food supplement.

Use of this preparation is unlikely to result in increased use of β -carotene as a food colour because the material is expected to be substituted for synthetic β -carotene.

A toxicological monograph was prepared. New specifications were prepared and designated as tentative, pending information on a suitable method for determination of residual ethyl acetate and isobutyl acetate used as solvents. This information is required by 2003.

3.1.3.2 Curcumin

Curcumin is obtained by solvent extraction of turmeric, which is in turn derived from ground rhizomes of *Curcuma longa* L. (*C. domestica* Valetton). In order to obtain concentrated curcumin powder, the extract is purified by crystallization. The commercial product consists predominantly of curcumins: the colouring principle (1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) and its desmethoxy and bisdesmethoxy derivatives, in varying proportions. Minor amounts of oils and resins that occur naturally in turmeric may be present.

Turmeric oleoresin and curcumin, the main colouring component of turmeric oleoresin, were evaluated by the Committee at its thirteenth, eighteenth, twenty-second, twenty-fourth, twenty-sixth, thirtieth, thirty-fifth, thirty-ninth, forty-fourth and fifty-first meetings (Annex 1, references 19, 35, 47, 53, 59, 73, 88, 101, 116 and 137). At its eighteenth meeting, the Committee established a temporary ADI of 0–0.1 mg/kg of body weight for curcumin on the basis of the ADI for turmeric oleoresin (0–2.5 mg/kg of body weight) and an assumed average concentration of 3% curcumin in turmeric. The temporary ADI for curcumin was extended at the twenty-second, twenty-fourth, twenty-sixth, thirtieth, thirty-fifth and thirty-ninth meetings of the Committee. At its thirty-ninth meeting, the Committee requested the results of studies of carcinogenicity in mice and rats fed turmeric

oleoresin and the results of a study of reproductive and developmental toxicity with curcumin.

At its forty-fourth meeting, the Committee evaluated the results of the studies of carcinogenicity in rats and mice given turmeric oleoresin containing 79–85% curcumin and new data on the biochemistry and genotoxicity of the compound. The Committee concluded that data on the developmental toxicity of curcumin were no longer required, but reiterated its request for a study of reproductive toxicity. On the basis of a NOEL of 220mg/kg of body weight per day in the study of carcinogenicity in mice and a safety factor of 200, the Committee increased the temporary ADI to 0–1 mg/kg of body weight and extended it, pending submission of the results of a study of reproductive toxicity with curcumin.

At its fifty-first meeting, the Committee evaluated the results of studies of fertility in rats and mice treated with turmeric oleoresin and concluded that they did not provide assurance that the potential reproductive effects of curcumin had been adequately investigated. The Committee again extended the temporary ADI, pending submission of the results of a study of reproductive toxicity with a substance that complied with the specifications for curcumin, for review in 2001.

The results of the requested study were not available to the Committee at its present meeting. Nevertheless, the Committee was informed that a multigeneration study of reproductive toxicity with a substance that complied with the specifications for curcumin was under way and would be completed within the next few months. In view of this information, the temporary ADI of 0–1 mg/kg of body weight for curcumin was extended until 2003, pending submission of the results of this study.

A toxicological monograph was not prepared. The existing specifications were revised, with minor changes.

3.1.4 **Food salts**

3.1.4.1 *Phosphates, diphosphates and polyphosphates*

Phosphates, diphosphates and polyphosphates were evaluated by the Committee at its sixth, seventh, eighth, ninth, thirteenth, fourteenth, seventeenth and twenty-sixth meetings (Annex 1, references 6–8, 11, 19, 22, 32 and 59). A maximum tolerable daily intake (MTDI) of 70mg/kg of body weight was established at the twenty-sixth meeting on the basis of the lowest concentration of phosphorus (6600mg/day) that caused nephrocalcinosis in rats. It was considered inappropriate to establish an ADI, as phosphorus (as phosphates) is an essential nutrient and an unavoidable constituent of food. The MTDI is

expressed as phosphorus and applies to the sum of phosphates naturally present in food and the phosphates derived from use of these food additives.

This MTDI was considered to cover a number of phosphate salts, according to the principle established by the Committee at its ninth, twenty-third and twenty-ninth meetings (Annex 1, references 11, 50 and 70) that the ADI (or MTDI) established for ionizable salts should be based on previously accepted recommendations for the constituent cations and anions. However, in this case, while an MTDI has been established for the class of phosphate salts, certain specific salts were not included because specifications were lacking and because information was not available to indicate whether they were being used as food-grade materials.

At its present meeting, the Committee established specifications for certain specific phosphate salts, pending further information, as indicated below.

- Calcium dihydrogen diphosphate is manufactured by calcination of calcium orthophosphate at a temperature of about 270°C, with a molar ratio of calcium:phosphorus of about 1:2. It is used in fine bakery wares at concentrations of up to 20 g/kg.
- Monomagnesium orthophosphate is manufactured by partial neutralization of phosphoric acid with magnesium oxide and drying the resultant product. It is used in fine bakery wares at concentrations of up to 20 g/kg. The Committee noted that this substance is more concisely denoted as monomagnesium phosphate. It therefore deleted the prefix “ortho” for the substance in the specifications. The specifications were made tentative, pending further information on loss on drying, loss on ignition, the test method for loss on ignition and the assay method for the dihydrate.
- Sodium calcium polyphosphate is manufactured by the fusion of sodium phosphate and calcium carbonate at temperatures greater than 1000°C. Phosphoric acid is neutralized with sodium and calcium hydroxides in a molar ratio of 5:1. The resulting mixture undergoes calcination and is cooled, milled and sieved. It is used in processed cheese and processed cheese analogues at concentrations of up to 20 g/kg.
- Trisodium diphosphate is manufactured by hydration and drying of mixtures of sodium diphosphate. It is used in meat products at concentrations of up to 5 g/kg. The specifications were made tentative, pending further information on loss on drying, loss on ignition, the test method for loss on ignition and the assay method for the monohydrate.

The Committee included these salts in the group MTDI for phosphates, diphosphates and polyphosphates.

A toxicological monograph was not prepared.

3.1.5 **Glazing agent**

3.1.5.1 *Hydrogenated poly-1-decene*

Hydrogenated poly-1-decene is obtained by catalytic hydrogenation of mixtures of trimers, tetramers, pentamers and hexamers of 1-decenes, produced by oligomerization of 1-decene in the presence of a catalyst. The product is purified by filtration through activated clay. Hydrogenated poly-1-decene consists of a mixture of branched isomeric hydrocarbons, predominantly with more than 30 carbon atoms. Minor amounts of molecules with fewer carbons may be present.

Hydrogenated poly-1-decene was first evaluated by the Committee at its forty-ninth meeting (Annex 1, reference 131) for use as a glazing and releasing agent. A 28-day range-finding study and a 90-day study in rats that were available at that time were considered inadequate to support use of this product as a food additive. Data were requested to demonstrate that the oily coats observed in rats fed hydrogenated poly-1-decene in the 90-day study were not the result of systemic absorption of the material. The Committee also requested that the results of a study demonstrating lack of absorption in humans be provided. In the absence of such data, the results of long-term studies of toxicity and reproductive toxicity and information on the metabolism, distribution and excretion of hydrogenated poly-1-decene would be required.

At its fifty-third meeting (Annex 1, reference 143), the Committee reviewed a study of the distribution and excretion of [³H]hydrogenated poly-1-decene conducted in rats. This study established that the oiliness of the fur observed within 1–6 h of administration of a bolus dose was associated with radiolabelled material originating from the anal region, which was spread by grooming. However, while the study indicated that very little hydrogenated poly-1-decene was absorbed after oral administration, it did not allow clear definition of the fate or disposition of any absorbed material. The Committee was therefore unable to establish an ADI and requested an adequate study of the absorption and deposition of hydrogenated poly-1-decene in order to determine whether further studies were required.

At its present meeting, the Committee re-evaluated the results of the study of the distribution and excretion of hydrogenated poly-1-decene. Although no additional studies on distribution and excretion were submitted, the Committee's attention was drawn to arguments

that supported the validity of the previous study. In addition, the Committee evaluated a study of the effect of hydrogenated poly-1-decene on the absorption, distribution and excretion of linoleic acid and glycerol trioleate that had been submitted for consideration.

The Committee also revised the existing specifications for hydrogenated poly-1-decene in order to take into account the decrease from 3% to 1.5% in the concentration of molecules with fewer than 30 carbon atoms in products on the market for food additive use.

In its re-evaluation, the Committee accepted that equivalent information can be obtained with ^3H and ^{14}C , provided that the label is located in a metabolically stable position, as was the case for [^3H]hydrogenated poly-1-decene. It also accepted that, for technical reasons, use of ^{14}C -labelled hydrogenated poly-1-decene might be less appropriate, as the synthetic ^{14}C -labelled compound might be different from the substance used in the studies of toxicity. The results of the study indicated that <1% of the dose of [^3H]hydrogenated poly-1-decene was absorbed from the gut. The absorbed radiolabel was present largely as $^3\text{H}_2\text{O}$, probably arising from tritium exchange between the labelled substance and body water. The Committee concluded that absorption of hydrogenated poly-1-decene was negligible. This conclusion was corroborated by the results of the 90-day study in rats, which provided no evidence of its accumulation in tissues. Furthermore, the revised specifications for the substance, which require that it contains a maximum of 1.5% of compounds with fewer than 30 carbon atoms should ensure that absorption of components of low relative molecular mass is kept to a minimum.

The Committee concluded that the available studies were adequate to assess the toxicity and safety of hydrogenated poly-1-decene. An ADI of 0–6 mg/kg of body weight was established on the basis of the NOEL of 550 mg/kg of body weight per day in the 90-day study in rats for effects on the condition of the fur, liver weight and histological appearance, and a safety factor of 100.

An additional study in rats submitted for consideration by the Committee suggested that hydrogenated poly-1-decene may decrease the bioavailability of linoleic acid, an essential fatty acid. However, the Committee concluded that a nutritionally relevant decrease in bioavailability would not occur if hydrogenated poly-1-decene was consumed at the level of the ADI, i.e. a maximum of 360 mg/person per day.

Hydrogenated poly-1-decene can be used as a release agent in bread prepared in commercial baking operations at concentrations of up to 300–500 mg/kg and in glazed fruit at concentrations of up to 2000 mg/kg. Bread is expected to be the major source of total intake of

this compound. If use only in bread is assumed, it can be calculated that up to 720g of bread containing hydrogenated poly-1-decene at a concentration of 500mg/kg could be consumed by a 60-kg person before the ADI of 0–6mg/kg of body weight was exceeded. However, it was considered highly unlikely that a person would consume this amount of bread containing hydrogenated poly-1-decene at the maximum level of use each day.

An addendum to the toxicological monograph was prepared.

3.1.6 **Preservative**

3.1.6.1 *Natamycin (pimaricin)*

Natamycin (pimaricin) is a polyene macrolide antibiotic produced by submerged aerobic fermentation of *Streptomyces natalensis* and related species. The fermentation process takes several days, after which the antibiotic is isolated by extraction from broth or by extraction of the mycelium.

Natamycin is used as a food additive to control the growth of yeasts and moulds on the surface of cheese and other non-sterile products, such as meat and sausages.

Natamycin was evaluated by the Committee at its twelfth and twentieth meetings (Annex 1, references 17 and 41). At its twentieth meeting, the Committee established an ADI of 0–0.3mg/kg of body weight. The present evaluation was conducted in response to a request by the Codex Committee on Food Additives and Contaminants at its Thirty-second Session (4).

The Committee considered information on the current uses of natamycin, data on its intake and biological data that had not been evaluated previously.

Uses. Because natamycin is active against yeasts and moulds, but not bacteria, it is used in foods that undergo a ripening period after processing. Its low solubility in water and most organic solvents makes it appropriate for the surface treatment of foods.

Natamycin is used topically in veterinary medicine to treat mycotic infections, such as ringworm in cattle and horses. Previously, it was used topically against fungal infections of the skin and mucous membranes in humans. Its medical use is now confined to topical treatment of fungal infections of the cornea and the prevention of such infections in contact lens wearers.

Assessment of intake. The Committee noted that as the draft Codex General Standard for Food Additives proposes restricted use of natamycin only in cheese and in dried, non-heat-treated meats, intake would not be expected to exceed the ADI.

Data submitted by Australia, Germany, New Zealand, the United Kingdom and the USA indicated that the intakes at mean and high percentiles of consumption were well below the ADI, although the estimates for the United Kingdom and the USA covered cheese consumption only. The estimated mean intakes by consumers ranged from 0.01 to 0.03 mg/kg of body weight per day (representing 3% and 9% of the ADI in Germany and the United Kingdom, respectively), and those by consumers at high percentiles were 0.03–0.08 mg/kg of body weight per day (representing 9% and 27% of the ADI in Australia and the United Kingdom, respectively), if it is assumed that natamycin was used at 40 mg/kg in all cheese products and 20 mg/kg in all cured meat products, as proposed in the draft Codex General Standard for Food Additives. The estimated intakes of natamycin were lower when national levels of use were assumed.

Toxicological studies. The Committee considered eight studies that had not been evaluated previously and had been conducted before the 1980s. A study of single intraperitoneal administration was considered to be irrelevant to the safety assessment of an ingested substance. The results of two studies of genotoxicity in three bacterial systems (*Bacillus subtilis*, *Salmonella typhimurium* and *Escherichia coli*) were negative.

Two studies in rats and one in dogs given radiolabelled material for investigation of the distribution and elimination of the compound supported the previous conclusion that natamycin is excreted primarily in the faeces, with minimal absorption. The only adverse effect reported in a short-term study of toxicity in dogs was diarrhoea, which occurred most frequently in animals given the highest dose (equivalent to 25 mg/kg of body weight per day); however, the usefulness of this study was limited, as only two dogs were tested.

In a study of developmental toxicity, an aqueous suspension of natamycin at 500 mg/l was given to groups of 20–26 rabbits at a dose of 0, 5, 15 or 50 mg/kg of body weight per day by gavage on days 6–18 of gestation. The maternal mortality rate was 0%, 5%, 9% and 19% at the four doses, respectively. No clinical signs of toxicity were observed in the does, and the cause of death was unknown. The mean maternal body weight, pregnancy rate, number of implantation sites, number of resorption sites, numbers of live and dead fetuses, proportion of viable fetuses and incidence of soft-tissue anomalies were comparable in the treated groups and a control group given the vehicle only. The fetal body weight in the group dosed at 15 mg/kg of body weight by gavage was lower than that of fetuses in the control group given the vehicle only. The incidence of extra sternebrae was increased at the two highest doses in comparison with the control

group, but not in a dose-related manner. However, in view of the unusual sensitivity of the gastrointestinal tract of rabbits to poorly absorbed substances and to compounds with antimicrobial activity, this study was considered unsuitable for deriving the ADI.

Microbiological studies. The antifungal activities of natamycin and other polyenes depend on their binding to cell membrane sterols, primarily ergosterol, the principal sterol in fungal membranes. Oomycetes fungi and bacteria are insensitive to these antibiotics because their membranes lack ergosterol.

Use of natamycin as an antifungal agent in food may result in exposure of the indigenous microflora to trace quantities of antimicrobial residues. The human intestinal microflora is a complex mixture of more than 400 bacterial species, consisting primarily of bacterial cells at a concentration of approximately 10^{11} – 10^{12} colony-forming units per gram (CFU/g). Fungi are much less abundant than bacteria in the human gastrointestinal tract, the concentration of yeast in stool samples from healthy subjects being up to 10^5 CFU/g. As bacteria are not affected by polyenes, natamycin residues should not harm them; as yeasts are found in low quantities, the consequences of exposure to traces of natamycin would be minimal.

Several studies in experimental animals indicated a lack of antimicrobial activity in the colon, suggesting that natamycin was degraded into microbiologically inactive compounds by bacterial flora. However, no data were available on the degradation of natamycin by human intestinal microflora. In one study, natamycin was present in faecal specimens of volunteers who ingested 500 mg of the compound, indicating that it is incompletely absorbed or degraded.

As emergence of resistance to antimicrobials is a concern, the Committee evaluated the possible development of resistance among microflora as a consequence of exposure to natamycin. A preparation containing 50% natamycin has been used since the 1980s to preserve cheese and sausages. Surveys in cheese warehouses and in dry-sausage factories where the preparation has been used showed no change in the composition or the sensitivity of the contaminating fungal flora. All but one of the species of yeasts and moulds isolated in cheese warehouses where natamycin was used were inhibited by similarly low concentrations (0.5–8 µg/ml). In another study, 26 strains of fungi were isolated in eight warehouses where natamycin was used and two warehouses where it had never been used, and were tested for sensitivity to the compound; no insensitive yeasts or moulds were found. The results of laboratory experiments to induce resistance to natamycin in strains of fungi isolated from cheese

warehouses indicated that, after 25–30 transfers to media with increasing concentrations of natamycin, none of the strains had become less sensitive. When the sensitivity of yeasts and moulds isolated from dry-sausage factories where natamycin had been used for several years was compared with that of isolates from factories where natamycin had never been used, no significant differences were demonstrated.

It has been found difficult to induce resistance to polyenes, especially natamycin, in fungi in vitro. Resistant isolates invariably show reduced metabolic and growth rates and, in the absence of polyenes, readily revert to normal metabolism, growth and sensitivity to natamycin. One means of obtaining isolates resistant to natamycin is successive subculturing in vitro in the presence of gradually increasing concentrations of the polyene. Typically, such isolates are resistant only up to the highest concentration to which they have been exposed. After 25 passages, the concentration that inhibited *Candida albicans* was minimally increased, from 2.5–12 µg/ml to 12–50 µg/ml.

Evaluation. Natamycin is a polyene macrolide antibiotic that is effective against yeasts and moulds but not against bacteria or oomycetes fungi. The antifungal activities of natamycin depend on its binding to cell membrane sterols, primarily ergosterol, the principal sterol in fungal membranes, which is absent in bacteria. The use of natamycin as an antifungal agent in food may result in exposure of the indigenous flora to trace quantities of antimicrobial residues. As bacteria in the human gastrointestinal tract are not affected by polyenes, the Committee concluded that natamycin would not have an effect and that disruption of the barrier to colonization of the intestinal tract was therefore not a concern. Fungi are much less prevalent than bacteria in the human gastrointestinal tract, and, in light of the negative results of the studies of acquired resistance, selection of natamycin-resistant fungi was not considered an issue.

The Committee noted the finding of extra sternebrae in the study of developmental toxicity in rabbits, in which a dose-related increase in the mortality rate was also reported. It considered, however, that administration of an antimicrobial agent to rabbits by gavage was an inappropriate way of testing for developmental toxicity. In addition, extra sternebrae have been described as a skeletal variation rather than a frank sign of teratogenicity. Thus, the Committee did not consider the finding of extra sternebrae to be evidence that natamycin is teratogenic.

The Committee confirmed the previously established ADI of 0–0.3 mg/kg of body weight for natamycin, which was based on observations of gastrointestinal effects in humans. The Committee noted that the estimated intakes of natamycin, based on maximum levels of use

in cheese and processed meats proposed in the draft Codex General Standard for Food Additives, do not exceed this ADI.

A toxicological monograph was prepared and the existing specifications were revised. The title of the specifications was changed from pimaricin to natamycin, the commonly used designation. The specifications were made tentative, pending the receipt of information on the level and determination of water content, limit for lead, specific rotation, assay value and method of assay for the commercial product. This information was required for evaluation in 2003.

3.1.7 **Sweetening agent**

3.1.7.1 *D-Tagatose*

D-Tagatose is a keto-hexose, an epimer of D-fructose inverted at C-4, with a sweet taste. It is obtained from D-galactose by isomerization under alkaline conditions in the presence of calcium.

D-Tagatose was evaluated by the Committee at its fifty-fifth meeting (Annex 1, reference 149), when it concluded that the available data indicated that D-tagatose is not genotoxic, embryotoxic or teratogenic. It noted that the increased liver weights and hepatocellular hypertrophy seen in Sprague-Dawley rats occurred concurrently with increased glycogen deposition; however, the reversal of increased glycogen storage after removal of D-tagatose from the feed was more rapid than regression of the liver hypertrophy. Although the gastrointestinal symptoms seen in adult humans with the expected daily intake of D-tagatose were minor, the Committee was concerned about the increased serum uric acid concentrations observed in a number of studies in humans after administration of either single or repeated doses of D-tagatose. Similar increases were observed with other sugars, such as fructose, but D-tagatose appeared to be a more potent inducer of this effect. The Committee also noted that this effect of D-tagatose had not been studied in persons prone to high serum uric acid concentrations. The Committee concluded that an ADI could not be allocated for D-tagatose because of concern about its potential to induce glycogen deposition in the liver and liver hypertrophy and to increase the serum concentration of uric acid.

Two studies of up to 7 days' duration in Wistar and Sprague-Dawley rats given repeated doses of D-tagatose were submitted to the Committee at its fifty-fifth meeting, but the reports were received only in draft form and were not suitable for consideration at that time. The Committee therefore asked for the final reports and for further data to clarify the extent, mechanism and toxicological consequences of the increased serum uric acid concentrations observed in humans exposed to D-tagatose. At its present meeting, the Committee reviewed the

reports of the two studies in rats, the results of a study in volunteers (on the relevance of the glycogen deposition and liver hypertrophy) and some published studies on the increased uric acid concentrations in serum after intake of D-tagatose, other sugars and other food components.

Biological data. Review of the results of the studies considered by the Committee at its fifty-fifth meeting and comparisons with the data reviewed at the present meeting revealed a difference in sensitivity between Wistar and Sprague-Dawley rats. Sprague-Dawley rats given D-tagatose at a concentration of 50 g/kg of diet for 28 days showed increased hepatic glycogen only when they had not been fasted the night before necropsy, and this effect was not associated with any microscopic changes in the liver. In a 90-day study in which Sprague-Dawley rats were killed after fasting overnight, administration of D-tagatose at a concentration of 50 g/kg of diet had no adverse effect on the liver. In a 6-month study in Wistar rats in which the animals were killed after fasting 3, 7, 14 and 28 days and 3 and 5 months after treatment, administration of D-tagatose at concentrations of up to 100 g/kg of diet had no adverse effects. Wistar rats are therefore less susceptible to the hepatic effects of D-tagatose than Sprague-Dawley rats. As D-tagatose stimulated glycogen deposition to a similar degree in the two rat strains in short-term studies, the difference is likely to occur at a later stage, during glycogen-induced or other stimulation of liver growth.

The authors suggested that the increase in normal liver mass seen in fasted rats fed diets containing 100 or 200 g/kg D-tagatose is triggered by increased postprandial storage of liver glycogen resulting from simultaneous feeding of D-tagatose and glucose equivalents. In order to test this hypothesis, the effects of separate and simultaneous administration of D-tagatose and glycogen precursors on liver weight and glycogen level were investigated in Wistar and Sprague-Dawley rats. The results neither supported nor invalidated the hypothesis.

As several studies have been performed in healthy volunteers and in patients with diabetes, the number of persons varying from 4 to 73, the Committee based its toxicological evaluation on the data from these studies. The length of these studies varied from several days to several weeks; one study of 12 months' duration included only a limited number of patients with type 2 diabetes. The toxicological aspects investigated included gastrointestinal effects, increased serum uric acid concentrations and hepatic effects.

Mild gastrointestinal symptoms were reported in only one study, in 3 of 10 patients with type 2 diabetes receiving D-tagatose at 10 g/day for several days, whereas in other studies diarrhoea was observed only in patients receiving 25 g three times daily for 8 weeks. In healthy individuals, administration of a single dose of 30 g induced diarrhoea in

some persons only, whereas other studies showed no laxative effect of single doses of D-tagatose as high as 75 g.

The serum or plasma concentration of uric acid was increased transiently in some studies, but the increased uric acid concentration was above the normal range for a number of days in only one study of persons receiving 75 g/day. The other studies showed either no increase or a transient increase in serum uric acid concentrations within the normal range.

In a 28-day study in which 15 g of D-tagatose or 15 g of sucrose were given three times daily to volunteers, magnetic resonance imaging was used to determine liver volume, and glycogen concentrations and several clinical chemical parameters were measured. The results did not reveal any relevant effect on the liver. In addition, no diarrhoea and no increase in serum uric acid concentration were observed. Therefore, the NOEL was 45 g/person per day, equivalent to 0.75 g/kg of body weight per day (for a person weighing 60 kg).

Evaluation. The Committee considered the 28-day study in which humans received a daily dose of 45 g of D-tagatose or sucrose in three divided doses as most representative of human dietary intake and therefore most relevant for assessing the acceptable intake of D-tagatose accurately. While effects were observed after administration of a single dose of 75 g, no effects were seen following administration of three daily doses of 15 g of D-tagatose, equivalent to 0.75 g/kg of body weight per day. The Committee established an ADI of 0–80 mg/kg of body weight on the basis of this NOEL and a safety factor of 10.

Assessment of intake. D-Tagatose is proposed for use as a bulk sweetener in low-energy foods, such as edible ices (at a concentration of 3 g/kg), chewing-gum and confectionery (at 15 g/kg), breakfast cereals (at 15 g/kg) and soft drinks (at 1 g/kg). At its present meeting, the Committee considered that the predicted intakes of D-tagatose determined at the fifty-fifth meeting, which were based on the manufacturers' proposed levels of use and individual dietary records in several countries, were conservative. This was because use had been assumed in the entire food category rather than only in the low-energy food component. The mean consumer intakes of D-tagatose from all proposed uses (except chewing-gum, dietary supplements and meal replacements) predicted for Australia, the Member States of the European Union and the USA ranged from 3 to 9 g/day (63–190% of the ADI), and the predicted intakes by persons at high percentiles of consumption were up to 18 g/day (375% of the ADI). On the basis of the information on possible uses, the Committee concluded that the ADI for D-tagatose may be exceeded by some groups of the population.

A toxicological monograph was prepared. The specifications prepared by the Committee at its fifty-fifth meeting were maintained.

3.1.8 **Thickening agents**

3.1.8.1 *Carrageenan and processed Eucheuma seaweed*

Carrageenan, a substance with hydrocolloid properties owing to the presence of sulfated polyglycans with average relative molecular masses well above 100000, is derived from a number of seaweeds of the family Rhodophyceae. It has no nutritional value and is used in food preparation for its gelling, thickening and emulsifying properties. Three main types of carrageenan, known as ι -, κ - and λ -carrageenan, are used commercially in the food industry. These names do not reflect definitive chemical structures but only general differences in the composition and degree of sulfation at specific locations in the polymer. Processed *Eucheuma* seaweed is derived from either *E. cottonii* (κ -carrageenan) or *E. spinosum* (λ -carrageenan), which are also Rhodophyceae.

Carrageenan is obtained by extraction of the seaweed into water or aqueous dilute alkali and may be recovered by precipitation with alcohol, by drying in a rotary drum or by precipitation with aqueous potassium chloride and subsequent freezing. In contrast, processed *Eucheuma* seaweed is prepared by soaking the cleaned seaweed in alkaline solution for a short time at elevated temperatures. The treated material is then thoroughly washed with water to remove residual salts and further washed with alcohol, dried and milled to a powder. For both carrageenan and processed *Eucheuma* seaweed, the alcohols that may be used during purification are restricted to methanol, ethanol and isopropanol. The articles of commerce may contain sugars added for standardization purposes, salts to obtain specific gelling or thickening characteristics, or emulsifiers carried over from the drum-drying process.

Carrageenan was reviewed by the Committee at its thirteenth, seventeenth, twenty-eighth and fifty-first meetings (Annex 1, references 19, 32, 66 and 137). At its twenty-eighth meeting, the Committee established an ADI “not specified”¹ on the basis of the results of a number

¹ ADI “not specified” is used to refer to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for reasons stated in the individual evaluation, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice, i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

of toxicological studies on carrageenans obtained from various sources.

Processed *Eucheuma* seaweed was considered by the Committee at its thirtieth, thirty-ninth, forty-first, forty-fourth and fifty-first meetings (Annex 1, references 73, 101, 107, 116 and 137). At its forty-fourth meeting, the Committee concluded that, because of the chemical relationship between processed *Eucheuma* seaweed and traditionally refined carrageenan, the toxicological data on carrageenan were relevant to the safety assessment of the carrageenan polysaccharide constituents of processed *Eucheuma* seaweed, but could not replace adequate toxicological studies on processed *Eucheuma* seaweed itself. At its fifty-first meeting, the Committee reviewed the results of a 90-day study on toxicity in rats fed processed *Eucheuma* seaweed from *E. cottonii* and *E. spinosum*. The Committee concluded that the toxicity of this material was sufficiently similar to that of carrageenan to allow extension of the previous ADI “not specified” for carrageenan to a group ADI that covered processed *Eucheuma* seaweed. The Committee also considered all studies on carrageenan that had been published since its twenty-eighth meeting and, for the earlier studies, noted the identity of the source material and the type of carrageenan, when these could be identified. It expressed concern about the potential promotion of colon carcinogenesis by carrageenans and processed *Eucheuma* seaweed and therefore made the group ADI “not specified” temporary, pending clarification of the significance of the promotion of colon cancer observed in studies in rats. At its present meeting, the Committee reviewed the available evidence for the tumour-promoting and related effects of these compounds in rat colon.

Assessment of intake. Carrageenan and processed *Eucheuma* seaweed are used as thickeners, gelling agents, stabilizers or emulsifiers in a wide range of foods at concentrations of up to 1500mg/kg. Per capita intakes in 1995 derived from “poundage” (disappearance) data in Europe and the USA ranged from 28 to 51 mg/day. These estimates corresponded to those reported for 1993 by the Seaweed Industry Association of the Philippines on the basis of sales of 44mg/person per day for the populations of Canada and the USA and 33 mg/person per day for European populations.

The estimates derived from poundage data were also consistent with those derived for the population of the USA from model diets, with reported mean intakes of carrageenan of 20mg/day for all consumers and 40mg/day for persons at the 90th percentile of consumption (derived by multiplying the mean by a factor of 2). The intakes were derived from data on the food consumption of individuals aged

2 years and over that were available in 1976 from nutrition surveys in the USA, combined with the results of a 2-week study by the Marketing Research Corporation of America on the frequency of food consumption.

Biological data. Two studies showed that carrageenan administered before, during and after administration of known carcinogens (dimethylhydrazine, azoxymethane, *N*-methyl-*N*-nitrosourea) enhanced the tumorigenicity of these carcinogens. One of the studies involved administration of carrageenan at 150 g/kg of diet, which resulted in decreased body-weight gain. In the second study, involving administration of carrageenan at 60 g/kg of diet, the body-weight gain of treated animals was comparable to that of controls. The increased incidence of tumours seen under these circumstances may have resulted from promotion but may also have resulted from altered toxicokinetics or biotransformation of the carcinogen. In addition, there were indications that the bacterial flora had been altered as a result of administration of carrageenan. In a separate study conducted according to a classical tumour initiation–promotion protocol, in which rats were given dimethylhydrazine, subsequent administration of carrageenan at dietary concentrations of up to 50 g/kg did not result in a statistically significant increase in the incidence of colon tumours over that seen with dimethylhydrazine alone.

Two further studies in rats involved use of a conventional tumour initiation–promotion protocol but in which formation of aberrant crypt foci was the end-point, instead of tumour formation. Rats were given azoxymethane with or without subsequent administration of carrageenan in their drinking-water. The higher concentration of carrageenan, 25 g/kg, was given in the form of a solid gel, which may have altered the food and water consumption patterns of the animals. The first study demonstrated that dietary administration of carrageenan after the carcinogen decreased the number of aberrant crypt foci seen relative to the number observed with the carcinogen alone, but significantly increased their size. A subsequent study in rats injected with human faecal microflora showed no effect of carrageenan on either the number or size of aberrant crypt foci. As the relationship between aberrant crypt foci and tumorigenesis is still unclear, it is difficult to interpret the biological significance of these results.

Increased cell proliferation has frequently been postulated as a mechanism of non-genotoxic carcinogenicity or tumour promotion. The preferred methods of assessing cell proliferation are based on histological techniques, which allow identification of the nature and location of proliferating cells. There was no consistent pattern of colon damage in rats treated with carrageenan for prolonged periods.

Some studies showed caecal enlargement, but most did not show histological damage. In one study in which rats underwent autoradiographic examination, no significant difference from controls in the number of cells per crypt or in the proportion of labelled cells was seen in rats fed a diet containing carrageenan at 74g/kg for 28 days.

Methods for measuring cell proliferation that are based on measurement of cell cycle-dependent enzyme activities, such as thymidine kinase activity, are cruder means of measuring overall cell proliferation in an entire tissue specimen. A significant increase in thymidine kinase activity, expressed relative to protein content, was found in homogenized mucosal scrapings from the colon of rats fed diets containing carrageenan at 26 or 50g/kg for 4 weeks; no significant effects were observed in the animals fed 0, 6.5 or 13g/kg carrageenan in the diet for 4 weeks. Histological examination revealed no evidence of infiltration by inflammatory cells in any of the treated groups. In another study, the increased thymidine kinase activity observed in rats fed diets containing carrageenan at 50g/kg returned to the basal level within 28 days when the animals were returned to a diet with no carrageenan. No increase in thymidine kinase activity was seen in animals receiving diets containing 2 or 15g/kg carrageenan for 28 days. Staining for proliferating cell nuclear antigen (PCNA) revealed a significant increase in PCNA-positive cells in the upper third of the crypts of rats receiving a diet containing carrageenan at 50g/kg for 91 days, but not after 28 or 64 days followed by a 28-day recovery period on a normal diet. No PCNA-positive cells were observed at the luminal surface. The pattern of staining for PCNA seen with carrageenan was considered indicative of an adaptive response, which would not contribute to an increased risk for colonic neoplasia.

In one study, carrageenan inhibited gap-junctional intercellular communication *in vitro*. However, the mechanism of action was different from that of a known tumour-promoting agent, phorbol ester, and the relevance of this observation is unclear for a substance that is not absorbed *in vivo*.

Evaluation. In a recent study with a classical tumour initiation-promotion protocol, administration of carrageenan at concentrations of up to 50 g/kg of diet did not promote colon carcinogenesis in rats given dimethylhydrazine. The Committee noted, however, that, in two studies that showed enhancement of colon carcinogenesis in rats, higher dietary concentrations of carrageenan were used and carrageenan was administered before, during and after the carcinogens. The enhanced carcinogenicity seen under these circumstances may have resulted from promotion or from altered toxicokinetics or bio-transformation of the carcinogen. Therefore, the mechanism of the

enhancement of colon carcinogenesis in these studies remains unresolved. Continuous feeding of high doses of carrageenan caused a generalized proliferative response, measured as increased thymidine kinase activity, in the mucosal tissue of the colon of male rats. This effect might play a role in the observed enhancement of the tumorigenicity of known colon carcinogens by high dietary concentrations of carrageenan. However, a proliferative effect of carrageenan on the mucosa of the colon was seen only at a dietary concentration of 26 g/kg or more. No effect was seen at a concentration of 15 g/kg in the diet, corresponding to 750 mg/kg of body weight per day, which greatly exceeded the estimated human intake of carrageenan and processed *Eucheuma* seaweed of 30–50 g/person per day from their use as food additives. Bearing in mind that the enhancement of colon carcinogenesis in rats was seen at much higher concentrations and that carrageenan at 50 g/kg of diet did not promote tumours in rat colon in a classical initiation–promotion study, the Committee considered that the intake of carrageenan and processed *Eucheuma* seaweed from their use as food additives was of no concern. It therefore allocated a group ADI “not specified”¹ to the sum of carrageenan and processed *Eucheuma* seaweed.

An addendum to the toxicological monograph was prepared. The existing specifications for both carrageenan and processed *Eucheuma* seaweed were revised by incorporating more complete descriptions of the analytical procedures for the determination of lead, cadmium and mercury and by raising the acceptable limit for lead from 2 mg/kg to 5 mg/kg and the acceptable limit for cadmium from 1 mg/kg to 2 mg/kg. These limits were raised to take into account new information on inadequacies of the analytical methods for determination of these elements, which are due to the high salt content of the polysaccharides of both processed *Eucheuma* seaweed and carrageenan. The changes were not made because of information about higher concentrations of lead and cadmium than those previously considered by the Committee. The Committee also observed that the new limits are consistent with the limits established for these heavy metals in specifications for other hydrocolloids, such as alginic acid.

3.1.8.2 *Curdlan*

Curdlan (synonym, β -1,3-glucan) is a linear polymer of high relative molecular mass, consisting of β -1,3-linked glucose units. Curdlan is produced by fermentation of pure cultures from a non-pathogenic, non-toxinogenic strain of *Agrobacterium* Biovar1 (identified as *Alcaligenes faecalis* var. *myxogenes* at the time of its isolation) or

¹ See footnote on page 32.

Alcaligenes radiobacter. Curdlan is recovered from the fermentation medium by addition of acid and alkali to disrupt the cells, which releases the curdlan into the medium, followed by separation by centrifugation. It is then washed with copious amounts of water to eliminate mineral salts and other water-soluble substances that may have been carried over from the fermentation broth. The commercial product is an odourless or nearly odourless, tasteless, white to nearly-white spray-dried powder.

The use of curdlan in a wide variety of foods is based on its ability to form an elastic gel upon heating in an aqueous suspension. Thus, it can be used in processed meat, fish and poultry products and in gelatins, puddings and fillings as a firming or gelling agent or as a stabilizer or thickener.

The Committee reviewed curdlan at its fifty-third meeting (Annex 1, reference 143), when it allocated a temporary ADI “not specified”,¹ pending information on the use of curdlan, including the maximum and typical expected levels in the food categories in which it is proposed for use in the draft Codex General Standard for Food Additives, and on the consumption in various regions of the world of foods that might contain curdlan.

Use of curdlan is based on its physical properties, which imply a self-limiting level of use in solid foods. A submission from the USA described a model constructed to predict the intakes of curdlan by a long-term consumer on the basis of a study of the frequency of consumption of foods in 1982–1988 from the Market Research Corporation of America, and average portion sizes from a 3-day national food consumption survey conducted in 1987–1988 by the United States Department of Agriculture. Intake was assessed on the basis of the self-limiting levels of use (20 mg/kg of processed meat, 15 mg/kg of processed poultry and fish, 10 mg/kg of dairy products, 35 mg/kg of egg products, 15 mg/kg of grain products and pasta, 30 mg/kg of cereals and starch desserts, 20 mg/kg of gravies and sauces and 40 mg/kg of gelatins). The resulting mean intake by consumers was estimated to be 3.6 g/person per day, corresponding to 60 mg/kg of body weight per day.

The sponsor submitted an estimate based on daily food intake per capita and typical levels of use in Japan (15mg/kg of processed meat, 10mg/kg of processed poultry and fish, 5mg/kg of dairy products, 30mg/kg of egg products, 10mg/kg of grain products and pasta, 10mg/kg of cereals and starch desserts, 10mg/kg of gravies and sauces and 30mg/kg of gelatins). The mean intakes were estimated to be

¹ See footnote on page 32.

0.77 g/person per day, corresponding to 13 mg/kg of body weight per day, for typical levels of use and 1.7 g/person per day, corresponding to 28 mg/kg of body weight per day, for maximum levels of use.

Estimates of the intake of curdlan based on individual dietary records were submitted by the USA on the basis of a survey by the Department of Agriculture and the Continuing Survey of Food Intakes by Individuals (1989–1992). When intake was estimated on the basis of the upper limit of the range of recommended use, the intake of curdlan by consumers was 20 mg/kg of body weight per day for consumption at the mean and 47 mg/kg of body weight per day for consumption at the 90th percentile. When intake was estimated on the basis of self-limiting levels of use, the intake of consumers was 30 mg/kg of body weight per day for consumption at the mean and 68 mg/kg of body weight per day for consumption at the 90th percentile.

The data on uses and intake requested by the Committee at its fifty-third meeting were provided and raised no safety concern. The Committee therefore established an ADI “not specified”¹ for use of curdlan as a food additive.

The existing specifications were revised, with minor changes.

3.1.9 **Miscellaneous substances**

3.1.9.1 *Acetylated oxidized starch*

Acetylated oxidized starch is a chemically modified root or grain starch. It is produced by oxidation of a slurry of starch granules in alkaline hypochlorite at low temperatures (21–38°C). The alkaline medium is neutralized with sodium bisulfite, and the resulting organic salts are removed by washing with water. The oxidized starch is then esterified with acetic anhydride under mildly alkaline conditions. The product is neutralized with hydrochloric acid, washed and dried.

Acetylated oxidized starch had not been evaluated previously by the Committee. At the present meeting, it was proposed for use as a binding agent in soft confectionery at a concentration of about 300 mg/kg — it is mixed with water, sugars and flavours in a batch process until a clear solution with a dry-solid content of 70% is obtained. The characteristics of the end-product important for confectionery use are gel strength and clarity. Acid hydrolysis results in starch products that are relatively unclear, and oxidized starch products result in overly soft confectionery. Acetylation of oxidized starch enhances the desired properties, resulting in a gummy, clear jelly. It

¹ See footnote on page 32.

can be used as a substitute for gelatin or gum arabic and would replace a large amount of sugar.

Acetylated oxidized starch has a stable configuration under normal conditions in food. It is hydrolysed slowly in the presence of strong acids, yielding glucose, gluconic acid and acetic acid. No degradation products are expected or known to result from storage or use of this substance in the preparation of foods at neutral pH. The substance is not known to sequester minerals, nor does it interact with proteins or vitamins. It has no known effect on other nutrients.

In a 14-day range-finding study in rats, administration of a diet containing acetylated oxidized starch at a concentration of 300 or 500 mg/kg increased the weights of full and empty caeca, and dilated caeca were found at autopsy. At the higher concentration, soft faeces also occurred. The NOEL was 100 mg/kg of diet.

In a 90-day study in rats given a diet containing acetylated oxidized starch, increased full and empty caecal weights were seen at the highest concentration of 300 mg/kg of diet. Macroscopic examination showed a dilated caecum in one male rat. Histological examination did not reveal changes in the caecal wall or other parts of the digestive tract. Increased caecal weights are a known response to high dietary concentrations of poorly digested carbohydrates in rats, due perhaps to an increased osmotic load of short-chain fatty acids produced by microbial degradation and the associated water retention. Focal hyperplasia of the urinary bladder epithelium was seen in 4 out of 10 male rats that received the highest dietary concentration but not in males given lower concentrations, in controls or in females. The change was probably treatment-related and a consequence of irritation of the urinary bladder by calculi. The NOEL was 100 mg/kg of diet, equivalent to 5900 mg/kg of body weight per day.

If acetylated oxidized starch was to be used only in jelly confectionery at a concentration of 300 g/kg and if the maximum consumption by consumers was 200 g of jelly confectionery per day, the maximum intake of acetylated oxidized starch would be 60 g/day.

The effects seen in the 14-day and 90-day studies in rats were similar to those observed with high dietary concentrations of other slowly digested carbohydrates and are commonly seen in rats given other modified starches in the diet. Because of the nature of acetylated oxidized starch and its similarity to other modified starches with non-systemic effects, the Committee established an ADI “not specified”,¹ on the basis of the known uses of acetylated oxidized starch as an ingredient in confectionery products.

¹ See footnote on page 32.

A toxicological monograph was prepared. New specifications for acetylated oxidized starch were prepared and incorporated into the specifications for modified starches.

3.1.9.2 *α-Cyclodextrin*

α-Cyclodextrin is a non-reducing cyclic saccharide composed of six glucose units linked by α -1,4 bonds. It is produced by the action of cyclodextrin glucosyltransferase (CGTase, EC 2.4.1.19) on hydrolysed starch syrups at neutral pH (6.0–7.0) and moderate temperatures (35–40°C). The annular structure of *α*-cyclodextrin provides a hydrophobic cavity that allows formation of inclusion complexes with a variety of non-polar organic molecules of appropriate size. The hydrophilic nature of the outer surface of the cyclic structure makes *α*-cyclodextrin water-soluble.

The principal method for the isolation and purification of *α*-cyclodextrin takes advantage of its complex-forming ability. At the end of the reaction, 1-decanol is added to the reaction mixture to form an insoluble 1:1 inclusion complex of *α*-cyclodextrin:1-decanol. The complex is continuously mixed with water and separated from the reaction mixture by centrifugation. The recovered complex is resuspended in water and dissolved by heating. Subsequent cooling leads to precipitation of the complex. The precipitate is recovered by centrifugation, and 1-decanol is removed by steam distillation. Upon cooling, *α*-cyclodextrin crystallizes from the solution. The crystals are removed by filtration and dried, yielding a white crystalline powder with a water content of less than 11%. The purity on a dried basis is at least 98%.

The hydrophobic cavity and the hydrophilic outer surface of *α*-cyclodextrin form the basis for its use in the food industry. *α*-Cyclodextrin, like its homologues β - and γ -cyclodextrin, can function as a carrier and stabilizer for flavours, colours and sweeteners; as an absorbent for suppression of undesirable flavours and odours in foods; as an absorbent for suppression of halitosis (in breath-freshening preparations); and as a water-solubilizer for fatty acids and vitamins.

α-Cyclodextrin had not been evaluated previously by the Committee, but the structurally related compound β -cyclodextrin was evaluated at the forty-first and forty-fourth meetings (Annex 1, references 107 and 116), and γ -cyclodextrin was evaluated at the fifty-first and fifty-third meetings (Annex 1, references 137 and 143). At its present meeting, the Committee noted the close structural similarity between *α*- and β -cyclodextrin (seven glucose units) and γ -cyclodextrin (eight glucose units), which permitted comparisons of the metabolism and toxicity of these compounds.

Biological data. α -Cyclodextrin, like β -cyclodextrin, is not digested in the gastrointestinal tract but is fermented by the intestinal microflora. In germ-free rats, α -cyclodextrin is almost completely excreted in the faeces, whereas γ -cyclodextrin is readily digested to glucose by the luminal and/or epithelial enzymes of the gastrointestinal tract. At low concentrations in the diet (about 20 g/kg), α -cyclodextrin is absorbed intact from the small intestine and is then excreted rapidly in the urine. The majority of the absorption takes place after metabolism of the substance by the microflora in the caecum. Although no studies of metabolism in humans in vivo were available, in vitro studies indicated that α - and β -cyclodextrin, unlike γ -cyclodextrin, cannot be hydrolysed by human salivary and pancreatic amylases.

The acute toxicity of α -cyclodextrin was studied in mice and rats that received the substance by intraperitoneal or intravenous injection. It caused osmotic nephrosis, probably because it was not degraded by lysosomal amylases. At high doses, this led to renal failure.

The results of short-term (28-day and 90-day) studies of the toxicity of α -cyclodextrin indicated that it had little effect when given orally to rats or dogs. After administration of a very high dietary concentration (200 g/kg), caecal enlargement and associated changes were seen in both species. This effect was probably the consequence of the presence of a high concentration of an osmotically active substance in the large intestine. No studies of intravenous administration were available to permit a comparison of the systemic toxicity of this compound with that of β - and γ -cyclodextrin.

Studies conducted in mice, rats and rabbits given α -cyclodextrin in the diet at concentrations of up to 200 g/kg did not indicate any teratogenic effects. Similarly, the results of assays for genotoxicity were negative. No long-term studies of toxicity, carcinogenicity or reproductive toxicity have been conducted with α -cyclodextrin, but the Committee concluded that, given the known fate of this compound in the gut, such studies were not required for an evaluation.

In vitro, α -cyclodextrin, like β -cyclodextrin, sequestered components of the membranes of erythrocytes, causing haemolysis. The threshold concentration for this effect was, however, higher than that observed with β -cyclodextrin.

While the potential interaction of α -cyclodextrin with lipophilic vitamins, which might impair their bioavailability, has not been studied directly, such an effect was considered unlikely, by analogy with the results of studies with β -cyclodextrin. Complexes between fat-soluble vitamins and β -cyclodextrin have been shown to have greater bioavailability than uncomplexed forms.

The enzyme cyclodextrin-glycosyltransferase, which is used in the production of α -cyclodextrin, is derived from a non-genotoxic, non-toxinogenic source and is completely removed from α -cyclodextrin during purification.

Assessment of intake. The predicted mean intake of α -cyclodextrin by consumers, based on individual dietary records for 1994–1998 in the USA and the proposed maximum levels of use in a variety of foods, would be 1.7 g/day (28 mg/kg of body weight per day) for the whole population and 1.6 g/day (87 mg/kg of body weight per day) for children aged 2–6 years. The main contributors to the total intake of α -cyclodextrin are likely to be soya milk and sweets. For persons at the 90th percentile of consumption, the predicted intake of α -cyclodextrin would be 3 g/day (50 mg/kg of body weight per day) for the whole population and 2.6 g/day (140 mg/kg of body weight per day) for children aged 2–6 years.

Evaluation. No studies of human tolerance to α -cyclodextrin were submitted to the Committee, despite the potentially high dietary intake. Nevertheless, the Committee was reassured by the relatively low toxicity of this compound in animals and the fact that it was less toxic than β -cyclodextrin, for which studies of human tolerance were available. Furthermore, the fact that it is fermented in the gastrointestinal tract in an analogous manner to β -cyclodextrin supported the conclusion that, as in laboratory animals, it would be fermented to innocuous metabolites before its absorption in the human gastrointestinal tract.

The Committee concluded that, on the basis of the available studies on α -cyclodextrin and studies on the related compounds β -cyclodextrin and γ -cyclodextrin, for which ADIs have been allocated, there was sufficient information to allocate an ADI “not specified”.¹ This ADI was based on the known current uses of α -cyclodextrin within good manufacturing practice as a carrier and stabilizer for flavours, colours and sweeteners; as a water-solubilizer for fatty acids and certain vitamins; as a flavour modifier in soya milk; and as an absorbent in confectionery.

A toxicological monograph and new specifications for α -cyclodextrin were prepared.

3.1.9.3 Sodium sulfate

Sodium sulfate was evaluated by the Committee at its fifty-third meeting (Annex 1, reference 143), when a temporary ADI “not specified”¹ was established. The ADI was made temporary because information was required on the functional effect and actual uses of

¹ See footnote on page 32.

sodium sulfate in food. This information was provided to the Committee at its fifty-fifth meeting (Annex 1, reference 149), and the “tentative” designation was removed from the specifications. At that time, the temporary ADI was not reconsidered.

Sodium sulfate is used as a colour adjuvant. Worldwide consumption from its use in food is approximately 100 tonnes per year.

At its present meeting, the Committee noted that the results of the few published studies conducted in experimental animals do not raise concern about the toxicity of sodium sulfate. Little is absorbed from the gut, and it is therefore used clinically as a laxative. The small amount absorbed remains in the extracellular fluid space and is rapidly excreted via the kidneys. Minor adverse effects have been reported in a small number of clinical trials and in case reports. All of the effects were seen with preparations containing sodium sulfate and may have resulted from other components of the preparations.

In the absence of evidence of toxicity and given the current uses of this substance, the Committee allocated an ADI “not specified”¹ for sodium sulfate.

A toxicological monograph was not prepared. The specifications prepared by the Committee at its fifty-fifth meeting were maintained.

3.2 Revision of specifications

3.2.1 Acesulfame K

Acesulfame K is prepared in a three-step process in which sulfamic acid and diketene are reacted to produce an adduct, which undergoes cyclization to the acid form of acesulfame. This product is neutralized with potassium hydroxide to form the potassium salt.

The specifications for acesulfame K were revised. In addition to editorial revisions, a new criterion for purity with regard to the pH value of the aqueous solution was introduced, and the limit for lead was lowered from 10mg/kg to 1 mg/kg.

3.2.2 Blackcurrant extract

Blackcurrant extract is obtained from blackcurrant pomace by aqueous extraction. The main colouring principles are four anthocyanins (cyanidin 3-rutinoside, delphinidin 3-rutinoside, cyanidin 3-glucoside and delphinidin 3-glucoside). Most of the extracted sugars are fermented to alcohol, and virtually all the alcohol is removed during concentration of the extract by vacuum evaporation. Sulfur dioxide is used during the extraction process, and residual sulfur dioxide may

¹ See footnote on page 32.

be present in the final product. The commercial products are concentrated liquids, pastes or spray-dried powders. Spray-dried powder may contain an added carrier such as maltodextrin or glucose syrup. At its present meeting, the Committee revised the specifications to include a chromatographic identification test which distinguishes blackcurrant extract from other anthocyanin colours and removed the “tentative” designation.

3.2.3 *L-Malic acid*

The Committee received no information about the uses of L-malic acid, other than its well-established use as a flavouring agent. As DL- and L-malic acid are different compounds made by different manufacturing processes, the specifications for DL-malic acid were corrected by removing the reference to the specifications for L-malic acid.

3.2.4 *Oxystearin*

The specifications for oxystearin were considered by the Committee at its fifty-fifth meeting (Annex 1, reference 149). At that meeting, the Committee maintained the “tentative” designation, with the stipulation that the specifications would be withdrawn if information on the levels of, and a suitable analytical method for, epoxides was not provided by 1 May 2001. The Committee noted that oxystearin was no longer in commercial use as a food additive.

At its present meeting, the Committee withdrew the specifications, as the requested information had not been received. The Committee also withdrew the ADI of 0–25 mg/kg of body weight for oxystearin established at its seventeenth meeting (Annex 1, reference 32), as it considered that there could not be an ADI for a substance for which there were no specifications.

3.2.5 *Pectins*

Pectins consist mainly of the partially methylated esters of polygalacturonic acid and its ammonium, sodium, potassium and calcium salts. Amidated pectins also contain amides of polygalacturonic acid. Pectins are obtained by extraction in an aqueous medium of an appropriate edible plant material, usually citrus fruits or apples. Amidated pectins are obtained by treating the extract with ammonia under alkaline conditions.

The specifications for pectins were revised. The four separate tests for the identification of pectins contained in the specifications prepared at the thirty-ninth meeting (Annex 1, reference 101) were replaced by a new test involving enzymatic degradation, which is specific for pectins, as the Committee had been informed that the previous tests were

not adequate for all commercial samples of pectins. The Committee was also informed that new separation techniques were used which could result in contamination of pectins with insoluble organic compounds. Therefore, a new criterion for purity, the percentage of “total insolubles”, was introduced. In addition, the limits for copper, zinc and arsenic were deleted, and the limit for lead was lowered from 10mg/kg to 5mg/kg.

3.2.6 **Smoke flavourings**

Smoke flavourings are complex mixtures of components of smoke obtained by subjecting untreated hardwoods to pyrolysis in a limited, controlled amount of air, dry distillation at 200–800°C or exposure to superheated steam at 300–500°C. The major flavouring principles are carboxylic acids, compounds with carbonyl groups and phenolic compounds.

During manufacture of smoke flavourings, hazardous constituents such as polycyclic aromatic hydrocarbons are removed by subjecting wood smoke to aqueous extraction or to distillation, condensation and separation for collection of the aqueous phase. The aqueous smoke fraction, containing water-soluble constituents, can be diluted with water or extracted with an edible vegetable oil to produce a smoke flavouring with a higher concentration of non-polar constituents, which may be further extracted with food-grade substances, such as propylene glycol or aqueous solutions of polysorbates.

The commercial products may also contain additives such as emulsifiers, antifoaming agents and gums. Smoke flavourings may also be prepared in dry form by the addition of carriers such as yeasts, flours, salt, phosphates, carbohydrates and anticaking agents.

The specifications for smoke flavourings were considered by the Committee at its fifty-fifth meeting (Annex 1, reference 149) and were maintained as “tentative”, pending the receipt of information on an alternative solvent to benzene for use in the analysis of the carbonyl content. At its present meeting, the Committee revised the existing tentative specifications and removed the “tentative” designation. The revised specifications apply only to water-soluble distillates of condensed wood smoke, to their aqueous, vegetable oil or polysorbate extracts and to concentrates of these products. They do not apply to products derived from the water-insoluble tars, to certain commercial products or to pyroligneous acid, a by-product of the manufacture of charcoal by carbonation of wood in the absence of air.

3.2.7 **Tagetes extract**

Tagetes extract is obtained by hexane extraction of dried petals of *Tagetes erecta* L., with subsequent removal of the solvent. The major

colouring principles are the xanthophyll lutein and its dipalmitate (helenien). Other hydroxy derivatives of carotenes may be present, together with other oxy derivatives, such as epoxides. The product may contain fats, oils and waxes that occur naturally in the plant material. The articles of commerce are usually further formulated, e.g. in order to standardize the colour content or to obtain water-soluble or dispersible products.

The specifications for tagetes extract were considered by the Committee at its fifty-fifth meeting (Annex 1, reference 149) and were designated as “tentative”, pending the receipt of information on the composition of the commercial products, a test for the identification of xanthophylls and a method of assay. As the Committee had received the requested information, it revised the existing tentative specifications and removed the “tentative” designation.

3.3 Revision of limits for metals in food additives

At its fifty-fifth meeting (Annex 1, reference 149), the Committee began to implement a systematic 5-year programme to replace the outdated test for heavy metals (as lead) in all existing food additive specifications with appropriate limits for individual metals of concern. Limits for lead and arsenic in 43 emulsifiers were proposed. As no alternative proposals were received by the Secretariat before the deadline for submission of data for the present meeting, the new published limits (Annex 1, reference 151) were adopted, replacing those published in the *Compendium of food additive specifications* and its addenda (Annex 1, references 103, 109, 118, 124, 133, 139, 145 and 151).

The second group of substances, considered at the present meeting, comprised 10 anticaking agents, 17 flavour enhancers, 10 sweetening agents and 13 thickening agents. In response to the call for data, proposed limits and data to support the proposals were received for sodium ferrocyanide. Comments and proposals only were received for calcium silicate, magnesium silicates (synthetic), silicon dioxide (amorphous), sodium aluminosilicate, monosodium L-glutamate, sorbitol, lactitol, xylitol, ammonium alginate, tara gum, methyl cellulose, ethyl cellulose, methylethyl cellulose, powdered cellulose, hydroxypropyl cellulose and hydroxypropylmethyl cellulose.

All the comments, proposals and supporting data were taken into account. Comments on the Committee’s new proposed limits (see Table 1) are invited. When higher limits are requested, analytical data in support of such limits must be provided. If alternative values and supporting data are not received by the deadline for submission of data for the fifty-ninth meeting of the Committee, the proposed limits will supersede the existing ones, replacing those published in the

Table 1

Limits for metals in food additives

Category	Additive name	INS No.	Limits (mg/kg)				
			Arsenic	Lead	Mercury	Cadmium	Other elements
Anticaking agents	Aluminium silicate	0559	—	5	—	—	
	Calcium aluminium silicate	0556	—	5	—	—	Iron <50
	Calcium silicate	0552	—	5	—	—	Iron <50
	Ferrocyanides of calcium, potassium and sodium	0538	3	5	—	—	Copper <10, zinc <25
	Magnesium oxide	0530	—	2	—	—	
	Magnesium silicates (synthetic)	0553a	—	5	—	—	Iron <10
	Silicon dioxide (amorphous)	0551	—	5	—	—	
	Sodium aluminosilicate	0554	—	5	—	—	
	Tricalcium phosphate	0341(iii)	—	4	—	—	Iron <50
	Trimagnesium phosphate	0342(iii)	—	4	—	—	Iron <5
	Calcium 5'-guanylate	0629	—	1			
	Calcium 5'-inosinate	0633	—	1			
Flavour enhancers	Calcium 5'-ribonucleotides	0634	—	1	—	—	
	Calcium di-L-glutamate	0623	—	1	—	—	
	Dipotassium 5'-guanylate	0628	—	1	—	—	
	Dipotassium 5'-inosinate	0632	—	1	—	—	
	Disodium 5'-guanylate	0627	—	1	—	—	
	Disodium 5'-inosinate	0631	—	1	—	—	
	Disodium 5'-ribonucleotides	0635	—	1	—	—	
	Ethyl maltol	0637	—	1	—	—	
	L-Glutamic acid	0620	—	1	—	—	
	5'-Guanylic acid	0626	—	1	—	—	
	5'-Inosinic acid	0630	—	1	—	—	
	Magnesium di-L-glutamate	0625	—	1	—	—	
	Monosodium L-glutamate	0624	—	1	—	—	
	Monopotassium L-glutamate	0622	—	1	—	—	
	Monosodium L-glutamate	0621	—	1	—	—	

Table 1 (continued)

Category	Additive name	INS No.	Limits (mg/kg)				Other elements
			Arsenic	Lead	Mercury	Cadmium	
Sweeteners	Alitame	0956	—	1	—	—	
	Aspartame	0951	—	1	—	—	
	Cyclohexylsulfamic acid	0952	—	1	—	—	Selenium <30
	Isomalt	0953	—	1	—	—	Nickel <2
	Lactitol	0966	—	1	—	—	Nickel <2
	Mannitol	0421	—	1	—	—	Nickel <2
	Saccharin and its sodium, potassium and calcium salts	0954	—	1	—	—	Selenium <30
	Sorbitol/sorbitol syrup	0420	—	1	—	—	Nickel <2
	Sucralose	0955	—	1	—	—	Nickel <2
	Xylitol	0967	—	1	—	—	
Thickeners	Ammonium alginate	0403	—	2	—	—	
	Ethyl cellulose	0462	—	2	—	—	
	Gum ghatti	0419	—	2	—	—	
	Hydroxypropyl cellulose	0463	—	2	—	—	
	Hydroxypropylmethyl cellulose	0464	—	2	—	—	
	Karaya gum	0416	—	2	—	—	
	Konjac flour	0425	—	2	—	—	
	Methylethyl cellulose	0465	—	2	—	—	
	Methyl cellulose	0461	—	2	—	—	
	Polyvinylpyrrolidone	1201	—	2	—	—	
	Powdered cellulose	0460(ii)	—	2	—	—	
	Tara gum	0417	—	2	—	—	
	Tragacanth gum	0413	—	2	—	—	

INS: International Numbering System.

Compendium of food additive specifications and its addenda (Annex 1, references 103, 109, 118, 124, 133, 139, 145 and 151).

In summary, the proposed changes to the current limits are as follows:

- The limits for arsenic are to be deleted, except in ferrocyanides of calcium, potassium and sodium, for which a limit of 3 mg/kg is proposed.
- The proposed limits for lead are 2 mg/kg in thickening agents and in the anticaking agent magnesium oxide, 1 mg/kg in flavour enhancers and sweeteners, 4 mg/kg in phosphates and 5 mg/kg in silicate and ferrocyanide anticaking agents.
- No limits were proposed for cadmium or mercury, as there was no concern that they are present in any of the substances under review.
- The limits for heavy metals (as lead) were deleted.

The Committee emphasized that the absence of a limit test for a particular metal from a specification which previously included the limit test for heavy metals (as lead) indicated that the level of contamination with that particular metal is so low as to be of no concern.

4. Flavouring agents

4.1 Substances evaluated by the Procedure for the Safety Evaluation of Flavouring Agents

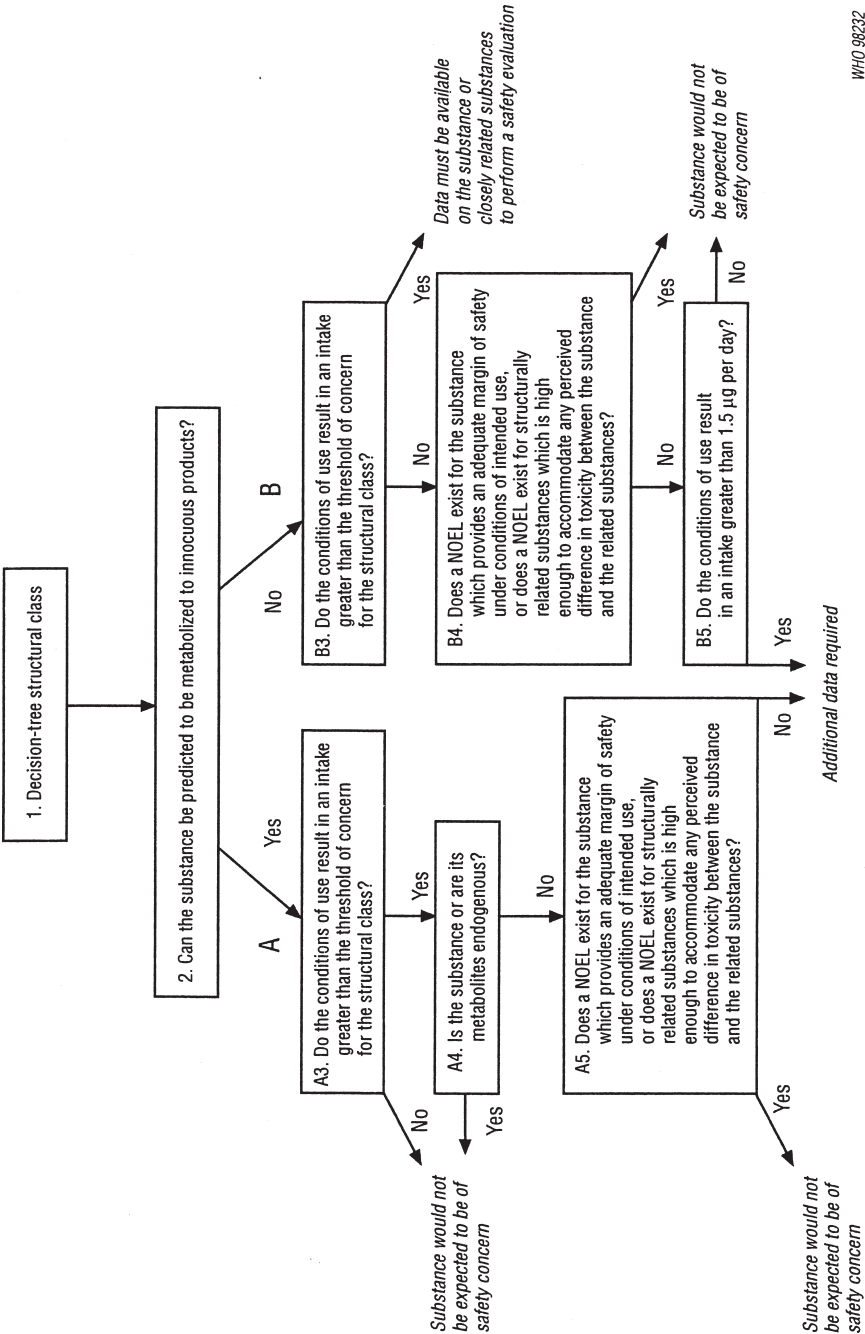
Six groups of flavouring agents were evaluated by the Procedure for the Safety Evaluation of Flavouring Agents, as outlined in Fig. 1 (Annex 1, references 116, 122, 131 and 137).

The Committee noted that, in applying the Procedure, a flavouring agent is first assigned to a structural class, as identified at the forty-sixth meeting (Annex 1, reference 122). The structural classes are as follows:

- Class I. Substances that have simple chemical structures and efficient modes of metabolism which would suggest a low order of toxicity when given by the oral route.
- Class II. Substances that have structural features that are less innocuous than those of substances in class I but are not suggestive of toxicity. Substances in this class may contain reactive functional groups.
- Class III. Substances that have structural features that permit no strong initial presumption of safety or may even suggest significant toxicity.

A key element of the Procedure involves determining whether a flavouring agent and the product(s) of its metabolism are innocuous and/or endogenous substances. For the purpose of the evaluations,

Figure 1
Procedure for the Safety Evaluation of Flavouring Agents



the Committee used the following definitions, adapted from the report of its forty-sixth meeting (Annex 1, reference 122):

Innocuous metabolic products are defined as products that are known or readily predicted to be harmless to humans at the estimated intake of the flavouring agent.

Endogenous substances are intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included. The estimated intake of a flavouring agent that is, or is metabolized to, an endogenous substance should be judged not to give rise to perturbations outside the physiological range.

Intake data

Estimates of the intake of flavouring agents by populations typically involve the acquisition of data on the amounts used in food. These data were derived from surveys in Europe and the USA. In Europe, a survey was conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring agent that had been incorporated into food sold in the European Union during the previous year. Manufacturers were requested to exclude use of flavouring agents in pharmaceutical, tobacco or cosmetic products.

In the USA, a series of surveys was conducted between 1970 and 1987 by the National Research Council of the National Academy of Sciences (under contract to the Food and Drug Administration), in which information was obtained from ingredient manufacturers and food processors on the amount of each substance destined for addition to the food supply and on the usual and maximal levels at which each substance was added to foods in a number of broad categories.

In using the data from these surveys to estimate intakes of flavouring agents, the Committee assumed that only 60% of the total amount used in Europe and 80% of that used in the USA is reported and that the total amount used in food is consumed by only 10% of the population. Intake was thus calculated from the following equation:

$$\text{Intake} \left(\frac{\mu\text{g}}{\text{person per day}} \right) = \frac{\text{Annual volume of production (kg)} \times 10^9 (\mu\text{g/kg})}{\text{Population of consumers} \times 0.6 \text{ (or } 0.8) \times 365 \text{ days}}$$

The population of consumers was assumed to be 32×10^6 in Europe and 26×10^6 in the USA.

4.1.1 **Pyrazine derivatives**

The Committee evaluated a group of 41 flavouring agents consisting of pyrazine and pyrazine derivatives (see Table 2) by the Procedure

Table 2

Summary of results of the safety evaluations of pyrazine derivatives used as flavouring agents^a

Flavouring agent	No.	CAS number and structure	Step A3 ^b Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current intake
Structural class II					
2-Methylpyrazine	761	109-08-0 	No Europe: 20 USA: 7	See note 1	No safety concern
2-Ethylpyrazine	762	13925-00-3 	No Europe: 3 USA: 6	See note 1	
2-Propylpyrazine	763	18138-03-9 	No Europe: 0.1 USA: 0.1	See note 1	
2-Isopropylpyrazine	764	29460-90-0 	No Europe: 0.1 USA: 0.1	See note 1	
2,3-Dimethylpyrazine	765	5910-89-4 	No Europe: 16 USA: 4	See note 1	
2,5-Dimethylpyrazine	766	123-32-0 	No Europe: 22 USA: 8	See note 1	
2,6-Dimethylpyrazine	767	108-50-9 	No Europe: 2 USA: 2	See note 1	

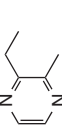
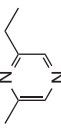
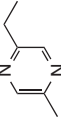
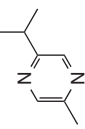
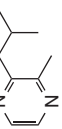
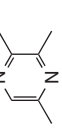
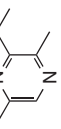
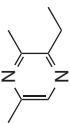
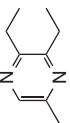
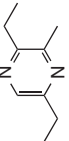
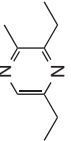
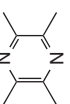
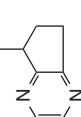
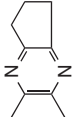
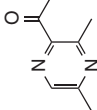
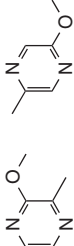
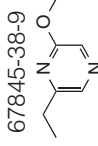
2-Ethyl-3-methylpyrazine	768	 15707-23-0	No Europe: 84 USA: 9	See note 1		No safety concern
2-Ethyl-6-methylpyrazine	769	 13925-03-6	No Europe: 0.4 USA: 0.4	See note 1		
2-Ethyl-5-methylpyrazine	770	 13360-64-0	No Europe: 5 USA: 1	See note 1		
2,3-Diethylpyrazine	771	 15707-24-1	No Europe: 2 USA: 1	See note 1		
2-Methyl-5-isopropylpyrazine	772	 13925-05-8	No Europe: ND USA: 0.4	See note 1		
2-Isobutyl-3-methylpyrazine	773	 13925-06-9	No Europe: 0.04 USA: 0.01	See note 1		
2,3,5-Trimethylpyrazine	774	 14667-55-1	No Europe: 120 USA: 46	See note 1		
2-Ethyl-3, (5 or 6)-dimethylpyrazine	775	 13360-65-1 13925-07-0	No Europe: 44 USA: 9	See note 1		

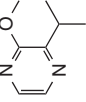
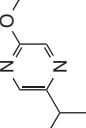
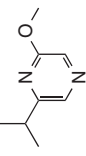
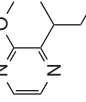
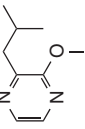
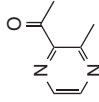
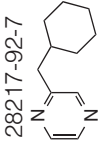
Table 2 (*continued*)

Flavouring agent	No.	CAS number and structure	Step A3 ^b Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current intake
3-Ethyl-2,6-dimethylpyrazine	776	13925-07-0 	No Europe: 2 USA: 0.3	See note 1	No safety concern
2,3-Diethyl-5-methylpyrazine	777	18138-04-0 	No Europe: 0.2 USA: 1	See note 1	
2,5-Diethyl-3-methylpyrazine	778	32736-91-7 	No Europe: 0.01 USA: 0.01	See note 1	
3,5-Diethyl-2-methylpyrazine	779	18138-05-1 	No Europe: 0.01 USA: 0.01	See note 1	
2,3,5,6-Tetramethylpyrazine	780	1124-11-4 	No Europe: 8 USA: 19	See note 1	
5-Methyl-6,7-dihydro-5H-cyclopentapyrazine	781	23747-48-0 	No Europe: 5 USA: 4	See note 1	
6,7-Dihydro-2,3-dimethyl-5H-cyclopentapyrazine	782	38917-63-4 	No Europe: 0.01 USA: 0.01	See note 1	

Acetylpyrazine	784	22047-25-2		No Europe: 14 USA: 120	See note 2
2-Acetyl-3-ethylpyrazine	785	32974-92-8		No Europe: 1 USA: 0.1	See note 2
2-Acetyl-3,(5 or 6)-dimethylpyrazine	786	54300-08-2		No Europe: 1 USA: 1	See note 2
Methoxypyrazine	787	3149-28-8		No Europe: 4 USA: 1	See note 3
(2 or 5 or 6)-Methoxy-3-methylpyrazine	788	2847-30-5		No Europe: ND USA: 15	See note 3
2-Ethyl-(3 or 5 or 6)-methoxypyrazine	789	25680-58-4		No Europe: ND USA: 1	See note 3
		67845-38-9			

No safety concern

Table 2 (continued)

Flavouring agent	No.	CAS number and structure	Step A3 ^b Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current intake
2-Methoxy-(3 or 5 or 6)-isopropylpyrazine	790	25773-40-4  56891-99-7  68039-46-3 	No Europe: ND USA: 0.1	See note 3	No safety concern
2-Methoxy-3-(1-methylpropyl)pyrazine	791	24168-70-5 	No Europe: 1 USA: 0.1	See note 3	
2-Isobutyl-3-methoxypyrazine	792	24683-00-9 	No Europe: 2 USA: 1	See note 3	
2-Acetyl-3-methylpyrazine	950	23787-80-6 	No Europe: 0.1 USA: 0.1	See note 2	
Structural class III (Cyclohexylmethyl)pyrazine	783	28217-92-7 	No Europe: ND USA: 0.01	See note 1	No safety concern

2-Methyl-(3 or 5 or 6)-ethoxypyrazine	793	<chem>CCOC1=CC=NC=C1C</chem> 32737-14-7 <chem>CCOC1=CC=NC=C1C</chem> 67845-34-5	No Europe: ND USA: 0.01	See note 3	No safety concern
		<chem>CCOC1=CC=NC=C1C</chem> 53163-97-6			
2-(Mercaptomethyl)pyrazine	794	<chem>SC1=CC=NC=C1</chem> 59021-02-2	No Europe: 0.01 USA: 0.01	See note 4	
2-Pyrazinylethane thiol	795	<chem>SCC1=CC=NC=C1</chem> 35250-53-4	No Europe: 0.2 USA: 1	See note 4	
Pyrazinylmethyl methyl sulfide	796	<chem>CSCC1=CC=NC=C1</chem> 21948-70-9	No Europe: ND USA: 0.01	See note 5	
(3 or 5 or 6)-(Methylthio)-2-methylpyrazine	797	<chem>CS1=CC=NC=C1C</chem> 2882-20-4 <chem>CS1=CC=NC=C1C</chem> 2884-14-2 <chem>CS1=CC=NC=C1C</chem> 2884-13-1	No Europe: 7 USA: 13	See note 5	

Table 2 (*continued*)

Flavouring agent	No.	CAS number and structure	Step A3 ^b Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current intake
5-Methylquinoxaline	798	13708-12-8 	No Europe: 26 USA: 1	See note 1	No safety concern
Pyrazine	951	290-37-9 	No Europe: 0.2 USA: 0.2	See note 1	
5,6,7,8-Tetrahydroquinoxaline	952	34413-35-9 	No Europe: 8 USA: ND	See note 1	

CAS: Chemical Abstracts Service; ND: no data on intake reported.

^a Step 2: All of the flavouring agents in this group are expected to be metabolized to innocuous products.

^b The thresholds for human intake for structural classes II and III are 540 µg/day and 90 µg/day, respectively. All intake values are expressed in µg/day.

Notes to Table 1

1. Detoxication by excretion in the urine unchanged, side-chain oxidation followed by conjugation and excretion, or ring hydroxylation followed by conjugation and excretion.
2. Detoxication as given in note 1 plus reduction to the corresponding alcohol and conjugation with glucuronic acid.
3. Detoxication as given in note 1 plus *O*-dealkylation followed by conjugation and excretion.
4. Detoxication as given in note 1 plus thiol oxidation, methylation, formation of mixed disulfides and conjugation with glucuronic acid.
5. Detoxication as given in note 1 plus *S*-oxidation to sulfoxide and sulfone analogues.

for the Safety Evaluation of Flavouring Agents (see Fig. 1). None of these agents has previously been evaluated by the Committee.

Thirty-four of the flavouring agents in this group are naturally occurring components of food. Members of this group have been detected in asparagus, potato, kohlrabi and wheaten bread.

4.1.1.1 Estimated daily per capita intake

The total annual volume of production of pyrazine and the 40 pyrazine derivatives in this group is approximately 2700kg in Europe and 2100kg in the USA. About 64% of the total annual volume of production in Europe is accounted for by 2,3,5-trimethylpyrazine (No. 774), 2-ethyl-3-methylpyrazine (No. 768) and 2-ethyl-3,(5 or 6)-dimethylpyrazine (No. 775). In the USA, about 66% of the total annual volume of production is accounted for by acetylpyrazine (No. 784), 2,3,5-trimethylpyrazine (No. 774) and 2,3,5,6-tetramethylpyrazine (No. 780). The estimated daily per capita intake of 2,3,5-trimethylpyrazine (No. 774) in Europe and of acetylpyrazine (No. 784) in the USA is about 120µg. The daily per capita intake of each agent in Europe and the USA is reported in Table 2.

4.1.1.2 Absorption, distribution, metabolism and elimination

Pyrazine is a weak base ($\log_{10}$ of the reciprocal of the dissociation constant, 13.4). Absorption of weak amine bases such as pyrazine derivatives is optimal at the pH of the intestine (5.0–7.0). In humans and laboratory rodents, orally administered substituted pyrazines are rapidly absorbed from the gut and excreted.

Alkyl-, alicyclic- and alkylaryl-substituted pyrazine derivatives. The biotransformation of alkyl-, alicyclic- and alkylaryl-substituted pyrazine derivatives (Nos 761–783 and 798) is expected to occur by oxidation of the alkyl side-chains. Methyl-substituted pyrazines are oxidized to yield the corresponding pyrazine-2-carboxylic acids. 5-Methylquinoxaline (No. 798) would be expected to be metabolized by the same pathway as the methyl- and ring-substituted pyrazine derivative 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), which is oxidized to yield the corresponding hydroxymethyl derivatives. An alternative pathway for the metabolism of pyrazine derivatives and the primary metabolic pathway for pyrazine (No. 951) itself involves hydroxylation of the pyrazine ring. Products of oxidative metabolism can be excreted unchanged or conjugated with glycine, glucuronic acid or sulfate before excretion.

Pyrazine derivatives containing an oxygenated functional group in the side-chain. In pyrazine derivatives containing a ring activator (e.g. a

methoxy substituent), significant ring hydroxylation may occur. Pyrazines with a methoxy side-chain, such as methoxypyrazine (No. 787), are more susceptible to nucleophilic attack, probably by molybdenum hydroxylases, and therefore primarily undergo ring hydroxylation. Additionally, the methoxy side-chain is *O*-demethylated. In rats, 3-acetylpyridine is reduced mainly to the secondary alcohol and excreted as the glucuronic acid conjugate. Therefore, acylated pyrazines (Nos 784–786 and 950) are expected to be metabolized mainly by reduction of the ketone functional group.

Pyrazine derivatives containing a thiol or sulfide functional group in the side-chain. Four pyrazine derivatives in this group contain either a thiol or a sulfide functional group in their side-chain. The possible metabolic pathways for the thiols, 2-(mercaptomethyl)pyrazine (No. 794) and 2-pyrazinylethane thiol (No. 795), include oxidation to form sulfinic acid (RSO_2H) and sulfonic acid (RSO_3H); methylation to yield methyl sulfides, which then form sulfoxides and sulfones; reaction with physiological thiols to form mixed disulfides and conjugation with glucuronic acid; or oxidation of the α -carbon, which results in desulfuration and formation of an aldehyde. Pyrazinylmethyl methyl sulfide (No. 796) and (3 or 5 or 6)-(methylthio)-2-methylpyrazine (No. 797) are predicted to be metabolized to sulfoxides and then to sulfones, which are the main urinary metabolites of simple sulfides. The Committee at its fifty-third meeting (Annex 1, reference 143) considered the pathways of metabolism of sulfur centres in its evaluation of a group of 137 flavouring agents that included aliphatic and aromatic sulfides and thiols, with and without an additional oxygenated functional group.

4.1.1.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents, the Committee assigned 32 of the 41 flavouring agents with one aromatic ring to structural class II on the basis of structural considerations and because they occur naturally (Nos 761–782, 784–792 and 950). Six flavouring agents with one aromatic ring were assigned to structural class III (Nos 783 and 793–797), as was 5,6,7,8-tetrahydroquinoxaline (No. 952). Pyrazine (No. 951) is the only agent in the group that bears no ring substituent, and it was therefore also assigned to structural class III. 5-Methylquinoxaline (No. 798) was assigned to structural class III because it is a polyheteroaromatic substance that does not contain sodium, potassium or calcium sulfonate or sulfamate.

Step 2. At current levels of intake, all 41 flavouring agents can be predicted to be metabolized to innocuous products, and the pathways

involved would not be expected to be saturated. The evaluation of these substances therefore proceeded via the left-hand side of the decision-tree.

Step A3. The estimated daily per capita intakes of all 32 flavouring agents in structural class II and all nine substances in structural class III are below the thresholds of concern for these classes (540 µg and 90 µg, respectively). The Committee concluded that these substances would not be expected to be of safety concern at the currently estimated levels of use.

Table 2 summarizes the evaluations of pyrazine and 40 pyrazine derivatives used as flavouring agents.

4.1.1.4 Consideration of combined intakes from use as flavouring agents

In the unlikely event that all 32 pyrazine derivatives in structural class II were to be consumed concurrently on a daily basis, the estimated combined intake would not exceed the threshold for human intake for this class (540 µg/day). In the unlikely event that all nine flavouring agents in structural class III were to be consumed concurrently on a daily basis, the estimated combined intake would not exceed the threshold for human intake for this class (90 µg/day). All the flavouring agents in this group are expected to be efficiently metabolized, and the available metabolic pathways would not be saturated. Evaluation of all the data indicated no safety concern associated with combined intake.

4.1.1.5 Conclusions

The Committee concluded that the safety of pyrazine and the 40 derivatives of pyrazine in this group would not raise concern at the currently estimated levels of intake.

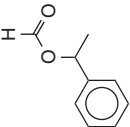
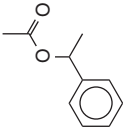
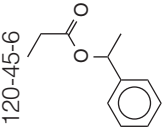
A monograph summarizing the data on the safety of this group of flavouring agents and specifications were prepared.

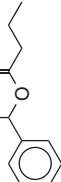
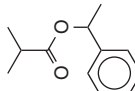
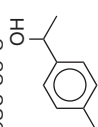
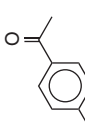
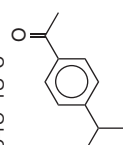
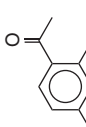
4.1.2 Aromatic substituted secondary alcohols, ketones and related esters

The Committee evaluated a group of flavouring agents that included α -methylbenzyl alcohol (No. 799), acetophenone (No. 806) and 36 structurally related aromatic secondary alcohols, ketones and related esters (Table 3) by the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1). All the members of this group are considered to be aromatic secondary alcohols, ketones or related esters. The aromatic ring may contain additional alkyl substituents or a methoxy group, and the aliphatic side-chain may be unsaturated or

Table 3

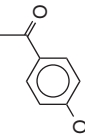
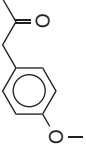
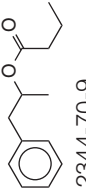
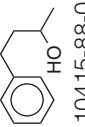
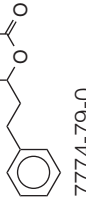
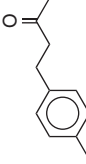
Summary of results of the safety evaluations of aromatic secondary alcohols, ketones and related esters used as flavouring agents^a

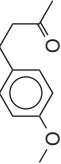
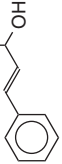
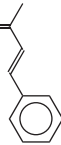
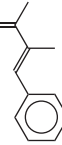
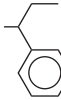
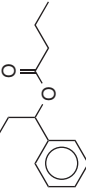
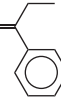
Flavouring agent	No.	CAS number and structure	Step A3/B3 ^b Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for substance or related substance?	Step B5 Does intake exceed 1.5µg/day?	Comments on predicted metabolism	Conclusions based on current intake
Structural class I							
α-Methylbenzyl alcohol ^c	799	98-85-1 	No Europe: 32 USA: 72	NR	NR	See note 1	No safety concern
α-Methylbenzyl formate	800	7775-38-4 	No Europe: 0.04 USA: 0.4	NR	NR	See note 2	
α-Methylbenzyl acetate	801	93-92-5 	No Europe: 200 USA: 650	NR	NR	See note 2	
α-Methylbenzyl propionate	802	120-45-6 	No Europe: 1 USA: 27	NR	NR	See note 2	

α -Methylbenzyl butyrate	803	3460-44-4		No Europe: 1 USA: 0.01	NR	NR	See note 2
α -Methylbenzyl isobutyrate	804	7775-39-5		No Europe: 29 USA: 1	NR	NR	See note 2
<i>p</i> , α -Dimethylbenzyl alcohol	805	536-50-5		No Europe: 0.2 USA: 1	NR	NR	See note 1
Acetophenone	806	98-86-2		No Europe: 18 USA: 170	NR	NR	See note 3
4-Methylacetophenone	807	122-00-9		No Europe: 26 USA: 37	NR	NR	See note 3
<i>p</i> -Isopropylacetophenone	808	645-13-6		No Europe: 0.01 USA: 0.4	NR	NR	See note 3
2,4-Dimethylacetophenone	809	89-74-7		No Europe: 0.3 USA: 0.01	NR	NR	See note 3

No safety
concern

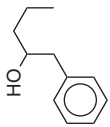
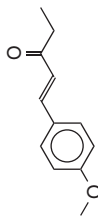
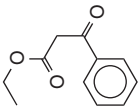
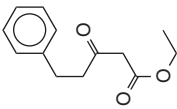
Table 3 (continued)

Flavouring agent	No.	CAS number and structure	Step A3/B3 ^b Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for substance or related substance?	Step B5 Does intake exceed 1.5µg/day?	Comments on predicted metabolism	Conclusions based on current intake
Acetanisole	810	100-06-1 	No Europe: 150 USA: 84	NR	NR	See notes 3 and 4	No safety concern
1-(<i>p</i> -Methoxyphenyl)-2-propanone	813	122-84-9 	No Europe: 0.2 USA: 0.1	NR	NR	See note 5	
α-Methylphenethyl butyrate	814	68922-11-2 	No Europe: 0.1 USA: 0.1	NR	NR	See note 6	
4-Phenyl-2-butanol	815	2344-70-9 	No Europe: 1 USA: 0.3	NR	NR	See note 7	
4-Phenyl-2-butyl acetate	816	10415-88-0 	No Europe: ND USA: 7	NR	NR	See note 7	
4-(<i>p</i> -Tolyl)-2-butanone	817	7774-79-0 	No Europe: 0.01 USA: 0.4	NR	NR	See notes 8 and 9	

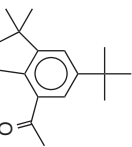
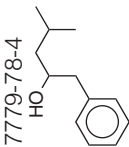
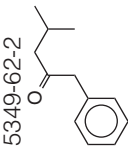
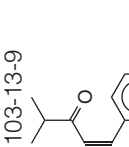
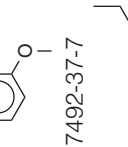
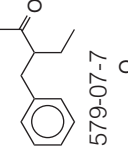
4-(p-Methoxyphenyl)-2-butanone	818		No Europe: 5 USA: 840	NR	NR	See notes 8 and 9
4-Phenyl-3-buten-2-ol	819		No Europe: 2 USA: 0.1	NR	NR	See note 10
4-Phenyl-3-buten-2-one	820		No Europe: 3 USA: 7	NR	NR	See note 10
3-Methyl-4-phenyl-3-buten-2-one	821		No Europe: 0.1 USA: 0.1	NR	NR	See note 10
1-Phenyl-1-propanol	822		No Europe: 0.3 USA: 0.1	NR	NR	See note 11
α-Ethylbenzyl butyrate	823		No Europe: ND USA: 0.3	NR	NR	See note 2
Propiophenone	824		No Europe: 0.01 USA: 0.03	NR	NR	See note 11

No safety
concern

Table 3 (continued)

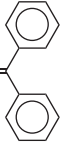
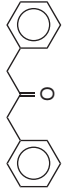
Flavouring agent	No.	CAS number and structure	Step A3/B3 ^b Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for substance or related substance?	Step B5 Does intake exceed 1.5µg/day?	Comments on predicted metabolism	Conclusions based on current intake
α-Propylphenethyl alcohol	825	705-73-7 	No Europe: 0.1 USA: 1	NR	NR	See note 6	No safety concern
1-(p-Methoxyphenyl)-1-penten-3-one	826	104-27-8 	No Europe: 0.5 USA: 110	NR	NR	See note 10	
Ethyl benzoylacetate	834	94-02-0 	No Europe: 0.01 USA: 140	NR	NR	See note 12	
Ethyl 2-acetyl-3-phenylpropionate	835	620-79-1 	No Europe: ND USA: 0.4	NR	NR	See note 12	

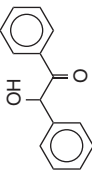
Structural class II

4-Acetyl-6- <i>tert</i> -butyl-1,1-dimethylindan	812		13171-00-1	No Europe: 6 USA: 1	No	Yes	Additional data required	
α -Isobutylphenethyl alcohol	827		7779-78-4	No Europe: 29 USA: 3	NR	NR		See note 6
4-Methyl-1-phenyl-2-pentanone	828		5349-62-2	No Europe: 10 USA: 0.3	NR	NR		See note 6
1-(4-Methoxyphenyl)-4-methyl-1-penten-3-one	829		103-13-9	No Europe: 33 USA: 0.3	NR	NR		See note 10
3-Benzyl-4-heptanone	830		7492-37-7	No Europe: ND USA: 1	NR	NR		See note 13
1-Phenyl-1,2-propanedione	833		579-07-7	No Europe: 6 USA: 0.1	NR	NR		See note 14

No safety
concern

Table 3 (continued)

Flavouring agent	No.	CAS number and structure	Step A3/B3 ^b Does intake exceed the threshold for human intake?	Step B4 Adequate margin of safety for substance or related substance?	Step B5 Does intake exceed 1.5µg/day?	Comments on predicted metabolism	Conclusions based on current intake
Structural class III							
Methyl β-naphthyl ketone	811	93-08-3 	No Europe: 7 USA: 48	Yes The NOEL of 33 mg/kg of body weight per day in a 90-day study in rats is >10 000 times the estimated intake of methyl β-naphthyl ketone when used as a flavouring agent	NR		No safety concern
Benzophenone	831	119-61-9 	No Europe: 27 USA: 11	NR	NR	See note 15	
1,3-Diphenyl-2-propanone	832	102-04-5 	No Europe: 0.1 USA: 0.1	NR	NR	See note 15	

Benzoin	836	119-53-9		No Europe: 7 USA: 21	NR	NR	See note 16	No safety concern

CAS: Chemical Abstracts Service; ND: no data on intake reported; NR: not required for evaluation.

^a Step 1: Twenty-eight of the flavouring agents in this group are in structural class I, six are in structural class II and four are in structural class III.

^b Step 2: All the flavouring agents in this group, except for Nos 811 and 812, are predicted to be metabolized to innocuous products.

^c The thresholds for human intake for structural classes I, II and III are 1800 µg/day, 540 µg/day and 90 µg/day, respectively. All intake values are expressed in µg/day.

^d An ADI of 0–0.1 mg/kg of body weight was established for α-methylbenzyl alcohol by the Committee at its forty-first meeting (Annex 1, reference 107), which was maintained at the present meeting.

Notes to Table 3

1. α-Methylbenzyl alcohol is conjugated with glucuronic acid and excreted in the urine. Oxidation of the methyl group to yield mandelic acid and hippuric acid may also occur.
2. α-Methylbenzyl esters are hydrolysed to simple aliphatic carboxylic acids and α-methylbenzyl alcohol. The acids are completely oxidized, and the alcohol is conjugated with glucuronic acid and excreted.
3. Acetophenone is reduced to α-methylbenzyl alcohol, conjugated with glucuronic acid and excreted primarily in the urine. The ketone may also undergo methyl group oxidation, eventually yielding mandelic acid and hippuric acid. These are also excreted in the urine.
4. Acetanilide may also undergo O-demethylation to yield the corresponding phenol. The phenol is excreted as the sulfate or glucuronic acid conjugate.
5. 1-(p-Methoxyphenyl)-2-propanone is reduced to the corresponding alcohol and excreted. O-Demethylation to yield the corresponding phenol may also occur.
6. α-Methylphenethyl butyrate is hydrolysed to butyric acid and α-methylphenethyl alcohol. The acid is completely metabolized and the alcohol is conjugated with glucuronic acid and excreted.
7. 4-Phenyl-2-butanol is conjugated with glucuronic acid and excreted. The corresponding acetate is predicted to be hydrolysed first to the alcohol and then to acetic acid.
8. 4-(p-Tolyl)-2-butanone is reduced to the corresponding alcohol. The alcohol may be conjugated with glucuronic acid and excreted primarily in the urine. The ketone may also undergo methyl group oxidation, eventually yielding a related carboxylic acid that is further oxidized to the phenylacetic acid derivative, which can be conjugated with glycine and excreted.
9. The alcohol may also be oxidized to the corresponding ketone and conjugated with glutathione.
10. 4-Phenyl-3-buten-2-one may be reduced to 4-phenyl-3-buten-2-ol. The alcohol is then further metabolized and excreted primarily as glycine conjugates. The ketone may also be conjugated with glutathione.
11. Propiophenone is reduced to the corresponding alcohol. The butyrate ester (No. 823) is predicted to be hydrolysed to the same alcohol (and butyric acid). The alcohol is conjugated with glucuronic acid and excreted primarily in the urine.
12. The esters are hydrolysed to ethanol and keto-carboxylic acids. The acids may be further oxidized and excreted as hippuric acid. The alcohol is completely metabolized.
13. Oxidation of side-chains is anticipated, primarily at the ω or ω-1 carbon atom. Carbonyl groups are reduced to alcohol, which can be conjugated with glucuronic acid. Oxidation of the side-chain can continue to benzoic or phenylacetic acid.
14. Reduced to the corresponding diol, which is conjugated with glucuronic acid.
15. Fifty per cent of a dose of 360 mg of benzophenone (No. 831) administered to rabbits by gavage was excreted as the glucuronide of the corresponding secondary alcohol within 48 h. 1,3-Diphenyl-2-propanone (No. 832), which is less sterically hindered than benzophenone, is also anticipated to be reduced to the alcohol and excreted as the glucuronide.
16. Benzoin is excreted as the glucuronic acid conjugate.

contain additional oxygenated functional groups. Of the 38 flavouring agents in this group, 34 are simple saturated or unsaturated methoxy- or alkyl-substituted benzene derivatives containing a secondary alcohol, corresponding ketone and/or related ester functional group.

The Committee had previously evaluated three members of this group. α -Methylbenzyl alcohol (No. 799) was evaluated at the forty-first meeting (Annex 1, reference 107), when an ADI of 0–0.1 mg/kg of body weight was established. At its twenty-fourth meeting, the Committee reviewed data on α -isobutylphenethyl alcohol (No. 827) (Annex 1, reference 53), and at its twenty-third and twenty-fifth meetings, it reviewed data on methyl β -naphthyl ketone (No. 811) (Annex 1, references 50 and 56). No ADI was allocated to either of these flavouring agents.

Of the 38 aromatic substituted secondary alcohols, ketones and related esters considered, 16 have been reported to occur naturally in foods. For instance, α -methylbenzyl alcohol (No. 799) has been detected in cheese, fruit and tea, and the corresponding ketone acetophenone (No. 806) is a natural component of berries, seafood, beef and nuts.

4.1.2.1 Estimated daily per capita intake

The total annual volume of production of the 38 aromatic secondary alcohols, ketones and related esters considered here is approximately 4.2 tonnes in Europe and 17 tonnes in the USA. Approximately 58% of the total annual volume of production in Europe is accounted for by α -methylbenzyl acetate (No. 801) and acetanisole (No. 810). The estimated daily per capita intakes of these two flavouring agents in Europe are 200 μ g and 150 μ g, respectively. In the USA, approximately 80% of the total volume of production arises from use of α -methylbenzyl acetate (No. 801), acetophenone (No. 806), 4-(*p*-methoxyphenyl)-2-butanone (No. 818) and ethyl benzoylacetate (No. 834). The estimated daily per capita intakes of these agents are 650 μ g, 170 μ g, 840 μ g and 140 μ g, respectively.

The estimated daily intake of each flavouring agent in the group is reported in Table 3.

4.1.2.2 Absorption, distribution, metabolism and elimination

Generally, the flavouring agents in this group are rapidly absorbed from the gut. The aromatic secondary alcohols (and aromatic ketones after reduction to the corresponding secondary alcohols) are then either conjugated with glucuronic acid and excreted primarily in the urine, or are further oxidized and excreted mainly as glycine conjugates. As aromatic esters are generally hydrolysed *in vivo* by the

catalytic activity of carboxylesterases, which are found predominantly in hepatocytes, it is anticipated that the 10 esters in this group of flavouring agents will be hydrolysed to their parent aromatic or aliphatic alcohols and carboxylic acids. The eight aromatic secondary alcohols formed as a result of this process are excreted as their glucuronides or are further metabolized and excreted in the urine. The corresponding eight simple aliphatic carboxylic acids are metabolized completely by well-known pathways. The two remaining esters (Nos 834 and 835) are hydrolysed to ethanol and aromatic keto-carboxylic acids (3-oxo-3-phenylpropanoic acid and 3-oxo-5-phenylpentanoic acid, respectively), which are anticipated to be further metabolized and excreted in the urine, like other aromatic ketones.

Simple aromatic ring substitution with methyl, isopropyl or methoxy groups (Nos 805, 807–810, 813, 817, 818, 826 and 829) is predicted to have little effect on the principal metabolic pathways. It is more difficult to predict the metabolic fate of Nos 811 and 812 on the basis of the available data, as it is not known to what extent they are distributed in the tissues and eliminated. One of these substances, 4-acetal-6-*tert*-butyl-1,1-dimethylindan (No. 812), might accumulate in human adipose tissue.

4.1.2.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1) to these 38 aromatic flavouring agents, the Committee assigned 28 to structural class I (Nos 799–810, 813–826, 834 and 835). Six flavouring agents were assigned to structural class II, one (No. 833) because it is a vicinal diketone and the other five because they contain a fused non-aromatic carbocyclic ring (No. 812) or aliphatic substituent chains with more than five carbon atoms (Nos 827–830). Four of the agents (Nos 811, 831, 832 and 836) were assigned to structural class III because they contain more than one aromatic ring and cannot be hydrolysed to mononuclear residues.

Step 2. At current levels of estimated intake, 36 of the 38 flavouring agents in this group are predicted to be metabolized to innocuous products and the available metabolic pathways would not be expected to be saturated. Evaluation of these substances therefore proceeded via the left-hand side of the decision-tree. The two remaining flavouring agents (Nos 811 and 812) cannot be predicted to be metabolized to innocuous products, and therefore their evaluation proceeded via the right-hand side of the decision-tree.

Step A3. The estimated daily per capita intakes of the 28 flavouring agents in structural class I, five of the six flavouring agents in

structural class II and three of the four agents in structural class III are below the thresholds of concern for these classes (1800 µg, 540 µg and 90 µg, respectively). The Committee concluded that these substances would not be expected to be of safety concern at their currently estimated levels of use as flavouring agents.

Step B3. The estimated daily per capita intakes of one agent in structural class II (No. 812) and one in structural class III (No. 811) are below the thresholds of concern for these classes (540 µg and 90 µg, respectively).

Step B4. The NOEL identified for methyl β-naphthyl ketone (No. 811) in a 90-day study in rats treated orally was the highest dose tested, 33 mg/kg of body weight per day. This dose provided safety margins >100 000 and >10 000 times the estimated daily per capita intakes in Europe and in the USA, respectively. The Committee concluded that methyl β-naphthyl ketone does not pose a safety concern at currently estimated levels of use as a flavouring agent.

No data were available on the toxicity of the remaining agent (No. 812) or of relevant structurally related substances. Accordingly, the evaluation of this substance proceeded to step B5.

Step B5. As the estimated daily per capita intake of 4-acetyl-6-*tert*-butyl-1,1-dimethylindan (No. 812) in Europe (6 µg) exceeds the threshold of 1.5 µg/person per day, further data are required for a safety evaluation. The Committee concluded that this flavouring agent cannot be classified as of “no safety concern at current level of intake”.

Table 3 summarizes the evaluations of α-methylbenzyl alcohol and acetophenone and 36 structurally related flavouring agents.

4.1.2.4 Consideration of combined intakes from use as flavouring agents

In the unlikely event that all foods containing all the flavouring agents in structural classes I and II were to be consumed simultaneously on a daily basis, the estimated combined intake would exceed the human intake threshold for class II (540 µg). However, the agents are expected to be metabolized efficiently and the available metabolic pathways would not be saturated. Evaluation of all the data indicated no safety concern associated with combined intake.

4.1.2.5 Conclusions

The Committee concluded that 37 of this group of 38 aromatic secondary alcohols, ketones and related esters would not pose a safety concern at currently estimated levels of use as flavouring agents.

The Committee noted that when data on toxicity were available, they were consistent with the results of the safety evaluation. Data on toxicity were required for two agents (Nos 811 and 812) in application of the Procedure. Relevant data were available for one of these substances (No. 811), which gave a large safety margin in relation to the estimated intake.

The Committee required additional data to evaluate the safety of 4-acetyl-6-*tert*-butyl-1,1-dimethylindan (No. 812), which could not be predicted to be metabolized to innocuous products, for which satisfactory data on toxicity were not available and of which the estimated daily intake, 6 µg/person in Europe, exceeded the threshold of 1.5 µg/person per day.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.1.3 ***Benzyl derivatives***

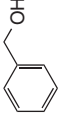
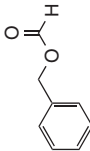
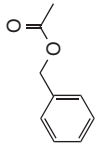
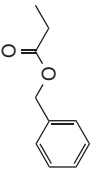
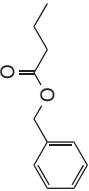
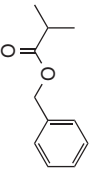
The Committee evaluated a group of 37 flavouring agents¹ that consisted of benzyl alcohol (No. 25), benzaldehyde (No. 22), benzoic acid (No. 850) and related substances (Table 4) by the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1). All members of this group are aromatic primary alcohols, aldehydes, carboxylic acids or related esters or acetals. The benzene ring may be ring-substituted with alkyl substituents (Nos 863–869).

The Committee had previously evaluated five members of the group. Benzyl alcohol (No. 25) was evaluated at the twenty-third and forty-sixth meetings (Annex 1, references 50 and 122); benzyl acetate (No. 23) was evaluated at the eleventh, twenty-seventh, twenty-ninth, thirty-first, thirty-fifth, forty-first and forty-sixth meetings (Annex 1, references 14, 62, 70, 77, 88, 107 and 122); benzyl benzoate (No. 24) was evaluated at the fifteenth and twenty-third meetings (Annex 1, references 26 and 50); benzaldehyde (No. 22) was evaluated at the eleventh and forty-sixth meetings (Annex 1, references 14 and 122; and benzoic acid (No. 850) was evaluated at the sixth, ninth, seventeenth, twenty-seventh and forty-sixth meetings (Annex 1, references 6, 11, 32, 62 and 122). At its forty-sixth meeting, the Committee evaluated benzyl acetate, benzyl alcohol, benzaldehyde, benzoic acid and the benzoate salts (calcium, potassium and sodium) as a group and maintained the group ADI of 0–5 mg/kg of body weight as benzoic acid equivalents (Annex 1, reference 122).

¹ During evaluation of these flavouring agents, the Committee questioned whether some substances in this group (Nos 850, 861 and 862) were used as flavouring agents and therefore appropriately evaluated by the Procedure. Information to address this question will be sought from the relevant manufacturers.

Table 4

Summary of results of the safety evaluations of benzyl derivatives^a

Flavouring agent	No.	CAS number and structure	Step A3/B3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step B4 Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Benzyl alcohol ^c	25	100-51-6 	Yes Europe: 16000 USA: 17000	Yes		See note 1	No safety concern
Benzyl formate	841	104-57-4 	No Europe: 41 USA: 51	NR		See note 2	
Benzyl acetate ^c	23	140-11-4 	No Europe: 1400 USA: 850	NR		See note 2	
Benzyl propionate	842	122-63-4 	No Europe: 49 USA: 99	NR		See note 2	
Benzyl butyrate	843	103-37-7 	No Europe: 120 USA: 290	NR		See note 2	
Benzyl isobutyrate	844	103-28-6 	No Europe: 15 USA: 21	NR		See note 2	

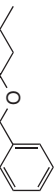
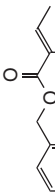
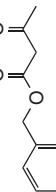
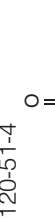
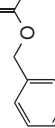
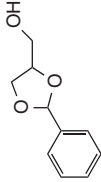
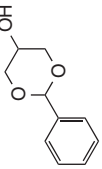
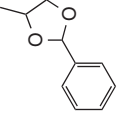
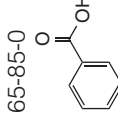
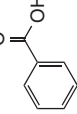
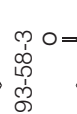
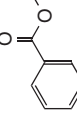
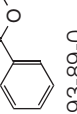
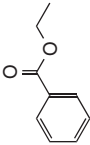
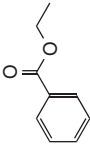
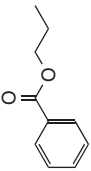
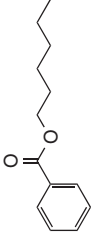
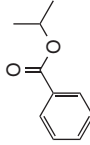
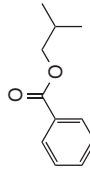
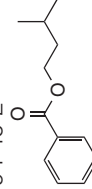
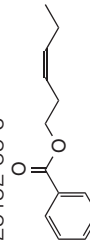
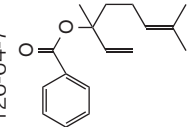
Benzyl isovalerate	845	103-38-8		No Europe: 14 USA: 19	NR	See note 2
Benzyl <i>trans</i> -2-methyl-2-butenate	846	37526-88-8		No Europe: 0.01 USA: 0.03	NR	See note 2
Benzyl 2,3-dimethylcrotonate	847	7492-69-5		No Europe: 0.01 USA: 1	NR	See note 2
Benzyl acetoacetate	848	5396-89-4		No Europe: 0.2 USA: 0.07	NR	See note 2
Benzyl benzoate ^c	24	120-51-4		Yes Europe: 1900 USA: 4200	Yes	No safety concern
Benzyl phenylacetate	849	102-16-9		No Europe: 5 USA: 57	NR	
Benzaldehyde ^c	22	100-52-7		Yes Europe: 9300 USA: 36000	Yes	See note 3
Benzaldehyde dimethyl acetal	837	1125-88-8		No Europe: 0.2 USA: 0.3	NR	See note 4

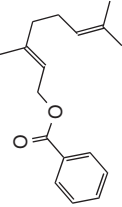
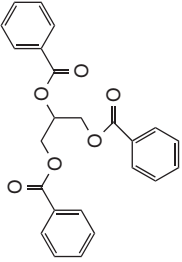
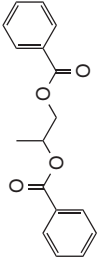
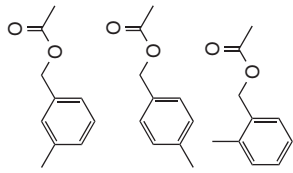
Table 4 (continued)

Flavouring agent	No.	CAS number and structure	Step A3/B3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step B4 Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Benzaldehyde glyceryl acetal	838	1319-88-6 	No Europe: 16 USA: 300	NR	See note 4		No safety concern
							
Benzaldehyde propylene glycol acetate	839	2568-25-4 	No Europe 0.04 USA: 110	NR	See note 4		No safety concern
							
Benzoic acid ^{c,d}	850	65-85-0 	No Europe: 39 USA: 340	NR	See note 5		Evaluation not finalized
							
Methyl benzoate	851	93-58-3 	No Europe: 47 USA: 230	NR	See note 6		No safety concern
							
Ethyl benzoate	852	93-89-0 	No Europe: 110 USA: 110	NR	See note 6		No safety concern
							

Propyl benzoate	853	2315-68-6		No Europe: 0.01 USA: 0.3	NR	See note 6
Hexyl benzoate	854	6789-88-4		No Europe: 380 USA: 1	NR	See note 6
Isopropyl benzoate	855	939-48-0		No Europe: 0.004 USA: 0.3	NR	See note 6
Isobutyl benzoate	856	120-50-3		No Europe: 0.4 USA: 1	NR	See note 6
Isoamyl benzoate	857	94-46-2		No Europe: 110 USA: 33	NR	See note 6
<i>cis</i> -3-Hexenyl benzoate	858	25152-85-6		No Europe: 8 USA: 0.1	NR	See note 6
Linalyl benzoate	859	126-64-7		No Europe: 10 USA: 2	NR	See note 6

No safety
concern

Table 4 (continued)

Flavouring agent	No.	CAS number and structure	Step A3/B3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step B4 Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Geranyl benzoate	860	94-48-4 	No Europe: 4 USA: 0.03	NR		See note 6	No safety concern
Glyceryl tribenzoate ^d	861	614-33-5 	No Europe: ND USA: 49	NR		See note 6	Evaluation not finalized
Propylene glycol dibenzoate ^d	862	19224-26-1 	No Europe: ND USA: 14	NR		See note 6	
Methylbenzyl acetate (mixed <i>ortho</i> -, <i>meta</i> - and <i>para</i> -isomers)	863	29759-11-3 	No Europe: ND USA: 3	NR		See note 2	No safety concern

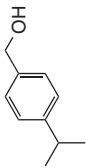
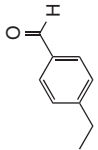
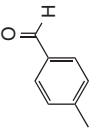
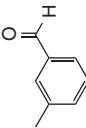
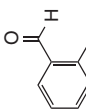
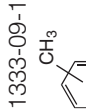
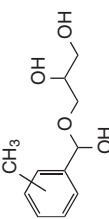
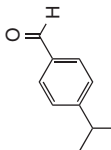
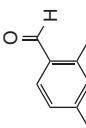
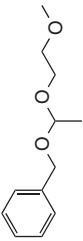
<i>p</i>-Isopropylbenzyl alcohol	864	536-60-7		No Europe: 0.3 USA: 0.3	NR	See note 1
	865	4748-78-1		No Europe: 0.4 USA: 6	NR	See note 3
	866	1334-78-7		No Europe: 260 USA: 1100	NR	See note 3
Tolualdehydes (mixed <i>ortho</i>-, <i>meta</i>- and <i>para</i>-isomers)				No safety concern		
						
						
Tolualdehyde glyceryl acetal	867	1333-09-1		No Europe: 0.01 USA: 1	NR	See note 4
	868	122-03-2		No Europe: 130 USA: 1	NR	See note 3
2,4-Dimethylbenzaldehyde	869	15764-16-6		No Europe: 0.4 USA: 0.1	NR	See note 3

Table 4 (continued)

Flavouring agent	No.	CAS number and structure	Step A3/B3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step B4 Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Benzyl 2-methoxyethyl acetal	840	7492-39-9 	No Europe: ND USA: 1		Yes The NOEL of 6 mg/kg of body weight per day in a two-generation study of reproductive toxicity in rats is > 10000 times the estimated intake of benzyl 2-methoxyethyl acetal when used as a flavouring agent	See note 7	No safety concern

CAS: Chemical Abstracts Service; ND: no data on intake reported; NR: not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the Procedure.

^a Step 1: All of the flavouring agents in this group are in structural class I.

^b Step 2: All of the flavouring agents in this group are expected to be metabolized to innocuous products, except for benzyl 2-methoxyethyl acetal (No. 840). The threshold for human intake for structural class I is 1800 µg/day. All intake values are expressed in µg/day.

^c A group ADI of 0–5 mg/kg of body weight for benzoic acid, the benzoate salts (calcium, potassium and sodium), benzaldehyde, benzyl acetate and benzyl alcohol, expressed as benzoic acid equivalents, was confirmed by the Committee at its forty-sixth meeting (Annex 1, reference 122) and extended to include benzyl benzoate at the present meeting.

^d Further information is required to determine whether this substance is in current use as a flavouring agent.

Notes to Table 4

1. Benzyl alcohols are oxidized to the corresponding acids, which are conjugated with glycine and excreted as hippuric acid.
2. Benzyl esters are hydrolysed to the corresponding acids and alcohols.
3. Benzyl aldehydes are oxidized to the corresponding acids.
4. Benzaldehyde acetals are hydrolysed to yield the aldehyde.
5. Benzoic acid is conjugated with glycine and excreted as hippuric acid.
6. Benzoate esters are hydrolysed to yield the corresponding alcohols and acids.
7. Hydrolysed to acetaldehyde, benzyl alcohol and 2-methoxyethanol.

Of the 37 substances in this group, 29 have been reported to occur naturally in foods. They have been detected in a wide variety of fruits, vegetables, meats, cheeses and wine.

4.1.3.1 Estimated daily per capita intake

The total annual volume of production of the 37 benzyl derivatives in this group is approximately 210 tonnes in Europe and 460 tonnes in the USA. About 91% of the total annual volume of production in Europe and 94% of that in the USA is accounted for by benzyl alcohol (No. 25), benzaldehyde (No. 22) and benzyl benzoate (No. 24). About 31% of the total annual volume of production in Europe is accounted for by benzaldehyde, 54% by benzyl alcohol and 6% by benzyl benzoate. About 59% of the total annual volume of production in the USA is accounted for by benzaldehyde, 28% by benzyl alcohol and 7% by benzyl benzoate. The estimated daily intake per capita of these three agents in Europe is 9300µg of benzaldehyde, 16000µg of benzyl alcohol and 1900µg of benzyl benzoate. The estimated daily intake per capita in the USA is 36000µg of benzaldehyde, 17000µg of benzyl alcohol and 4200µg of benzyl benzoate. The estimated daily per capita intake of each flavouring agent in Europe and the USA is reported in Table 4.

Benzoic acid is not only present in food and flavours but is also endogenous in the human body. Endogenous benzoic acid is formed through the phenylalanine–tyrosine pathway (Annex 1, reference 123).

4.1.3.2 Absorption, distribution, metabolism and elimination

In general, aromatic esters are hydrolysed *in vivo* by the catalytic activity of carboxylesterases, which are found predominantly in hepatocytes. All the benzyl and benzoate esters and acetals of benzaldehyde (or acetaldehyde) are anticipated to be hydrolysed readily under acidic conditions to yield benzyl alcohol (and carboxylic acids) and to benzaldehyde (and alcohols), respectively, followed by oxidation to yield benzoic acid. Benzoate esters are hydrolysed to benzoic acid (and alcohols). The remaining alcohol or acid components formed by hydrolysis are simple aliphatic substances, which are either oxidized to polar metabolites and excreted or metabolized in the fatty acid pathway and tricarboxylic acid cycle.

Benzyl derivatives have been shown to be absorbed rapidly in the gut, metabolized primarily in the liver and excreted in the urine as glycine conjugates of benzoic acid derivatives. Once absorbed, benzyl derivatives are oxidized and excreted primarily as the glycine conjugate of benzoic acid (hippurate). When high doses of benzyl derivatives are

given, formation of the glycine conjugate is limited; when glycine is depleted, free benzoic acid may sequester acetyl coenzyme A or be excreted unchanged or as the glucuronic acid conjugate. Aromatic ring substitution is anticipated to have little effect on the principal pathway of metabolism.

Oxidation of the alcohol or aldehyde group may be accompanied by oxidation of the alkyl side-chain.

4.1.3.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1) to the 37 benzyl derivatives, the Committee assigned all of them to structural class I.

Step 2. At currently estimated levels of intake, 36 of the 37 substances in this group can be predicted to be metabolized to innocuous products. The evaluation of these substances therefore proceeded via the left-hand side of the decision-tree. One compound, benzyl 2-methoxyethyl acetal (No. 840), cannot be predicted to be metabolized to innocuous products, and its evaluation therefore proceeded via the right-hand side of the decision-tree.

Step A3. The estimated daily per capita intakes of 33 of the flavouring agents in this group are below the threshold of concern for structural class I (1800µg). The Committee concluded that these substances would not be expected to be of safety concern at current estimated levels of use as flavouring agents. The estimated daily per capita intakes of the remaining three substances are above the threshold of concern for this class, that of benzyl alcohol (No. 25) being 16 000µg in Europe and 17 000µg in the USA, that of benzyl benzoate (No. 24) being 1900µg in Europe and 4200µg in the USA, and that of benzaldehyde (No. 22) being 9300µg in Europe and 36 000µg in the USA. Accordingly, the evaluation of these three substances proceeded to step A4.

Step A4. Benzyl alcohol, benzyl benzoate and benzaldehyde are readily metabolized to benzoic acid, which is endogenous in humans. These agents would therefore not be expected to be of safety concern.

Step B3. For benzyl 2-methoxyethyl acetal (No. 840), no data on intake were reported for Europe and an intake of 1µg/person per day was reported for the USA, which is below the threshold of concern for substances in structural class I (1800µg/person per day). The evaluation of this substance therefore proceeded to step B4.

Step B4. The NOEL of 6 mg/kg of body weight per day for benzyl 2-methoxyethyl acetal (No. 840) in a two-generation study of reproductive toxicity in rats provides a margin of safety >10000 times the estimated daily per capita intake in the USA. The Committee concluded that this substance would not pose a safety concern at the currently estimated level of intake.

Table 4 summarizes the evaluations of the 37 benzyl derivatives used as flavouring agents.

4.1.3.4 Consideration of combined intakes from use as flavouring agents

In the unlikely event that all the benzyl derivatives used as flavouring agents, except benzyl 2-methoxyethyl acetal (No. 840), were to be consumed concurrently on a daily basis, the estimated combined intake would exceed the threshold for human intake for structural class I. However, these agents are expected to be efficiently detoxicated and the available detoxication pathways would not be saturated. Evaluation of all the data indicated no safety concern associated with combined intake.

Furthermore, the total combined daily intake per kilogram of body weight of all benzyl derivatives (0.5 mg in Europe and 1 mg in the USA) is lower than the group ADI of 0–5 mg/kg of body weight for benzoic acid, the benzoate salts (calcium, potassium and sodium), benzaldehyde, benzyl acetate and benzyl alcohol, expressed as benzoic acid equivalents, which was maintained by the Committee at its forty-sixth meeting (Annex 1, reference 122). The three benzyl derivatives that account for more than 90% of the total intake of this group of substances in Europe and the USA are benzyl benzoate (No. 24), which is rapidly hydrolysed to benzyl alcohol and benzoic acid, benzaldehyde (No. 22) and benzyl alcohol (No. 25). All these substances are readily metabolized to benzoic acid, which is endogenous in humans. The Committee considered that the endogenous concentration of this substance would not give rise to perturbations outside the physiological range. Therefore, these three substances were considered to be of no safety concern at currently estimated levels of intake.

4.1.3.5 Conclusions

The Committee concluded that the safety of the flavouring agents in the group of benzyl derivatives would not present concern at currently estimated levels of use as flavouring agents. No data on toxicity were required in application of the Procedure to 36 of the 37 benzyl derivatives in the group, and the Committee noted that the available information was consistent with the results of the safety evaluation.

The necessary data on toxicity were available for benzyl 2-methoxyethyl acetal (No. 840).

A monograph summarizing the safety data on this group of flavouring agents and specifications were prepared.

4.1.4 ***Hydroxy- and alkoxy-substituted benzyl derivatives***

The Committee evaluated a group of flavouring agents¹ comprising 46 structurally related substances by the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1). All members of this group are aromatic primary alcohols, aldehydes, carboxylic acids or their corresponding esters or acetals. The structural feature common to all members of the group is a primary oxygenated functional group bound directly to a benzene ring. The ring also contains hydroxy or alkoxy substituents (see Table 5).

The Committee had previously evaluated four members of this group. Ethyl vanillin (No. 893) was evaluated by the Committee at its eleventh meeting (Annex 1, reference 14), when a conditional ADI of 0–10 mg/kg of body weight was established. At its thirty-fifth meeting, the Committee converted this ADI to a temporary ADI of 0–5 mg/kg of body weight (Annex 1, reference 88). At its thirty-ninth meeting, the Committee extended the temporary ADI (Annex 1, reference 101). At its forty-fourth meeting, the Committee allocated an ADI of 0–3 mg/kg of body weight to ethyl vanillin (Annex 1, reference 116). Vanillin (No. 889) was evaluated by the Committee at its eleventh meeting (Annex 1, reference 14), when an ADI of 0–10 mg/kg of body weight was established. Methyl salicylate (No. 899) was evaluated by the Committee at its eleventh meeting (Annex 1, reference 14), and an ADI of 0–0.5 mg/kg of body weight was established. Piperonal (No. 896) was also evaluated at the eleventh meeting, and an ADI of 0–2.5 mg/kg of body weight was established (Annex 1, reference 14).

Twenty-nine of the 46 substances in this group of flavouring agents have been reported to occur naturally in food. Vanillin (No. 889), a major constituent of natural vanilla, is also present in strawberries and milk. Methyl salicylate (No. 899), the predominant substituent of oil of wintergreen, is also found in tomatoes and grilled beef. Ethyl vanillin (No. 893) has been detected in raspberries and ginger, while piperonal (No. 896) is found in cooked chicken and pepper.

¹ During evaluation of these flavouring agents, the Committee questioned whether one substance in this group (No. 870) was currently used as a flavouring agent and therefore appropriately evaluated by the Procedure. Information to address this question will be sought from relevant manufacturers.

Table 5

Summary of results of the safety evaluations of hydroxy- and alkoxy-substituted benzyl derivatives used as flavouring agents^a

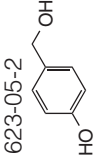
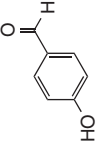
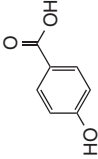
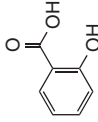
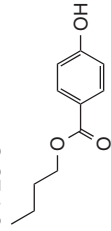
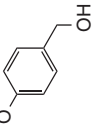
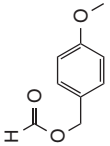
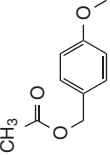
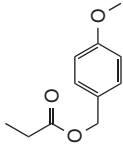
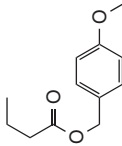
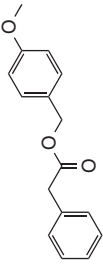
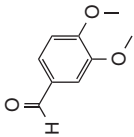
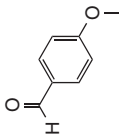
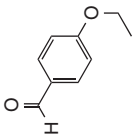
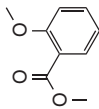
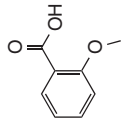
Flavouring agent	No.	CAS number and structure	Step A3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Structural class I							
4-Hydroxybenzyl alcohol	955		No Europe: 6 USA: 0.06	NR	NR	See note 1	No safety concern
4-Hydroxybenzaldehyde	956		No Europe: 64 USA: 59	NR	NR	See note 1	
4-Hydroxybenzoic acid	957		No Europe: 19 USA: 17	NR	NR	See note 1	
2-Hydroxybenzoic acid	958		No Europe: 0.03 USA: 0.03	NR	NR	See note 1	
Butyl <i>p</i> -hydroxybenzoate ^c	870		No Europe: ND USA: 0.03	NR	NR	See note 2	Evaluation not finalized

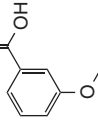
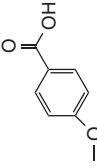
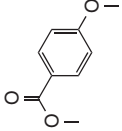
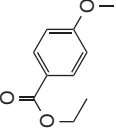
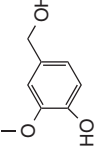
Table 5 (continued)

Flavouring agent	No.	CAS number and structure	Step A3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Anisyl alcohol	871	105-13-5 	No Europe: 150 USA: 58	NR	NR	See note 1	No safety concern
Anisyl formate	872	122-91-8 	No Europe: 46 USA: 24	NR	NR	See note 2	
Anisyl acetate	873	104-21-2 	No Europe: 59 USA: 300	NR	NR	See note 2	
Anisyl propionate	874	7549-33-9 	No Europe: ND USA: 5	NR	NR	See note 2	
Anisyl butyrate	875	6963-56-0 	No Europe: 34 USA: 0.1	NR	NR	See note 2	

Anisyl phenylacetate	876	102-17-0		No Europe: 0.003 USA: 0.1	NR	NR	See note 3
Veratraldehyde	877	120-14-9		No Europe: 140 USA: 55	NR	NR	See note 1
p-Methoxybenzaldehyde	878	123-11-5		No Europe: 440 USA: 580	NR	NR	See note 1
p-Ethoxybenzaldehyde	879	10031-82-0		No Europe: 0.1 USA: 0.01	NR	NR	See note 1
Methyl o-methoxybenzoate	880	606-45-1		No Europe: 57 USA: 8	NR	NR	See note 2
2-Methoxybenzoic acid	881	579-75-9		No Europe: ND USA: 0.01	NR	NR	See note 1

No safety concern

Table 5 (continued)

Flavouring agent	No.	CAS number and structure	Step A3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
3-Methoxybenzoic acid	882	586-38-9 	No Europe: ND USA: 0.01	NR	NR	See note 1	No safety concern
4-Methoxybenzoic acid	883	100-09-4 	No Europe: ND USA: 0.1	NR	NR	See note 1	
Methyl anisate	884	121-98-2 	No Europe: 1 USA: 0.01	NR	NR	See note 2	
Ethyl p-anisate	885	94-30-4 	No Europe: 11 USA: 2	NR	NR	See note 2	
Vanillyl alcohol	886	498-00-0 	No Europe: 6 USA: 6	NR	NR	See note 1	

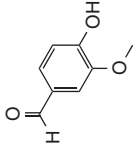
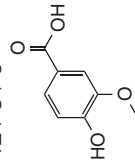
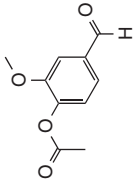
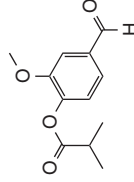
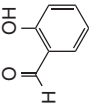
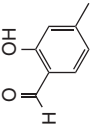
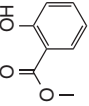
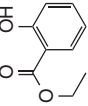
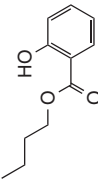
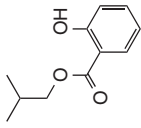
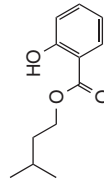
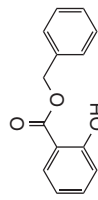
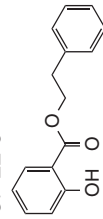
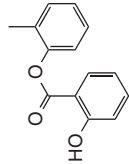
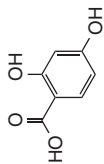
Vanillin ^d	889	121-33-5		Yes Europe: 55 000 USA: 150 000	No	Yes The NOEL of 1000mg/kg of body weight per day in a 2-year study in rats is >100 times the estimated daily intake of vanillin when used as a flavouring agent NR	See note 1	No safety concern
4-Hydroxy-3-methoxybenzoic acid	959	121-34-6		No Europe: 29 USA: 26	NR	NR	See note 1	
Vanillin acetate	890	881-68-5		No Europe: 2 USA: 1	NR	NR	See note 2	
Vanillin isobutyrate	891	20665-85-4		No Europe: 64 USA: 0.04	NR	NR	See note 2	

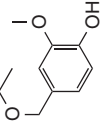
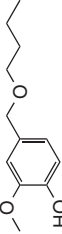
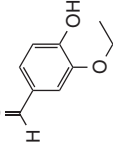
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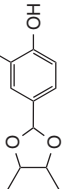
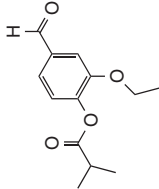
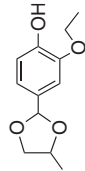
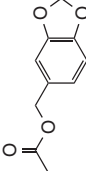
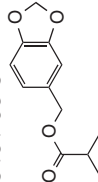
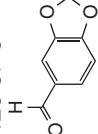
Flavouring agent	No.	CAS number and structure	Step A3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Salicylaldehyde	897	90-02-8 	No Europe: 98 USA: 16	NR	NR	See note 1	No safety concern
2-Hydroxy-4-methylbenzaldehyde	898	698-27-1 	No Europe: 1 USA: 0.3	NR	NR	See note 1	
Methyl salicylate ^e	899	119-36-8 	Yes Europe: 490 USA: 44000	No	Yes The NOEL of 50 mg/kg of body weight per day in a 2-year study in dogs is >100 times the estimated daily intake of methyl salicylate when used as a flavouring agent	See note 2	
Ethyl salicylate	900	118-61-6 	No Europe: 31 USA: 1700	NR	NR	See note 2	

Butyl salicylate	901	2052-14-4		No Europe: 0.01 USA: 0.0007	NR	NR	See note 2
Isobutyl salicylate	902	87-19-4		No Europe: 1 USA: 6	NR	NR	See note 2
Isoamyl salicylate	903	87-20-7		No Europe: 49 USA: 7	NR	NR	See note 2
Benzyl salicylate	904	118-58-1		No Europe: 30 USA: 29	NR	NR	See note 3
Phenethyl salicylate	905	87-22-9		No Europe: 0.2 USA: 4	NR	NR	See note 3
o-Tolyl salicylate	907	617-01-6		No Europe: ND USA: 30	NR	NR	See note 3
2,4-Dihydroxybenzoic acid	908	89-86-1		No Europe: ND USA: 6	NR	NR	See note 1

No safety concern

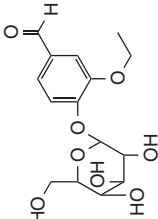
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Flavouring agent	No.	CAS number and structure	Step A3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Structural class II							
Vanillyl ethyl ether	887	13184-86-6 	No Europe: 22 USA: 22	NR	NR	See note 1	No safety concern
Vanillyl butyl ether	888	82654-98-6 	No Europe: ND USA: 0.1	NR	NR	See note 1	
Ethyl vanillin ^f	893	121-32-4 	Yes Europe: 6200 USA: 43000	No	Yes The NOEL of 500mg/kg of body weight per day in a 14-week study in rats is >100 times the estimated daily intake of ethyl vanillin when used as a flavouring agent	See note 1	

Vanillin <i>erythro</i> - and <i>threo</i> -butan-2,3-diol acetal	960	63253-24-7		No Europe: 4 USA: 3	NR	NR	See note 2
Ethyl vanillin isobutyrate	953	188417-26-7		No Europe: 64 USA: ND	NR	NR	See note 2
Ethyl vanillin propylene glycol acetal	954	68527-76-4		No Europe: 39 USA: 36	NR	NR	See note 2
Piperonyl acetate	894	326-61-4		No Europe: 41 USA: 11	NR	NR	See note 4
Piperonyl isobutyrate	895	5461-08-5		No Europe: 0.1 USA: 3	NR	NR	See note 4
Piperonal ⁹	896	120-57-0		Yes Europe: 1700 USA: 3200	No	Yes The NOEL of 250mg/kg of body weight per day in a 2-year study in rats is >1000 times the estimated daily intake of piperonal when used as a flavouring agent	See note 4

No safety concern

Table 5 (continued)

Flavouring agent	No.	CAS number and structure	Step A3 ^a Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Step A5 Adequate margin of safety for substance or related substance?	Comments on predicted metabolism	Conclusion based on current intake
Ethyl vanillin β -D-glucopyranoside	892	122397-96-0 	No Europe: ND USA: 30	NR	NR	See note 2	No safety concern

CAS: Chemical Abstracts Service; ND: no data on intake reported; NR: not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the Procedure.

- ^a Step 2: All of the flavouring agents in this group are expected to be metabolized to innocuous products.
- ^b The thresholds for human intake for structural classes I and II are 1800 µg/day and 540 µg/day, respectively. All intake values are expressed in µg/day.
- ^c Further information is required to determine whether this substance is in current use as a flavouring agent.
- ^d An ADI of 0–10 mg/kg of body weight was established for vanillin by the Committee at its eleventh meeting (Annex 1, reference 14), which was maintained at the present meeting.
- ^e An ADI of 0–0.5 mg/kg of body weight was established for methyl salicylate by the Committee at its eleventh meeting (Annex 1, reference 14), which was maintained at the present meeting. The estimated daily per capita intake of methyl salicylate is 0.7 mg/kg of body weight when calculated on the basis of the usual 10% proportion of eaters; however, a survey of intake showed that >50% of the population would be expected to consume methyl salicylate. When this measured proportion of eaters was used, the calculated intake was 0.1 mg/kg of body weight.
- ^f An ADI of 0–3 mg/kg of body weight was established for ethyl vanillin by the Committee at its forty-fourth meeting (Annex 1, reference 116), which was maintained at the present meeting.
- ^g An ADI of 0–2.5 mg/kg of body weight was established for piperonal by the Committee at its eleventh meeting (Annex 1, reference 14), which was maintained at the present meeting.

Notes to Table 5

- Detoxication by excretion in the urine unchanged or as glucuronic acid, glycine or sulfate conjugates; aldehyde groups will undergo oxidation or reduction to the corresponding carboxylic acid or alcohol, respectively, followed by conjugation and excretion; *O*-dealkylation followed by conjugation and excretion; other, minor metabolic routes, which probably occur in the intestinal microflora after biliary excretion of conjugates, include decarboxylation and reduction of benzyl groups to the methyl analogues.
- Detoxication as described in note 1 plus hydrolysis of esters to the corresponding benzyl alcohol or benzoic acid derivatives, acetal hydrolysis to the parent benzaldehyde derivative and simple aliphatic alcohol, or glycosidic bond hydrolysis to the corresponding phenolic derivative.
- Detoxication as described in note 1, preceded by hydrolysis to yield mononuclear residues, each of which would be detoxicated as described in note 1.
- Detoxication as described in note 1 plus limited oxidation of the methylenedioxyphenyl group to a catechol, which would undergo conjugation.

4.1.4.1 Estimated daily per capita intake

The total annual volume of production of the 46 flavouring agents in this group is 450 tonnes in Europe and 1800 tonnes in the USA. Vanillin (No. 889), ethyl vanillin (No. 893), methyl salicylate (No. 899) and piperonal (No. 896), for which ADIs were previously established by the Committee, account for approximately 98% of the total annual volume in Europe and 99% in the USA. In Europe, the estimated daily per capita intakes of these compounds are 55 mg of vanillin, 6.2 mg of ethyl vanillin, 0.5 mg of methyl salicylate and 1.7 mg of piperonal. In the USA, the estimated daily per capita intakes are 150 mg of vanillin, 43 mg of ethyl vanillin, 44 mg of methyl salicylate and 3.2 mg of piperonal. The estimated daily per capita intakes of the other flavouring agents in this group are lower. Ethyl salicylate (No. 900) and *p*-methoxybenzaldehyde (No. 878) have the next highest daily per capita intakes; that of ethyl salicylate is 1.7 mg in the USA and that of *p*-methoxybenzaldehyde is 0.5 mg in both Europe and the USA. The remaining 40 flavouring agents have estimated daily per capita intakes of <100 µg, 10 of which are under 1 µg. The daily per capita intake of each substance in Europe and the USA is shown in Table 5.

4.1.4.2 Absorption, distribution, metabolism and elimination

The aromatic esters in this group can be expected to be hydrolysed extensively through the catalytic activity of the intestinal carboxylesterases, especially β -esterases, to benzyl alcohol or benzoic acid derivatives before absorption. Likewise, acetals of substituted benzaldehyde derivatives will be hydrolysed in gastric and intestinal fluids to yield benzaldehyde and aliphatic alcohols. The resulting hydroxy- and alkoxy-substituted benzyl derivatives are rapidly absorbed in the gut, metabolized in the liver and excreted in the urine.

Once absorbed, benzyl derivatives are oxidized to the corresponding benzoic acid derivative, which is subsequently excreted unchanged or as sulfate or glucuronide conjugates. Minor metabolic detoxication pathways include *O*-demethylation, reduction and decarboxylation. These pathways are used during enterohepatic cycling of conjugated benzyl metabolites and subsequent intestinal bacterial action. Piperonal is oxidized to piperonylic acid and excreted mainly as the glycine conjugate.

4.1.4.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1), the Committee assigned 36 of the 46 flavouring agents in this group to structural class I. These flavouring

agents are all either simple substituted aromatic compounds, cyclic acetals of benzaldehydes which are expected to be hydrolysed to aromatic aldehydes and simple aliphatic alcohols (Nos 870–875, 877–886, 889–891, 897–903, 908, 955–959), or compounds containing two aromatic rings which are expected to be hydrolysed to mononuclear residues with simple functional groups (Nos 876, 904, 905 and 907). The remaining 10 flavouring agents (Nos 887, 888, 892–896, 953, 954 and 960) are ethyl vanillin or piperonal derivatives that contain either an ethoxy or a methylene dioxy substituent. They are common components of food, or closely structurally related to common components of food, and were assigned to structural class II.

Step 2. At current levels of intake, the metabolic pathways of these flavouring agents can be predicted not to be saturated, and all can be predicted to be metabolized to innocuous products. The evaluation of these substances therefore proceeded down the left-hand side of the decision-tree.

Step A3. The estimated daily per capita intakes of 34 of the flavouring agents in structural class I and eight flavouring agents in structural class II were below the thresholds of concern for these classes (1800µg and 540µg, respectively). The Committee concluded that these 42 flavouring agents would not be expected to be of safety concern at currently estimated levels of use.

The estimated daily per capita intakes of vanillin (No. 889) and methyl salicylate (No. 899), which are in structural class I, exceed the threshold of concern for this class (1800µg). The estimated daily per capita intakes of vanillin are 55 000µg in Europe and 150 000µg in the USA, and that of methyl salicylate is 44 000µg in the USA. The estimated daily per capita intakes of ethyl vanillin (No. 893) and piperonal (No. 896), which are in structural class II, exceed the threshold of concern for this class (540µg). The estimated daily per capita intakes of ethyl vanillin are 6200µg in Europe and 43 000µg in the USA, and those of piperonal are 1700µg in Europe and 3200µg in the USA.

The estimated daily per capita intakes of these four substances are below their respective ADI values. The daily intakes of vanillin in Europe and the USA, approximately 0.9 and 2.5 mg/kg of body weight respectively, do not exceed the ADI of 0–10 mg/kg of body weight for vanillin. The highest estimated daily intakes of ethyl vanillin (0.7 mg/kg of body weight in the USA) and piperonal (0.05 mg/kg of body weight in the USA) do not exceed the ADIs of 0–3 mg/kg of body weight for ethyl vanillin and 0–2.5 mg/kg of body weight for piperonal. The highest estimated daily intake of the remaining substance, methyl salicylate, is 0.7 mg/kg of body weight, which is approximately equal to its ADI of 0–0.5 mg/kg of body weight.

The estimates of intake derived from total annual volume of production are based on the assumption that only 10% of the population consumes the substance under consideration. The Committee reviewed an analysis of the intake of methyl salicylate which was based on individual dietary records of consumption of baked goods, chewing-gums, hard and soft sweets and beverages in which this agent is used in the USA. The analysis showed that more than 50% of the population would be expected to consume methyl salicylate. Use of this *measured* proportion of eaters in place of the default assumption of 10% yields an estimated intake of methyl salicylate of 0.1 mg/kg of body weight, which is still below the current ADI of 0–0.5 mg/kg of body weight.

Step A4. Vanillin (No. 889), methyl salicylate (No. 899), ethyl vanillin (No. 893) and piperonal (No. 896) are not endogenous in humans. The evaluation of these substances therefore proceeded to step A5.

Step A5. The ADI of 0–10 mg/kg of body weight for vanillin is based on a NOEL of 1000 mg/kg of body weight per day in a 2-year feeding study in rats. This NOEL provides a margin of safety, as it is more than 100 times the per capita intake of vanillin from its currently estimated use as a flavouring agent in Europe (0.9 mg/kg of body weight per day) or in the USA (2.5 mg/kg of body weight per day).

The ADI of 0–0.5 mg/kg of body weight for methyl salicylate is based on a NOEL of 50 mg/kg of body weight per day reported in a 2-year study in dogs. This NOEL is more than 1000 times greater than the intake of methyl salicylate from its currently estimated use as a flavouring agent in Europe (0.008 mg/kg of body weight per day) and is more than 100 times greater than the intake of methyl salicylate in the USA when intake is calculated on the basis of the measured portion of eaters of 50% (0.1 mg/kg of body weight per day).

A NOEL of 500 mg/kg of body weight per day for ethyl vanillin was reported in a 14-week feeding study in rats. This NOEL is more than 100 times greater than the intake of ethyl vanillin from its use as a flavouring agent in Europe (0.1 mg/kg of body weight per day) or in the USA (0.7 mg/kg of body weight per day).

A NOEL of 250 mg/kg of body weight per day for piperonal was reported in a 2-year study in rats. This NOEL is more than 1000 times the intake of piperonal from its use as a flavouring agent in Europe (0.03 mg/kg of body weight per day) and in the USA (0.05 mg/kg of body weight per day).

Table 5 summarizes the evaluations of the 46 hydroxy- and alkoxy-substituted benzyl derivatives used as flavouring agents.

4.1.4.4 *Consideration of combined intakes from use as flavouring agents*

In the unlikely event that all 36 flavouring agents in structural class I were to be consumed on a daily basis, the estimated combined intake would exceed the threshold for human intake for this class (1800 µg/day). In the unlikely event that all 10 flavouring agents in structural class II were to be consumed on a daily basis, the estimated combined intake would exceed the threshold for human intake for this class (540 µg/day). However, all 46 flavouring agents in this group are expected to be efficiently detoxicated, and the available detoxication pathways would not be saturated. Evaluation of all the data indicated no safety concern associated with combined intake.

4.1.4.5 *Conclusions*

The Committee retained the previously established ADIs of 0–10 mg/kg of body weight for vanillin (No. 889), 0–3 mg/kg of body weight for ethyl vanillin (No. 893), 0–2.5 mg/kg of body weight for piperonal (No. 896) and 0–0.5 mg/kg of body weight for methyl salicylate (No. 899). The Committee noted that the estimated daily intake of 0.7 mg/kg of body weight of methyl salicylate, based on the production volume used, is approximately equal to its ADI of 0–0.5 mg/kg of body weight, within the precision of the intake estimates. The Committee reviewed an analysis of intake based on individual dietary records of consumption of mint-flavoured baked goods, chewing-gums, hard and soft sweets and beverages in which methyl salicylate is potentially used. This analysis showed that more than 50% of the population would be expected to consume methyl salicylate. The use of this *measured* proportion of eaters in place of the default assumption of 10% yields an estimated intake of methyl salicylate of 0.1 mg/kg of body weight, which is below the ADI of 0–0.5 mg/kg of body weight.

On the basis of the available data on metabolism and toxicity, the Committee concluded that the safety of the flavouring agents in this group would not raise concern at the currently estimated levels of use. Other data on toxicity, including studies on developmental toxicity and genotoxicity, were consistent with the results of the safety evaluations.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.1.5 ***Aliphatic acyclic diols, triols and related substances***

The Committee evaluated a group of 31 flavouring agents¹ that included aliphatic acyclic diols, triols and related substances

¹ During evaluation of these flavouring agents, the Committee questioned whether some substances in this group (Nos 909 and 914–926) were used as flavouring agents and therefore appropriately evaluated by this Procedure. Information to address this question will be sought from relevant manufacturers.

(Table 6) by the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1). All members of this group are aliphatic acyclic primary alcohols, aldehydes, acids or related esters with one or more additional oxygenated functional groups. The group comprised four subgroups: glycerol (No. 909) and 15 related glycerol esters and acetals (Nos 910–924); propylene glycol (No. 925) and four related esters, acetals and ketals (Nos 926–929); lactic acid (No. 930) and four lactate esters (Nos 931, 932, 934 and 935); and pyruvic acid (No. 936), its corresponding aldehyde (No. 937), two pyruvate esters (Nos 938 and 939) and one acetal of pyruvic acid (No. 933).

The Committee had previously evaluated three members of the group. Glycerol (No. 909) was considered at the twentieth meeting (Annex 1, reference 41), when an ADI “not specified”¹ was established. Propylene glycol (No. 925) was considered at the seventh meeting (Annex 1, reference 7), when an ADI of 0–20 mg/kg of body weight was established; it was further considered at the seventeenth meeting (Annex 1, reference 32), when the ADI was increased to 0–25 mg/kg of body weight. Ethyl lactate (No. 931) was considered at the eleventh, twenty-third, twenty-fourth and twenty-sixth meetings (Annex 1, references 14, 50, 53 and 59). At its twenty-sixth meeting, the Committee included ethyl lactate in the group ADI “not specified”¹ with lactic acid.

Nine of the 31 substances (Nos 909, 929–932, 934 and 936–938) have been detected as natural components of foods, in cocoa, milk, cider, cognac, asparagus, tomatoes and mushrooms.

4.1.5.1 Estimated daily per capita intake

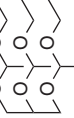
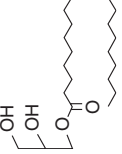
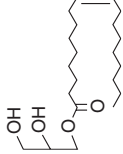
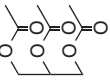
The total annual volume of production of the 31 flavouring agents in this group is 140 tonnes in Europe and 21000 tonnes in the USA. These values are equivalent to a total estimated daily per capita intake of 20000 µg in Europe and 2800000 µg in the USA. The large difference in the annual volume of production in Europe and the USA is due to the inclusion in the USA of figures on the use of glycerol, triacetin and propylene glycol as solvents in the preparation of flavour mixtures.

In Europe, 97% of the total daily per capita intake of this group of flavouring agents was accounted for by glycerol (17000 µg), ethyl lactate (1900 µg) and butyl lactate (380 µg). In the USA, 96% of the total daily per capita intake was accounted for by glycerol (220000 µg), triacetin (83000 µg) and propylene glycol (240000 µg).

¹ See footnote on page 32.

Table 6

Summary of results of the safety evaluations of aliphatic acyclic diols, triols and related substances^a

Flavouring agent	No.	CAS number and structure	Step A3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Conclusion based on current intake
Structural class I					
Glycerol ^{c,d}	909	56-81-5 	Yes Europe: 17 000 USA: 220 000	Yes Glycerol is endogenous	Evaluation not finalized
1,2,3-Tris[(1'-ethoxy)-ethoxy]propane	913	67715-82-6 	No Europe: 0 USA: 140	NR	No safety concern
Glyceryl monostearate ^c	918	123-94-4 	No Europe: 0 USA: 230	NR	Evaluation not finalized
Glyceryl monooleate ^c	919	111-03-5 	No Europe: ND USA: 860	NR	
Triacetin ^c	920	102-76-1 	Yes Europe: ND USA: 83 000	Yes Expected to be hydrolysed to glycerol, which is endogenous	

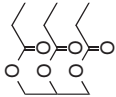
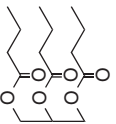
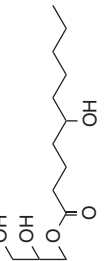
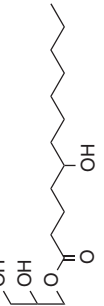
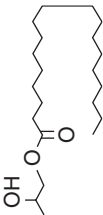
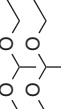
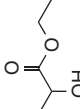
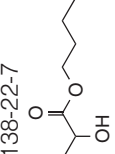
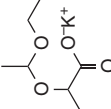
Glycerol tripropanoate ^c	921	139-45-7 	No Europe: 0.1 USA: 280	NR	Evaluation not finalized
Tributylin ^c	922	60-01-5 	No Europe: 31 USA: 2	NR	
Glycerol 5-hydroxy-decanoate ^c	923	26446-31-1 	No Europe: 4 USA: 0	NR	
Glycerol 5-hydroxy-dodecanoate ^c	924	26446-32-2 	No Europe: 4 USA: 0	NR	
Propylene glycol ^{c,e}	925	57-55-6 	Yes Europe: ND USA: 2400000	Yes Expected to be oxidized to lactic acid, which is endogenous	
Propylene glycol stearate ^c	926	142-75-6 	Yes Europe: ND USA: 66000	Yes Expected to be hydrolysed to propylene glycol and subsequently oxidized to lactic acid	

Table 6 (continued)

Flavouring agent	No.	CAS number and structure	Step A3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Conclusion based on current intake
1,2-Di[(1-ethoxy)- ethoxy]propane	927	67715-79-1 	No Europe: 7 USA: 150	NR	No safety concern
Lactic acid	930	598-82-3 	Yes Europe: ND USA: 47 000	Yes Lactic acid is endogenous	
Ethyl lactate ⁱ	931	97-64-3 	Yes Europe: 1900 USA: 760	Yes Expected to be hydrolysed to lactic acid, which is endogenous	
Butyl lactate	932	138-22-7 	No Europe: 380 USA: 24	NR	
Potassium 2-(1'-ethoxy)- ethoxypropanoate	933	100743-68-8 	No Europe: ND USA: 1400	NR	
cis-3-Hexenyl lactate	934	61931-81-5 	No Europe: 38 USA: 5	NR	

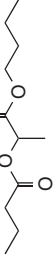
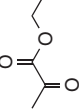
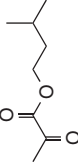
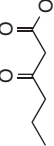
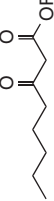
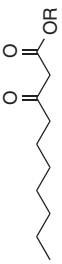
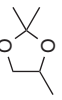
Butyl butyryllactate	935	7492-70-8		No Europe: 280 USA: 1400	NR	No safety concern
Pyruvic acid	936	127-17-3		No Europe: 35 USA: 69	NR	
Pyruvaldehyde	937	78-98-8		No Europe: 120 USA: 3	NR	
Ethyl pyruvate	938	617-35-6		No Europe: 1 USA: 20	NR	
Isoamyl pyruvate	939	7779-72-8		No Europe: 0 USA: 0	NR	
Structural class III 3-Oxohexanoic acid glyceride	910	91052-72-1		Yes Europe: 0 USA: 270	Yes Expected to be hydrolysed to glycerol, which is endogenous NR	No safety concern
3-Oxooctanoic acid glyceride	911	91052-68-5		No Europe: 34 USA: 0	NR	
Heptanal glyceryl acetal (mixed 1,2 and 1,3 acetals)	912	1708-35-6		No Europe: 4 USA: 0	NR	

Table 6 (continued)

Flavouring agent	No.	CAS number and structure	Step A3 ^b Does intake exceed the threshold for human intake?	Step A4 Is the substance or are its metabolites endogenous?	Conclusion based on current intake
3-Oxodecanoic acid glyceride ^c	914	91052-69-6 	Yes Europe: 0 USA: 270	Yes Expected to be hydrolysed to glycerol, which is endogenous NR	Evaluation not finalized
3-Oxododecanoic acid glyceride ^c	915	91052-70-9 	No Europe: 73 USA: 0		
3-Oxotetradecanoic acid glyceride ^c	916	91052-73-2 	Yes Europe: 0 USA: 270	Yes Expected to be hydrolysed to glycerol, which is endogenous NR	
3-Oxohexadecanoic acid glyceride ^c	917	91052-71-0 	No Europe: 43 USA: 0		No safety concern
4-Methyl-2-pentyl-1,3-dioxolane	928	26563-74-6 	No Europe: 0 USA: 0.2	NR	
2,2,4-Trimethyl-1,3-oxacyclopentane	929	1193-11-9 	No Europe: 0.3 USA: 0.2	NR	

CAS: Chemical Abstracts Service; ND: no data on intake reported; NR: not required for evaluation because consumption of the substance was determined to be of no safety concern at step A3 of the Procedure.

^a Step 2: All of the flavouring agents in this group are predicted to be metabolized to innocuous products.

^b The threshold for human intake for structural classes I and III are 1800 µg/day and 90 µg/day, respectively. All intake values are expressed in µg/day.

^c Further information is required to determine whether this substance is in current use as a flavouring agent.

^d An ADI "not specified" was established for glycerol by the Committee at its twentieth meeting (Annex 1, reference 41), which was maintained at the present meeting.

^e An ADI of 0–25 mg/kg of body weight was established for propylene glycol by the Committee at its seventeenth meeting (Annex 1, reference 32), which was maintained at the present meeting.

^f Ethyl lactate was included in the group ADI "not specified" for lactic acid and its salts that was established by the Committee at its twenty-sixth meeting (Annex 1, reference 59), which was maintained at the present meeting.

The daily per capita intake of each substance in Europe and the USA is shown in Table 6.

4.1.5.2 Absorption, distribution, metabolism and elimination

The aliphatic esters of propylene glycol (No. 925), lactic acid (No. 930) and pyruvic acid (No. 936) and their parent compounds would all be expected to be readily absorbed from the gut. Hydrolysis of the aliphatic esters is catalysed largely by hepatic esterases, to give the component alcohol and carboxylic acid or aldehyde. After hydrolysis of the glycerol esters in the intestine, glycerol is readily absorbed. Glycerol, pyruvic acid and lactic acid are endogenous in humans. Glycerol and pyruvic acid are metabolized completely and are not excreted. Lactic acid is also largely metabolized, although urinary excretion may occur if the blood concentration is high. Propylene glycol can be metabolized, but high doses are likely to be excreted largely unchanged in the urine.

Glycerol (No. 909) is converted in the liver to glycerol-3-phosphate, which is metabolized via the glycolytic pathway, by oxidation, to yield dihydroxyacetone phosphate, which is isomerized to glyceraldehyde-3-phosphate, eventually yielding pyruvic acid.

Pyruvic acid follows two primary routes of metabolism. Under aerobic conditions, it is converted to acetyl coenzyme A and enters the citric acid cycle. Under anaerobic conditions, primarily in muscles as a result of strenuous physical activity, pyruvic acid is reduced by lactic dehydrogenase to lactic acid.

Lactic acid diffuses through muscle tissue and is transported to the liver in the bloodstream. In the liver, it is converted to glucose by gluconeogenesis. Lactic acid can also be further catabolized in the lactic acid cycle (also known as the Cori cycle).

Propylene glycol can be oxidized to lactic acid via two biochemical pathways. If propylene glycol is phosphorylated, it can be converted to acetol phosphate, lactaldehyde phosphate, lactyl phosphate and then lactic acid. If it is not phosphorylated, propylene glycol is successively oxidized to lactaldehyde, methylglyoxal and lactic acid.

4.1.5.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. Twenty-eight of the 31 flavouring agents in this group are linear, simple-branched aliphatic compounds. In applying the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1), the Committee assigned 22 of the agents to structural class I because they contain fewer than three types of functional group (Nos 909, 913,

918–927 and 930–939). The Committee assigned six of the substances to structural class III because they contain three or more types of functional group (Nos 910, 911 and 914–917). The three remaining substances were also assigned to structural class III because they are cyclic acetals and ketals (Nos 912, 928 and 929).

Step 2. The data on the metabolism of individual members of the group were sufficient to allow conclusions about their probable metabolic fate. The aliphatic esters of propylene glycol (No. 926), lactic acid (Nos 931, 932, 934 and 935) and pyruvic acid (Nos 938 and 939) can be expected to be hydrolysed to their component alcohols and carboxylic acids. The glycerol esters (Nos 910, 911 and 914–924) can be expected to be hydrolysed to glycerol and carboxylic acids. Esters of propylene glycol can be expected to be hydrolysed to propylene glycol and its component acid. Esters of lactic acid and pyruvic acid can be expected to be hydrolysed to lactic acid and pyruvic acid, respectively, and the corresponding alcohols. The acetals (Nos 912, 913, 927 and 933) can be expected to be hydrolysed to their component alcohols and aldehydes, while the ketals (Nos 928 and 929) can be expected to be hydrolysed to their component ketones and alcohols. Glycerol (No. 909), lactic acid (No. 930) and pyruvic acid (No. 936) are endogeneous and are metabolized through the glycolytic and citric acid pathways. Propylene glycol (No. 925) is oxidized to lactic acid. Because they are predicted to be metabolized to innocuous products, the evaluation of all the substances in this group proceeded via the left-hand side of the decision-tree.

Step A3. The estimated daily per capita intakes of 17 of the substances in structural class I and five substances in structural class III are below the threshold of concern for these classes (1800 µg and 90 µg, respectively). The Committee concluded that these substances would not be expected to be of safety concern at their currently estimated levels of use. The daily per capita intakes of six substances in structural class I (Nos 909, 920, 925, 926, 930 and 931) and three substances in structural class III (Nos 910, 914 and 916) exceed the threshold of concern for these classes, and their evaluation therefore proceeded to step A4.

Step A4. Glycerol (No. 909), lactic acid (No. 930) and the hydrolysis products of ethyl lactate (No. 931) are endogenous in humans and their use as flavouring agents is therefore not expected to be of safety concern. Triacetin (No. 920), 3-oxohexanoic acid glyceride (No. 910), 3-oxodecanoic acid glyceride (No. 914) and 3-oxotetradecanoic acid glyceride (No. 916) are glycerol esters and are hydrolysed to glycerol.

Propylene glycol (No. 925) and propylene glycol stearate (No. 926) are not endogenous in humans; however, the ester is expected to be hydrolysed to propylene glycol and stearic acid. Propylene glycol is known to be oxidized to lactic acid in mammals. The safety of these substances would therefore not be expected to be of concern.

4.1.5.4 Consideration of combined intakes from use as flavouring agents

In the unlikely event that all 23 substances in structural class I were to be consumed concurrently on a daily basis, the estimated combined intake would exceed the threshold for human intake for this class (1800µg). In the unlikely event that all eight substances in structural class III were to be consumed concurrently on a daily basis, the estimated combined intake would exceed the threshold for human intake for this class (90µg). Given that the substances can be expected to be efficiently metabolized by known metabolic pathways, the Committee considered that the combined intake would not give rise to concern about safety.

4.1.5.5 Conclusions

On the basis of the predicted metabolism, the Committee concluded that the safety of the 31 aliphatic acyclic diols, triols and related substances in this group would not raise concern at the currently estimated levels of use as flavouring agents. The Committee noted that all of the available data on toxicity were consistent with the results of the safety evaluations.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

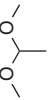
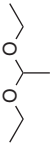
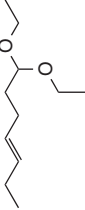
4.1.6 Aliphatic acyclic acetals

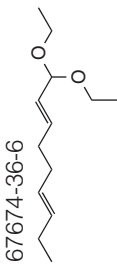
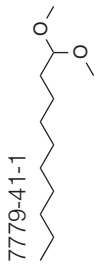
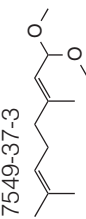
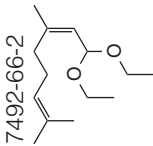
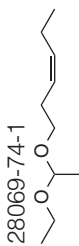
The Committee evaluated a group of 10 flavouring agents consisting of aliphatic acyclic acetals (Table 7) by the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1). They all have acyclic structures that vary only in the length of their hydrocarbon chains and the number and placement of double bonds. None of these flavouring agents had been evaluated previously by the Committee.

Aliphatic acetals are geminal diethers in which two molar equivalents of alcohol are condensed with an aldehyde. Three of the 10 acetals in this group are formed from acetaldehyde and simple aliphatic alcohols (Nos 940, 941 and 943); the remaining seven acetals are formed from methanol or ethanol and aldehydes of carbon chain-length C7–C10 (Nos 942 and 944–949). Acetals are known to be hydrolysed *in vivo* to yield the corresponding alcohols and aldehydes. Of the component alcohols (methanol, ethanol and *cis*-3-hexen-1-ol)

Table 7

Summary of results of the safety evaluations of aliphatic acyclic acetals used as flavouring agents^a

Flavouring agent	No.	CAS number and structure	Step A3 ^b Does intake exceed the threshold for human intake?	Comments on predicted metabolism	Conclusion based on current intake
1,1-Dimethoxyethane	940	534-15-6 	No Europe: 71 USA: 11	Metabolized to acetaldehyde and methanol	No safety concern
Acetal	941	105-57-7 	No Europe: 240 USA: 640	Metabolized to acetaldehyde and ethanol	
Heptanal dimethyl acetal	947	10032-05-0 	No Europe: 0.04 USA: 0.26	Metabolized to heptanal and methanol	
4-Heptenal diethyl acetal	949	18492-65-4 	No Europe: 0.04 USA: 0	Metabolized to 4-heptenal and ethanol	
Octanal dimethyl acetal	942	10022-28-3 	No Europe: 1.1 USA: 0	Metabolized to octanal and methanol	

2,6-Nonadienal diethyl acetal	946		No Europe: 0.04 USA: 0.01	Metabolized to 2,6-nonadienal and ethanol
Decanal dimethyl acetal	945		No Europe: 0.03 USA: 0	Metabolized to decanal and methanol
Citral dimethyl acetal	944		No Europe: 3 USA: 5	Metabolized to citral and methanol
Citral diethyl acetal	948		No Europe: 4 USA: 0	Metabolized to citral and ethanol
Acetaldehyde ethyl cis-3-hexenyl acetal	943		No Europe: ND USA: 0	Metabolized to acetaldehyde, ethanol and cis-3-hexen-1-ol

No safety concern

CAS: Chemical Abstracts Service; ND: no data on intake reported.

^a Step 1: All of the flavouring agents in this group are in structural class I.

^b Step 2: All of the flavouring agents in this group are expected to be metabolized to innocuous products.

^c The threshold for human intake for structural class I is 1800 µg/day. All intake values are expressed in µg/day.

and aldehydes, acetaldehyde, heptanal, 4-heptenal, octanal and decanal had been considered previously by the Committee at its forty-ninth and fifty-first meetings (Annex 1, references 131 and 137), when it concluded that their safety was of no concern at currently estimated levels of use as flavouring agents.

Three of the 10 flavouring agents in this group, 1,1-dimethoxyethane (No. 940), acetal (No. 941) and acetaldehyde ethyl *cis*-3-hexenyl acetal (No. 943), have been reported to occur as natural components of foods. They have been detected in orange juice, strawberries, cider, peas, coffee and cognac.

4.1.6.1 Estimated daily per capita intake

The total annual volume of production of the 10 aliphatic acyclic acetals is approximately 2.2 tonnes in Europe and 4.9 tonnes in the USA. About 97% of the total annual volume of production in Europe and 99% of that in the USA is accounted for by 1,1-dimethoxyethane (No. 940), formed from acetaldehyde and methanol, and acetal (No. 941), formed from acetaldehyde and ethanol.

4.1.6.2 Absorption, distribution, metabolism and elimination

In general, aliphatic acetals undergo acid-catalysed hydrolysis to their component aldehydes and alcohols. They are hydrolysed within 1–5 h in simulated gastric fluid *in vitro* and to a lesser extent in simulated intestinal fluid. Indirect evidence, from a study in which rabbits were given aliphatic acetals in aqueous suspension by stomach tube, indicated that rapid hydrolysis occurs in the stomach. The acetals formed from the reaction of alkyl-substituted pentanal with methanol, ethanol and isopropyl alcohol are metabolized to the corresponding alcohols and acids in rat liver homogenates by an oxidative mechanism involving cytochrome P450 enzymes. Aliphatic acetals can be expected to undergo similar metabolism in humans to the corresponding alcohols and acids. There are insufficient data to exclude the possibility that significant amounts of the parent acetals reach the general circulation; however, the parent compounds are all in structural class I. The low intake resulting from uses of these substances as flavours would not be expected to saturate metabolic enzyme pathways, and the acetals are metabolized to innocuous compounds by hydrolysis or oxidation.

On the basis of their recognized or presumed metabolic fate, the component alcohols and aldehydes can be grouped into three structural classes: linear, aliphatic, primary, saturated and unsaturated alcohols and aldehydes; α,β -unsaturated aldehydes; and branched-chain aliphatic aldehydes. The metabolic detoxication of linear, ali-

phatic, primary alcohols in vivo occurs primarily by oxidation to the corresponding aldehyde, with subsequent oxidation of the aldehyde to the corresponding carboxylic acid. The acid can serve as a substrate for fatty acid oxidation pathways and the citric acid cycle. In general, α,β -unsaturated aldehydes are metabolized by oxidation to the corresponding carboxylic acid, which may then participate in the fatty acid pathway. The aldehyde can also be conjugated with glutathione in a Michael-type addition. Branched-chain aliphatic aldehydes are oxidized primarily to polar metabolites, which are excreted mainly in the urine. The main urinary metabolites of branched aldehydes are diacids and hydroxyacids resulting from ω -oxidation, reduction and hydration of the alkene function and oxidation of the aldehyde function.

Although few studies on the absorption, distribution and elimination of aliphatic acyclic acetals have been reported, the metabolism of the component alcohols and aldehydes has been investigated. These studies are considered relevant to the safety evaluation of orally administered acetals which are expected to be hydrolysed in the acid environment of the stomach.

Citral is predicted to be a metabolite of citral dimethyl acetal (No. 944) and citral diethyl acetal (No. 948). The absorption, distribution and excretion of citral have been studied extensively in rats and mice. Citral underwent rapid absorption from the gut and was distributed uniformly throughout the body. Rapid elimination of citral and its metabolites occurred primarily in the urine and to a minor extent in exhaled air and faeces.

4.1.6.3 Application of the Procedure for the Safety Evaluation of Flavouring Agents

Step 1. In applying the Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1), the Committee assigned all 10 of the aliphatic acetals to structural class I.

Step 2. At current levels of intake, all of these flavouring agents can be predicted to be metabolized to their component aldehydes and alcohols, which are then metabolized to innocuous products,¹ and the pathways involved would not be expected to be saturated. Evaluation of these substances therefore proceeded via the left-hand side of the decision-tree.

¹ Some aldehydes, including acetaldehyde, were genotoxic in vitro in a number of test systems, and acetaldehyde has been reported to produce tumours of the respiratory tract in rats and hamsters exposed to high doses by inhalation. The relevance of this observation to oral administration is questionable, as various metabolic processes in the intestinal wall and liver (i.e. oxidation and conjugation) are predicted to result in extensive first-pass metabolic inactivation, especially at the low concentrations expected from use of these substances as flavours.

Step A3. The estimated daily per capita intakes of all 10 substances in this group are below the threshold of concern for structural class I (1800µg). The Committee concluded that their safety raises no concern at their currently estimated levels of use as flavouring agents.

Table 7 summarizes the results of the evaluations of the aliphatic acetals used as flavouring agents.

4.1.6.4 Consideration of combined intakes from use as flavouring agents

In the unlikely event that all 10 aliphatic acetals were consumed concurrently on a daily basis, the estimated combined intake would not exceed the threshold for human intake for structural class I (1800µg/day). All flavouring agents in this group are expected to be efficiently metabolized and the available metabolic pathways would not be saturated. Evaluation of all the data indicated there would be no safety concern associated with combined intake.

4.1.6.5 Conclusions

The Committee concluded that the safety of aliphatic acetals would not raise concern at the currently estimated levels of intake. Other data on the toxicity of aliphatic acetals were consistent with the results of the safety evaluation.

A monograph summarizing the safety data on this group of flavouring agents was prepared.

4.2 Revision of certain specifications for purity

4.2.1 *Flavouring agents with specifications designated as “tentative” at previous meetings*

At its forty-sixth, forty-ninth, fifty-first, fifty-third and fifty-fifth meetings (Annex 1, references 122, 131, 137, 143 and 149), the Committee evaluated a total of 143 flavouring agents for which further information was required in order to complete the specifications. At its present meeting, the Committee reviewed new data on 95 of these flavouring agents and revised the specifications to take account of the new information. For 83 substances, the “tentative” designation was removed; for the remaining 12, the revised specifications were classified as “tentative” (see Annex 2).

For the remaining 47 substances, no further data were provided that would permit the preparation of satisfactory specifications, according to the criteria identified by the Committee at its fifty-third meeting (Annex 1, reference 143). The flavour industry has indicated that many of these substances are highly noxious, sulfur-containing compounds which are not routinely handled in the pure form. It is there-

fore impracticable to provide information on, for instance, boiling-point or specific gravity. The Committee concluded that, in such cases, the additional criteria identified at its fifty-third meeting for the establishment of satisfactory specifications should not apply and that only the basic data on chemical identity, minimum assay and identity test were required.

The Committee reiterated that the evaluation of flavouring agents relies on adequate information about the identity and composition of products in commercial use. In future, specifications for flavouring agents will be withdrawn within 2 years of publication of tentative specifications, if the necessary information is not provided.

4.2.2 *Flavouring agents with minimum assay values less than 95%*

At its fifty-third meeting (Annex 1, reference 143), the Committee announced its intention to re-examine specifications for flavouring agents for which the minimum assay values were <95% (see also section 2.6 of the present report), which were designated as “tentative”. At its present meeting, the Committee considered information on all 62 remaining substances in this category with regard to the secondary components that might be present in commercial products.

The available information was sufficient for the Committee to revise the specifications for 27 flavouring agents (including two flavouring agents for which the specifications were revised for other reasons). The Committee therefore removed the “tentative” designations, on the basis of the general principle that no more than 5% of any commercial product should remain undefined after taking account of the flavouring agent and named secondary components.

The Committee will review this group of flavouring agents at a future meeting to confirm whether all the named secondary components are covered by existing safety evaluations. At the same time, the Committee will determine the need for further information to complete those specifications that remain tentative.

4.2.3 *Specifications for flavouring agents being reviewed for safety*

A total of 203 flavouring agents were reviewed for safety at the present meeting. For four of them, specifications had been drawn up at the forty-sixth meeting (Annex 1, reference 122), and these were revised for use of these substances as flavouring agents. For six flavouring agents, information on specifications was not submitted. These will be considered at a future meeting when such data become available.

New specifications were drawn up for the remaining 193 flavouring agents. For 27 substances, the new specifications were designated as “tentative”, either because they failed to meet the criteria drawn up at the fifty-third meeting (Annex 1, reference 143) or because certain aspects required clarification.

5. Contaminants

5.1 Chloropropanols

Certain chlorinated propanols occur as contaminants in hydrolysed vegetable proteins. Processing of defatted vegetable proteins by traditional hydrochloric acid hydrolysis leads to the formation of 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol. These two substances were evaluated by the Committee at its forty-first meeting (Annex 1, reference 107), when it concluded that they are undesirable contaminants in food and expressed the opinion that their concentrations in hydrolysed vegetable proteins should be reduced as far as is technically achievable. The present evaluations were conducted in response to a request by the Codex Committee on Food Additives and Contaminants at its Thirty-second Session (4) for the Expert Committee to re-evaluate 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol on the basis of new data that had become available since the forty-first meeting.

5.1.1 **3-Chloro-1,2-propanediol**

5.1.1.1 Absorption, distribution, metabolism and excretion

3-Chloro-1,2-propanediol crosses the blood–testis barrier and the blood–brain barrier and is widely distributed in the body fluids. The parent compound is partly detoxified by conjugation with glutathione, resulting in excretion of the corresponding mercapturic acid, and is partly oxidized to β -chlorolactic acid and further to oxalic acid. Approximately 30% is broken down to carbon dioxide and exhaled. In the studies from which these data were derived, however, much of the administered dose was not accounted for. Intermediate formation of an epoxide has been postulated but not proven. There is some indication that microbial enzymes can dehalogenate halogenated alcohols to produce glycidol, a known genotoxin in vitro and in vivo.

5.1.1.2 Toxicological studies

The median lethal dose of 3-chloro-1,2-propanediol in rats after oral administration was 150mg/kg of body weight.

In several studies in which 3-chloro-1,2-propanediol was administered orally to rats as repeated doses of >1 mg/kg of body weight per day, it

decreased sperm motility and impaired male fertility. At doses of ≥ 10 – 20 mg/kg of body weight per day, alterations in sperm morphology and epididymal lesions (spermatocoele) were found. The compound reduced fertility in males of several other mammalian species at slightly higher doses than in the rat.

In rats and mice, oral administration of 3-chloro-1,2-propanediol at doses of ≥ 25 mg/kg of body weight per day was associated with the development of dose-related lesions of the central nervous system, particularly in the brain stem.

In several short-term studies in rats and mice, the kidney was shown to be the target organ for toxicity. In a 4-week study in rats treated by gavage at 30 mg/kg of body weight per day and in a 13-week study in rats given an oral dose of 9 mg/kg of body weight per day, 3-chloro-1,2-propanediol increased the weight of the kidneys relative to body weight.

In the pivotal long-term study in Fischer 344 rats, the absolute weight of the kidney was reported to be significantly increased by administration of 3-chloro-1,2-propanediol in drinking-water, at all doses tested. The incidence of tubule hyperplasia in the kidneys of treated animals of both sexes was also higher than in controls. Although the incidence did not reach statistical significance at the lowest dose tested (1.1 mg/kg of body weight per day), the Committee concluded that it represented part of a compound-related dose–response relationship. Overt nephrotoxicity was seen at higher doses (5.2 and 28 mg/kg of body weight per day).

The results of most assays for mutagenicity in bacteria *in vitro* were reported to be positive, although negative results were obtained in the presence of an exogenous metabolic activation system from mammalian tissue. The results of assays in mammalian cells *in vitro* were also generally positive. It should be noted, however, that the concentrations used in all these assays were very high (0.1–9 mg/ml), so that their relevance might be questionable. The weight of the evidence indicates that 3-chloro-1,2-propanediol is not genotoxic *in vitro* at concentrations at which other toxic effects are not observed. The results of assays conducted *in vivo*, including a test for micronucleus formation in mouse bone marrow and an assay for unscheduled DNA synthesis in rats, were negative. The Committee concluded that 3-chloro-1,2-propanediol is not genotoxic *in vivo*.

Four long-term studies of toxicity and carcinogenicity were available. Three (two in mice and one in rats) did not meet modern standards of quality; nevertheless, none of these three studies indicated

carcinogenic activity. In the fourth study, conducted in Fischer 344 rats, oral administration of 3-chloro-1,2-propanediol was associated with increased incidences of benign tumours in some organs. These tumours occurred only at doses greater than those causing renal tubule hyperplasia, which had been selected as the most sensitive end-point.

5.1.1.3 Occurrence

3-Chloro-1,2-propanediol has been detected at concentrations >1 mg/kg in only two food ingredients: acid-hydrolysed vegetable protein and soya sauce. In both ingredients, a range of concentrations has been reported, from below the limit of quantification (0.01 mg/kg) with an analytical method that has been validated in various foods and food ingredients, up to 100 mg/kg in some samples of acid-hydrolysed vegetable protein and more than 300 mg/kg in some samples of soya sauce.

Formation of 3-chloro-1,2-propanediol in acid-hydrolysed vegetable protein has been found to be related to production processes, and the concentration can be reduced markedly by modifying the processes suitably. The source of 3-chloro-1,2-propanediol in soya sauce is being investigated; by analogy with hydrolysed vegetable protein, however, it may arise during acid hydrolysis in the manufacture of some products. Traditionally fermented soya sauces would not be contaminated with 3-chloro-1,2-propanediol.

3-Chloro-1,2-propanediol has also been quantified at concentrations generally <0.1 mg/kg in other foods and food ingredients, notably a number of cereal products that have been subjected to high temperatures during roasting or toasting. Concentrations ≤0.5 mg/kg have been found in food ingredients such as malt extracts, but the resulting concentrations in finished foods were <0.01 mg/kg.

5.1.1.4 Estimates of dietary intake

Information on the concentrations of 3-chloro-1,2-propanediol in foods, food ingredients and protein hydrolysates was submitted by the United Kingdom and the USA and by the International Hydrolyzed Protein Council. The USA supplied a national estimate of the intake of 3-chloro-1,2-propanediol, and information on the consumption of soya sauce in Australia, Japan and the USA was received.

The toxicological studies summarized above indicate that 3-chloro-1,2-propanediol would not be expected to have acute effects at any level of intake that might reasonably be expected. This analysis therefore addressed only long-term intake of 3-chloro-1,2-propanediol from foods.

Intake of 3-chloro-1,2-propanediol would be due predominantly to consumption of contaminated soya sauces. In a survey of 90 samples of commercial soya sauces, 50 samples contained <1 mg/kg, and the average concentration was 18 mg/kg. The results of this survey were taken as representative for all soya sauces for the purposes of the intake assessment.

The mean daily per capita consumption of soya sauce in Australia by persons consuming this product was about 11 g, and that of persons at the 95th percentile of consumption was about 35 g. Thus, the daily per capita intake of 3-chloro-1,2-propanediol would be 200 µg for mean consumption of soya sauce and 630 µg at the 90th percentile of consumption. The estimated mean daily per capita consumption of soya sauce in Japan (equivalent to consumption by consumers only, in view of its widespread use in that country) was about 30 g, resulting in a mean daily per capita intake of 3-chloro-1,2-propanediol of about 540 µg. Intake at the 95th percentile in Japan was estimated to be 1100 µg by assuming twice the mean consumption of soya sauce. The estimated mean daily per capita consumption of soya sauce in the USA by consumers of this product was 8 g, and that of consumers at the 90th percentile of consumption was 16 g. The resulting estimated daily per capita intake of 3-chloro-1,2-propanediol was 140 µg for mean consumption and 290 µg for consumption at the 90th percentile.

The data submitted by the United Kingdom showed that 3-chloro-1,2-propanediol occurs in some savoury foods, with about 30% of samples containing concentrations above the limit of detection of 0.01 mg/kg. The mean residual concentration in these savoury foods was 0.012 mg/kg.

In estimating the intake of 3-chloro-1,2-propanediol from foods other than soya sauce, the Committee assumed that about one-eighth of the diet, i.e. 180 g (on the basis of 1500 g/day of solid food), consists of savoury foods that might contain 3-chloro-1,2-propanediol and that the mean residual concentration of the compound in those foods is 0.012 mg/kg. On this basis, the daily per capita intake of 3-chloro-1,2-propanediol from foods other than soya sauce was estimated to be 2 µg.

5.1.1.5 Evaluation

The Committee chose tubule hyperplasia in the kidney as the most sensitive end-point for deriving a tolerable intake. This effect was seen in the long-term study of toxicity and carcinogenicity in rats in a dose-related manner, although the effect did not reach statistical significance at the lowest dose. The Committee concluded that the

lowest-observed-effect level (LOEL) was 1.1 mg/kg of body weight per day and considered this to be close to a NOEL.

The Committee established a provisional maximum tolerable daily intake (PMTDI) of 2 µg/kg of body weight for 3-chloro-1,2-propanediol on the basis of the LOEL of 1.1 mg/kg of body weight per day and a safety factor of 500, which included a factor of 5 for extrapolation from a LOEL to a NOEL. This factor was considered to be adequate to allow for the absence of a clear NOEL and to account for the effects on male fertility and for inadequacies in the studies of reproductive toxicity. Data available to the Committee indicated that the estimated mean intake of 3-chloro-1,2-propanediol by consumers of soya sauce would be at or above this PMTDI.

5.1.1.6 Impact of regulatory limits

As 3-chloro-1,2-propanediol is found infrequently in foods, a regulatory limit would be unlikely to have much effect on the overall intake by persons who do not consume soya sauce. However, because the distribution of residual 3-chloro-1,2-propanediol in soya sauce is highly skewed and because it is likely that brand loyalty could result in regular consumption of highly contaminated brands of soya sauce, a regulatory limit on the concentration of 3-chloro-1,2-propanediol in soya sauce could markedly reduce the intake by consumers of this commodity.

5.1.2 1,3-Dichloro-2-propanol

5.1.2.1 Absorption, distribution, metabolism and excretion

Approximately 5% of an oral dose of 1,3-dichloro-2-propanol was excreted in the urine of rats as β -chlorolactate and about 1% of the dose as 2-propanol-1,3-dimercapturic acid. In another experiment, the urine of rats contained the parent compound (2.4% of the dose), 3-chloro-1,2-propanediol (0.35% of the dose) and 1,2-propanediol (0.43% of the dose). Epoxy-chloropropane (epichlorohydrin) was postulated to be an intermediate, and this compound may either undergo conjugation with glutathione to form mercapturic acid or be hydrolysed to 3-chloro-1,2-propanediol. The latter undergoes oxidation to β -chlorolactate, which is further oxidized to oxalic acid.

5.1.2.2 Toxicological studies

The median lethal dose of 1,3-dichloro-2-propanol in rats treated orally was 120–140 mg/kg of body weight.

In several short-term studies in rats, oral administration of 1,3-dichloro-2-propanol at doses of ≥ 10 mg/kg of body weight per day

caused significant hepatic toxicity. This was associated with oxidative metabolism, which yielded intermediates that reacted with and depleted glutathione.

In a 13-week study in rats, overt hepatotoxicity, including increased liver weights, histological changes and/or increased activity of serum alanine and aspartate transaminases, was seen after oral administration of 1,3-dichloro-2-propanol at doses of ≥ 10 mg/kg of body weight per day. These doses also caused histopathological changes in the kidney, increased kidney weights and alterations in urinary parameters. The NOEL was 1 mg/kg of body weight per day.

1,3-Dichloro-2-propanol has been reported to be hepatotoxic in humans exposed occupationally.

1,3-Dichloro-2-propanol was clearly mutagenic and genotoxic in various bacterial and mammalian test systems in vitro. The only available study in vivo showed no mutagenic effect in a wing spot test in *Drosophila melanogaster*.

The results of the one long-term study of toxicity and carcinogenicity in rats confirmed the hepatotoxicity and nephrotoxicity seen in the 13-week study. Furthermore, it demonstrated a clear carcinogenic effect of 1,3-dichloro-2-propanol at the highest dose tested, 19 mg/kg of body weight per day. The tumours (adenomas and carcinomas) occurred in liver, kidney, the oral epithelium and tongue and the thyroid gland. No increase in tumour incidence was seen at the lowest dose tested, 2.1 mg/kg of body weight per day. Treatment-related non-neoplastic lesions of the liver were observed, sinusoidal peliosis being found in all treated groups.

5.1.2.3 Occurrence

Information on the concentrations of 1,3-dichloro-2-propanol in soya sauce was submitted by the USA. Additional information was derived from a published report on the concomitant occurrence of 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol in soya sauces, which showed that 1,3-dichloro-2-propanol may be present at concentrations >1 mg/kg in samples of hydrolysed vegetable protein and soya sauce that contain 3-chloro-1,2-propanediol. In those products in which 1,3-dichloro-2-propanol was quantifiable, the ratio of the concentrations of 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol was at least 20.

5.1.2.4 Estimates of dietary intake

A report from the USA was used by the Committee to estimate the intake of 1,3-dichloro-2-propanol present in soya sauces. Information

about the consumption of soya sauce was received from Australia, Japan and the USA.

The toxicological studies summarized above indicate that 1,3-dichloro-2-propanol would not be expected to have acute effects at any level of intake that might reasonably be expected. This analysis therefore addressed only long-term intake of the compound from foods.

The upper-bound 20:1 ratio of 3-chloro-1,2-propanediol:1,3-dichloro-2-propanol was used by the Committee to estimate the intake of 1,3-dichloro-2-propanol from consumption of soya sauce. As the average concentration of 3-chloro-1,2-propanediol in a survey of 90 commercially obtained soya sauce samples was 18mg/kg, the residual concentration of 1,3-dichloro-2-propanol was assumed to be 0.9mg/kg.

The mean daily per capita consumption of soya sauce in Australia was approximately 11g, and that of persons at the 95th percentile of consumption was 35g. The estimated daily per capita intake was therefore 10µg for consumers at the mean and 30µg at the 95th percentile. The daily per capita intake of soya sauce in Japan (equivalent to the consumption by consumers only, in view of its widespread use in that country) was 30g, resulting in an estimated daily per capita intake of 1,3-dichloro-2-propanol of 27µg. An intake of 55µg/person per day was estimated for consumers in the upper percentile of consumption by assuming twice the mean consumption of soya sauce. The estimated mean daily per capita consumption of soya sauce in the USA by consumers of this product was 8g, and that of consumers at the 90th percentile of consumption was 16g. The resulting estimated daily per capita intake of 1,3-dichloro-2-propanol was 7µg at the mean level of consumption and 14µg at the 90th percentile.

The intake of 1,3-dichloro-2-propanol from foods other than soya sauce can be estimated roughly from data on residual concentrations of 3-chloro-1,2-propanediol in savoury foods and the upper-bound 20:1 ratio of 3-chloro-1,2-propanediol:1,3-dichloro-2-propanol. If it is assumed that about one-eighth of the diet, i.e. 180g (on the basis of 1500g/day of solid food), consists of savoury foods that might contain 1,3-dichloro-2-propanol and that the mean residual concentration of the compound in those foods is 0.6µg/kg, the background daily per capita intake is approximately 0.1µg.

5.1.2.5 Evaluation

Although only a few studies of kinetics, metabolism, short- and long-term toxicity and reproductive toxicity were available for evaluation,

the results clearly indicated that 1,3-dichloro-2-propanol was genotoxic in vitro, was hepatotoxic and induced a variety of tumours in various organs in rats. The Committee concluded that it would be inappropriate to estimate a tolerable intake because of the nature of the toxicity observed:

- The results of the long-term study of toxicity and carcinogenicity showed significant increases in the incidences of both benign and malignant neoplasms in at least three different tissues.
- It has been shown unequivocally that this contaminant can interact with chromosomes and/or DNA; however, the tests were confined to bacterial and mammalian test systems in vitro, and there were no data on intact mammalian organisms or humans.

The Committee noted that the dose that caused tumours in rats (19 mg/kg of body weight per day) was about 20 000 times the highest estimated intake of 1,3-dichloro-2-propanol by consumers of soya sauce (1 µg/kg of body weight per day).

The available evidence suggests that 1,3-dichloro-2-propanol is associated with high concentrations of 3-chloro-1,2-propandiol in food. Regulatory control of the latter would therefore obviate the need for specific controls on 1,3-dichloro-2-propanol.

5.2 Polychlorinated dibenzodioxins, polychlorinated dibenzofurans and coplanar polychlorinated biphenyls

5.2.1 Introduction

Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are by-products of combustion and of various industrial processes, and they are found widely in the environment. Polychlorinated biphenyls (PCBs) were manufactured in the past for a variety of industrial uses, notably as electrical insulators or dielectric fluids and specialized hydraulic fluids. Most countries banned manufacture and use of PCBs in the 1970s; however, past improper handling of PCBs constitutes a continuing source of these compounds in the environment, and disposal of equipment containing these compounds poses some risk of further contamination.

Neither PCDDs nor PCDFs have been evaluated previously by the Committee. PCBs were evaluated by the Committee at its thirty-fifth meeting (Annex 1, reference 88), when it concluded that it was impossible to establish a precise numerical value for a tolerable intake in humans because of limitations in the available data and the ill-defined nature of the materials that were used in feeding studies.

PCDDs, PCDFs and coplanar PCBs were evaluated at the present meeting in response to a request by the Codex Committee on Food Additives and Contaminants at its Thirty-second Session (4) for the Expert Committee to evaluate the risks associated with their presence in food.

The Committee evaluated the PCDDs, PCDFs and coplanar PCBs for which toxic equivalency factors (TEFs) for mammals have been derived by WHO. Table 8 lists the compounds that were considered

Table 8

Compounds considered and their toxic equivalency factor assigned by WHO

Compound	Abbreviation	Toxic equivalency factor
Polychlorinated dibenzodioxins		
2,3,7,8-Tetrachlorodibenzodioxin	TCDD	1
1,2,3,7,8-Pentachlorodibenzodioxin	1,2,3,7,8-PeCDD	1
1,2,3,4,7,8-Hexachlorodibenzodioxin	1,2,3,4,7,8-HxCDD	0.1
1,2,3,6,7,8-Hexachlorodibenzodioxin	1,2,3,6,7,8-HxCDD	0.1
1,2,3,6,7,9-Hexachlorodibenzodioxin	1,2,3,6,7,9-HxCDD	0.1
1,2,3,4,6,7,8-Heptachlorodibenzodioxin	1,2,3,4,6,7,8-HpCDD	0.01
Octachlorodibenzodioxin	OCDD	0.0001
Polychlorinated dibenzofurans		
2,3,7,8-Tetrachlorodibenzofuran	2,3,7,8-TCDF	0.1
1,2,3,7,8-Pentachlorodibenzofuran	1,2,3,7,8-PeCDF	0.05
2,3,4,7,8-Pentachlorodibenzofuran	2,3,4,7,8-PeCDF	0.5
1,2,3,4,7,8-Hexachlorodibenzofuran	1,2,3,4,7,8-HxCDF	0.1
1,2,3,6,7,8-Hexachlorodibenzofuran	1,2,3,6,7,8-HxCDF	0.1
1,2,3,7,8,9-Hexachlorodibenzofuran	1,2,3,7,8,9-HxCDF	0.1
2,3,4,6,7,8-Hexachlorodibenzofuran	2,3,4,6,7,8-HxCDF	0.1
1,2,3,4,6,7,8-Heptachlorodibenzofuran	1,2,3,4,6,7,8-HpCDF	0.01
1,2,3,4,7,8,9-Heptachlorodibenzofuran	1,2,3,4,7,8,9-HpCDF	0.01
Octachlorodibenzofuran	OCDF	0.0001
“Non-ortho” polychlorinated biphenyls		
3,3',4,4'-Tetrachlorobiphenyl	3,3',4,4'-TCB	0.0001
3,4,4',5'-Tetrachlorobiphenyl	3,4,4',5'-TCB	0.0001
3,3',4,4',5-Pentachlorobiphenyl	3,3',4,4',5-PeCB	0.1
3,3',4,4',5,5'-Hexachlorobiphenyl	3,3',4,4',5,5'-HxCB	0.01
“Mono-ortho” polychlorinated biphenyls		
2,3,3',4,4'-Pentachlorobiphenyl	2,3,3',4,4'-PeCB	0.0001
2,3,4,4',5-Pentachlorobiphenyl	2,3,4,4',5-PeCB	0.0005
2,3',4,4',5-Pentachlorobiphenyl	2,3',4,4',5-PeCB	0.0001
2,3',4,4',5'-Pentachlorobiphenyl	2,3',4,4',5'-PeCB	0.0001
2,3,3',4,4',5-Hexachlorobiphenyl	2,3,3',4,4',5-HxCB	0.0005
2,3,3',4,4',5'-Hexachlorobiphenyl	2,3,3',4,4',5'-HxCB	0.0005
2,3',4,4',5,5'-Hexachlorobiphenyl	2,3',4,4',5,5'-HxCB	0.00001
2,3,3',4,4',5,5'-Heptachlorobiphenyl	2,3,3',4,4',5,5'-HpCB	0.00001

and their assigned TEF values. In the TEF approach, the toxicity of all chemicals in the series is related to that of 2,3,7,8-tetrachlorodibenzodioxin (TCDD), one of the most potent of the dioxins and that for which most toxicological and epidemiological information is available. Use of the TEF approach is based on the assumption that PCDDs, PCDFs and coplanar PCBs have a common mechanism of action, which involves binding to the aryl hydrocarbon (Ah) receptor, an intracellular receptor protein. This binding is considered to be the necessary, but not sufficient, first step in the expression of the toxicity of these compounds. Many uncertainties exist in applying the TEF approach to the assessment of human risk, but it is the most feasible approach currently available.

PCDDs, PCDFs and coplanar PCBs were considered by a WHO consultation held in 1998 (5), which established a tolerable daily intake (TDI) of 1–4pg/kg of body weight, applicable to the toxic equivalents of these compounds. The TDI was based on a number of studies of developmental toxicity and immunological toxicity. At its present meeting, the Committee used the assessment of the consultation as the starting point for its evaluation, taking into account the following additional data:

- the results of a study on the toxicokinetics of TCDD after single and repeated dosing;
- two new studies of developmental toxicity;
- new information on a study in rhesus monkeys that had been evaluated by the Committee at its fifty-fifth meeting (Annex 1, reference 149).

5.2.2 *Toxicokinetics*

5.2.2.1 *Absorption and biotransformation*

Coplanar compounds in dietary fat pass easily from the gut into the blood. Indeed, experiments in humans and laboratory animals given an oral dose of TCDD showed 50–90% absorption. This figure is comparable with the near-complete absorption of PCDDs, PCDFs and PCBs by nursing infants from their mothers' milk.

After absorption from the gut, TCDD enters the lymph in the form of chylomicrons and is cleared from the blood within 1 h, to appear mainly (74–81% of an administered dose) in the liver and adipose tissue. After clearance from the blood, coplanar compounds remain mainly in serum lipoproteins (very low density, low density and high density), and some are bound to serum proteins.

The Committee used the results of a study in which [³H]TCDD was given to pregnant Long-Evans rats by gavage as a single dose of 50,

200, 800 or 1000 ng/kg of body weight on day 15 of gestation, and the concentration of the radiolabel measured in tissues 1 day after treatment. The average maternal body burdens (with the percentage of the dose in the four treatment groups) were 31 (60%), 97 (48%), 520 (65%) and 580 (59%) ng/kg of body weight, respectively. On the basis of this study, the Committee used a value of 60% for the percentage of TCDD retained in pregnant rats 1 day after administration of a single dose by gavage on day 15 of gestation.

The distribution of PCDDs and PCDFs between the blood and organs is governed by lipid partitioning and binding to plasma proteins. The concentrations of PCDDs and PCDFs in blood and adipose tissue are closely correlated. TCDD is distributed between blood and adipose tissue by lipid partitioning, whereas the distribution of hexachlorodibenzodioxins (HxCDDs), hexachlorodibenzofurans (HxCDFs), octachlorodibenzodioxins (OCDDs) and octachlorodibenzofurans (OCDFs) is also governed by binding to plasma proteins.

Binding to plasma proteins plays an important role in the uptake of coplanar compounds from the blood in the liver, even for lower chlorinated congeners. When rodents are exposed to increasing doses of TCDD, it is preferentially sequestered in the liver. After entering liver cells, TCDD either dissolves in the lipid fraction or binds to the Ah receptor or to cytochrome P450 (CYP) proteins, probably microsomal CYP 1A2. As the amounts of CYP 1A and CYP 1B proteins in cells are regulated by formation of the TCDD–Ah receptor complex, exposure to increasing amounts of TCDD results in increased formation of this complex, which leads to increased production of CYP 1A and CYP 1B mRNA and proteins (enzyme induction), and accumulation of TCDD by increased binding to the induced CYP proteins. Similar sequestration has been observed with higher chlorinated PCDDs and PCDFs and with coplanar PCBs.

The hepatic sequestration of coplanar compounds markedly affects their distribution in the body. For example, whereas the liver usually contributes 10% and the adipose tissue 60% of the body burden of TCDD in mice, these fractions may increase to 67% in liver and decrease to 23% in adipose tissue in mice in which hepatic CYP proteins have been fully induced. Similar results were found in rats, clearly indicating the non-linear character of the kinetics of TCDD at concentrations that induce hepatic CYP proteins.

As in rodents, preferential sequestration of PCDDs and PCDFs in the liver rather than in adipose tissue has been observed in humans exposed to background concentrations of these compounds. Although Ah receptor-dependent CYP induction has been observed in

human liver cells in vitro after exposure to TCDD, it occurred at concentrations that were several orders of magnitude higher than those observed in human blood. It is therefore likely that the sequestration is due to binding to constitutive CYP proteins.

5.2.2.2 Metabolism and excretion

In laboratory animals, PCDDs and PCDFs are excreted almost exclusively in the bile, excretion in the urine being a minor route. Whereas the parent compound is found primarily in the organs of rodents, only metabolites of PCDDs and PCDFs occur in bile, indicating hepatic metabolism, including hydroxylation and conjugation, of these compounds. Similar reactions were found in vitro when recombinant human CYP 1A1 was incubated with TCDD. Faecal excretion of unmetabolized PCDDs and PCDFs is also an important route of elimination in humans.

In rodents, the half-life of TCDD ranges from 8–24 days in mice to 16–28 days in rats. Humans eliminate PCDDs and PCDFs more slowly, the estimated mean half-life of TCDD ranging from 5.5 to 11 years. The half-lives of other PCDD congeners and of PCDFs and coplanar PCBs vary widely. These differences in the half-lives of different congeners are reflected in their TEFs (see Table 8).

5.2.2.3 Relationship between human intake and doses used in studies in laboratory animals

The biochemical and toxicological effects of PCDDs, PCDFs and coplanar PCBs are directly related to their concentrations in tissues, and not to the daily dose. The most appropriate measure of dose would therefore be the concentration at the target tissue; however, this is seldom known. The body burden, which is strongly correlated with the concentrations in tissue and serum, integrates the differences in half-lives between species. Thus, rodents require appreciably higher daily doses (100–200-fold) to achieve a body burden at steady state that is equivalent to that recorded in humans exposed to background concentrations. Toxicokinetically, estimates of body burden are therefore more appropriate measures of dose for interspecies comparisons than is the daily dose.

The long half-lives of PCDDs, PCDFs and coplanar PCBs in humans have several implications for the period of intake that is relevant to the assessment. First, the concentration of toxic equivalents in the body (or the internal toxic equivalents to which a target organ is exposed) will increase over time as more of the compounds are ingested. Second, after cessation of exposure, the body's concentration of stored toxic equivalents (and the exposure of internal organs) will

decline slowly, only half of the accumulated toxic equivalents disappearing over about 7 years, resulting in a pseudo-steady state only after decades. Third, because of this long-term storage in the body and the consequent daily exposure to the body's stored toxic equivalents, intake on a particular day will have a small or even negligible effect on the overall body burden. For example, in the unlikely event of food contamination that leads to an intake 100 times the amount present in a typical meal, the body burden of the adult eating that meal would increase by <3%. The rest of the body burden would be made up of the PCDDs, PCDFs and coplanar PCBs consumed in many thousands of meals over the previous decade or more.

Therefore, the Committee concluded that the appropriate period for evaluating the mean intake of these compounds is 1 month.

In order to transform an animal body burden into an equivalent human monthly intake (EHMI) that on a long-term basis would result in a similar body burden (at steady state), simple, classical toxicokinetic calculations can be used. The elimination of low doses of PCDDs was considered to follow first-order kinetics and to be independent of the body burden or dose. The Committee calculated the total body burden at steady-state using the following equation:

$$\text{Body burden at steady state (ng/kg of body weight)} = \frac{f \times \text{intake (ng/kg of body weight per day)} \times \text{half-life (days)}}{\ln(2)}$$

where *f* is the fraction of dose absorbed from food (assumed to be 50% in humans) and the estimated half-life of TCDD is 2774 days (7.6 years). For compounds that follow first-order kinetics, 4–5 half-lives will be required to approach steady state. For TCDD, this would be equivalent to more than 30 years.

This model is based on the assumption that PCDDs are distributed in only one compartment: the whole body. Although most of the body burden of PCDD is distributed in the lipid stores, at higher doses the liver also sequesters these compounds in both humans and animals. Predictions of body burden after intake of high doses that are based on lipid concentrations may therefore be underestimates of the total body burden (and the intake leading to that body burden), because of hepatic sequestration. Use of physiologically based pharmacokinetic models may be more appropriate under these circumstances. In order to transform the body burdens resulting from intake of the low concentrations to which the general population is exposed and from the low doses used in the pivotal toxicological studies into estimated

human daily intake, the Committee considered use of a less complicated, classical pharmacokinetic model to be appropriate.

5.2.2.4 Determinants of dose received by fetuses in studies of developmental toxicity

The time of dosing in several of the studies considered by the Committee, day 15 of gestation, marks the onset of the sensitive phase of sexual differentiation in rats and represents a critical time of fetal exposure. The determinant of the reproductive effects is the fetal concentration on days 15–16 of gestation, which in turn is determined by the maternal serum concentration. The latter concentration differs with a bolus dose (as in these studies) and with repeated doses providing the same total intake. As the serum concentration of TCDD after a bolus dose rises before distribution to the tissue compartments, the serum concentration is likely to be higher than that after long-term intake of a lower concentration.

The difference in the fetal body burden after a single bolus dose and after repeated administration of low doses resulting in a similar maternal body burden was addressed in a study in Long-Evans rats treated on day 16 of gestation (6, 7). The rats were given [³H]TCDD at 1, 10 or 30 ng/kg of body weight per day by gavage in corn oil, on 5 days per week for 13 weeks. They were then mated, and dosing was continued daily throughout gestation. The regimen produced a steady-state concentration of TCDD in the dams. The average maternal and fetal body burdens on day 16 of gestation after this treatment and after administration of a single dose of TCDD by gavage on day 15 of gestation are shown in Table 9.

As expected, a single dose on day 15 of gestation by gavage resulted in considerably higher fetal concentrations on day 16 than short-term administration of low daily doses leading to maternal steady-state body burdens of similar magnitude.

Using the data in Table 9, the Committee conducted least-squares linear fits of dose versus maternal and fetal body burdens. Since radiolabelled TCDD was used in both studies, a zero intercept was assumed for the fitted line. None of these fits showed what appeared to be any significant deviation from linearity. These data indicate that the ratio of fetal:maternal body burden resulting from a bolus dose would be 1.7 times that from multiple doses providing the same total dose. Kinetic data indicate that a linear dose–response relationship would be expected at the doses used in these studies. The fetal and maternal body burdens in both data sets were also fitted to power equations, which provided a better fit of the data obtained at the low end of the range of single doses. The factor used to convert maternal

Table 9

Average maternal and fetal body burdens after a single dose and after administration of repeated doses of TCDD to pregnant Long-Evans rats

Dose (ng/kg of body weight per day)	Body burden on day 16 of gestation (ng/kg of body weight per day)	
	Maternal	Fetal
Single dose		
50	30	5.3
200	97	13
800	520	39
1000	520	56
Repeated doses^a		
0.71	20	1.4
7.1	120	7.5
21	300	15

Source: references 6 and 7; used by permission.

^a Daily dose, adjusted for continuous administration from 5 to 7 days per week.

body burden after single doses to a corresponding steady-state body burden with the power equations was 2.6.

5.2.3 *Toxicological studies*

5.2.3.1 *Acute toxicity studies*

In laboratory animals, the acute toxicity of TCDD and related PCDDs and PCDFs substituted in at least the C-2, C-3, C-7 and C-8 positions varies widely between and among species. For example, the median lethal dose in guinea-pigs treated orally was 0.6 µg/kg of body weight, while that in hamsters was >5000 µg/kg of body weight. Explanations for this variation include differences in Ah receptor functionality (size, transformation and binding of the PCDD response element), toxicokinetics (metabolic capacity and tissue distribution) and body fat content. While data on acute toxicity were available for various commercial PCB mixtures (median lethal doses usually >100 mg/kg of body weight), the data on individual coplanar PCB congeners in mammals were limited.

One of the more common symptoms associated with lethality induced by PCDDs is a generalized delayed wasting syndrome characterized by inhibition of gluconeogenesis, reduced feed intake and loss of body weight. Other toxic effects observed after a single exposure to PCDDs include haemorrhages in a number of organs, thymic atrophy, reduced bone-marrow cellularity and loss of body fat and lean muscle mass, although some differences in the frequency of these effects was seen among species.

5.2.3.2 Carcinogenicity studies

TCDD and other PCDDs induced tumours at multiple sites in laboratory animal species of each sex. In a series of assays *in vivo* and *in vitro*, TCDD promoted the growth of transformed cells (e.g. rat tracheal epithelium cells treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine), consistent with observations of cancer promotion in whole animals *in vivo*. In a long-term study of carcinogenicity with TCDD in rats, the LOEL for hepatic adenomas in females was 10 ng/kg of body weight per day, and the NOEL was 1 ng/kg of body weight per day. Several studies have shown that TCDD promotes tumours in laboratory animals, in particular liver tumours. Several other PCDDs, PCDFs and non-*ortho*- and mono-*ortho*-PCBs also promoted liver tumours. In a long-term study in rats in which the incidence of liver tumours was increased over that in controls, the LOEL of 10 ng/kg of body weight per day corresponded to a steady-state body burden of 290 ng/kg of body weight. In order for humans to attain a similar steady-state body burden, they would have to have a daily intake of 150 pg/kg of body weight (see the equation on page 126).

5.2.3.3 Genotoxicity studies

The results of several short-term assays for genotoxicity with TCDD, covering various end-points, were negative. Furthermore, TCDD did not bind covalently to DNA from the liver of mice. The Committee concluded that TCDD does not initiate carcinogenesis.

5.2.3.4 Developmental toxicity studies

A number of biochemical changes, including enzyme induction, altered expression of growth factors and enhanced oxidative stress, have been noted in laboratory animals with body burdens of TCDD within a lower range of 3–10 ng/kg of body weight. The Committee considered these biochemical effects to be early markers of exposure to PCDDs, PCDFs and coplanar PCBs, or events induced by these compounds in animals and in humans that may or may not result in adverse effects at higher body burdens.

The Committee reviewed the relevant studies (8–14) considered by the WHO consultation held in 1998 (4), as well as three recent studies (15–17). The Committee noted that the most sensitive adverse effects reported were on development in the male offspring of rats and immunological deficits in rats after prenatal exposure to TCDD (see Table 10).

The WHO consultation identified a study in which endometriosis was found after long-term administration of TCDD to rhesus monkeys. The Committee stressed that the findings in this study should be

Table 10

Summaries of studies in which the lowest NOELs and LOELs were identified for the most sensitive adverse effects of TCDD on developmental end-points in rats^a

Dosing regimen	Strain	End-point	NOEL body burden (ng/kg of body weight)	LOEL body burden (ng/kg of body weight)	Reference no.
Single bolus by gavage on day 14 of gestation	Fischer 344	Immune suppression in offspring		50	8, 15
Single bolus by gavage on day 15 of gestation	Holtzman	Reduced ventral prostate weight; decreased anogenital distance in male offspring	13	51	17
Single bolus by gavage on day 15 of gestation	Holtzman	Decreased sperm count in offspring		28	13
Single bolus by gavage on day 15 of gestation	Long-Evans	Accelerated eye opening and decreased sperm count in offspring		28	9
Loading and maintenance doses by subcutaneous injection	Wistar	Decreased sperm production and altered sexual behaviour in male offspring		25	16

^a Body burdens estimated from a linear fit to the data in Table 9.

interpreted with caution, as the daily intake was not adequately reported. In addition, analyses conducted 13 years after the end of exposure showed high concentrations of coplanar PCBs in the blood of the monkeys with endometriosis, possibly from an unknown source (14). The Committee also noted that the LOELs in some of the pivotal studies in rats (Table 10) would result in EHMI that were similar to or lower than that derived from the LOEL for endometriosis in monkeys.

In a recent study (17), pregnant Holtzman rats were given a single oral dose of TCDD at 0–800 ng/kg of body weight on day 15 of gestation, and the male offspring were examined on days 49 and 120 after birth. No changes were seen in testicular or epididymal weight nor in daily sperm production or sperm reserve at any dose. However, the weight of the urogenital complex, including the ventral prostate, was significantly reduced at doses of 200 and 800 ng/kg of body weight in rats killed on day 120. Moreover, the anogenital distance of male rats receiving doses ≥ 50 ng/kg of body weight and killed on day 20 was significantly decreased. The Committee noted that administration of TCDD at any dose resulted in a dose-dependent increase in 5 α -reductase type 2 mRNA and a decrease in androgen receptor mRNA in the ventral prostate of rats killed at day 49 but not in those killed at day 120, with no adverse sequelae at the lowest dose of 12.5 ng/kg of body weight. On the basis of 60% absorption and an assumption of a linear relationship for the data in Table 9, the equivalent maternal body burden after multiple doses at this NOEL would be 13 ng/kg of body weight. Fitting the data in Table 9 into the power equation, the Committee estimated the body burden NOEL to be 19 ng/kg of body weight. The LOEL of 50 ng/kg of body weight per day corresponds to an equivalent body burden of 51 ng/kg of body weight with the linear model and 76 ng/kg of body weight with the power model.

The lowest LOEL reported for the reproductive system of male offspring was found in an experiment with Wistar rats (16). In this study, the dams were treated subcutaneously before mating and throughout mating, pregnancy and lactation. They received an initial loading dose of [^{14} C]TCDD at 25, 60 or 300 ng/kg of body weight 2 weeks before mating, and then a weekly maintenance dose of TCDD at 5, 12 or 60 ng/kg of body weight. The size of the maintenance doses was determined on the basis of a reported elimination half-life for TCDD of 3 weeks in adult rats. The effects on male reproductive end-points were studied on days 70 and 170 after birth. The number of sperm per cauda epididymis at puberty and in adulthood was lower in the offspring of all treated dams than in those of controls. Daily sperm production was permanently lower in offspring of treated dams than

in those of controls, as was the sperm transit rate, thus increasing the time required by the sperm to pass through the cauda epididymis. Moreover, the offspring of the treated groups showed increased numbers of abnormal sperm when investigated in adulthood. The latency periods to mounting and intromission were significantly greater in offspring of dams at the lowest and highest doses, but not of those at the intermediate dose, than in offspring of controls. The Committee noted the lack of clear dose–response relationships for most of these effects in the treated groups. In the male offspring of dams at the highest dose, the concentration of serum testosterone was decreased in adulthood, and permanent changes found in the testicular tubuli included pyknotic nuclei and the presence of cell debris in the lumen. The fertility of the male offspring was not affected in any of the treated groups.

In computing the long-term dose required to produce the fetal concentration found in the group given the initial loading dose of 25 ng/kg of body weight, the Committee noted that the dose would have been reduced to 20 ng/kg of body weight before the maintenance dose of 5 ng/kg of body weight given on day 14. On the basis of the linear fit to the data in Table 9, the fetal body burden resulting from the maternal body burden of 20 ng/kg of body weight would be 1.04 ng/kg of body weight. The maintenance dose of 5 ng/kg of body weight administered on day 14 of gestation would make an additional contribution to the fetal body burden of 0.27 ng/kg of body weight, resulting in a total fetal body burden of 1.31 ng/kg of body weight. On the basis of a linear fit to the data in Table 9, a maternal body burden of TCDD of 25 ng/kg of body weight at steady state would be required to produce this fetal body burden.

The studies summarized in Table 10 provide evidence that adverse effects on the reproductive system are induced in male offspring of pregnant rats given TCDD. The studies show reductions in daily sperm production, in the number of sperm in the cauda epididymides and in epididymal weight as well as accelerated eye opening, a reduction in anogenital distance and feminized sexual behaviour in male offspring associated with maternal steady-state body burdens of TCDD of ≥ 25 ng/kg of body weight. Reductions in the weights of the testes and the size of the sex accessory glands, such as the ventral prostate, in male offspring, development of external malformations of the genitalia in female offspring and reduced fertility in males and females required higher maternal body burdens.

The Committee noted that the most sensitive end-points differed between studies, perhaps reflecting strain differences in sensitivity and even minor differences in the experimental conditions, e.g. the

diet. The Committee also noted that, in one study, administration of a single dose of TCDD at 12.5 ng/kg of body weight to dams by gavage decreased the amount of androgen receptor mRNA in the ventral prostate of offspring at puberty on day 49 after birth, indicating reduced androgenic responsiveness. However, none of the other above-mentioned adverse effects were seen in male offspring at this dose, which corresponds to an estimated maternal steady-state body burden of TCDD of approximately 19 ng/kg of body weight (Table 10). The Committee considered the effect on androgenic responsiveness to be an early marker of exposure to TCDD, like enzyme induction, altered expression of growth factors and enhanced oxidative stress, or an event that may or may not result in adverse effects in animals at higher body burdens.

5.2.4 **Observations in humans**

5.2.4.1 Effects other than cancer

In two episodes of food poisoning in China (Province of Taiwan) and Japan, in which infants were exposed in utero to heat-degraded PCBs, a variety of adverse physical developmental abnormalities was observed, including decreased penis length and alterations of spermatozoa; neurodevelopmental abnormalities were also seen. The affected children in Taiwan, China, were born to mothers with estimated body burdens of toxic equivalents of PCBs of 2–3 µg/kg of body weight.

Environmental or background exposure of infants in Germany, the Netherlands and the USA was evaluated in several studies; for example, the mean concentration of toxic equivalents in human milk was 60 pg/g of lipid (range 25–155 pg/g) in a study in Groningen and Rotterdam, the Netherlands. Low birth weight and detriments in neurological development and alterations in thyroid hormones, the distribution of lymphocyte subpopulations and the frequency of infections and respiratory symptoms were observed. The observed neurodevelopmental deficits were subtle and the prevalence within the normal range; their potential consequences for future intellectual function are unknown. The associations observed were considered to be due to prenatal exposure rather than to postnatal intake (from milk). In one study of breastfed and bottle-fed infants, the intake of PCDDs and PCBs was inversely related to performance in neurobehavioural tests, breastfed infants having better scores than bottle-fed infants. These studies of low exposure related primarily to PCBs, and fewer data were available on the effects of PCDDs and PCDFs.

In adults, most of the effects other than cancer observed after exposure to PCDDs, PCDFs and coplanar PCBs, such as chloracne,

appeared only at doses several orders of magnitude greater than those generally received from background contamination of foods. In Seveso, Italy, more female children than expected were born to fathers who had serum TCDD concentrations $>80\text{ pg/g}$ of lipid ($16\text{--}20\text{ ng/kg}$ of body weight) at the time of conception.

5.2.4.2 Carcinogenicity

A working group convened by the International Agency for Research on Cancer (IARC) classified TCDD as a human carcinogen (Group 1). Other PCDDs and PCDFs were considered not to be classifiable as to their carcinogenicity to humans (Group 3).

The most informative studies for evaluating the carcinogenicity of TCDD are four cohort studies of herbicide producers (two in Germany and one each in the Netherlands and the USA) and one cohort study of residents of a contaminated area in Seveso, Italy. A multi-country cohort study from IARC included three of these four cohorts, other industrial cohorts, many of which had not been reported in separate publications, and a cohort of professional herbicide applicators.

In most of the epidemiological studies considered, exposure had been primarily to TCDD, with some exposure to mixtures of other PCDDs, as contaminants of phenoxy herbicides and chlorophenols. The studies involved persons with the highest recorded exposure to TCDD, the estimated geometric mean blood lipid concentrations after the last exposure ranging from 1100 to 2300 pg/g of lipid in the industrial cohorts; lower average concentrations were found in the population exposed in Seveso.

Low excess risks of the order of 40% were found for all neoplasms combined in all the studies of industrial cohorts in which the exposure assessment was adequate. The risks for cancers at specific sites were increased in some of the studies, but the results were not consistent between studies, and no single cancer site seemed to predominate. The results of tests for trends for increasing excess risks for all neoplasms with increasing intensity of exposure were statistically significant. Increasing risks for all neoplasms with time since first exposure were observed in those studies in which latency was evaluated. The follow-up of the Seveso cohort has so far been shorter than that of the industrial cohorts; however, the rate of death from all cancers has not been found to differ significantly from that expected in the general population. Excess risks were seen for cancers at some specific sites among persons in the most heavily contaminated zones at the time of the accident, but there were few cases.

In these well-conducted cohort studies, the intensity of exposure could be ascertained with precision because of the long biological half-life of TCDD in human tissues, and the relative risks increased significantly with increasing exposure. Although the excess cancer risk at the highest exposure was statistically significant, these results must be evaluated with caution, as the overall risks are not high and the strongest evidence is for industrial populations whose exposure was two to three orders of magnitude greater than that of the general population, and who also had heavy exposure to other chemicals; furthermore, lifestyle factors such as smoking were not evaluated. There are few precedents of carcinogens that increase the risk for cancer at all sites combined, with no excess risk for any specific tumour predominating.

A “benchmark dose” was calculated from the effective dose estimated to result in a 1% increase in cancer mortality (ED_{01}), on the basis of a meta-analysis of data for three industrial cohorts with well-documented exposure and comparison with the doses required for effects other than cancer. A statistically significant linear trend in risk with intensity of exposure was observed, which persisted even after exclusion of the groups with the greatest exposure. Within the range of reasonable assumptions, the ED_{01} differed quite widely and depended strongly on the assumptions made. Furthermore, a number of uncertainties would influence the predicted ED_{01} , including the exact exposure of the occupational cohorts and, to a lesser extent, the potential confounding effects of factors not considered in the studies.

5.2.5 ***Sampling and analytical methods***

As no specific guidelines have been drawn up for sampling foods to be analysed for their PCDD, PCDF and coplanar PCB content, the basic guidelines for sampling for organic contaminants or pesticides should be used. The objective is to obtain a representative, homogeneous laboratory sample without introducing secondary contamination. Although PCDDs, PCDFs and coplanar PCBs are chemically stable, the samples should be stored and transported in such a way that they do not deteriorate. PCDDs, PCDFs and coplanar PCBs are usually found as complex mixtures of varying composition in different matrices. Their identification and quantification require a highly sophisticated method of analysis in order to separate the toxic congeners listed in Table 8 from the more prevalent, less toxic congeners. Usually, PCDDs, PCDFs and coplanar PCBs are determined by capillary gas chromatography with mass spectrometry.

No official method exists for the determination of these compounds in food. Reliable results have been obtained in the absence of official

methods when the method used has been shown to be suitable and to fulfil analytical quality criteria developed in other fields of residue analyses. The methods used to determine PCDDs and PCDFs in food must provide sufficient information to allow calculation of the results as toxic equivalents, at concentrations of 0.1–1 pg/g of fat in milk, meat and eggs, 10 pg/g of fat in fish or ≥ 100 pg/g of fat in cases of heavier contamination, and 0.1–0.5 pg/g of dry matter in food of vegetable origin. The patterns of congeners can vary between regions and foods.

When the method used is of insufficient sensitivity, the concentrations of PCDDs, PCDFs and coplanar PCBs in many foods may be near or below the limit of quantification. The method used to derive the concentrations of undetected congeners (the imputation method) can therefore have a variable effect on the summary toxic equivalent value for a food sample. In the most commonly used imputation methods, the contribution of each undetected congener to the toxic equivalent is considered to be either 0 (“lower-bound concentrations”), the limit of detection or limit of quantification (“upper-bound concentrations”) or half the limit of detection or limit of determination. In methods with insufficient sensitivity, the lower- and upper-bound concentrations can differ by a factor of 10–100 or even more. If the sensitivity is appropriate, the differences between lower- and upper-bound concentrations are negligible. Therefore, low estimates of PCDDs, PCDFs and coplanar PCBs in a sample may represent truly low concentrations or be the result of use of zero as the value for undetected congeners in a food sample. Conversely, high estimates may be the result either of contamination or of use of the upper-bound concept with insufficient sensitivity.

Application of upper-bound or lower-bound concentrations leads to over- and underestimates of intake, respectively. Therefore, the Committee recommended that laboratories report their results as lower-bound, upper-bound and half-detection limits, in addition to values for individual congeners, thus providing all the necessary information for interpreting the results for specific requirements. Experts who are summarizing results based on toxic equivalents should indicate the way in which the toxic equivalents were calculated.

For analysis of food samples with normal background contamination by PCDDs, PCDFs or PCBs, gas chromatography with high-resolution mass spectrometry has been validated in collaborative studies and has been shown to provide the required sensitivity and specificity. Bioanalytical assays have been developed for rapid screening of sediments, soil, fly ash and various foods, but only the chemical-

activated luciferase gene expression (CALUX) assay has been used for food; validation of this assay has begun. While gas chromatography with mass spectrometry is the most powerful method for identifying and quantifying congeners and for recognizing congener-specific patterns, it does not allow direct measurement in a matrix of all congeners present that act through the Ah receptor pathway. The CALUX assay provides an indication of the toxic equivalents present in a certain matrix, including interactive (synergistic or antagonistic) effects; however, it cannot provide information on the pattern of congeners.

The Committee recognized that the available analytical data on PCDDs, PCDFs and coplanar PCBs are limited by the lack of generally accepted criteria for intra- and inter-laboratory validation. Mutual acceptance of analytical methods would be facilitated by international collaborative studies and proficiency testing programmes. For reliable analysis of concentrations in the range of normal background contamination, control laboratories must use sufficiently sensitive methods. General statistical parameters that have been established in other fields of residue analysis could be used. The requirements for acceptable analytical methods clearly need to be harmonized, so that data are comparable and can be used for risk management purposes.

5.2.6 *Levels and patterns of contamination of food commodities*

Data were submitted by Belgium, Canada, Japan, New Zealand, Poland and the USA and by the European Commission in a report containing data on Belgium, Denmark, Finland, France, Germany, Italy, the Netherlands, Norway, Sweden and the United Kingdom. In all countries in which a substantial number of samples had been analysed, the concentrations of PCDDs, PCDFs and coplanar PCBs in food were found to have decreased up to the late 1990s, but the decrease had slowed or was even partly reversed in some food categories in several countries owing to contamination of animal feed. For the present assessment of intake at the international level, only data collected after 1995 were considered.

As the Committee did not have access to the original analytical results, it was not possible to ascertain whether the results had been obtained by the lower- or upper-bound approach, and the concentrations used in the assessment were expressed as sums of congeners.

Insufficient individual data were available from most countries to allow construction of a full curve of the distribution of concentrations. Most data were submitted in an aggregated format. As recommended by a FAO/WHO workshop on assessing exposure to contaminants

Table 11

Weighted mean and derived median concentrations of PCDDs, PCDFs and coplanar PCBs in six food groups, expressed as toxic equivalents (pg/g whole food)

Region or country	Food category	PCDDs/PCDFs		Coplanar PCBs	
		Weighted mean	Derived median	Weighted mean	Derived median
North America	Dairy	0.10	0.07	0.02 ^a	0.01 ^a
	Eggs	0.17	0.14	0.04 ^a	0.02 ^a
	Fish	0.56	0.28	0.13 ^a	0.08 ^a
	Meat	0.13	0.10	0.14 ^a	0.05 ^a
Western Europe	Dairy	0.07	0.04	0.08	0.07
	Eggs	0.16	0.15	0.07	0.06
	Fish	0.47	0.31	2.55	0.90
	Meat	0.08	0.06	0.41	0.08
	Vegetable products	0.04	0.03	0.04	LOD
Japan	Dairy	0.06	0.04	0.04	0.02
	Eggs	0.07	0.03	0.06	0.04
	Fish	0.37	0.11	0.69	0.19
	Meat	0.09	0.01	0.04	0.009
	Vegetable products	0.003	0.002	0.02	0.003
New Zealand	Dairy	0.02	0.02	0.01	0.008
	Fish	0.06	0.05	0.09	0.07
	Meat	0.01	0.01	0.02	0.01
	Vegetable products	0.008	0.008	<LOD	<LOD
All	Fats and oils	0.21	0.10	0.07 ^a	0.02

LOD: limit of detection.

^a Data on PCBs frequently did not include mono-*ortho* PCBs.

(18), aggregated data were weighted as a function of the number of initial samples and then used to obtain a weighted mean concentration of PCDDs, PCDFs and PCBs in six major food groups: meat and meat products, eggs, fish and fish products, milk and milk products, vegetables and vegetable products, and fats and oils. National data were aggregated by region or country (North America, Western Europe, Japan and New Zealand) and are summarized in Table 11. Insufficient data were available for the rest of the world to permit a realistic estimate of the distribution of contaminants. The Committee recognized that there are significant differences within the food categories in Table 11, and that the data used in this analysis may not reflect the true mean for a food category. For example, the mean concentrations of PCDDs, PCDFs and coplanar PCBs and the rate of consumption vary considerably in different fish species, and it was not possible to determine if the mean represents the fish species most

commonly consumed. However, the data received were not sufficient to allow an analysis that might account for such variation.

In a second step, a log-normal distribution of contaminants in foods was assumed, and a model of distribution was constructed from the weighted mean and a geometric standard deviation of 3 derived from the concentrations in six broad food groups. On the basis of these derived distributions, the percentiles of consumption were determined. The derived median values (50th percentiles) are presented in Table 11.

5.2.7 *Estimated dietary intake*

Because of the long half-lives of PCDDs, PCDFs and coplanar PCBs, their hazard to health can be estimated only after consideration of intake over a period of months. Short-term variations in PCDD, PCDF and coplanar PCB concentrations in foods have much less effect on overall intake than might be the case for other food contaminants.

The distribution of long-term mean intake in various populations was calculated by the following procedure:

- The distributions of concentrations were constructed for various regions and food groups from the available data. The distributions were assumed to be log-normal.
- Data on food consumption from the GEMS/Food regional diets and national surveys were used to estimate mean consumption of six major food groups in each diet. A log-normal distribution was constructed from these data with a geometric standard deviation of 1.3 extrapolated from the results of the food consumption survey in the Netherlands in order to account for inter-individual variation in consumption. The average contributions of the six basic food groups to total food consumption were derived for each diet.
- The dietary intake of a particular population was assessed by combining the concentrations in foods and food consumption distributions for that population using a Monte Carlo approach. In each Monte Carlo trial, the dietary intake was estimated by multiplying random values for food consumption and concentrations in various food groups. The concentrations were weighted according to the contribution of the food group to total food consumption. The estimates of intake were combined to form a distribution of long-term mean dietary intake for each population studied. The distributions are characterized by a median and a 90th percentile intake. Calculations were performed for the sum of the toxic equivalents of PCDDs and PCDFs and for the sum of coplanar PCBs separately, because the data on occurrence of PCBs were obtained independently.

Table 12

Median and 90th percentile values of estimated long-term intake of PCDDs, PCDFs and coplanar PCBs,^a based on the GEMS/Food regional diets

Source of data on concentrations ^b	Source of data on food consumption	Intake of PCDDs and PCDFs		Intake of coplanar PCBs	
		Median	90th percentile	Median	90th percentile
North America	Europe	68	160	14	35
Western Europe	Europe	54	130	57	150
Japan	Far East	7	15	7	19
New Zealand	Europe	18	36	10	22

^a Expressed as toxic equivalents, pg/kg of body weight per month, assuming 60kg of body weight.

^b For North America, the data on concentrations in vegetables in Western Europe were used; for New Zealand, the data on concentrations in eggs in Japan were used.

Table 13

Median and 90th percentile values of estimated long-term intake of PCDDs, PCDFs and coplanar PCBs,^a based on national food consumption data

Source of data on concentrations ^b	Source of data on food consumption	Intake of PCDDs and PCDFs		Intake of coplanar PCBs	
		Median	90th percentile	Median	90th percentile
North America	USA	42	100	9	25
Western Europe	France	40	94	47	130
	Netherlands	33	81	30	82
	United Kingdom	39	91	41	110

^a Expressed as toxic equivalents, pg/kg of body weight per month, assuming 60kg of body weight.

^b For North America, the data on concentrations in vegetables in Western Europe were used.

The simulated intakes of PCDDs, PCDFs and coplanar PCBs in the GEMS/Food regional diets are presented in Table 12. These intakes are, however, likely to be overestimates, as the data on concentrations were derived from surveys (without random sampling) and from the GEMS/Food regional diets, which are based on data on food supply (apparent consumption) and which are known to overestimate food consumption by at least 15%.

More reliable estimates of intake (Table 13) were obtained by using national food consumption data rather than data on the food supply (apparent consumption) from the GEMS/Food regional diets. The simulated intakes presented in Table 13 are not strictly national estimates and are somewhat higher than the national estimates submitted by the European Commission.

The calculated contributions of various food categories to the intake of PCDDs, PCDFs and coplanar PCBs showed that the largest fraction (>70%) is from food of animal origin in both the GEMS/Food regional diets and the national diets.

Information was lacking on both the quality of data and geographical representativeness for some regions. More data are required on the occurrence of coplanar compounds in food products, particularly from geographical regions other than Europe, so that more representative estimates of intake can be made for all regions.

Breastfed infants have higher intakes of these compounds than bottle-fed infants or adults on a body-weight basis, although for only a small portion of their lives. Breast milk has beneficial effects, despite the risk of contamination. WHO has therefore repeatedly evaluated the health significance of contamination of breast milk with coplanar compounds. WHO recommends and supports breastfeeding but has concluded that continued and enhanced efforts should be directed towards identifying and controlling environmental sources of these substances.

5.2.8 **Evaluation**

In view of the long half-lives of PCDDs, PCDFs and coplanar PCBs, the Committee concluded that it would not be appropriate to establish an acute reference dose for these compounds.

The Committee concluded that a tolerable intake could be established for TCDD on the basis of the assumption that there is a threshold for all effects, including cancer. Carcinogenicity due to TCDD was not linked to mutagenicity or DNA binding, and it occurred at higher body burdens in animals than other toxic effects. The Committee concluded that the establishment of a tolerable intake based on effects other than cancer would also address any carcinogenic risk.

The studies listed in Table 10 were those considered by the Committee in choosing the lowest LOELs and NOELs for assessment of tolerable intake. The lowest LOEL and NOEL were provided by the studies of Faqi et al. (16) and Ohsako et al. (17), respectively. With the toxicokinetic conversions described in Table 9, these two studies indicate maternal body-burden LOELs and NOELs for effects on male rat offspring of 25ng/kg of body weight and 13ng/kg of body weight, respectively.

5.2.8.1 *Background body burdens in laboratory animals*

In the studies used to estimate body burden on the basis of the distribution of TCDD after multiple dosing, radiolabelled material

was used. Therefore, the known background concentrations of TCDD and other PCDDs and PCDFs in the tissues of laboratory rodents resulting from traces of these compounds in rat feed were ignored. The Committee identified two studies that could be used to predict the body burdens of rats resulting from the presence of coplanar compounds in laboratory feed. These studies were mutually consistent and predicted that “unexposed” laboratory rats had toxic equivalent body burdens of 3–12 ng/kg of body weight, depending on age. Thus, the maternal body burdens of TCDD seen in studies with radiolabelled material should be adjusted upwards by a minimum of 3 ng/kg of body weight to account for the background concentrations of unlabelled PCDDs and PCDFs. The maternal toxic equivalent body burden may still be underestimated, as 3 ng/kg of body weight was the minimum in the two studies, and in one of the studies coplanar PCBs were not included.

Addition of 3 ng/kg of body weight to the body burdens calculated from the linear model and the data in Table 9 resulted in estimated total toxic equivalent body burdens of 16 ng/kg of body weight for the NOEL and 28 ng/kg of body weight for the LOEL. These body burdens correspond to EHMI of 240 and 420 pg/kg of body weight, respectively. Fitting the data in Table 9 into the power-model equation gave EHMI of 330 pg/kg and 630 pg/kg of body weight, respectively.

5.2.8.2 Identification of safety factors

The safety factors considered in establishing acceptable levels of intake on the basis of the results of studies in laboratory animals usually include the following: a factor to convert a LOEL to a NOEL (if needed); a factor to extrapolate from animals to humans; and factors to account for inter-individual variations in susceptibility. Typically, factors of 10 have been used for extrapolation between species and for accounting for the human variation in susceptibility, and a factor of 3–10 for extrapolating from a LOEL to a NOEL.

As a NOEL was identified for effects in the male offspring of rats, no factor for conversion from a LOEL to a NOEL was needed for the EHMI derived from the study described above (17).

As concluded by the WHO consultation (5), use of body burdens to scale doses from studies in laboratory animals to equivalent human doses removes the need for safety factors to account for differences in toxicokinetics between animals and humans.

To account for inter-individual differences in toxicokinetics among humans, a safety factor should be applied. The Committee noted that limited data were available on the toxicokinetics of TCDD in humans and considered that the default factor of 3.2 was appropriate.

The Committee observed that humans may be less sensitive than rats to some effects. However, the conclusion is less certain for other effects and the possibility that the most sensitive humans might be as sensitive to the adverse effects of TCDD as rats were in the pivotal studies cannot be excluded. Therefore, the Committee concluded that no safety factor in either direction need be applied for differences in toxicodynamics among humans.

Use of a LOEL instead of a NOEL indicates the need for an additional safety factor. As the LOEL for the sensitive end-point was considered to be close to a NOEL and represented marginal effects, the Committee applied a factor of 3 to account for use of a LOEL instead of a NOEL. This resulted in an overall safety factor of 9.6 (3×3.2).

The Committee concluded that a total safety factor of 3.2 should be applied to the EHMI associated with the NOEL, and a total safety factor of 9.6 should be applied to the EHMI associated with the LOEL.

5.2.8.3 Tolerable intake

As stated in the discussion of toxicokinetics, the long half-lives of PCDDs, PCDFs and coplanar PCBs mean that each daily ingestion has a small or even a negligible effect on overall intake. In order to assess long- or short-term risks to health due to these substances, total or average intake should be assessed over months, and tolerable intake should be assessed over a period of at least 1 month. To encourage this view, the Committee decided to express the tolerable intake as a monthly value in the form of a provisional tolerable monthly intake¹ (PTMI).

As shown in Table 14, use of the linear model to extrapolate the maternal body burden at the NOEL, obtained with a single dose, to that expected at multiple doses gives a EHMI of 237 pg/kg of body weight, which would be expected to result in a body burden that is lower than that which had effects in animals. The PTMI derived by application of the safety factor of 3.2 to this EHMI is 74 pg/kg of body weight.

Similarly, as shown in Table 14, the PTMI derived by application of the safety factor of 9.6 to the EHMI derived from the study that

¹ By analogy with the provisional tolerable weekly intake (PTWI), the end-point used for safety evaluations by the Committee for food contaminants with cumulative properties. Its value represents the permissible human monthly exposure to those contaminants unavoidably associated with otherwise wholesome and nutritious foods.

Table 14

Summary of four calculations of PTMI

	Linear model		Power model	
	NOEL	LOEL	NOEL	LOEL
Administered dose (ng/kg of body weight)	12.5 ^a		12.5 ^a	
Maternal body burden (ng/kg of body weight)	7.6	25 ^b	7.6	25 ^b
Equivalent maternal body burden with long-term dosing (ng/kg of body weight)	13 ^c	25 ^c	19 ^d	39 ^d
Body burden from feed (ng/kg of body weight)	3	3	3	3
Total body burden (ng/kg of body weight)	16 ^e	28 ^e	22 ^e	42 ^e
EHMI (pg/kg of body weight per month)	237	423	330	630
Safety factor	3.2	9.6	3.2	9.6
PTMI (pg/kg of body weight per month)	74	44	103	66

^a Bolus dose (NOEL).^b Target maternal body burden from repeated dosing (LOEL).^c Assuming a linear relationship between fetal and maternal body burden (based on data in Table 9).^d Assumes a non-linear relationship between fetal and maternal body burden (based on data in Table 9).^e Assuming, for humans, a half-life of 7.6 years and 50% uptake from food (see equation on page 126).

provided the LOEL is 44pg/kg of body weight. As also shown in Table 14, use of the power model to extrapolate the maternal body burden with single doses to multiple doses would result in PTMIs of 103pg/kg of body weight for the NOEL and 66pg/kg of body weight for the LOEL. The range of PTMIs derived from the two studies, with either the linear or the power model to extrapolate the maternal body burden with single to multiple doses, is thus 40–100pg/kg of body weight per month. The Committee chose the mid-point of this range, 70pg/kg of body weight per month, as the PTMI. Furthermore, in accordance with the conclusions of the WHO consultation (5), the Committee concluded that this tolerable intake should be applied to intake of PCDDs, PCDFs and coplanar PCBs expressed as TEFs.

5.2.8.4 Comparison of PTMI with estimated intake from food

In the GEMS/Food regional diets, the range of estimated intake of toxic equivalents of PCDDs and PCDFs is 7–68pg/kg of body weight per month at the median and 15–160pg/kg of body weight per month at the 90th percentile of mean lifetime exposure, and that for coplanar PCBs is 7–57pg/kg of body weight per month at the median and 19–150pg/kg of body weight per month at the 90th percentile of consumption. The intakes estimated from national food consumption data were lower: 33–42pg/kg of body weight per month at the median and 81–100pg/kg of body weight per month at the 90th percentile for PCDDs and PCDFs, and 9–47pg/kg of body weight per month at the

median and 25–130 pg/kg of body weight per month at the 90th percentile for coplanar PCBs. Estimates could not be made for the sum of PCDDs, PCDFs and coplanar PCBs, because data on concentrations were submitted separately by countries.

The median and 90th percentile of the derived distribution of intakes were considered to describe long-term intake. A Monte Carlo calculation was used to predict these intakes for coplanar PCBs on the basis of two sets of distribution curves generated from information on mean concentrations in six major food groups and corresponding data on mean food consumption from several sources, by applying geometric standard deviations of 3 and 1.3 to the respective means. The geometric standard deviation for the food consumption curves accounted for long-term consumption patterns. As the mean intakes of the whole population tend not to change with the duration of a survey, use of mean consumer intakes to generate the curves for major food groups, rather than individual commodities, approximates the mean intakes of the whole population, as nearly all respondents were consumers.

5.2.8.5 Uncertainties

Several sources of uncertainty were identified in the data used to assess intake, which suggest that they are likely to be overestimates at both the median and the 90th percentile levels of consumption. Despite the uncertainties, the results suggest that a considerable fraction of the population will have a long-term mean intake above the PTMI.

Furthermore, despite the large amount of information on toxicity, substantial uncertainties remain which should be considered in applying the risk assessment and in interpreting the estimates of intake of PCDDs, PCDFs and coplanar PCBs. The Committee used the overall data to identify a level of intake of coplanar compounds in food that represents no appreciable risk to humans. The safety assessment includes adjustment for a number of uncertainties, including estimates of TEFs within orders of magnitude in order to relate the potency of 28 relatively poorly studied compounds to that of one well-studied compound, TCDD. Moreover, the relative proportion of TCDD and the other 28 compounds varies; TCDD typically constitutes a small percentage of the total toxic equivalents in foods.

The PTMI is not a limit of toxicity and does not represent a boundary between safe intake and intake associated with a significant increase in body burden or risk. Long-term intakes slightly above the PTMI would not necessarily result in adverse health effects but would erode the safety factor built into the calculations of the PTMI. It is not

possible, given current knowledge, to define the magnitude and duration of excess intake that would be associated with adverse health effects.

5.2.8.6 Effect of maximum limits on intake, risk and food availability

The concentrations of PCDDs, PCDFs and coplanar PCBs vary among foods. In establishing regulatory limits, the possible undesired consequences of their enforcement should be taken into account, such as reductions in the food supply. The Committee explored the theoretical effects of various maximum regulatory limits on compliance and on long-term average reduction of intake. On the basis of this analysis, the Committee concluded that, in order to achieve, for example, a 20% reduction in intake of coplanar compounds from food, the intake of a wide range of foods would have to be reduced by a similar percentage. This relationship exists because these contaminants are present at relatively high levels in major food types. Furthermore, in view of the half-times of these compounds in humans, setting regulatory limits on the basis of the PTMI would have no discernible effect on body burdens for several years.

In contrast, long-term reductions could be gained by identifying and eliminating the routes by which these compounds pass from the environment into food supplies. The Committee was informed that studies of environmental concentrations over time in several countries suggest that measures to control emissions to the environment generally have had a substantial impact on both the amounts of PCDDs and PCDFs present in the environment and the body burdens of the general public.

6. Future work

1. The Committee has been asked on several occasions to advise the Codex Committee on Food Additives and Contaminants on the relative risks associated with alternative proposed maximum limits for contaminants in foods. However, a maximum limit will not, in many cases, have a substantial effect on the long-term intake of the contaminant by the general population, nor will it have a measurable impact on public health unless a substantial proportion of the food supply is removed from the market. Nevertheless, maximum limits could have a positive influence on agricultural and industrial practices and contribute to reducing the intake of some contaminants for which the distribution is highly skewed. The Committee recommended more detailed consideration of this issue at a future meeting.

2. The Committee strongly reiterated its recommendation made at its fifty-fifth meeting for revision of the *Guide to specifications* (Annex 1, reference 100). This revision is urgently required, so that significant developments in methods of analysis can be included.
3. The Committee recommended continuation of its activity to update limits for heavy metals in food additives and concluded that acidity regulators and colours should be reviewed at its next meeting on food additives and contaminants.
4. The Committee recommended that the monograph that covers specifications for 16 modified starches should be divided into smaller monographs, as changes to one specification mean that the entire monograph must be changed.

7. Recommendations

1. In view of the large number of food additives and contaminants requiring evaluation or re-evaluation, the important role that the recommendations of the Committee play in the development of international food standards and of regulations in many countries, and the need for maintaining consistency and continuity within the Committee, it is strongly recommended that meetings of the Joint FAO/WHO Expert Committee on Food Additives continue to be held at least once yearly to evaluate these substances.
2. The Codex Alimentarius Commission has adopted International Numbering System (INS) numbers 472e and 472f for diacetyltartaric and fatty acid esters of glycerol and for tartaric, acetic and fatty acid esters of glycerol, mixed, respectively. At its fifty-first meeting (Annex 1, reference 137), the Committee established one specification under the name “diacetyltartaric and fatty acid esters of glycerol” to cover these two substances, and at the present meeting a temporary ADI was established. The specifications have been combined because, even if diacetyltartaric and fatty acid esters of glycerol and tartaric, acetic and fatty acid esters of glycerol, mixed, are manufactured from different raw materials, they meet all the criteria of the specifications and cannot be distinguished from each other by currently available analytical methods. The Committee recommended that the Codex Committee on Food Additives and Contaminants consider whether it would be more appropriate to have only one INS number for labelling purposes.
3. The Committee recognized that the revised “General specifications and recommendations for enzyme preparations used in food processing” in the *Compendium of food additive specifications, addendum 9* (FAO Food and Nutrition Paper, No. 52, Add. 9, 2001)

- contain many criteria for safety evaluations that are more appropriate for inclusion elsewhere. The Committee recommended that the project to update and consolidate principles and methods for the assessment of chemicals in food (see section 2.3) include the safety assessment of enzymes intended for use in food and a subsequent removal of these guidelines from the general specifications.
4. In view of the complexity of the analytical methods for determining PCDDs, PCDFs and coplanar PCBs, the Committee recommended that a specific validation protocol be developed. Laboratories involved in such analytical work should be encouraged by FAO/WHO to participate in collaborative studies and proficiency testing.
 5. A clear definition of “flavouring agent” has not been elaborated by the Committee, which recommended to FAO and WHO that such a definition be developed when updating principles for the assessment of chemicals in food.

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Annex 1

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Annex 2

Acceptable Daily Intakes, other toxicological information and information on specifications

Food additives evaluated toxicologically

Food additive	Specifications ^a	Acceptable daily intake (ADI in mg/kg of body weight) and other toxicological recommendations
Emulsifiers		
Diacetyltartaric and fatty acid esters of glycerol	R	0–50 (temporary) ^b
Tartaric, acetic and fatty acid esters of glycerol, mixed	W ^c	ADI “not limited” withdrawn ^c
Quillaia extracts	R, T ^b	0–5 (temporary) ^{b,d}
Enzyme preparation		
Invertase from <i>Saccharomyces cerevisiae</i>	N	Acceptable ^e
Food colours		
β-Carotene from <i>Blakeslea trispora</i>	N, T ^b	0–5 (group ADI) ^f
Curcumin	R	0–1 (temporary) ^b
Food salts		
Calcium dihydrogen diphosphate	N	Included in the maximum tolerable daily intake of 70 mg/kg of body weight for phosphates, diphosphates and polyphosphates
Monomagnesium phosphate	N, T ^b	
Sodium calcium polyphosphate	N	
Trisodium diphosphate	N, T ^b	
Glazing agent		
Hydrogenated poly-1-decene	R	0–6
Preservative		
Natamycin (pimaricin)	R, T ^b	0–0.3
Sweetening agent		
D-Tagatose	S	0–80
Thickening agents		
Carrageenan	R	ADI “not specified” ^g (group ADI) ^h
Processed <i>Eucheuma</i> seaweed	R	
Curdlan	R	ADI “not specified” ^g
Miscellaneous substances		
Acetylated oxidized starch	N, R ⁱ	ADI “not specified” ^g
α-Cyclodextrin	N	
Sodium sulfate	S	

^a N, new specifications prepared; R, existing specifications revised; S, specifications exist, revision not considered or required; T, the existing, new or revised specifications are tentative and new information is needed; W, existing specifications withdrawn.

^b See Annex 3.

- ^c The ADI was withdrawn because the specifications for tartaric, acetic and fatty acid esters of glycerol, mixed, were combined with those for diacetyltartaric and fatty acid esters of glycerol under the latter name at the fifty-first meeting (Annex 1, reference 137).
- ^d Applicable only to the unpurified extract.
- ^e Invertase from *Saccharomyces cerevisiae* that meets the specifications developed at the present meeting was considered to be acceptable because *S. cerevisiae* is commonly used in the preparation of food. Its use should be limited by good manufacturing practice.
- ^f Group ADI for β -carotene from *Blakeslea trispora* and synthetic β -carotene.
- ^g ADI "not specified" is used to refer to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice, i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.
- ^h Group ADI for carrageenan and processed *Eucheuma* seaweed.
- ⁱ The new specifications for acetylated oxidized starch were incorporated into the revised specifications for modified starches.

Food additives considered for specifications only

Food additive	Specification ^a
Acesulfame K (potassium salt)	R
Blackcurrant extract	R
D,L-Malic acid	R
Oxystearin	W
Pectins	R
Smoke flavourings	R
Tagetes extract	R

^a R, existing specifications revised; W, existing specifications withdrawn.

Flavouring agents evaluated by the Procedure for the Safety Evaluation of Flavouring Agents

Flavouring agent	No.	Specifications ^a	Conclusion based on current intake

Pyrazine derivatives

Structural class II

2-Methylpyrazine	761	N	No safety concern
2-Ethylpyrazine	762	N	
2-Propylpyrazine	763	N	
2-Isopropylpyrazine	764	N	
2,3-Dimethylpyrazine	765	N	
2,5-Dimethylpyrazine	766	N	
2,6-Dimethylpyrazine	767	N	

Flavouring agent	No.	Specifications ^a	Conclusion based on current intake	
2-Ethyl-3-methylpyrazine	768	N	No safety concern	
2-Ethyl-6-methylpyrazine	769	N		
2-Ethyl-5-methylpyrazine	770	N		
2,3-Diethylpyrazine	771	N		
2-Methyl-5-isopropylpyrazine	772	N		
2-Isobutyl-3-methylpyrazine	773	N		
2,3,5-Trimethylpyrazine	774	N		
2-Ethyl-3,(5 or 6)-dimethylpyrazine	775	N		
3-Ethyl-2,6-dimethylpyrazine	776	N		
2,3-Diethyl-5-methylpyrazine	777	N		
2,5-Diethyl-3-methylpyrazine	778	N		
3,5-Diethyl-2-methylpyrazine	779	N		
2,3,5,6-Tetramethylpyrazine	780	N		
5-Methyl-6,7-dihydro-5 <i>H</i> -cyclopentapyrazine	781	N		
6,7-Dihydro-2,3-dimethyl-5 <i>H</i> -cyclopentapyrazine	782	N		
Acetylpyrazine	784	N		
2-Acetyl-3-ethylpyrazine	785	N		
2-Acetyl-3,(5 or 6)-dimethylpyrazine	786	N		
Methoxypyrazine	787	N		
(2 or 5 or 6)-Methoxy-3-methylpyrazine	788	N		
2-Ethyl-(3 or 5 or 6)-methoxypyrazine	789	N		
2-Methoxy-(3 or 5 or 6)-isopropylpyrazine	790	N		
2-Methoxy-3-(1-methylpropyl)pyrazine	791	N		
2-Isobutyl-3-methoxypyrazine	792	N		
2-Acetyl-3-methylpyrazine	950	N		
Structural class III				
(Cyclohexylmethyl)pyrazine	783	N	No safety concern	
2-Methyl-(3 or 5 or 6)-ethoxypyrazine	793	N		
2-(Mercaptomethyl)pyrazine	794	N		
2-Pyrazinylethane thiol	795	N		
Pyrazinylmethyl methyl sulfide	796	N		
(3 or 5 or 6)-(Methylthio)-2-methylpyrazine	797	N		
5-Methylquinoxaline	798	N		
Pyrazine	951	N		
5,6,7,8-Tetrahydroquinoxaline	952	N		
Aromatic substituted secondary alcohols, ketones and related esters				
Structural class I				
α -Methylbenzyl alcohol ^b	799	N	No safety concern	
α -Methylbenzyl formate	800	N		
α -Methylbenzyl acetate	801	N		
α -Methylbenzyl propionate	802	N		
α -Methylbenzyl butyrate	803	N		
α -Methylbenzyl isobutyrate	804	N		
<i>p</i> . α -Dimethylbenzyl alcohol	805	N		

Flavouring agent	No.	Specifications ^a	Conclusion based on current intake
Acetophenone	806	N	No safety concern
4-Methylacetophenone	807	N	
<i>p</i> -Isopropylacetophenone	808	N	
2,4-Dimethylacetophenone	809	N	
Acetanisole	810	N	
1-(<i>p</i> -Methoxyphenyl)-2-propanone	813	N	
α -Methylphenethyl butyrate	814	N, T	
4-Phenyl-2-butanol	815	N	
4-Phenyl-2-butyl acetate	816	N	
4-(<i>p</i> -Tolyl)-2-butanone	817	N, T	
4-(<i>p</i> -Methoxyphenyl)-2-butanone	818	N	
4-Phenyl-3-buten-2-ol	819	N	
4-Phenyl-3-buten-2-one	820	N	
3-Methyl-4-phenyl-3-buten-2-one	821	N	
1-Phenyl-1-propanol	822	N	
α -Ethylbenzyl butyrate	823	N	
Propiophenone	824	N	
α -Propylphenethyl alcohol	825	N	
1-(<i>p</i> -Methoxyphenyl)-1-penten-3-one	826	N	
Ethyl benzoylacetate	834	N	
Ethyl 2-acetyl-3-phenylpropionate	835	N	
Structural class II			
4-Acetal-6- <i>tert</i> -butyl-1,1-dimethylindan	812	N	Additional data required
α -Isobutylphenethyl alcohol	827	N	No safety concern
4-Methyl-1-phenyl-2-pentanone	828	N	
1-(4-Methoxyphenyl)-4-methyl-1-penten-3-one	829	N	
3-Benzyl-4-heptanone	830	N	
1-Phenyl-1,2-propanedione	833	N	
Structural class III			
Methyl β -naphthyl ketone	811	N	No safety concern
Benzophenone	831	N	
1,3-Diphenyl-2-propanone	832	N	
Benzoin	836	N	

Benzyl derivatives

Structural class I

Benzyl alcohol ^c	25	R	No safety concern
Benzyl formate	841	N	
Benzyl acetate ^c	23	R	
Benzyl propionate	842	N	
Benzyl butyrate	843	N	
Benzyl isobutyrate	844	N	
Benzyl isovalerate	845	N	
Benzyl <i>trans</i> -2-methyl-2-butenolate	846	N	
Benzyl 2,3-dimethylcrotonate	847	N, T	

Flavouring agent	No.	Specifications ^a	Conclusion based on current intake
Benzyl acetoacetate	848	N	No safety concern
Benzyl benzoate ^c	24	R	
Benzyl phenylacetate	849	N	
Benzaldehyde ^c	22	R	
Benzaldehyde dimethyl acetal	837	N	
Benzaldehyde glyceryl acetal	838	N	
Benzaldehyde propylene glycol acetal	839	N	
Benzoic acid ^c	850	N	Evaluation not finalized ^d
Methyl benzoate	851	N	No safety concern
Ethyl benzoate	852	N	
Propyl benzoate	853	N	
Hexyl benzoate	854	N	
Isopropyl benzoate	855	N	
Isobutyl benzoate	856	N	
Isoamyl benzoate	857	N	
<i>cis</i> -3-Hexenyl benzoate	858	N	
Linalyl benzoate	859	N	
Geranyl benzoate	860	N	Evaluation not finalized ^d
Glyceryl tribenzoate	861	N, T	
Propylene glycol dibenzoate	862	N, T	
Methylbenzyl acetate (mixed <i>ortho</i> -, <i>meta</i> - and <i>para</i> -isomers)	863	N	No safety concern
<i>p</i> -Isopropylbenzyl alcohol	864	N	
4-Ethylbenzaldehyde	865	N	
Tolualdehydes (mixed <i>ortho</i> -, <i>meta</i> - and <i>para</i> -isomers)	866	N, T	
Tolualdehyde glyceryl acetal	867	N	
Cuminaldehyde	868	N	
2,4-Dimethylbenzaldehyde	869	N	
Benzyl 2-methoxyethyl acetal	840	N	

Hydroxy- and alkoxy-substituted benzyl derivatives

Structural class I

4-Hydroxybenzyl alcohol	955	— ^e	No safety concern
4-Hydroxybenzaldehyde	956	— ^e	
4-Hydroxybenzoic acid	957	— ^e	
2-Hydroxybenzoic acid	958	— ^e	
Butyl <i>p</i> -hydroxybenzoate	870	N, T	Evaluation not finalized ^d
Anisyl alcohol	871	N	No safety concern
Anisyl formate	872	N, T	
Anisyl acetate	873	N	
Anisyl propionate	874	N	
Anisyl butyrate	875	N	
Anisyl phenylacetate	876	N	

Flavouring agent	No.	Specifications ^a	Conclusion based on current intake
Veratraldehyde	877	N	No safety concern
<i>p</i> -Methoxybenzaldehyde	878	N	
<i>p</i> -Ethoxybenzaldehyde	879	N	
Methyl <i>o</i> -methoxybenzoate	880	N	
2-Methoxybenzoic acid	881	N	
3-Methoxybenzoic acid	882	N	
4-Methoxybenzoic acid	883	N	
Methyl anisate	884	N	
Ethyl <i>p</i> -anisate	885	N	
Vanillyl alcohol	886	N	
Vanillin ^f	889	N	
4-Hydroxy-3-methoxybenzoic acid	959	— ^e	
Vanillin acetate	890	N	
Vanillin isobutyrate	891	N	
Salicylaldehyde	897	N	
2-Hydroxy-4-methylbenzaldehyde	898	N	
Methyl salicylate ^g	899	N	
Ethyl salicylate	900	N	
Butyl salicylate	901	N	
Isobutyl salicylate	902	N	
Isoamyl salicylate	903	N	
Benzyl salicylate	904	N	
Phenethyl salicylate	905	N	
<i>o</i> -Tolyl salicylate	907	N	
2,4-Dihydroxybenzoic acid	908	N	
Structural class II			
Vanillyl ethyl ether	887	N	No safety concern
Vanillyl butyl ether	888	N	
Ethyl vanillin ^h	893	N	
Vanillin <i>erythro</i> - and <i>threo</i> -butan-2,3-diol acetal	960	— ^e	
Ethyl vanillin isobutyrate	953	N	
Ethyl vanillin propylene glycol acetal	954	N, T	
Piperonyl acetate	894	N	
Piperonyl isobutyrate	895	N	
Piperonal ⁱ	896	N	
Ethyl vanillin β-D-glucopyranoside	892	N	
Aliphatic acyclic diols, triols and related substances			
Structural class I			
Glycerol ^j	909	N, T	Evaluation not finalized ^d
1,2,3-Tris[(1'-ethoxy)ethoxy]propane	913	N	No safety concern
Glyceryl monostearate	918	N, T	Evaluation not finalized ^d
Glyceryl monooleate	919	N, T	
Triacetin	920	N, T	
Glyceryl tripropanoate	921	N, T	

Flavouring agent	No.	Specifications ^a	Conclusion based on current intake
Tributyrin	922	N, T	Evaluation not finalized ^d
Glycerol 5-hydroxydecanoate	923	N, T	
Glycerol 5-hydroxydodecanoate	924	N, T	
Propylene glycol ^k	925	N, T	
Propylene glycol stearate	926	N, T	
1,2-Di[(1-ethoxy)ethoxy]propane	927	N	No safety concern
Lactic acid	930	N	
Ethyl lactate ^l	931	N	
Butyl lactate	932	N	
Potassium 2-(1'-ethoxy)ethoxypropanoate	933	N	
<i>cis</i> -3-Hexenyl lactate	934	N	
Butyl butyryllactate	935	N	
Pyruvic acid	936	N	
Pyruvaldehyde	937	N, T	
Ethyl pyruvate	938	N	
Isoamyl pyruvate	939	N	
Structural class III			
3-Oxohexanoic acid glyceride	910	N, T	No safety concern
3-Oxooctanoic acid glyceride	911	N, T	
Heptanal glyceryl acetal (mixed 1,2 and 1,3 acetals)	912	N	
3-Oxodecanoic acid glyceride	914	N, T	Evaluation not finalized ^d
3-Oxododecanoic acid glyceride	915	N, T	
3-Oxotetradecanoic acid glyceride	916	N, T	
3-Oxohexadecanoic acid glyceride	917	N, T	
4-Methyl-2-pentyl-1,3-dioxolane	928	N	No safety concern
2,2,4-Trimethyl-1,3-oxacyclopentane	929	N	
Aliphatic acyclic acetals			
Structural class I			
1,1-Dimethoxyethane	940	N	No safety concern
Acetal	941	N	
Heptanal dimethyl acetal	947	N	
4-Heptenal diethyl acetal	949	N	
Octanal dimethyl acetal	942	N	
2,6-Nonadienal diethyl acetal	946	N	
Decanal dimethyl acetal	945	N	
Citral dimethyl acetal	944	N	
Citral diethyl acetal	948	N	
Acetaldehyde ethyl <i>cis</i> -3-hexenyl acetal	943	N, T	

^a N, new specifications prepared; R, existing specifications revised; T, the existing, new or revised specifications are tentative and new information is needed.

^b An ADI of 0–0.1 mg/kg of body weight was established for α -methylbenzyl alcohol by the Committee at its forty-first meeting (WHO Technical Report Series, No. 837, 1993), which was maintained at the present meeting.

^c A group ADI of 0–5 mg/kg of body weight for benzoic acid, the benzoate salts (calcium, potassium and sodium), benzaldehyde, benzyl acetate and benzyl alcohol, expressed as

benzoic acid equivalents, was confirmed by the Committee at its forty-sixth meeting (WHO Technical Report Series, No. 868, 1997) and extended to include benzyl benzoate at the present meeting.

^d Further information is required to determine whether this substance is in current use as a flavouring agent.

^e Specifications will be considered at the fifty-ninth meeting of the Committee.

^f An ADI of 0–10 mg/kg of body weight was established for vanillin by the Committee at its eleventh meeting (WHO Technical Report Series, No. 383, 1968), which was maintained at the present meeting.

^g An ADI of 0–0.5 mg/kg of body weight was established for methyl salicylate by the Committee at its eleventh meeting (WHO Technical Report Series, No. 383, 1968), which was maintained at the present meeting.

^h An ADI of 0–3 mg/kg of body weight was established for ethyl vanillin by the Committee at its forty-fourth meeting (WHO Technical Report Series, No. 859, 1995), which was maintained at the present meeting.

ⁱ An ADI of 0–2.5 mg/kg of body weight was established for piperonal by the Committee at its eleventh meeting (WHO Technical Report Series, No. 383, 1968), which was maintained at the present meeting.

^j An ADI “not specified” was established for glycerol by the Committee at its twentieth meeting (WHO Technical Report Series, No. 599, 1976), which was maintained at the present meeting.

^k An ADI of 0–25 mg/kg of body weight was established for propylene glycol by the Committee at its seventeenth meeting (WHO Technical Report Series, No. 539, 1974), which was maintained at the present meeting.

^l Ethyl lactate was included in the group ADI “not specified” for lactic acid and its salts that was established by the Committee at its twenty-sixth meeting (WHO Technical Report Series, No. 683, 1982), which was maintained at the present meeting.

Flavouring agents considered for specifications only

Flavouring agent	No.	Specifications ^a
Allyl tiglate	10	R
Allyl cyclohexane acetate	12	R
Allyl cyclohexane butyrate	14	R
Allyl cyclohexane valerate	15	R
Allyl cyclohexane hexanoate	16	R
Isoamyl formate	42	R
Isoamyl 2-methylbutyrate	51	R
Geranyl acetate	58	R
Rhodiny l propionate	64	R
Geranyl hexanoate	70	R
Geranyl isobutyrate	72	R
Rhodiny l isobutyrate	74	R
Rhodiny l isovalerate	77	R
3,7-Dimethyl-2,6-octadien-1-yl 2-ethylbutanoate	78	R
Heptanal	95	R
Nonanal	101	R
Undecanal	107	R
Lauric acid	111	R, T
Myristic acid	113	R, T
Palmitic acid	115	R, T
Stearic acid	116	R, T
Propyl formate	117	R
<i>n</i> -Amyl formate	119	R
Isobutyl formate	124	R
<i>n</i> -Amyl heptanoate	170	R

Flavouring agent	No.	Specifications ^a
Isobutyl heptanoate	172	R
Nonyl octanoate	178	R
Methyl laurate	180	R
Isoamyl laurate	182	R, T
Butyl stearate	184	R
<i>trans</i> -3-Heptenyl 2-methyl propanoate	191	R
Methyl 2-methylbutyrate	205	R
2-Methylbutyl 2-methylbutyrate	212	R
ω -6-Hexadecenlactone	240	R
<i>cis</i> -4-Hydroxy-6-dodecenoic acid lactone	249	R
2-Methylpentanal	260	R
2-Methylhexanoic acid	265	R
5-Methylhexanoic acid	266	R
2-Methyloctanal	270	R
2,6-Dimethyloctanal	273	R
2-Methylundecanal	275	R
Isopropyl formate	304	R
Isopropyl propionate	306	R
Isopropyl hexanoate	308	R
<i>cis</i> -5-Octen-1-ol	322	R
<i>cis</i> -5-Octenal	323	R
<i>cis</i> -6-Nonenal	325	R
4-Decenal	326	R
9-Decenoic acid	328	R
10-Undecenal	330	R
Methyl 3-hexenoate	334	R
Butyl 10-undecenoate	344	R
2-Methyl-3-pentenoic acid	347	R
2,6-Dimethyl-6-hepten-1-ol	348	R
Ethyl 2-methyl-3-pentenoate	350	R
Hexyl 2-methyl-3(4)-pentenoate (mixture)	352	R
Terpinyl formate	367	R
Terpinyl butyrate	370	R
Terpinyl isovalerate	372	R
<i>p</i> -Menth-8-en-1-ol	374	R
α -Ionone	388	R
γ -Ionone	390	R, T
Allyl α -ionone	401	R
α -iso-Methylionone	404	R
5-Hydroxy-4-octanone	416	R
2-Hydroxy-2-cyclohexen-1-one	424	R
(+)-Neo-menthol	428	R
<i>p</i> -Menth-1-en-3-ol	434	R
2-Ethyl-1,3,3-trimethyl-2-norbornanol	440	R
Methyl 1-acetoxycyclohexyl ketone	442	R
1-Ethylhexyl tiglate	448	R
(1-Buten-1-yl) methyl sulfide	457	R
3-(Methylthio)propanol	461	R
3-(Methylthio)propyl acetate	478	R
Allyl thiopropionate	490	R, T

Flavouring agent	No.	Specifications ^a
2-Propanethiol	510	R
2-Naphthalenethiol	531	R
Trithioacetone	543	R
2,5-Dimethyl-2,5-dihydroxy-1,4-dithiane	562	R
2-Methyl-2-(methylthio)propanal	580	R
Ethyl 2-(methylthio)propionate	581	R
Methyl 2-oxo-3-methylpentanoate	591	R
Geranyl acetoacetate	599	R
3-(Hydroxymethyl)-2-heptanone	604	R, T
1,4-Nonanediol diacetate	609	R, T
Aconitic acid	627	R, T
3-Phenylpropyl hexanoate	642	R, T
3-Phenylpropionaldehyde	645	R
Cinnamaldehyde ethylene glycol acetal	648	R
Cinnamyl butyrate	652	R
Cinnamaldehyde	656	R
Propyl cinnamate	660	R
Butyl cinnamate	663	R
Heptyl cinnamate	666	R
Phenethyl cinnamate	671	R
3-Phenylpropyl cinnamate	672	R
Cinnamyl cinnamate	673	R
α -Amylcinnamyl formate	676	R
α -Amylcinnamyl acetate	677	R
α -Amylcinnamyl isovalerate	678	R, T
α -Amylcinnamaldehyde dimethyl acetal	681	R
<i>o</i> -Tolyl acetate	698	R
<i>p</i> -Vinylphenol	711	R
Guaiacyl phenylacetate	719	R
Hydroquinone monoethyl ether	720	R
4-Ethyl-2,6-dimethoxyphenol	723	R
4-Propyl-2,6-dimethoxyphenol	724	R
4-Allyl-2,6-dimethoxyphenol	726	R
Dihydroxyacetophenone	729	R, T
Vanillylidene acetone	732	R
Furfuryl propionate	740	R
Furfuryl pentanoate	741	R
Furfuryl octanoate	742	R
Furfuryl 3-methylbutanoate	743	R
Amyl 2-furoate	748	R
Hexyl 2-furoate	749	R
Octyl 2-furoate	750	R
2-Phenyl-3-carboethoxyfuran	752	R, T
Furfuryl butyrate	759	R
Cinnamyl benzoate	760	R

^a R, existing specifications revised; T, the existing, new or revised specifications are tentative and new information is required.

Contaminants

Contaminant	Tolerable intake and other toxicological recommendations
3-Chloro-1,2-propanediol	Provisional maximum tolerable daily intake: 2 µg/kg of body weight
1,3-Dichloro-2-propanol	Establishment of a tolerable intake was considered to be inappropriate because of the nature of the toxicity observed (tumorigenic in various organs in rats and interacts with chromosomes and/or DNA); the Committee noted that the dose that caused tumours in rats (19 mg/kg of body weight per day) was about 20 000 times the highest estimated intake of 1,3-dichloro-2-propanol by consumers of soya sauce (1 µg/kg of body weight per day).
Polychlorinated dibenzodioxins, polychlorinated dibenzofurans and coplanar polychlorinated biphenyls	Provisional tolerable monthly intake: 70 pg/kg of body weight

Annex 3

Further information required or desired

Toxicological information

Diacetyltartaric and fatty acid esters of glycerol

The following information relating to the 2-year study on toxicity in rats is required for evaluation in 2003:

1. In order to determine whether some of the adverse effects that were observed were treatment-related, the groups treated with diacetyltartaric and fatty acid esters of glycerol should be compared with both untreated and monoglyceride-treated controls, and the control groups should be compared with one another.
2. Additional information on the incidence of myocardial fibrosis and adrenal medullary hyperplasia in animals at the lowest and intermediate doses should be provided.

Curcumin

The results of a study on reproductive toxicity with a substance complying with the specifications for curcumin, known to be in progress, is required for evaluation in 2003.

Information on specifications

β -Carotene from *Blakeslea trispora*

Information is required on the method of analysis for residual solvents (ethyl acetate and isobutyl acetate). This information is required for evaluation in 2003.

Monomagnesium phosphate and trisodium diphosphate

Information is required on the loss on drying, loss on ignition, test method for loss on ignition and assay method for the hydrates. This information is required for evaluation in 2003.

Natamycin

Information is required on the level and determination of water content, limit for lead, specific rotation, assay value and method of assay for the commercial product. Comments on other aspects of the monograph are invited. This information is required for evaluation in 2003.

Quillaia extracts

The existing specifications for quillaia extracts were revised in order to clarify the differences between unpurified and semi-purified extracts. As additional information on composition (minimum and

maximum percentages of saponins in unpurified and semi-purified extracts) is necessary, the specifications were designated as tentative. Once the requested information has been received, the Committee will consider whether separate specifications for unpurified and semi-purified extracts are required. This information is required for evaluation in 2003.

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

Evaluation of certain mycotoxins in food.

Fifty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives.

WHO Technical Report Series, No. 906, 2002 (70 pages)

Safety evaluation of certain mycotoxins in food.

WHO Food Additives Series, No. 47, 2001 (707 pages)

Evaluation of certain food additives and contaminants.

Fifty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives.

WHO Technical Report Series, No. 901, 2001 (117 pages)

Safety evaluation of certain food additives and contaminants.

WHO Food Additives Series, No. 46, 2001 (392 pages)

Evaluation of certain food additives and contaminants.

Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives.

WHO Technical Report Series, No. 896, 2000 (136 pages)

Safety evaluation of certain food additives and contaminants.

WHO Food Additives Series, No. 44, 2000 (539 pages)

Evaluation of certain food additives.

Fifty-first report of the Joint FAO/WHO Expert Committee on Food Additives.

WHO Technical Report Series, No. 891, 2000 (176 pages)

Safety evaluation of certain food additives.

WHO Food Additives Series, No. 42, 1999 (494 pages)

Evaluation of certain food additives and contaminants.

Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives.

WHO Technical Report Series, No. 884, 1999 (104 pages)

This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives and contaminants, with a view to recommending Acceptable Daily Intakes (ADIs) and tolerable intakes, respectively, and to prepare specifications for the identity and purity of food additives.

The first part of the report contains a general discussion of the principles governing the toxicological evaluation of food additives (including flavouring agents) and contaminants, assessments of intake, and the establishment and revision of specifications for food additives. A summary follows of the Committee's evaluations of toxicological and intake data on various specific food additives (diacetyltartaric and fatty acid esters of glycerol, quillaia extracts, invertase from *Saccharomyces cerevisiae*, β -carotene from *Blakeslea trispora*, curcumin, phosphates, diphosphates and polyphosphates, hydrogenated poly-1-decene, natamycin, D-tagatose, carrageenan, processed *Eucheuma* seaweed, curdlan, acetylated oxidized starch, α -cyclodextrin and sodium sulfate), flavouring agents and contaminants (3-chloro-1,2-propanediol, 1,3-dichloro-2-propanol, and a large number of polychlorinated dibenzodioxins, polychlorinated dibenzofurans and coplanar polychlorinated biphenyls). Annexed to the report are tables summarizing the Committee's recommendations for ADIs of the food additives and tolerable intakes of the contaminants considered, changes in the status of specifications of these food additives and specific flavouring agents, and further information required or desired.

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SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006

Version 6.7

Revision Date 17.03.2023

Print Date 08.05.2023

GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name : Benzyl alcohol

Product Number : PHR1019

Brand : Sigma-Aldrich

Index-No. : 603-057-00-5

REACH No. : 01-2119492630-38-XXXX

CAS-No. : 100-51-6

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich Chemie GmbH
Industriestrasse 25
CH-9471 BUCHS

Telephone : +41 81 755 2511

Fax : +41 81 756 5449

E-mail address : technischerservice@merckgroup.com

1.4 Emergency telephone

Emergency Phone # : +41 43-508-2011 (CHEMTREC)
+41 44-251-5151 (Tox-Zentrum)
145(Tox Info Suisse)

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008

Acute toxicity, Oral (Category 4), H302
Acute toxicity, Inhalation (Category 4), H332
Eye irritation (Category 2), H319

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008

Pictogram



Signal Word

Warning



Hazard statement(s)	
H302 + H332	Harmful if swallowed or if inhaled.
H319	Causes serious eye irritation.
Precautionary statement(s)	
P261	Avoid breathing mist or vapors.
P264	Wash skin thoroughly after handling.
P271	Use only outdoors or in a well-ventilated area.
P301 + P312	IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell.
P304 + P340 + P312	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor if you feel unwell.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Supplemental Hazard Statements	none

Reduced Labeling (<= 125 ml)

Pictogram



Signal Word	Warning
Hazard statement(s)	none
Precautionary statement(s)	none
Supplemental Hazard Statements	none

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Formula	: C ₇ H ₈ O
Molecular weight	: 108,14 g/mol
CAS-No.	: 100-51-6
EC-No.	: 202-859-9
Index-No.	: 603-057-00-5

Component		Classification	Concentration
Benzyl alcohol			
CAS-No.	100-51-6	Acute Tox. 4; Eye Irrit. 2; H302, H332, H319	<= 100 %
EC-No.	202-859-9		
Index-No.	603-057-00-5		

For the full text of the H-Statements mentioned in this Section, see Section 16.



SECTION 4: First aid measures

4.1 Description of first-aid measures

General advice

Show this material safety data sheet to the doctor in attendance.

If inhaled

After inhalation: fresh air. If breathing stops: mouth-to-mouth breathing or artificial respiration. Oxygen if necessary. Immediately call in physician.

In case of skin contact

In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower.

In case of eye contact

After eye contact: rinse out with plenty of water. Call in ophthalmologist. Remove contact lenses.

If swallowed

After swallowing: immediately make victim drink water (two glasses at most). Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed

The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed

No data available

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media

Water Foam Carbon dioxide (CO₂) Dry powder

Unsuitable extinguishing media

For this substance/mixture no limitations of extinguishing agents are given.

5.2 Special hazards arising from the substance or mixture

Carbon oxides

Combustible.

Vapors are heavier than air and may spread along floors.

Forms explosive mixtures with air on intense heating.

Development of hazardous combustion gases or vapours possible in the event of fire.

5.3 Advice for firefighters

In the event of fire, wear self-contained breathing apparatus.

5.4 Further information

Prevent fire extinguishing water from contaminating surface water or the ground water system.



SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

Advice for non-emergency personnel: Do not breathe vapors, aerosols. Avoid substance contact. Ensure adequate ventilation. Evacuate the danger area, observe emergency procedures, consult an expert.
For personal protection see section 8.

6.2 Environmental precautions

Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up

Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up with liquid-absorbent material (e.g. Chemizorb®). Dispose of properly. Clean up affected area.

6.4 Reference to other sections

For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

Advice on safe handling

Work under hood. Do not inhale substance/mixture. Avoid generation of vapours/aerosols.

Hygiene measures

Change contaminated clothing. Preventive skin protection recommended. Wash hands after working with substance.

For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions

Tightly closed.

Store at Room Temperature. Light sensitive. Hygroscopic. Store under inert gas. Air sensitive.

Storage class

Storage class (TRGS 510): 10: Combustible liquids

7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

Ingredients with workplace control parameters

Derived No Effect Level (DNEL)

Application Area	Routes of exposure	Health effect	Value
Worker DNEL, acute	inhalation	Systemic effects	450 mg/m ³
Worker DNEL, acute	dermal	Systemic effects	
Worker DNEL, longterm	inhalation	Systemic effects	90 mg/m ³



Worker DNEL, longterm	dermal	Systemic effects	
Consumer DNEL, acute	oral	Systemic effects	
Consumer DNEL, acute	inhalation	Systemic effects	95,5 mg/m3
Consumer DNEL, acute	dermal	Systemic effects	
Consumer DNEL, longterm	oral	Systemic effects	
Consumer DNEL, longterm	inhalation	Systemic effects	19,1 mg/m3
Consumer DNEL, longterm	dermal	Systemic effects	

Predicted No Effect Concentration (PNEC)

Compartment	Value
Fresh water	1 mg/l
Fresh water sediment	5,27 mg/kg
Sea water	0,1 mg/l
Sea sediment	0,527 mg/kg
Soil	0,456 mg/kg
Sewage treatment plant	39 mg/l
Aquatic intermittent release	2,3 mg/l

8.2 Exposure controls

Personal protective equipment

Eye/face protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses

Skin protection

This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN374 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).

Full contact

Material: butyl-rubber

Minimum layer thickness: 0,7 mm

Break through time: 480 min

Material tested: Butoject® (KCL 898)

This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN374 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).

Splash contact

Material: Viton®

Minimum layer thickness: 0,7 mm

Break through time: 120 min



Material tested: Vitoject® (KCL 890 / Aldrich Z677698, Size M)

Body Protection

protective clothing

Respiratory protection

Recommended Filter type: Filter A (acc. to DIN 3181) for vapours of organic compounds

The entrepreneur has to ensure that maintenance, cleaning and testing of respiratory protective devices are carried out according to the instructions of the producer. These measures have to be properly documented.

Control of environmental exposure

Do not let product enter drains.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a) Physical state	liquid
b) Color	No data available
c) Odor	No data available
d) Melting point/freezing point	No data available
e) Initial boiling point and boiling range	205 °C at 1.013 hPa
f) Flammability (solid, gas)	No data available
g) Upper/lower flammability or explosive limits	Upper explosion limit: 13 %(V) Lower explosion limit: 1,3 %(V)
h) Flash point	101 °C - DIN 51758
i) Autoignition temperature	No data available
j) Decomposition temperature	No data available
k) pH	No data available
l) Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: No data available
m) Water solubility	No data available
n) Partition coefficient: n-octanol/water	log Pow: 1,05 at 20 °C - Bioaccumulation is not expected.
o) Vapor pressure	No data available
p) Density	1,05 g/cm ³ at 20 °C
Relative density	No data available
q) Relative vapor density	No data available



r) Particle characteristics No data available

s) Explosive properties No data available

t) Oxidizing properties none

9.2 Other safety information

Dissociation constant 15,4 at 25 °C

SECTION 10: Stability and reactivity

10.1 Reactivity

Forms explosive mixtures with air on intense heating.
A range from approx. 15 Kelvin below the flash point is to be rated as critical.

10.2 Chemical stability

The product is chemically stable under standard ambient conditions (room temperature) .
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions

No data available

10.4 Conditions to avoid

Strong heating.

10.5 Incompatible materials

various plastics

10.6 Hazardous decomposition products

In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity

LD50 Oral - Rat - male - 1.620 mg/kg

Remarks: (ECHA)

LC50 Inhalation - Rat - male and female - 4 h - > 4,178 mg/l - aerosol

(OECD Test Guideline 403)

Remarks: (Regulation (EC) No 1272/2008, Annex VI)

Dermal: No data available

Skin corrosion/irritation

Skin - Rabbit

Result: No skin irritation - 4 h

(OECD Test Guideline 404)

Serious eye damage/eye irritation

Eyes - Rabbit

Result: irritating



(OECD Test Guideline 405)

Respiratory or skin sensitization

Maximization Test

Result: negative

(OECD Test Guideline 406)

Germ cell mutagenicity

No data available

Carcinogenicity

No data available

Reproductive toxicity

No data available

Specific target organ toxicity - single exposure

No data available

Specific target organ toxicity - repeated exposure

No data available

Aspiration hazard

No data available

11.2 Additional Information

Endocrine disrupting properties

Product:

Assessment

The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

RTECS: DN3150000

Central nervous system depression

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Liver - Irregularities - Based on Human Evidence

SECTION 12: Ecological information

12.1 Toxicity

Toxicity to fish	static test LC50 - Pimephales promelas (fathead minnow) - 460 mg/l - 96 h (US-EPA)
Toxicity to daphnia and other aquatic invertebrates	Immobilization EC50 - Daphnia magna (Water flea) - 230 mg/l - 48 h (OECD Test Guideline 202)
Toxicity to algae	static test ErC50 - Pseudokirchneriella subcapitata (green algae) - 700 mg/l - 72 h (OECD Test Guideline 201)
Toxicity to daphnia	semi-static test NOEC - Daphnia magna (Water flea) - 51 mg/l - 21

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The life science business of Merck operates as MilliporeSigma in the US and Canada



and other aquatic d
invertebrates(Chronic (OECD Test Guideline 211)
toxicity)

12.2 Persistence and degradability

Biodegradability aerobic - Exposure time 14 d
Result: 92 - 96 % - Readily biodegradable.
(OECD Test Guideline 301C)
aerobic - Exposure time 21 d
Result: 95 - 97 % - Readily biodegradable.
(OECD Test Guideline 301A)

Biochemical Oxygen Demand (BOD) 1.550 mg/g
Remarks: (Lit.)

Theoretical oxygen demand 2.515 mg/g
Remarks: (IUCLID)

Ratio BOD/ThBOD 62 %
Remarks: (Lit.)

12.3 Bioaccumulative potential

No data available

12.4 Mobility in soil

No data available

12.5 Results of PBT and vPvB assessment

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

12.6 Endocrine disrupting properties

Product:

Assessment : The substance/mixture does not contain components considered to have endocrine disrupting properties according to REACH Article 57(f) or Commission Delegated regulation (EU) 2017/2100 or Commission Regulation (EU) 2018/605 at levels of 0.1% or higher.

12.7 Other adverse effects

Additional ecological information No data available

SECTION 13: Disposal considerations

13.1 Waste treatment methods

Product

See www.retrologistik.com for processes regarding the return of chemicals and containers, or contact us there if you have further questions.

SECTION 14: Transport information

14.1 UN number

ADR/RID: -

IMDG: -

IATA: 3334



14.2 UN proper shipping name

ADR/RID: Not dangerous goods

IMDG: Not dangerous goods

IATA: Aviation regulated liquid, n.o.s. (Benzyl alcohol)

14.3 Transport hazard class(es)

ADR/RID: -

IMDG: -

IATA: 9

14.4 Packaging group

ADR/RID: -

IMDG: -

IATA: III

14.5 Environmental hazards

ADR/RID: no

IMDG Marine pollutant: no

IATA: no

14.6 Special precautions for user

No data available

Further information : No data available

SECTION 15: Regulatory information**15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture**

This material safety data sheet complies with the requirements of Regulation (EC) No. 1907/2006.

Other regulations

Observe work restrictions regarding maternity protection in accordance to Dir 92/85/EEC or stricter national regulations where applicable.

Take note of Dir 94/33/EC on the protection of young people at work.

15.2 Chemical Safety Assessment

A Chemical Safety Assessment has been carried out for this substance.

SECTION 16: Other information**Full text of H-Statements referred to under sections 2 and 3.**

H302	Harmful if swallowed.
H302 + H332	Harmful if swallowed or if inhaled.
H319	Harmful if swallowed or if inhaled.
H332	Causes serious eye irritation.



Full text of other abbreviations

ADN - European Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways; ADR - Agreement concerning the International Carriage of Dangerous Goods by Road; AIIC - Australian Inventory of Industrial Chemicals; ASTM - American Society for the Testing of Materials; bw - Body weight; CMR - Carcinogen, Mutagen or Reproductive Toxicant; DIN - Standard of the German Institute for Standardisation; DSL - Domestic Substances List (Canada); ECx - Concentration associated with x% response; ELx - Loading rate associated with x% response; EmS - Emergency Schedule; ENCS - Existing and New Chemical Substances (Japan); ErCx - Concentration associated with x% growth rate response; GHS - Globally Harmonized System; GLP - Good Laboratory Practice; IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; IBC - International Code for the Construction and Equipment of Ships carrying Dangerous Chemicals in Bulk; IC50 - Half maximal inhibitory concentration; ICAO - International Civil Aviation Organization; IECSC - Inventory of Existing Chemical Substances in China; IMDG - International Maritime Dangerous Goods; IMO - International Maritime Organization; ISHL - Industrial Safety and Health Law (Japan); ISO - International Organisation for Standardization; KECI - Korea Existing Chemicals Inventory; LC50 - Lethal Concentration to 50 % of a test population; LD50 - Lethal Dose to 50% of a test population (Median Lethal Dose); MARPOL - International Convention for the Prevention of Pollution from Ships; n.o.s. - Not Otherwise Specified; NO(A)EC - No Observed (Adverse) Effect Concentration; NO(A)EL - No Observed (Adverse) Effect Level; NOELR - No Observable Effect Loading Rate; NZIoC - New Zealand Inventory of Chemicals; OECD - Organization for Economic Co-operation and Development; OPPTS - Office of Chemical Safety and Pollution Prevention; PBT - Persistent, Bioaccumulative and Toxic substance; PICCS - Philippines Inventory of Chemicals and Chemical Substances; (Q)SAR - (Quantitative) Structure Activity Relationship; REACH - Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals; RID - Regulations concerning the International Carriage of Dangerous Goods by Rail; SADT - Self-Accelerating Decomposition Temperature; SDS - Safety Data Sheet; TCSI - Taiwan Chemical Substance Inventory; TECI - Thailand Existing Chemicals Inventory; TSCA - Toxic Substances Control Act (United States); UN - United Nations; UNRTDG - United Nations Recommendations on the Transport of Dangerous Goods; vPvB - Very Persistent and Very Bioaccumulative

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

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