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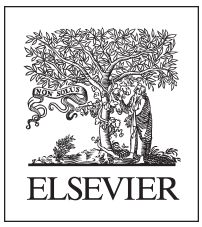

Food and Chemical Toxicology

Toxicologic and Dermatologic Assessments for Three Groups of Fragrance Ingredients:

1) Related Esters and Alcohols of Cinnamic Acid and Cinnamic Alcohol
2) Ionones
3) Salicylates

FOOD AND CHEMICAL TOXICOLOGY

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Reports on the following studies are encouraged: food, water, functional foods, neutraceuticals, herbal extracts, phytochemicals, food supplements, cosmetics and other consumer products, cosmeceuticals, pharmaceuticals, and industrial and agricultural chemicals and interactions among these. Methodologies employed may be traditional or new and novel. For example, in the food area reports related to the safety evaluation of novel foods, biotechnologically derived products and the effects of novel ways of preparing or conserving foods will be considered.

In addition to original research papers, Brief Communications will also be considered, as will concise relevant critical Reviews of toxicological topics of contemporary significance and papers that discuss theoretical models relating safety, transitional issues and science. On occasion, the Editors may request editorial comment for controversial issues. Letters to the Editor will be limited to comments on contributions already published in the journal; if a letter is accepted, a response (for simultaneous publication) will be invited from the authors of the original contribution.

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FOOD AND CHEMICAL TOXICOLOGY

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Review

A toxicologic and dermatologic assessment of related esters and alcohols of cinnamic acid and cinnamyl alcohol when used as fragrance ingredients ☆

The RIFM Expert Panel
D. Belsito [a], D. Bickers [b], M. Bruze [c], P. Calow [d], H. Greim [e], J.M. Hanifin [f], A.E. Rogers [g], J.H. Saurat [h], I.G. Sipes [i], H. Tagami [j]

[a] *University of Missouri (Kansas City), Division of Dermatology, 6333 Long Avenue, Shawnee, KS 66216, USA*
[b] *College of Physicians and Surgeons of Columbia University, Department of Dermatology, 161 Fort Washington Avenue, New York, NY 10032, USA*
[c] *Lund University, Malmo University Hospital, Department of Occupational and Environmental Dermatology, Sodra Forstadsgatan 101 Malmo SE-20502, Sweden*
[d] *Department of Environmental, Social and Spatial Change, Roskilde University, DK 4000, Denmark*
[e] *Institute of Toxicology and Environmental Hygiene, Technical University of Munich, Hohenbachernstrasse 15-17, D-85354 Freising, Germany*
[f] *Oregon Health Sciences University, Department of Dermatology L468, 3181 SW Sam Jackson Park Road, Portland, Oregon 97201-3098, USA*
[g] *Boston University School of Medicine, Department of Pathology and Laboratory Medicine, 715 Albany Street, Boston, MA 02118-2394, USA*
[h] *University Hospital Geneva, Department of Dermatology, CH-1211 Geneva 14, Switzerland*
[i] *University of Arizona, Health Sciences Center, College of Pharmacy, 1703 East Mable Street, Tucson, AZ 85721, USA*
[j] *Tohoku University School of Medicine, 1-1 Seiryo-machi, Aoba-ku Sendai 980, Japan*

Abstract

An evaluation and review of a structurally related group of fragrance materials.

Keywords: Safety; Review; Fragrance; Esters; Alcohols; Cinnamic

Contents

☆ All correspondence should be addressed to: A.M. Api, 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: amapi@rifm.org

0278-6915/$ - see front matter
doi:10.1016/j.fct.2007.09.087

1. Introduction

This report summarizes scientific data relevant to the risk assessment of related esters and alcohols of cinnamic acid and cinnamyl alcohol (see Table 1). These substances are all used as fragrance ingredients. This report uses data from animals and humans by various routes of exposure, but emphasizes the risk assessment for the use of related esters and alcohols of cinnamic acid and cinnamyl alcohol as fragrance ingredients. The scientific evaluation focuses on dermal exposure, which is considered to be the primary route for fragrance materials. Where relevant, toxicity, metabolism and biological fate data from other exposures have been considered. This assessment, therefore, addresses the use of the material as a fragrance ingredient.

The current format includes a group summary evaluation paper and individual Fragrance Materials Reviews on discrete chemicals. The group summary is an evaluation of relevant data selected from the large bibliography of studies and reports on the individual chemicals. The selected data were deemed to be relevant based on protocols that conform with current guidelines, quality of the data, statistical significance and appropriate exposure. These are identified in tabular form in the group summary. The Fragrance Material Reviews (available online at www.rifm.org) contain a comprehensive summary of published and non-published reports including complete bibliographies.

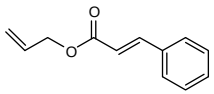
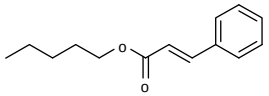
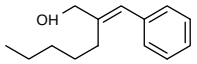
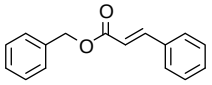
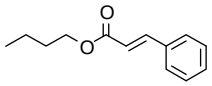
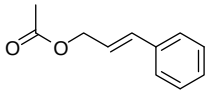
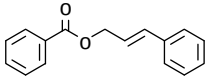
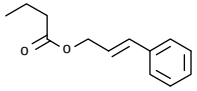
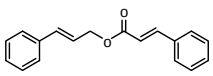
2. Chemical identity and exposure (Table 1)

In the United States, the regulatory status of these materials includes approval of 21 substances (21 CFR 172.515) by the Food and Drug Administration (FDA) and 20 materials by the Flavor and Extract Manufacturers' Association (FEMA, 1965) as Generally Recognized as Safe (GRAS) as flavor ingredients [Numbers 2022, 2063, 2064, 2065, 2142, 2192, 2193, 2293, 2296, 2297, 2298, 2299, 2300, 2301, 2302, 2430, 2641, 2698, 2863, 2939]. Twenty one of these materials were also included in the Council of Europe's list of substances [Numbers 79, 208, 216, 235, 279, 323, 325, 326, 327, 328, 329, 331, 332, 333, 335, 336, 352, 414, 454, 496, 743] which may be used in foodstuffs (Council of Europe, 2000). Finally, the International Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2000) has evaluated 19 of these materials and found them to have no safety concerns based on current levels of intake as food flavors.

Seven of the 23 substances have been reported as common components of food occurring mainly in a wide variety of fruits, vegetables, herbs and spices in varying concentrations. For example, concentrations of 2800–51,000 ppm cinnamyl acetate in cinnamon (*Cinnamomum zeylanicum* Blume and other *Cinnamomum* species), and trace-278,000 ppm methyl cinnamate in basil (*Ocimum basilicum* varieties) have been reported (TNO, 2006). Quantitative natural occurrence data have been reported for methyl cinnamate and ethyl cinnamate, and indicate that intake of these substances is predominately from food (i.e., consumption ratio >1) (Stofberg and Grundschober, 1987).

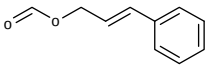
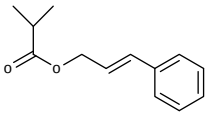
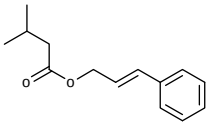
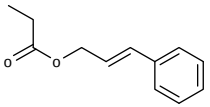
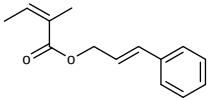
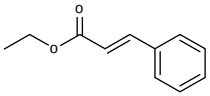
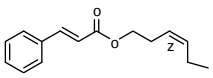
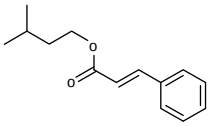
Data from a survey conducted in the year 2004 indicate that the annual worldwide use of benzyl cinnamate, cinnamyl acetate and methyl cinnamate is between 10 and 100 metric tons (see Table 1) and the annual worldwide

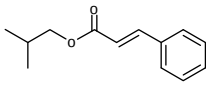
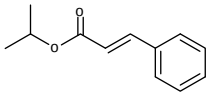
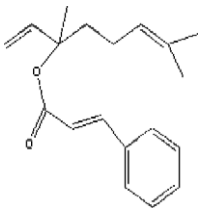
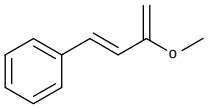
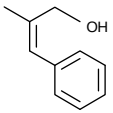
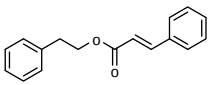
Table 1
Material identification and summary of volume of use and dermal exposure

Material	Synonyms	Structure	Annual worldwide (metric tons)[a]	Dermal systemic exposure in cosmetic products (mg/kg/day)	Maximum skin Level[b]
Allyl cinnamate CAS# 1866-31-5 Molecular weight: 188.23 Log K_{ow} (calculated): 3.2	• Allyl β-phenylacrylate • Allyl 3-phenyl-2-propenoate • 2-Propenoic acid, 3-phenyl-, 2-propenyl ester • Propenyl cinnamate • 2-Propen-1-yl 3-phenyl-2-propenoate • Vinyl carbinyl cinnamate		<0.1	0.0127	0.10%
Amyl cinnamate CAS# 3487-99-8 Molecular weight: 218.3 Log K_{ow} (calculated): 4.32	• Pentyl cinnamate • Pentyl 3-phenyl-2-propenoate • 2-Propenoic acid, 3-phenyl-, pentyl ester		<0.1	0.0127	0.10%
***alpha*-Amylcinnamyl alcohol** CAS# 101-85-9 Molecular weight: 204.31 Log K_{ow} (calculated): 4.35	• α-Amylcinnamic alcohol • 2-Amyl-3-phenyl-2-propen-1-ol • 2-Benzylideneheptanol • 1-Heptanol, 2-(phenylmethylene)- • α-Pentylcinnamyl alcohol		0.1–1	0.0038	0.04%
Benzyl cinnamate CAS# 103-41-3 Molecular weight: 238.29 Log K_{ow} (calculated): 4.06	• Benzyl β-phenylacrylate • Benzyl 3-phenylpropenoate • Cinnamein • 2-Propenoic acid, 3-phenyl-, phenylmethyl ester		10–100	0.0022	0.89%
Butyl cinnamate CAS# 538-65-8 Molecular weight: 204.27 Log K_{ow} (calculated): 3.83	• *n*-Butyl cinnamate • Butyl β-phenylacrylate • *n*-Butyl phenylacrylate • Butyl 3-phenylpropenoate • 2-Propenoic acid, 3-phenyl-, butyl ester		<0.1	0.0127	0.10%
Cinnamyl acetate CAS# 103-54-8 Molecular weight: 176.22 Log K_{ow} (calculated): 2.85	• 3-Phenylallyl acetate • 3-Phenyl-2-propen-1-yl acetate • 2-Propen-1-ol, 3-phenyl-, acetate		10–100	0.0115	0.62%
Cinnamyl benzoate CAS# 5320-75-2 Molecular weight: 238.29 Log K_{ow} (calculated): 4.3	• 3-Phenyl-2-propen-1-yl benzoate • 2-Propen-1-ol, 3-phenyl-, benzoate		<0.1	0.0127	0.10%
Cinnamyl butyrate CAS# 103-61-7 Molecular weight: 204.27 Log K_{ow} (calculated): 3.83	• Butanoic acid, 3-phenyl-2-propenyl ester • 3-Phenylallyl butyrate • 3-Phenyl-2-propen-1-yl butanoate		<0.1	0.0025	0.02%
Cinnamyl cinnamate CAS# 122-69-0 Molecular weight: 264.33 Log K_{ow} (calculated): 4.83	• Phenylallyl cinnamate • 3-Phenylallyl cinnamate • 3-Phenyl-2-propen-1-yl 3-phenylpropenoate • 2-Propenoic acid, 3-phenyl-, 3-phenyl-2-propenyl-ester		0.1–1	0.0061	0.24%

(*continued on next page*)

Table 1 (*continued*)

Material	Synonyms	Structure	Annual worldwide (metric tons)[a]	Dermal systemic exposure in cosmetic products (mg/kg/day)	Maximum skin Level[b]
Cinnamyl formate CAS# 104-65-4 Molecular weight: 162.19 Log K_{ow} (calculated): 2.3	• 3-Phenylallyl formate • 3-Phenyl-2-propen-1-yl formate • 2-Propen-1-ol, 3-phenyl-, formate		0.1–1	0.0010	0.01%
Cinnamyl isobutyrate CAS# 103-59-3 Molecular weight: 204.27 Log K_{ow} (calculated): 3.76	• Cinnamyl 2-methylpropanoate • 3-Phenyl-2-propen-1-yl isobutyrate • 3-Phenyl-2-propen-1-yl 2-methylpropanoate • Propanoic acid, 2-methyl-, 3-phenyl-2-propenyl ester		0.1–1	0.0005	0.02%
Cinnamyl isovalerate CAS# 140-27-2 Molecular weight: 218.39 Log K_{ow} (calculated): 4.25	• Butanoic acid, 3-methyl-, 3-phenyl-2-propenyl ester • Cinnamyl 3-methylbutanoate • 3-Phenylallyl isovalerate • 3-Phenylallyl 3-methylbutanoate • 3-Phenyl-2-propen-1-yl 3-methylbutanoate		<0.1	0.0008	0.002%
Cinnamyl propionate CAS# 103-56-0 Molecular weight: 190.24 Log K_{ow} (calculated): 3.34	• 3-Phenylallyl propionate • 3-Phenyl-2-propenyl propanoate • 3-Phenyl-2-propen-1-yl propionate • 2-Propen-1-ol, 3-phenyl-, propanoate		0.1–1	0.0023	0.02%
Cinnamyl tiglate CAS# 61792-12-9 Molecular weight: 216.28 Log K_{ow} (calculated): 4.16	• 2-Butenoic acid, 2-methyl-, 3-phenyl-2-propenyl-ester, (2E)- • Cinnamyl *trans*-2-methyl-2-butenoate • Cinnamyl 2-methylcrotonate • Cinnamyl α-methylcrotonate		<0.1	0.0003	0.002%
Ethyl cinnamate CAS# 103-36-6 Molecular weight: 176.22 Log K_{ow} (calculated): 2.85	• Ethyl phenylacrylate • Ethyl 3-phenylpropenoate • 2-Propenoic acid, 3-phenyl-, ethyl ester		1–10	0.0003	0.13%
***cis*-3-Hexenyl cinnamate** CAS# 68133-75-5 Molecular weight: 230.07 Log K_{ow} (calculated): 4.6	• (Z)-3-Hexenyl cinnamate • 2-Propenoic acid, 3-phenyl-, (3Z)-3-hexenyl ester • 2-Propenoic acid, 3-phenyl-, 3-hexenyl ester, (?,Z)-		<0.1	0.0178	0.08%
Isoamyl cinnamate CAS# 7779-65-9 Molecular weight: 218.3 Log K_{ow} (calculated): 4.25	• Amyl(iso) cinnamate • Isoamyl β-phenylacrylate • Isopentyl cinnamate • Isopentyl β-phenylacrylate • Isopentyl 3-phenylpropenoate • 2-Propenoic acid, 3-phenyl-, 3-methylbutyl ester		0.1–1	0.0029	0.05%

Isobutyl cinnamate CAS# 122-67-8 Molecular weight: 204.27 Log K_{ow} (calculated): 3.76	• Isobutyl β-phenylacrylate • Isobutyl 3-phenylpropenoate • Labdanol • 2-Methylpropyl cinnamate • 2-Methylpropyl β-phenylacrylate • 2-Methylpropyl 3-phenylpropenoate • 2-Propenoic acid, 3-phenyl-, 2-methylpropyl ester		0.1–1	0.0127	0.10%
Isopropyl cinnamate CAS# 7780-06-5 Molecular weight: 190.24 Log K_{ow} (calculated): 3.27	• Isopropyl 3-phenylpropenoate • 1-Methylethyl 3-phenylpropenoate • 2-Propenoic acid, 3-phenyl-, 1-methylethyl ester		0.1–1	0.0008	0.01%
Linalyl cinnamate CAS# 78-37-5 Molecular weight: 284.4 Log K_{ow} (calculated): 6.37	• Cinnamic acid, linalyl ester • 3,7-Dimethyl-1,6-octadien-3-yl cinnamate • 3,7-Dimethyl-1,6-octadien-3-yl β-phenylacrylate • 3,7-Dimethyl-1,6-octadien-3-yl 3-phenylpropenoate • Linalyl 3-phenylpropenoate • 2-Propenoic acid, 3-phenyl-, 1-ethenyl-1,5-dimethyl-4-hexenyl ester		0.1–1	0.0268	0.42%
Methyl cinnamate CAS# 103-26-4 Molecular weight: 162.19 Log K_{ow} (calculated): 2.36	• Methyl 3-phenylpropenoate • 2-Propenoic acid, 3-phenyl-, methyl ester		10–100	0.0054	0.31%
α-Methylcinnamic alcohol CAS# 1504-55-8 Molecular weight: 148.21 Log K_{ow} (calculated): 2.39	• Cinnamyl alcohol, α-methyl- • Methylcinnamic alcohol • α-Methylcinnamyl alcohol • 3-Phenyl-2-methyl-2-propen-1-ol		0.1–1	0.0051	0.01%
Phenethyl cinnamate CAS# 103-53-7 Molecular weight: 252.32 Log K_{ow} (calculated): 4.56	• Benzylcarbinyl cinnamate • β-Phenethyl β-phenylacrylate • Phenylethyl cinnamate • 2-Phenylethyl cinnamate • 2-Phenylethyl 3-phenylpropenoate • 2-Propenoic acid, 3-phenyl-, 2-phenylethyl ester		1–10	0.0196	0.22%

[a] 2004 IFRA volume of use survey.

[b] The maximum skin levels are based on the assumption that the fragrance mixture is used at 20% in a consumer product (IFRA Use Level Survey).

use of ethyl cinnamate and phenethyl cinnamate is between 1 and 10 metric tons and the annual worldwide use of the other cinnamyl materials range from <0.1 to 1 metric ton (Table 1).

The most recent total annual volume and exposure data for these compounds in fine fragrances, personal care products, and household products comes from a 2004 survey. Data from this survey indicates that the annual worldwide use of these materials ranges from a high of approximately 27 metric tons for methyl cinnamate to a low of 0.001 metric tons for cinnamyl benzoate and *cis*-3-hexenyl cinnamate with a majority of the materials being used at less than one metric ton (see Table 1).

2.1. Estimated consumer exposure

The availability of fragrance ingredients for potential exposure by consumers is estimated in two ways (see Table 1). One is for estimating potential percutaneous absorption from the entire body due to the use of many different fragranced products. The other is for estimating potential dermal exposure due to the use of products, such as fine fragrances, that usually contain higher concentrations and are used on smaller localized skin sites. Thus potential systemic exposure to linalyl cinnamate from ten types of cosmetic products (body lotion, face cream, eau de toilette, fragrance cream, anti-perspirant, shampoo, bath products, shower gel, toilet soap and hair spray) using an average 97.5 percentile concentration of 1.05% is calculated as 0.0268 mg/kg body weight/day (IFRA, 2001). The calculated exposures for the other cinnamyl materials range from 0.0003 mg/kg body weight/day for cinnamyl tiglate to 0.0196 mg/kg body weight/day for phenethyl cinnamate (IFRA, 2001) (see Table 1). For consideration of potential sensitization, the exposure is calculated as a per cent concentration used on the skin. Thus exposure to linalyl cinnamate used in fine fragrance products is reported as 0.42% based on the use of 20% of the fragrance mixture containing the fragrance material in the fine fragrance consumer product (IFRA, 2001). The comparable exposures for the other cinnamyl materials range from 0.002% for cinnamyl tiglate to 0.89% for benzyl cinnamate (IFRA, 2001) (see Table 1). Exposure data are provided by the fragrance industry. An explanation of how the data are obtained and how exposure is determined has been reported by Cadby et al. (2002) and Ford et al. (2000).

3. Biological data

3.1. Absorption, distribution and metabolism

3.1.1. Percutaneous absorption

There are no absorption studies on these cinnamyl materials. However, there are limited data on the absorption of cinnamyl alcohol, cinnamaldehyde and cinnamic acid through the skin. The data that exist suggest that there is significant absorption through the skin. A conservative estimate from in vitro studies on human skin is that 61% cinnamic acid, 52% cinnamaldehyde and 66% cinnamyl alcohol are absorbed through the skin (Bickers et al., 2005).

3.1.2. Pharmacokinetics

Cinnamyl alcohol, cinnamaldehyde and cinnamic acid have all been shown to be rapidly absorbed from the gut, metabolized and excreted primarily in the urine and, to a minor extent, in the feces. Results of studies beginning in 1909 indicate that cinnamyl derivatives are absorbed, metabolized and excreted as polar metabolites within 24 h. Recent studies in laboratory animals on the effects of dose, species, sex, and mode of administration on the absorption, metabolism and excretion of cinnamyl alcohol, cinnamaldehyde and cinnamic acid are discussed in detail in Bickers et al. (2005). After oral or intraperitoneal administration to rats and mice, 76–77%, 69–98% and 73–94% [^{14}C] of the dose of cinnamyl alcohol, cinnamaldehyde and cinnamic acid, respectively, were recovered in the urine and feces within 24 h (Bickers et al., 2005). In human subjects, plasma was cleared of cinnamic acid within 20 minutes after a single intravenous dose; 100% of a dose of cinnamaldehyde was recovered as metabolites in the urine within 8 h (Bickers et al., 2005).

In rats, 1.5 mmol/kg body weight dose of methyl cinnamate was rapidly and almost completely (95%) absorbed from the gut after oral administration. Methyl cinnamate was hydrolyzed to some extent in the stomach (approximately 9% of the administered methyl cinnamate was detected in the stomach of the rat as cinnamic acid) and approximately 40% of the administered ester was detected in the lower part of the gut as cinnamic acid. The rates of absorption for cinnamic acid and methyl cinnamate from the gut was similar. No ester was detected in the peripheral blood of dosed rabbits or rats. Only traces were detected in portal and heart blood samples taken from dosed rats, indicating that almost complete hydrolysis of methyl cinnamate occurred upon or during absorption from the gut (Fahelbum and James, 1977).

3.1.3. Metabolism

These substances are simple aromatic compounds and they participate in common routes of absorption, distribution, and metabolic detoxication, and exhibit similar toxicological endpoints. The members of this group are expected to be hydrolyzed to yield the component alcohol, aldehyde, or acid. If the product is an alcohol or aldehyde, it is oxidized to yield the corresponding 3-phenylpropenoic acid or a 3-phenylpropanoic acid derivative which undergoes further side-chain β-oxidation and cleavage to yield mainly the corresponding benzoic acid derivatives (Williams, 1959). The benzoic acid derivatives are conjugated with glycine and/or glucuronic acid and excreted primarily in the urine (Snapper et al., 1940). To a minor extent, the presence of *o*-alkyl- and *o*-alkoxy-ring substituents may lead to alternative metabolic pathways (Solheim and

Scheline, 1973; Solheim and Scheline, 1976; Samuelsen et al., 1986).

In general, esters containing an aromatic ring system are expected to be hydrolyzed in vivo. Hydrolysis is catalyzed by classes of enzymes recognized as carboxylesterases or esterases (Heymann, 1980), the most important of which are the A-esterases. In mammals, A-esterases occur in most tissues throughout the body (Anders, 1989; Heymann,

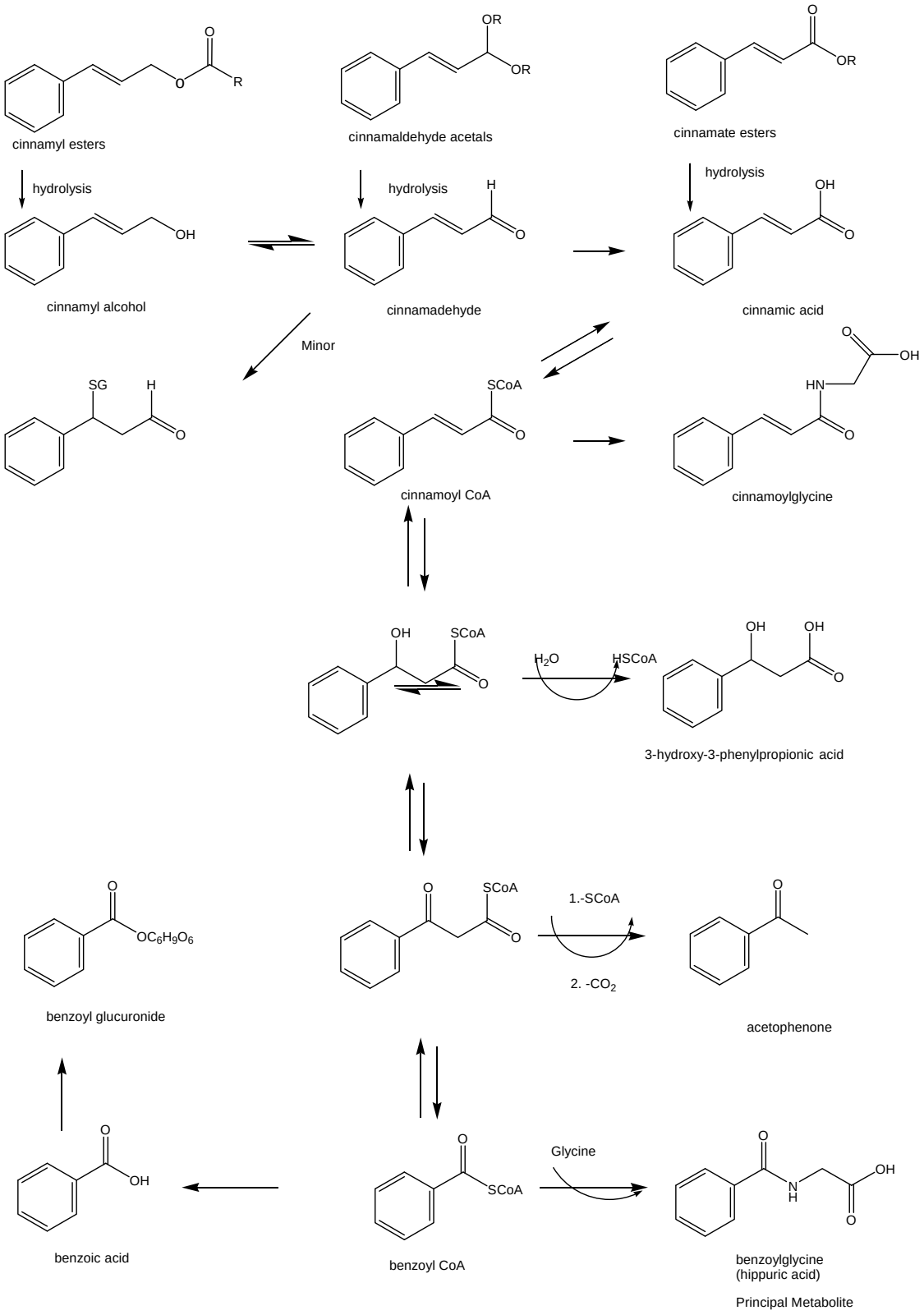


Fig. 1. Metabolism of cinnamyl derivatives.

1980) but predominate in the hepatocytes (Heymann, 1980).

Esters of cinnamic acid and structurally related aromatic esters have been shown to hydrolyze rapidly to the component acid and alcohol. Oral administration of methyl cinnamate (50 mg/kg body weight) resulted in the urinary excretion, after 24 h, of hippuric acid (66%) and benzoylglucuronide (5%). This distribution of metabolites, nearly identical to that for cinnamic acid, indicates that rapid hydrolysis of the ester in vivo precedes metabolism of the acid (Fahelbum and James, 1977). Ethyl cinnamate administered subcutaneously to a cat produced cinnamic acid metabolites that were excreted in the urine (Dakin, 1909). Eighty percent hydrolysis was measured when benzyl cinnamate was incubated with simulated intestinal fluid (pH 7.5; pancreatin) at 37° for 2 h (Grundschober, 1977).

The aromatic primary alcohols used as flavoring substances or formed by the hydrolysis of esters and acetals are readily oxidized to a cinnamic acid derivative (see Fig. 1). Human NAD^+ dependent alcohol dehydrogenase (ADH) catalyzes oxidation of primary alcohols to aldehydes (Pietruszko et al., 1973). Aromatic alcohols have been reported to be excellent substrates for ADH (Sund and Theorell, 1963). The aldehydes that are formed are

Table 2A
Acute toxicity oral studies

Material	Species	No. animals/dose group	LD_{50}[a]	References
Allyl cinnamate	Rat	10 (5/sex)	1.52 g/kg body weight (95% C.I. 0.79–1.29 g/kg body weight)	Jenner et al. (1964)
α-Amylcinnamyl acetate[b]	Rat	10	>5.0 g/kg body weight	RIFM (1974a)
α-Amylcinnamyl alcohol	Rat	10	4.0 g/kg body weight (95% C.I. 3.08–5.20 g/kg body weight)	RIFM (1973a)
Benzyl cinnamate	Rat	10 (5/sex)	3.28 g/kg body weight (95% C.I. 2.62–4.10 g/kg body weight)	RIFM (1972a)
Benzyl cinnamate	Rat	10 (5/sex)	5.53 g/kg body weight (95% C.I. 3.10–7.74 g/kg body weight)	Jenner et al. (1964)
Benzyl cinnamate	Guinea pig	Not specified	3.760 g/kg body weight (95% C.I. 2.340–6.055 g/kg body weight)	Jenner et al. (1964)
Butyl cinnamate	Rat	10	>5.0 g/kg body weight	RIFM (1977a)
Cinnamyl acetate	Rat	10	3.3 g/kg body weight (95% C.I. 2.9–3.7 g/kg body weight)	RIFM (1972b)
Cinnamyl benzoate	Rat	10	4.0 g/kg body weight (95% C.I. 3.56–4.44 g/kg body weight)	RIFM (1975a)
Cinnamyl butyrate	Rat	10	>5.0 g/kg body weight	RIFM (1976a)
Cinnamyl cinnamate	Rat	10	4.2 g/kg body weight	RIFM (1974b)
Cinnamyl formate	Rat	10	2.9 g/kg body weight (95% C.I. 2.38–3.54 g/kg body weight)	RIFM (1973b)
Cinnamyl isobutyrate	Rat	10	>5.0 g/kg body weight	RIFM (1977a)
Cinnamyl isovalerate	Rat	10	≥5.0 g/kg body weight	RIFM (1973c)
Cinnamyl propionate	Rat	10	3.4 g/kg body weight (95% C.I. 3.2–3.6 g/kg body weight)	RIFM (1973d)
Cinnamyl tiglate	Rat	10	>5.0 g/kg body weight	RIFM (1975b)
Ethyl cinnamate	Guinea pig	6	4.0 g/kg body weight	Zaitsev and Rakhmanina (1974)
Ethyl cinnamate	Rat	6	4.0 g/kg body weight	Zaitsev and Rakhmanina (1974)
Ethyl cinnamate	Mouse	6	4.0 g/kg body weight	Zaitsev and Rakhmanina (1974)
Ethyl cinnamate	Rat	Not reported	1.52 g/kg body weight	Bar and Griepentrog (1967)
Isoamyl cinnamate	Rat	10	>5.0 g/kg body weight	RIFM (1974a)
Isobutyl cinnamate	Rat	10	>5.0 g/kg body weight	RIFM (1975b)
Isopropyl cinnamate	Rat	10	>5.0 g/kg body weight	RIFM (1982a)
Isopropyl cinnamate	Guinea pig	10	2.7 g/kg body weight	Draize et al. (1948)
Linalyl cinnamate	Rat	10 (5/sex)	9.96 g/kg body weight (95% C.I. 8.23–12.05 g/kg body weight)	Jenner et al. (1964)
Linalyl cinnamate	Mouse	10	>39.04 g/kg body weight	RIFM (1967)
Methyl cinnamate	Rat	5 male and female	2.61 g/kg body weight (95% C.I. 2.00–3.41 g/kg body weight)	RIFM (1971a)
α-Methylcinnamic alcohol	Rat	10	2.4 g/kg body weight (95% C.I. 1.9–3.0 g/kg body weight)	RIFM (1974c)
Phenethyl cinnamate	Rat	10	~5.0 g/kg body weight	RIFM (1975a)
Phenethyl cinnamate	Mouse	10	>5.0 g/kg body weight	RIFM (1975b)
Propyl cinnamate[b]	Guinea pig	10	3 g/kg body weight	Draize et al. (1948)
Propyl cinnamate[b]	mouse	10	7 g/kg body weight	Draize et al. (1948)

[a] Units have been converted to make easier comparisons; original units are in the Fragrance Material Reviews.
[b] α-Amylcinnamyl acetate and propyl cinnamate are not materials that are being reviewed, but they are included in this table because they are structurally related.

Table 2B
Acute toxicity dermal studies

Material	Species	No. animals/dose group	LD_{50}[a]	References
Allyl cinnamate	Rabbit	4	<5.0 g/kg body weight	RIFM (1975b)
α-Amylcinnamyl acetate[b]	Rabbit	10	>5.0 g/kg body weight	RIFM (1974a)
α-Amylcinamyl alcohol	Rabbit	6	>5.0 g/kg body weight	RIFM (1973a)
Benzyl cinnamate	Rabbit	4	>3.0 g/kg body weight	RIFM (1972a)
Butyl cinnamate	Rabbit	10	>5.0 g/kg body weight	RIFM (1977a)
Cinnamyl acetate	Rabbit	10	>5.0 g/kg body weight	RIFM (1972b)
Cinnamyl benzoate	Rabbit	10	>5.0 g/kg body weight	RIFM (1975a)
Cinnamyl butyrate	Rabbit	4	>5.0 g/kg body weight	RIFM (1976a)
Cinnamyl cinnamate	Rabbit	10	>5.0 g/kg body weight	RIFM (1974b)
Cinnamyl formate	Rabbit	6	>5.0 g/kg body weight	RIFM (1973b)
Cinnamyl isobutyrate	Rabbit	10	>5.0 g/kg body weight	RIFM (1977a)
Cinnamyl isovalerate	Rabbit	10	>5.0 g/kg body weight	RIFM (1973c)
Cinnamyl propionate	Rabbit	10	>5.0 g/kg body weight	RIFM (1973d)
Cinnamyl tiglate	Rabbit	4	>5.0 g/kg body weight	RIFM (1975b)
Ethyl cinnamate	Rabbit	10	>5.0 g/kg body weight	RIFM (1973e)
Isoamyl cinnamate	Rabbit	7	>5.0 g/kg body weight	RIFM (1974a)
Isobutyl cinnamate	Rabbit	4	>5.0 g/kg body weight	RIFM (1975b)
Isopropyl cinnamate	Rabbit	10	>5.0 g/kg body weight	RIFM (1982a)
Isopropyl cinnamate	Rabbit	Not specified	>10 g/kg body weight	Draize et al. (1948)
Linalyl cinnamate	Rabbit	10	>5.0 g/kg body weight	RIFM (1973b)
Methyl cinnamate	Rabbit	4 (male and female)	>5.0 g/kg body weight	RIFM (1971a)
α-Methylcinnamic alcohol	Rabbit	4	>5.0 g/kg body weight	RIFM (1974c)
Phenethyl cinnamate	Rabbit	10	>5.0 g/kg body weight	RIFM (1975a)
Phenethyl cinnamate	Rabbit	4	>5.0 g/kg body weight	RIFM (1975b)

[a] Units have been converted to make easier comparisons; original units are in the Fragrance Material Reviews.
[b] α-Amylcinnamyl acetate is not one of the materials being reviewed, but it is included in this table because it is structurally related.

Table 2C
Acute toxicity miscellaneous studies

Material	Route	Species	No. animals/dose group	LD_{50}	References
Cinnamyl acetate	Intraperitoneal	Mouse	Not specified	1.2 g/kg body weight	Powers et al. (1961)

further metabolized by aldehyde dehydrogenase to yield the acid (Feldman and Weiner, 1972). The urinary metabolites of cinnamyl alcohol are mainly those derived from metabolism of cinnamic acid.

In animals, aromatic carboxylic acids, such as cinnamic acid, that enter the cell are converted to acyl CoA esters (Nutley et al., 1994). Cinnamoyl CoA either conjugates with glycine, a reaction catalyzed by *N*-acyl transferase, or undergoes β-oxidation eventually leading to the formation of benzoyl CoA. The reactions, which form benzoic acid from cinnamic acid, are reversible, but the equilibrium favors formation of the benzoic acid CoA ester (Nutley et al., 1994). Benzoyl CoA is in turn conjugated with glycine, yielding hippuric acid, or the CoA thioester is hydrolyzed to yield free benzoic acid which is then excreted (Nutley et al., 1994). CoA thioesters of carboxylic acids are obligatory intermediates in amino acid conjugation reactions (Hutt and Caldwell, 1990). Regardless of dose or species, the β-oxidation pathway is the predominant pathway of metabolic detoxication of cinnamic acid in animals.

The position and size of the substituents play a role in the metabolism of cinnamyl derivatives. Cinnamyl derivatives containing α-methyl substituents are extensively metabolized via β-oxidation and cleavage to yield mainly the corresponding hippuric acid derivative. A benzoic acid metabolite was isolated from the urine of dogs given α-methylcinnamic acid (Kay and Raper, 1924). Larger substituents located at the α- or β-position to some extent inhibit β-oxidation (Kassahun et al., 1991; Deuel, 1957), in which case there may be direct conjugation of the carboxylic acid with glucuronic acid followed by excretion. While α-methylcinnamic acid undergoes oxidation to benzoic acid, α-ethyl- and α-propylcinnamic acids are excreted unchanged (Carter, 1941). α-Ethylcinnamic alcohol administered orally to rabbits resulted in the urinary excretion of α-ethylcinnamic acid, in addition to small amounts of benzoic acid (Fischer and Bielig, 1940).

4. Toxicological studies

4.1. Acute toxicity (Tables 2A–2C)

Twenty one cinnamyl materials have been evaluated for acute toxicity (see Tables 2A–2C). Dermal LD_{50} values in rabbits exceeded 5000 mg/kg body weight for 20 of these materials; benzyl cinnamate was non-toxic at 3000 mg/kg body weight which was the highest dose tested. Oral

Table 3
Subchronic toxicity

Material	Method	Concentration	Species	Results	References
Benzyl cinnamate	Oral 19-week study	50 & 500 mg/kg body weight/day	Rats (5/sex/dose)	NOEL 500 mg/kg body weight/day	Hagan et al. (1967)
Benzyl cinnamate	Oral 19-week study	50 & 500 mg/kg body weight/day	10 rats (5/sex/dose)	NOEL 500 mg/kg body weight/day	FDA (1954)
Cinnamyl benzoate	Oral 14-day study	~750, 1500 and 3000 mg/kg body weight/day	24 male albino rats (6/dose)	No deaths and no gross abnormalities were reported; significantly depressed growth, food intake and food efficiency were noted	RIFM (1958b)
Cinnamyl cinnamate (as part of a mixture containing 5 cinnamic flavoring agents)	Oral 12-week study	~3 mg/kg body weight/day cinnamyl cinnamate (tested in a mixture containing 5 cinnamic flavoring agents)	24 rats (12/sex)	Depressed growth; food utilization significantly decreased in both sexes	RIFM (1958a)
Ethyl cinnamate	Oral 12-week study	~3 mg/kg body weight/day ethyl cinnamate (tested in a mixture containing 5 cinnamic flavoring agents)	24 rats (12/sex)	Depressed growth; food utilization significantly decreased in both sexes	RIFM (1958a)
Isopropyl cinnamate	Dermal 90-day study	500, 1000, 2000 and 4000 mg/kg body weight/day	Rabbits (no further details reported)	NOAEL 1000 mg/kg body weight/day	Draize et al. (1948)
Linalyl cinnamate	Oral 17 week study	50, 125 & 500 mg/kg body weight/day	rats (10/sex/dose)	NOEL 500 mg/kg body weight/day	Hagan et al. (1967)
Propyl cinnamate[a]	Dermal 90-day study	500, 1000, 2000 and 4000 mg/kg body weight/day	Rabbits (no further details reported)	Inanition; moderate atrophy of testis; inconsistent slight bone marrow hyperplasia (no further details reported)	Draize et al. (1948)
Methyl cinnamate	12-week study	~3 mg/kg body weight/day methyl cinnamate (tested in a mixture containing 5 cinnamic flavoring agents)	24 rats (12/sex)	Depressed growth; food utilization significantly decreased in both sexes	RIFM (1958a)

[a] Propyl cinnamate is not one of the materials being reviewed, but is included in this table because it is structurally related.

LD_{50} values have been reported for 21 materials and were in the range from 1520 mg/kg body weight for allyl cinnamate to 39,040 mg/kg for linalyl cinnamate with a majority of the materials in the 2500–5000 mg/kg body weight range. An intraperitoneal LD_{50} value of 1200 mg/kg body weight was reported for cinnamyl acetate.

4.2. Subchronic toxicity (Table 3)

Toxicological studies have been reported for benzyl cinnamate, cinnamyl benzoate, cinnamyl cinnamate, ethyl cinnamate, isopropyl cinnamate, linalyl cinnamate, and methyl cinnamate. Results of these studies are summarized in Table 3 and are described below.

4.2.1. Dermal studies

Isopropyl cinnamate applied daily to rabbit's skin for 90 days at dose levels of 0.5, 1.0, 2.0 and 4.0 ml/kg body weight [~ equivalent to 500, 1000, 2000 and 4000 mg/kg body weight] produced moderate chronic dermatitis; at the two highest dose levels, atrophy of the testes, hyperplasia of the bone marrow, slight inanition and severe skin irritation were also observed. The 90-day LD_{50} was reported to exceed 4000 mg/kg body weight. The No-Observed-Adverse-Effect Level (NOAEL) was concluded to be 1000 mg/kg body weight. (Draize et al., 1948). A related material, propyl cinnamate, also tested in the same manner, produced mild irritation, moderate atrophy of the testes, slight but inconsistent bone marrow hyperplasia and minimal splenitis; the 90-day LD_{50} was reported to be 2000 mg/kg body weight (Draize et al., 1948).

4.2.2. Oral studies

Osborne–Mendel rats received a dietary admixture containing linalyl cinnamate at dose levels of 0, 1000, 2500 or 10000 ppm [~ equivalent to 0, 50, 125 and 500 mg/kg body weight/day] for 17 weeks. There were no deaths and no adverse clinical signs were observed. There were no effects on growth or hematology, and no macroscopic or microscopic changes in the tissues were observed. The No-Observed-Effect Level (NOEL) was concluded to be 500 mg/kg body weight/day (Hagan et al., 1967).

Table 4
Mutagenicity and genotoxicity bacterial studies

Material	Test system in vitro	Species	Dose[a]	Results	References
Allyl cinnamate	Ames with and without S9 activation	*Salmonella typhimurium* TA1535, TA100, TA1537, TA1538 and TA98	Doses up to 3600 μg/plate	Negative	Wild et al. (1983)
α-Amylcinnamyl alcohol	Ames with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Doses up to 3600 μg/plate	Negative	Wild et al. (1983)
Benzyl cinnamate	Modified Ames (spot test) with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535, TA1537	715 μg/plate	Sample precipitated (questionable results)	Florin et al. (1980)
Benzyl cinnamate	Rec assay	*Bacillus subtilis* H17(rec+) & M45(rec−)	1000 μg/plate	Negative	Yoo (1986)
Cinnamyl acetate	Ames with and without S9 activation	*S. typhimurium* strains TA1535, TA1537, TA98, TA100, and TA102	Doses up to 5000 μg/plate	Negative	RIFM (2003a)
Ethyl cinnamate	Ames with and without S9 activation	*S. typhimurium* TA92, TA1535, TA100, TA1537, TA94, TA98	Doses up to 5000 μg/plate	Negative	Ishidate et al. (1984)
Ethyl cinnamate	Rec assay	*B. subtilis* H17(rec+) & M45(rec−)	20 μg/plate	Negative	Oda et al. (1978)
Isoamyl cinnamate	Ames assay with and without S9 activation	*S. typhimurium* TA98 & TA100 with/without activation and TA97, TA1535	Not reported	Negative	Zeiger and Margolin (2000)
Linalyl cinnamate	Ames assay with and without S9 activation	*S. typhimurium* TA98, TA100, TA102, TA1535, with/without S9 activation	Doses up to 5000 μg/plate	Negative	RIFM (2003b)
Methyl cinnamate	Rec assay	*B. subtilis* H17(rec+) & M45(rec-)	20 μg/plate	Negative	Oda et al. (1978)
α-Methyl cinnamic alcohol	Ames assay	*S. typhimurium* TA98,TA100, TA102, TA1535 and TA1537	Doses up to 5000 μg/plate	Weakly mutagenic	RIFM (1997a)

[a] Units have been converted to make easier comparisons; original units are in the Fragrance Material Reviews.

In another study, Osborne–Mendel rats received a dietary admixture containing benzyl cinnamate at dose levels of 0, 1000, or 10000 ppm [∼ equivalent to 0, 50 and 500 mg/kg body weight/day] for 19 weeks. There were no deaths and no adverse clinical signs were observed. There were no effects on growth or hematology and no macroscopic or microscopic changes in the tissues were observed. The NOEL was concluded to be 500 mg/kg body weight/day (FDA, 1954; Hagan et al., 1967).

A mixture of 897 ppm cinnamaldehyde and 25 ppm each of methyl cinnamate, ethyl cinnamate, cinnamyl cinnamate and α-methylcinnamaldehyde was added to the diet of rats for 12 weeks at levels calculated to result in average daily intakes of 103 mg/kg body weight for cinnamaldehyde and 3 mg/kg body weight for the other components. A slight retardation of growth was observed only in the females. Measurements of hematology, clinical chemistry, and urinalysis at weeks 6 and 12 showed no significant differences between test and control groups. Histopathology revealed no dose-related lesions. Food utilization was significantly decreased in both males and females. Depressed growth was observed in male rats which was not considered to be statistically significant, however, it may have been biologically relevant (RIFM, 1958a).

Male albino rats received a dietary admixture for 14 days containing cinnamyl benzoate at dose levels of 0.5% (∼750 mg/kg body weight/day), 1.0% (∼1500 mg/kg/day) and 2.0% (3000 mg/kg body weight/day). Behavior and appearance were normal and gross observations at necropsy were normal. Significantly depressed growth rates, food intake and efficiency of food utilization were observed at all dose levels due in part to poor palatability of the diet (RIFM, 1958b).

4.3. Chronic toxicity

There are no long term studies on these materials; however, since the members of this group may be hydrolyzed to yield the component alcohol, aldehyde, or acid, chronic studies for cinnamaldehyde provide a basis for the estimation of the toxic potential of the group.

The National Toxicology Program (NTP, 2003) has conducted a 2-year feeding assay with trans-cinnamaldehyde in rats and mice. In rats, they identified a No-Observed-Adverse-Effect-Level (NOAEL) as 200 mg/kg body weight/day; in mice the NOAEL was identified as 550 mg/kg body weight/day.

4.4. Mutagenicity and genotoxicity

Studies evaluating mutagenicity/genotoxicity have been performed on eight cinnamyl materials in this group. The results of these tests are summarized in Tables 4–6 and are described below.

4.4.1. Bacterial studies (Table 4)

Five cinnamyl materials were tested in bacterial assays using *Salmonella typhimurium,* and/or *Bacillus subtilis.*

Allyl cinnamate, α-amylcinnamyl alcohol, benzyl cinnamate, ethyl cinnamate, isoamyl cinnamate and linalyl cinnamate were inactive in *Salmonella typhimurium*, including strains TA92, TA94, TA98, TA100, TA1535, TA1537, and TA1538. The assays were performed at concentrations ranging up to the level of cytotoxicity, both in the absence and presence of metabolic activation (S9 fraction) obtained from the livers of Aroclor 1254- or methylcholanthrene-induced Sprague–Dawley rats or

Table 5
Mutagenicity and genotoxicity insect studies

Material	Test system in vitro	Test object	Concentration	Results	References
Allyl cinnamate	Basc test	*Drosophila melanogaster.* Berlin K (wild type) and Basc	1 mM	Negative	Wild et al. (1983)
α-Amylcinnamyl alcohol	Basc test	*Drosophila melanogaster* Berlin K (wild type) and Basc	45 mM	Negative	Wild et al. (1983)

Table 6
Mutagenicity and genotoxicity mammalian studies

Material	Test system in vitro	Test object	Concentration	Results	References
Allyl cinnamate	Bone marrow micronucleus assay	Male and female NMRI mice	94–282 mg/kg body weight	Negative	Wild et al. (1983)
α-Amylcinnamyl alcohol	Bone marrow micronucleus assay	Male and female NMRI mice	204–510 mg/kg body weight	Negative	Wild et al. (1983)
Cinnamyl acetate	Sister chromatid exchange	Chinese hamster ovary cells (CHO-K1)	1.0–100 μM	Negative (highest dose tested was toxic)	Sasaki et al. (1989)
Ethyl cinnamate	Sister chromatid exchange	Chinese hamster ovary cells (CHO-K1)	1.0, 3.3 and 10 μM	Negative (highest dose tested was toxic)	Sasaki et al. (1989)
Ethyl cinnamate	Chromosomal aberration	Chinese hamster fibroblast cell line	0.063 mg/ml (tested at three doses, only maximum dose reported)	Equivocal increases in chromosome aberrations and polyploidization effects were observed	Ishidate et al. (1984)
Methyl cinnamate	Sister chromatid exchange	Chinese hamster ovary cells (CHO-K1)	1.0, 3.3, 10 and 33.3 μM	Negative (highest dose tested was toxic)	Sasaki et al. (1989)
α-Methyl cinnamic alcohol	L5178Y TK+/− assay	Mouse L5178Y TK+/− cells	600 nl/ml	No effects	RIFM (1998)

Syrian hamsters (Wild et al., 1983; Florin et al., 1980; Ishidate et al., 1984; RIFM, 2003; Zeiger and Margolin, 2000).

Benzyl cinnamate, ethyl cinnamate and methyl cinnamate gave negative results in the Rec assay in *Bacillus subtilis* (Oda et al., 1978; Yoo, 1986).

4.4.2. Insect studies (Table 5)

No significant increases in sex-linked recessive lethal (SRL) mutations were observed with 1 mM allyl cinnamate or with 45 mM α-Amylcinnamyl alcohol in a Basc test using *Drosophila melanogaster* Berlin K and Basc strains (Wild et al., 1983).

4.4.3. Mammalian cell systems (Table 6)

Sister chromatid exchange (SCE) were not observed in Chinese hamster ovary (CHO) cells treated with cinnamyl acetate at doses of 1.0–100 μM or with ethyl cinnamate at doses of 3.3 and 10 μM, or with methyl cinnamate at doses of 3.3, 10 and 33.3 μM (Sasaki et al., 1989). Ethyl cinnamate produced equivocal increases in chromosome aberrations in a Chinese hamster fibroblast cell line without metabolic activation; polyploidization effects were also observed (Ishidate et al., 1984).

4.4.4. In vivo studies

In a micronucleus assay, groups of male and female mice received a single ip injection of allyl cinnmate or amylcinnamyl alcohol at dose levels of 94, 188 or 282 mg/kg body weight (allyl cinnamate), or 204, 357 or 510 mg/kg body weight (amylcinnamyl alcohol). At 30 h, the mice were sacrificed, the bone marrow extracted and polychromatic and normochromatic erythrocytes were scored for the presence of micronuclei. No evidence of genotoxic activity was produced (Wild et al., 1983).

Both in vitro tests in mammalian cells and in vivo studies in rats and mice have been carried out with cinnamyl alcohol, cinnamaldehyde and cinnamic acid. After an in depth review of all available data on these three materials, Bickers et al. (2005) concluded that based on a weight of evidence evaluation of all genotoxicity and mutagenicity studies as well as the metabolism and detoxification of these three materials, that they would have no significant genotoxic potential under their current conditions of use.

4.5. Carcinogenicity

There are no definitive long term studies that directly evaluate the carcinogenicity of these cinnamyl ester or alcohol derivatives. However, cinnamaldehyde has been evaluated by the National Toxicology Program (NTP, 2003) in a 2-year assay feeding microencapsulated cinnamaldehyde to rats and mice at dose levels of 50, 100 or 200 mg/kg body weight/day and 125, 270 and 550 mg/kg body weight/day, respectively. There was no evidence of carcinogenic activity (or other lesions) in rats or mice. Also, no significant carcinogenic effects (Wiseman et al., 1987) were produced by cinnamaldehyde when it was evaluated for

Table 7
Skin irritation humans

Material	Method	Concentration	Subjects	Results	References
Allyl cinnamate	Maximization pre-test. 48-h closed patch test	4% in petrolatum	22 male volunteers	irritant reactions observed in 20/22	RIFM (1975c)
Allyl cinnamate	48-h patch test	0.10%, 0.25%, 0.50% and 4% in petrolatum	11 male volunteers	No irritant reactions at 0.1% 5 irritant reactions at 0.25% 9 irritant reactions at 0.50% 10 irritant reactions at 4%	RIFM (1975c)
α-Amylcinnamyl acetate[a]	Maximization pre-test. 48-h closed patch test	8% in petrolatum	5 male and female volunteers	No irritation	RIFM (1975d)
α-Amylcinnamyl alcohol	Induction phase of an HRIPT	3% concentration in 3:1 diethyl phthalate: ethanol	105 male and female volunteers	No irritation	RIFM (2004a)
α-Amylcinnamyl alcohol	Maximization pre-test. 48-h closed patch test	8% in petrolatum	5 male volunteers	No irritation	RIFM (1973d)
Benzyl cinnamate	Induction phase of an HRIPT	4% in ethanol:diethyl phthalate (1:3)	101 male and female volunteers	No irritation	RIFM (2005a)
Benzyl cinnamate	Maximization pre-test. 48-h closed patch test	8% in petrolatum	5 male volunteers	No irritation	RIFM (1972c)
Benzyl cinnamate	Maximization pre-test. 48-h closed patch test	8% in petrolatum	5 male and female volunteers	No irritation	RIFM (1975d)
Butyl cinnamate	Maximization pre-test. 48-h closed patch test	4% in petrolatum	25 male and female volunteers	No irritation	RIFM (1977b)
Cinnamyl acetate	Maximization pre-test. 48-h closed patch test	5% in petrolatum	5 male volunteers	No irritation	RIFM (1972c)
Cinnamyl acetate	48-h semi-occluded patch test	32% in acetone	50 male volunteers	Irritation observed in 10–40% of subjects (no further details reported)	Motoyoshi et al. (1979)
Cinnamyl benzoate	Maximization pre-test. 48-h closed patch test	5% in petrolatum	5 male and female volunteers	No irritation	RIFM (1975d)
Cinnamyl butyrate	Maximization pre-test. 48-h closed patch test	4% in petrolatum	29 male volunteers	No irritation	RIFM (1976b)
Cinnamyl cinnamate	Maximization pre-test. 48-h closed patch test	4% in petrolatum	5 male and female volunteers	No irritation	RIFM (1974d)
Cinnamyl formate	Maximization pre-test. 48-h closed patch test	4% in petrolatum	5 male volunteers	No irritation	RIFM (1973d)
Cinnamyl isobutyrate	Maximization pre-test. 48-h closed patch test	4% in petrolatum	31 male volunteers	No irritation	RIFM (1977c)
Cinnamyl isovalerate	Maximization pre-test. 48-h closed patch test	2% in petrolatum	5 male volunteers	No irritation	RIFM (1973d)
Cinnamyl propionate	Maximization pre-test. 48-h closed patch test	4% in petrolatum	5 male volunteers	No irritation	RIFM (1973d)
Cinnamyl tiglate	Maximization pre-test. 48-h closed patch test	4% in petrolatum	24 male volunteers	No irritation	RIFM (1975c)
Ethyl cinnamate	Maximization pre-test. 48-h closed patch test	4% in petrolatum	5 male volunteers	No irritation	RIFM (1973d)
Ethyl cinnamate	24-h closed patch test	100%	22 male and female volunteers	1/22 irritant reactions	Katz (1946)
Isoamyl cinnamate	Maximization pre-test. 48-h closed patch test	8% in petrolatum	5 male and female volunteers	No irritation	RIFM (1974d)
Isobutyl cinnamate	Maximization pre-test. 48-h closed patch test	8% in petrolatum	29 male volunteers	No irritation	RIFM (1975c)
Isopropyl cinnamate	Maximization pre-test. 48-h closed patch test	6% in petrolatum	28 male and female volunteers	No irritation	RIFM (1982b)
Linalyl cinnamate	Maximization pre-test. 48-h closed patch test	8% in petrolatum	5 male volunteers	No irritation	RIFM (1973d)

(continued on next page)

Table 7 (*continued*)

Material	Method	Concentration	Subjects	Results	References
Methyl cinnamate	Maximization pre-test. 48-h closed patch test	10% in petrolatum	5 male and female volunteers	No irritation	RIFM (1975d)
α-Methylcinnamic alcohol	Maximization pre-test. 48-h closed patch test	2% in petrolatum	5 male and female volunteers	No irritation	RIFM (1974d)
Phenethyl cinnamate	Maximization pre-test. 48-h closed patch test	2% in petrolatum	5 male and female volunteers	No irritation	RIFM (1975d)
Phenethyl cinnamate	Maximization pre-test. 48-h closed patch test	2% in petrolatum	5 male and female volunteers	No irritation	RIFM (1975d)

[a] α-Amylcinnamyl acetate is not one of the materials being reviewed, but it is included in this table because it is structurally related.

hepatocarcinogenic potential in 44 B6C3F1 mice that had received intraperitoneal injections once a week for 4 weeks (total cumulative dose, 0.0006 g). While hemangiosarcomas were observed in three treated animals in this study, they were also observed in two control animals and the authors concluded that no significant carcinogenic effects were produced by cinnamaldehyde.

In addition, both cinnamyl alcohol (total cumulative intraperitoneal doses were 1.4 and 7.0 g/kg body weight) and cinnamaldehyde (total cumulative intraperitoneal doses, 0.8 and 4.0 g/kg body weight) did not induce primary lung tumors in female A/He mice under the conditions of the test (Stoner et al., 1973).

4.6. Reproductive and developmental toxicity

There are no reproductive studies on these cinnamyl materials. However in a review of cinnamyl alcohol, cinnamaldehyde and cinnamic acid, Bickers et al. (2005) reported on developmental toxicity studies conducted in rats and mice on cinnamyl alcohol, cinnamaldehyde and cinnamic acid that showed that these materials do not possess any significant potential for developmental effects under current conditions of use as fragrance ingredients.

4.7. Skin irritation

4.7.1. Human studies (Table 7)

Twenty-one cinnamyl materials were evaluated for skin irritation in 537 male and female volunteers. Allyl cinnamate produced irritation in a majority of its' test subjects (which was thought to be caused by its allyl component) at dose levels ranging from 0.25% to 4% in petrolatum. Irritation was not observed with the other cinnamyl materials tested at dose levels up to 10%. Mild irritation was observed with 32% cinnamyl acetate (see Table 7).

4.7.2. Animal studies (Table 8)

Fifteen materials that were tested for skin irritation at 100% in rabbits produced reactions that ranged from non-irritating to very slight irritation to moderate irritation. Linalyl cinnamate was also tested at 5% in rabbits and produced slight irritation. Benzyl cinnamate(3%), cinnamyl acetate(100%) and methyl cinnamate(3%) were also tested for irritation in guinea pigs and/or miniature swine and produced minimal irritation (see Table 8).

4.8. Mucous membrane (eye) irritation (Table 9)

Undiluted linalyl cinnamate and 5% linalyl cinnamate produced very slight to well-defined irritation to the rabbit eye which cleared by 24 h; undiluted methyl cinnamate produced no irritation to the rabbit eye (see Table 9).

4.9. Skin sensitization

4.9.1. Human studies (Table 10)

Bickers et al. (2005) reported that cinnamyl alcohol and cinnamaldehyde were sensitizers in humans, with NOELs of approximately 4% for the alcohol. More recent studies (RIFM, 2004d,e) show that the NOEL is 2.5% (3000 μg/cm^2) for the alcohol and 0.5% (591 μg/cm^2) for the aldehyde. Dermal sensitization was not observed for 21 cinnamyl esters and alcohols that were tested in maximization tests at concentrations ranging from 2% (1380 μg/cm^2) to 10% (6900 μg/cm^2) in 608 volunteers (see Table 10). In a modified Draize test, which was considered to be a non-standard test in which the induction consisted of continuous 48 h occluded patches and the challenge consisted of a 72 h occluded patch application, 8% (2481 μg/cm^2) α-amylcinnamyl alcohol produced one reaction in 78 volunteers when tested in an alcohol vehicle but not when tested in petrolatum. However, in a standard repeated insult patch test, 3% (3543 μg/cm^2)α-amylcinnamyl alcohol did not produce sensitization when tested in 105 volunteers. When 4% (4720 μg/cm^2) benzyl cinnamate was tested in a standard repeated insult patch test in 101 volunteers, it also did not produce sensitization. It is likely that their slow hydrolysis does not produce sensitizing levels of cinnamaldehyde.

However, as a result of recent studies and this review, IFRA (2007) has established Standards on α-amylcinnamyl alcohol and benzyl cinnmate using a Quantitative Risk Assessment (QRA) for dermal sensitization (see the individual Fragrance Material Reviews on these materials for more information).

Table 8
Skin irritation animals

Material	Method	Concentration	Species	Results	References
Allyl cinnamate	Irritation evaluated during an associated LD_{50} study	100%	4 rabbits	No irritation was observed	RIFM (1975b)
α-Amylcinnamyl acetate[a]	Irritation evaluated during an associated LD_{50} study	100%	10 rabbits	Slight to moderate irritation	RIFM (1974a)
Benzyl cinnamate	Irritation evaluated during an associated LD_{50} study	100%	4 rabbits	Moderate irritation which cleared by 48 h	RIFM (1972a)
Benzyl cinnamate	Preliminary irritation screen for an open epicutaneous test	A range of concentrations (vehicle not specified)	6–8 guinea pigs	3% = minimal irritating concentration (defined as lowest concentration producing mild erythema in at least 25% of animals)	Klecak et al. (1977)
Benzyl cinnamate	Induction phase of open epicutaneous test	A range of concentrations (vehicle not specified)	6–8 guinea pigs	3% = minimal irritating concentration	Klecak et al. (1977)
Butyl cinnamate	Irritation evaluated during an associated LD_{50} study	100%	10 rabbits	Mild to moderate irritation	RIFM (1977a)
Cinnamyl acetate	A 48-h closed patch test	100%	6 miniature swine	No irritation was observed	Motoyoshi et al. (1979)
Cinnamyl acetate	A 24-h open application to clipped dorsal skin; 30 minutes after reading, cinnamyl acetate was applied again. A 2nd set of readings and applications was made 48 h later. After 72-h reading, Evans blue was injected intravenously	100%	6 guinea pigs	Mild irritation	Motoyoshi et al. (1979)
Cinnamyl acetate	A 24-h open application to clipped dorsal skin; 30 minutes after reading, cinnamyl acetate was applied again. A 2nd set of readings and applications was made 48 h later. After 72-h reading, Evans blue was injected intravenously	100%	6 rabbits	Moderate irritation	Motoyoshi et al. (1979)
Cinnamyl benzoate	Irritation evaluated during an associated LD_{50} study	100%	10 rabbits	Slight to moderate irritation	RIFM (1975a)
Cinnamyl butyrate	Irritation evaluated during an associated LD_{50} study	100%	4 rabbits	Irritation lasting 24 h was observed	RIFM (1976a)
Cinnamyl isobutyrate	Irritation evaluated during an associated LD_{50} study	100%	10 rabbits	Mild to moderate irritation	RIFM (1977a)
Cinnamyl propionate	Irritation evaluated during an associated LD_{50} study	100%	10 rabbits	Slight to moderate irritation	RIFM (1973d)
Cinnamyl tiglate	Irritation evaluated during an associated LD_{50} study	100%	4 rabbits	No irritation	RIFM (1975b)
Isoamyl cinnamate	Irritation evaluated during an associated LD_{50} study	100%	7 rabbits	Slight irritation in one animal	RIFM (1974a)
Isobutyl cinnamate	Irritation evaluated during an associated LD_{50} study	100%	4 rabbits	Mild irritation lasting 24 h	RIFM (1975b)
Isopropyl cinnamate	Irritation evaluated during an associated LD_{50} study	100%	10 rabbits	Very slight to well-defined irritation	RIFM (1982a)
Linalyl cinnamate	Irritation evaluated during an associated LD_{50} study	100%	10 rabbits	Slight to moderate irritation	RIFM (1973b)
Linalyl cinnamate	Single application to intact or abraded skin	100%	3 rabbits	Very slight irritation	RIFM (1967)
Linalyl cinnamate	Single application to intact or abraded skin	5% in diethyl phthalate	3 rabbits	Very slight irritation	RIFM (1967)

(*continued on next page*)

Table 8 (*continued*)

Material	Method	Concentration	Species	Results	References
Methyl cinnamate	Irritation evaluated during an associated LD_{50} study	100%	6 rabbits	No irritation	RIFM (1971a)
Methyl cinnamate	Preliminary irritation screen for an open epicutaneous test	A range of concentrations (vehicle not specified)	6–8 guinea pigs	30% = minimal irritating concentration	Klecak et al. (1977)
Methyl cinnamate	Induction phase of open epicutaneous test	A range of concentrations (vehicle not specified)	6–8 guinea pigs	3% = minimal irritating concentration	Klecak et al. (1977)
α-Methylcinnamic alcohol	Intradermal pre-screen test for a maximization test	1%, 5%, 10% and 25% concentration w/v in arachis oil BP	4 guinea pigs	5% = highest concentration that caused a mild to moderate skin irritation	RIFM (1997b)
α-Methylcinnamic alcohol	A 48-h occluded patch test	100%, and 25%, 50%, and 75% v/v in arachis oil BP	2 guinea pigs	Very slight erythema was observed in 1/2 at 25%, 75% and 100%	RIFM (1997b)
α-Methylcinnamic alcohol	A 24-h occluded patch test	100%, and 25%, 50%, and 75% v/v in arachis oil BP	2 guinea pigs	No irritation observed at any dose level	RIFM (1997b)
α-Methylcinnamic alcohol	Irritation evaluated during an associated LD_{50} study	100%	4 rabbits	Mild irritation	RIFM (1974c)
Phenethyl cinnamate	Irritation evaluated during an associated LD_{50} study	100%	10 rabbits	Slight irritation	RIFM (1975a)
Phenethyl cinnamate	Irritation evaluated during an associated LD_{50} study	100%	4 rabbits	No irritation	RIFM (1975b)

[a] α-Amylcinnamyl acetate is not one of the materials being reviewed, but it is included in this table because it is structurally related.

4.9.2. Animal studies (Table 11)

Weak sensitization effects were observed with cinnamyl cinnamate in a modified Freund's Complete Adjuvant test at 3% and 10%. Sensitization was also observed with benzyl cinnamate (6 studies) and methyl cinnamate (4 studies) when they were tested in several studies using various test methods. Ethyl cinnamate did not produce sensitization at 4% in an open epicutaneous test (see Table 11).

4.10. Phototoxicity and photoallergy

4.10.1. Phototoxicity (Table 12)

UV spectra have been obtained on 19 cinnamyl esters and alcohols. All 19 peaked within a 245–278 nm range and all showed minute absorption in 290–320 nm region (see Table 12). In addition, 1% cinnamaldehyde and 20% cinnamic acid were evaluated for phototoxicity and photoallergy in guinea pigs and showed no potential for phototoxic or photoallergic activity (Bickers et al., 2005). Based on these data, it is not expected that the cinnamyl ester and alcohol derivatives would have a potential to produce phototoxic or photoallergic effects.

4.11. Environmental data

In addition to a human health assessment, environmental assessment of fragrance materials is performed according to a standard framework (Salvito et al., 2002). This screens chemicals in the RIFM/FEMA Database for their potential to present a hazard to the aquatic environment by considering their removal in wastewater treatment, minimal dilution in the mixing zone, and the application of a large uncertainty factor to ecotoxicological endpoints determined using quantitative structure-activity relationships. This screening, based on conservative assumptions, identifies priority materials that may require further study to quantitatively assess potential environmental risks. None of the materials in the Substituted Cinnamyl Alcohols and Esters of Cinnamic Acid and Alcohol group was identified as priority material for risk assessment refinement.

Table 9
Mucous membrane (eye) irritation

Material	Species	Concentration	Vehicle	Results	References
α-Amylcinnamyl alcohol	3 rabbits	1.25%	Not specified	Mild conjunctival irritation in 3/3 which cleared by day 7	RIFM (1964)
Linalyl cinnamate	3 rabbits	100%	N/A	Very slight to well-defined irritation in 3/3	RIFM (1967)
Linalyl cinnamate	3 rabbits	5%	Diethyl phthalate	Very slight to well-defined irritation in 3/3	RIFM (1967)
Methyl cinnamate	Rabbits	100%	N/A	No irritation	RIFM (1971b)
Methyl cinnamate	Rabbits	15%	Not specified	No irritation	RIFM (1971b)
Methyl cinnamate	6 Rabbits	100%	N/A	No irritation	RIFM (1971a)

Table 10
Skin sensitization humans

Material	Method	Concentration	Subjects	Results	References
Allyl cinnamate	MAX	4% (2760 μg/cm^2) in petrolatum	22 male volunteers	No reactions	RIFM (1975c)
α-Amylcinnamyl acetate[a]	MAX	8% (5520 μg/cm^2) in petrolatum	25 male and female volunteers	No reactions	RIFM (1975d)
α-Amylcinnamyl alcohol	HRIPT	3% (3543 μg/cm^2) in 3:1 diethyl phthalate:ethanol	31 male and 74 female volunteers	No reactions	RIFM (2004a)
α-Amylcinnamyl alcohol	MAX	8% (5520 μg/cm^2) in petrolatum	25 male volunteers	No reactions	RIFM (1973d)
α-Amylcinnamyl alcohol	Modified Draize	8% in petrolatum 8% in ethyl alcohol	78 volunteers	No (0/78) reactions with 8% in petrolatum 1/78 reactions with 8% in ethyl alcohol	Marzulli and Maibach (1980)
Benzyl cinnamate	HRIPT	4% (4720 μg/cm^2) in 1:3 ethanol: diethyl phthalate	25 male and 76 female volunteers	No reactions	RIFM (2005a)
Benzyl cinnamate	MAX	8% (5520 μg/cm^2) in petrolatum	25 male volunteers	No reactions	RIFM (1972c)
Benzyl cinnamate	MAX	8% (5520 μg/cm^2) in petrolatum	25 male and female volunteers	No reactions	RIFM (1975d)
Butyl cinnamate	MAX	4% (2760 μg/cm^2) in petrolatum	25 male and female volunteers	No reactions	RIFM (1977b)
Cinnamyl acetate	MAX	5% (3450 μg/cm^2) in petrolatum	25 male volunteers	No reactions	RIFM (1972c)
Cinnamyl benzoate	MAX	5% (3450 μg/cm^2) in petrolatum	25 male and female volunteers	No reactions	RIFM (1975d)
Cinnamyl butyrate	MAX	4% (2760 μg/cm^2) in petrolatum	29 male volunteers	No reactions	RIFM (1976b)
Cinnamyl cinnamate	MAX	4% (2760 μg/cm^2) in petrolatum	25 male and female volunteers	No reactions	RIFM (1974d)
Cinnamyl formate	MAX	4% (2760 μg/cm^2) in petrolatum	25 male volunteers	No reactions	RIFM (1973d)
Cinnamyl isobutyrate	MAX	4% (2760 μg/cm^2) in petrolatum	31 male volunteers	No reactions	RIFM (1977c)
Cinnamyl isovalerate	MAX	2% (1380 μg/cm^2) in petrolatum	25 male volunteers	No reactions	RIFM (1973d)
Cinnamyl propionate	MAX	4% (2760 μg/cm^2) in petrolatum	25 male volunteers	No reactions	RIFM (1973d)
Cinnamyl tiglate	MAX	4% (2760 μg/cm^2) in petrolatum	24 male volunteers	1 questionable reaction which was negative at re-test 5 months later	RIFM (1975c)
Ethyl cinnamate	MAX	4% (2760 μg/cm^2) in petrolatum	25 male volunteers	No reactions	RIFM (1973d)
Isoamyl cinnamate	MAX	8% (5520 μg/cm^2) in petrolatum	25 male and female volunteers	No reactions	RIFM (1974d)
Isobutyl cinnamate	MAX	8% (5520 μg/cm^2) in petrolatum	24 male volunteers	No reactions	RIFM (1975c)
Isopropyl cinnamate	MAX	6% (4140 μg/cm^2) in petrolatum	28 male and female volunteers	No reactions	RIFM (1982b)
Linalyl cinnamate	MAX	8% (5520 μg/cm^2) in petrolatum	25 male volunteers	No reactions	RIFM (1973d)
Methyl cinnamate	MAX	2% (1380 μg/cm^2) in petrolatum	25 male volunteers	No reactions	RIFM (1970a)
Methyl cinnamate	MAX	10% (6900 μg/cm^2) in petrolatum	25 male and female volunteers	No reactions	RIFM (1975d)
α-Methylcinnamic alcohol	MAX	2% (1380 μg/cm^2) in petrolatum	25 male and female volunteers	No reactions	RIFM (1974d)
Phenethyl cinnamate	MAX	2% (1380 μg/cm^2) in petrolatum	25 male and female volunteers	No reactions	RIFM (1975d)
Phenethyl cinnamate	MAX	2% (1380 μg/cm^2) in petrolatum	25 male and female volunteers	No reactions	RIFM (1975d)

[a] α-Amylcinnamyl acetate is not one of the materials being reviewed, but it is included in this table because it is structurally related.

However, there are environmental data in the RIFM/FEMA Database for materials within the Substituted Alcohols and Esters of Cinnamic Acid and Cinnamic Alcohol group. These include biodegradation and acute invertebrate studies. Data are available for four materials. Values for ready biodegradation (minimum 28-day studies) for the 3 materials tested range from 50% to 106%; the acute invertebrate toxicities range from 2.8 to 13 mg/L (48 h Geometric Mean EC_0/EC_{100} and 96 h LC_{50}, respectively).

The Substituted Cinnamyl Alcohols and Esters of Cinnamic Acid and Cinnamic Alcohol, as used in fragrance compounds, present a negligible environmental risk as indicated by applying the RIFM framework (Salvito et al., 2002) and reviewing the available environmental data.

5. Summary

1. Based on data from cinnamyl alcohol, cinnamaldehyde and cinnamic acid, these cinnamyl materials are anticipated to be significantly absorbed through the skin.
2. Cinnamyl ester and alcohol derivatives are anticipated to be extensively hydrolyzed by tissue esterases. The cinnamyl alcohol, aldehyde or ester formed all

Table 11
Sensitization animals

Material	Method	Concentration	Species	Results	References
α-Amylcinnamyl alcohol	Open Epicutaneous Test	8% (vehicle not specified by material)	male & female guinea pigs	No reactions (no further data reported)	Klecak (1979, 1985)
α-Amylcinnamyl alcohol	Local Lymph Node Assay	1%, 2.5%, 5%, 10% & 25% in 1:3 ethanol:diethyl phthalate	4 female CBA/Ca/Ola/Hsd mice per group	Negative EC_3 > 25% (6250 μg/cm^2)	RIFM (2004b)
α-Amylcinnamyl alcohol	Local Lymph Node Assay	7.5%, 15% & 30% in 1:3 diethyl phthalate:ethanol	5 female CBA/J f mice per group	Negative EC_3 > 30% (7500 μg/cm^2)	RIFM (2004c)
Benzyl cinnamate	Maximization test	A subirritant concentration in petrolatum	Male & female outbred Himalayan guinea pigs	Sensitization was observed (no further data reported)	Klecak et al. (1977)
Benzyl cinnamate	Freund's Complete Adjuvant Test	A subirritant concentration in petrolatum	Male & female outbred Himalayan guinea pigs	Sensitization was observed (no further data reported)	Klecak et al. (1977)
Benzyl cinnamate	Open Epicutaneous Test	0.3% & 3.0% (vehicle not specified)	Male & female outbred Himalayan guinea pigs	Sensitization was observed (no further data reported)	Klecak et al. (1977)
Benzyl cinnamate	Open Epicutaneous Test	3.0% (vehicle not specified)	6–8 guinea pigs	No reactions (no further data reported)	Klecak (1979)
Benzyl cinnamate	Modified Draize test	0.1% in saline	Male & female outbred Himalayan guinea pigs	Sensitization was observed (no further data reported)	Klecak et al. (1977)
Benzyl cinnamate	Modified Freund's Complete Adjuvant Test	3% and 10% in acetone	10 female Pirbright guinea pigs	1/10 reactions plus 3 questionable reactions at 10% 1/10 reactions plus 2 questionable reactions at 3%	Hausen and Wollenweber (1988) Hausen et al. (1995)
Benzyl cinnamate	Local Lymph Node Assay	2.5%, 5%, 10%, 25% & 50% in 1:3 ethanol: diethyl phthalate	4 female CBA/Ca mice	EC_3 = 18.44% (4610 μg/cm^2)	RIFM (2005b)
Cinnamyl cinnamate	Modified Freund's complete adjuvant test	3% & 10% in acetone	Guinea pigs	Weak sensitization was observed at both concentrations (no further details given)	Hausen et al. (1992) Hausen et al. (1995)
Ethyl cinnamate	Open epicutaneous test	4% (vehicle not specified)	6–8 guinea pigs	No reactions	Klecak (1979) Klecak (1985)
Methyl cinnamate	Maximization test	a subirritant concentration in petrolatum	Male & female outbred Himalayan guinea pigs	Sensitization effects were observed (no further details given)	Klecak et al. (1977)
Methyl cinnamate	Freund's complete adjuvant test	a subirritant concentration in petrolatum	Male & female outbred Himalayan guinea pigs	Sensitization effects were observed (no further details given)	Klecak et al. (1977)
Methyl cinnamate	Modified Freund's complete adjuvant test	10% in acetone	guinea pigs	No reactions (no further details given)	Hausen et al. (1992) Hausen et al. (1995)
Methyl cinnamate	Open epicutaneous test	30% (vehicle not specified)	Male & female outbred Himalayan guinea pigs	Sensitization effects were observed (no further details given)	Klecak et al. (1977)
Methyl cinnamate	Open epicutaneous test	2.0% (vehicle not specified)	6–8 guinea pigs	No reactions (no further details given)	Klecak (1979)
Methyl cinnamate	Open epicutaneous test	10% (vehicle not specified)	6–8 guinea pigs	No reactions (no further details given)	Klecak (1985)
Methyl cinnamate	Intradermal sensitization test	0.1% in 5% ethyl alcohol in distilled water	Male albino guinea pigs	No reactions	RIFM (1971b)
Methyl cinnamate	Modified Draize test	0.1% in saline	Male & female outbred Himalayan guinea pigs	Sensitization effects were observed (no further details given)	Klecak et al. (1977)
α-Methyl cinnamic alcohol	Maximization test	75% test material v/v in arachis oil BP	Dunkin Hartley albino guinea pigs	No reactions	RIFM (1997b)

Table 12
Summary of UV Spectra Data for Cinnamyl Esters and Substituted Alcohols

Material	UV Spectra Range of Absorption (nm)
Allyl cinnamate	peaked at 245–278 minor absorption in 290–320 nm range
α-Amylcinnamyl alcohol	peaked at 245–278 minor absorption in 290–320 nm range
Benzyl cinnamate	peaked at 245–278 minor absorption in 290–320 nm range
Cinnamyl acetate	peaked at 245–278 minor absorption in 290–320 nm range
Cinnamyl benzoate	peaked at 245–278 minor absorption in 290–320 nm range
Cinnamyl butyrate	peaked at 245–278 minor absorption in 290–320 nm range
Cinnamyl cinnamate	peaked at 245–278 minor absorption in 290–320 nm range
Cinnamyl formate	peaked at 245–278 minor absorption in 290–320 nm range
Cinnamyl isobutyrate	peaked at 245–278 minor absorption in 290–320 nm range
Cinnamyl isovalerate	peaked at 245–278 minor absorption in 290–320 nm range
Cinnamyl propionate	peaked at 245–278 minor absorption in 290–320 nm range
Ethyl cinnamate	peaked at 245–278 minor absorption in 290–320 nm range
Isoamyl cinnamate	peaked at 245–278 minor absorption in 290–320 nm range
Isobutyl cinnamate	peaked at 245–278 minor absorption in 290–320 nm range
Linalyl cinnamate	peaked at 245–278 minor absorption in 290–320 nm range
Methyl cinnamate	peaked at 245–278 minor absorption in 290–320 nm range
Phenethyl cinnamate	peaked at 245–278 minor absorption in 290–320 nm range

follow the same metabolic pathway in that the alcohol is transformed into the aldehyde, which is metabolized to the acid. The final major urinary metabolite is hippuric acid.

3. Based on acute toxicity data, these cinnamyl materials can be considered to range from practically nontoxic to moderately toxic.
4. Based on a subchronic dermal study, the NOAEL for isopropyl cinnamate is 1000 mg/kg body weight/day. Based on oral studies, the NOELs for benzyl cinnamate and linalyl cinnamate are 500 mg/kg body weight/day. Based on the results of oral chronic studies (2 years) available for trans-cinnamaldehyde, NOAELs for it and related materials have been identified as 200 mg/kg body weight/day in rats and 550 mg/kg/body weight per day in mice. All of these NOAELs greatly exceed the expected dose absorbed from dermal exposure in humans from the use of these compounds as fragrance ingredients. Such exposures are estimated at 0.0003–0.0268 mg/kg body weight/day.
5. Based on a weight of evidence evaluation of the available mutagenicity and genotoxicity data on these cinnamyl materials, as well as the metabolism and detoxification of cinnamyl alcohol, cinnamaldehyde and cinnamic acid, it can be concluded that this group of fragrance materials has no significant genotoxic potential under the current conditions of use as fragrance ingredients.
6. Oral chronic studies (2 years) with trans-cinnamaldehyde in rats and mice produced no evidence of carcinogenic activity.
7. Based on the available data on developmental toxicity studies on cinnamyl alcohol, cinnamaldehyde and cinnamic acid, it is not expected that these cinnamyl materials possess any significant potential for developmental effects under the current conditions of use as fragrance ingredients.
8. Based on human studies, these cinnamyl materials are not considered to be primary irritants under the recommended current conditions of use as fragrance ingredients with the exception of allyl cinnamate which produced irritation due to its allyl component.
9. Although slight to well-defined eye irritation was observed in animals with linalyl cinnamate, these cinnamyl materials are not considered to be eye irritants in humans under the recommended current conditions of use as fragrance ingredients.
10. Weak sensitization reactions were observed in animals, but no reactions were observed in human studies. While, IFRA (2007) has established Standards on α-amylcinnamyl alcohol and benzyl cinnamate using a Quantitative Risk Assessment (QRA) for dermal sensitization (see the individual fragrance material reviews on α-amylcinnamyl alcohol and benzyl cinnamate for more information); the weight of evidence supports the conclusion that these cinnamyl materials present no significant risk of sensitization under the recommended current conditions of use as fragrance ingredients.
11. Based on UV spectra and phototoxicity and photoallergy studies with cinnamaldehyde and cinnamic acid, it is not expected that these materials would produce phototoxic or photoallergic effects.

6. Conclusion

After a review of all available data on the related esters and alcohols of cinnamic acid and cinnamyl alcohol and on the parent materials, cinnamyl alcohol, cinnamaldehyde and cinnamic acid, the Panel has determined that there

are unlikely to be safety concerns regarding these materials under the present conditions of use and exposure for the following reasons:

- In acute studies, these materials have a low to moderate order of oral toxicity (LD50 values of 1.5–39 g/kg body weight), and a low order of dermal toxicity based on dermal LD50 values that exceeded 3–5 g/kg body weight.
- Dermal and oral subchronic NOAELs greatly exceed the expected dose absorbed in humans from their use as fragrance ingredients.
- While there are no long-term studies on these materials, a 2-year oral chronic study with trans-cinnamaldehyde provides a basis for the estimation of toxic potential for these materials; NOAELs from this study also greatly exceed the expected dose absorbed in humans from their use as fragrance ingredients.
- These materials have no significant potential to produce genotoxic effects in vivo based on a weight of evidence evaluation of all mutagenicity and genotoxicity data.
- These materials are expected to be extensively hydrolyzed by tissue esterases and the alcohols and acids that are formed are expected to undergo further oxidation, conjugation and excretion. The metabolic fate of the parent materials, cinnamyl alcohol, cinnamaldehyde and cinnamic acid are well known and toxic or persistent metabolites are not formed from their metabolism.
- In Human Dermatological Studies: Allyl cinnamate has a potential to produce irritation; with the remaining cinnamyl materials, no irritation was observed at dose levels up to 10%.
- These materials pose no significant risk of sensitization based on studies with 22 materials.
- Phototoxicity and photoallergic effects have not been evaluated in humans for these materials; however, 1% cinnamaldehyde and 20% cinnamic acid did not produce phototoxicity or photoallergy. It is not expected that these materials would have a potential to produce phototoxicity or photoallergy.
- These materials are used at low levels of exposure relative to doses that elicit adverse effects in laboratory animals via systemic exposure. The estimated dermal systemic exposure is greatest for linalyl cinnamate (0.03 mg/kg body weight/day). If one looks at the NOAEL in mice for cinnamaldehyde (550 mg/kg body weight/day), the margin of safety for systemic exposure based on this NOAEL is 18, 333 times the maximum daily exposure for linalyl cinnamate.

Conflicts of interest statement

D. Belsito, D. Bickers, M. Bruze, P. Calow, H. Greim, J.M. Hanifin, A.E. Rogers and J.H. Saurat are members of the Expert Panel of the Research Institute for Fragrance Materials, an independent group of experts who evaluate the safety of fragrance materials that is supported by the manufacturers of fragrances and consumer products containing fragrances. I.G. Sipes and H. Tagami are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on allyl cinnamate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

A toxicologic and dermatologic review of allyl cinnamate when used as a fragrance ingredient is presented.

Keywords: Fragrance; Review; Allyl cinnamate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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In 2006, a complete literature search was conducted on allyl cinnamate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Allyl β-phenylacrylate; Allyl 3-phenyl-2-Propenoate; 2-Propenoic acid, 3-phenyl-, 2-propenyl ester; Propenyl cinnamate; 2-Propen-1-yl 3-phenyl-2-propenoate; Vinyl carbinyl cinnamate.
1.2 CAS Registry Number: 1866-31-5.
1.3 EINECS Number: 217-477-8.
1.4 Formula: $C_{12}H_{12}O_2$.
1.5 Molecular weight: 188.23.
1.6 FDA: Allyl cinnamate was approved by the Food and Drug Administration as a flavor (21 CFR 172.515).
1.7 FEMA: Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient - GRAS 3. (2202) (FEMA, 1965).
1.8 Joint Expert Committee on Food Additives (JECFA): The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 19) concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent (JECFA, 2000).
1.9 IFRA guideline specification: Use only when the level of free allyl alcohol is less than 0.1%. Based on the delayed irritant potential of allyl alcohol (IFRA, 1977).

2. Physical properties

2.1 Physical form: An almost colorless or pale straw-colored, slightly viscous liquid with a peach, apricot-type odor.
2.2 Boiling point: 105–108 °C; >250 °C.
2.3 Log K_{OW} (calculated): 3.2.
2.4 Henry's law (calculated): 00000544 atm m^3/mol 25 °C.
2.5 Flash point: >200 °F; CC.
2.6 Refractive index: 1.5661.
2.7 Specific gravity: 1.100.
2.8 Vapor pressure (calculated): 0.003 mm Hg at 20 °C.
2.9 Water solubility (calculated): 92.3 mg/l at 25 °C.

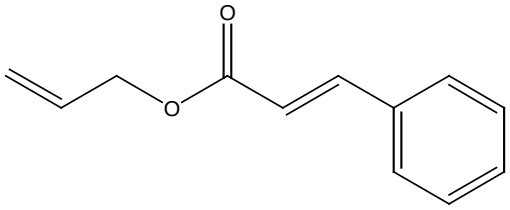

Fig. 1. Allyl cinnamate.

3. Usage (Table 1)

Allyl cinnamate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of less than 0.1 metric tonnes per annum.

The maximum skin level that results from the use of allyl cinnamate in formulae that go into fine fragrances has been reported to be 0.10% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.5% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0127 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Ten Osborne-Mendel rats (5/sex) were dosed orally via gavage with allyl cinnamate. The rats were observed for 14 days. Deaths occurred between four hours and eight days. The acute oral LD_{50} was calculated to be 1.52 g/kg (95% C.I. 0.79–1.29 g/kg). The major clinical sign observed was scrawny appearance (Jenner et al., 1964).

4.1.2. Dermal studies

4.1.2.1. The approximate dermal LD_{50} in rabbits was reported to be less than 5.0 g/kg based on 3/4 deaths at that dose. Four rabbits received a dermal application of neat allyl cinnamate at 5.0 g/kg/bodyweight. Observations were made over a 14-day period. Deaths occurred within the first 24 h. No clinical effects were observed (RIFM, 1975a).

4.2. Skin irritation

4.2.1. Human studies (Table 3)

4.2.1.1. In a pre-test for a human maximization study, irritation was observed in 20/22 volunteers, after a 48-h closed patch test with allyl cinnamate at 4% in petrolatum on the backs of 22 healthy male volunteers (RIFM, 1975b).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing allyl cinnamate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product (%)	Ingredient/mixture[a]	Ingredient[b] (mg/kg/day)
Body lotion	8.00	0.71	1.000	0.004	0.5	0.0019
Face cream	0.80	2.00	1.000	0.003	0.5	0.0004
Eau de toilette	0.75	1.00	1.000	0.080	0.5	0.0050
Fragrance cream	5.00	0.29	1.000	0.040	0.5	0.0048
Antiperspirant	0.50	1.00	1.000	0.010	0.5	0.0004
Shampoo	8.00	1.00	0.010	0.005	0.5	0.0000
Bath products	17.00	0.29	0.001	0.020	0.5	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.5	0.0001
Toilet soap	0.80	6.00	0.010	0.015	0.5	0.0001
Hair spray	5.00	2.00	0.010	0.005	0.5	0.0000
Total						0.0127

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity data

Route	Species	No. animals/ dose group	LD_{50}	References
Oral	Rat	10 (5/sex)	1.52 g/kg (95% C.I. 0.79–1.29 g/kg)	Jenner et al. (1964)
Dermal	Rabbit	4	<5.0 g/kg	RIFM (1975a)

Table 3
Summary of human skin irritation studies

Method	Dose (%)	Results	Reference
Maximization pre-test	4% in petrolatum	Irritation observed in 20/22	RIFM (1975b)
Primary irritation screen	0.10%	0.10% – no irritation	RIFM (1975c)
	0.25%	0.25% – 5 irritant reactions	
	0.50%	0.50% – 9 irritant reactions	
	4% in petrolatum	4% – 10 irritant reactions	

4.2.1.2. A 48-h closed patch test was conducted with allyl cinnamate at 0.10%, 0.25%, 0.50% and 4% in petrolatum on 11 healthy male volunteers. The test material was applied to normal sites on the backs of all the volunteers. Reactions were read at 48 and 72 h after application. At 0.25%, 0.5% and 4%, irritation was observed in the majority of the subjects. At 0.1%, questionable reactions were observed at the 48-h reading. By the 72-h reading, these reactions had cleared and were determined to be non-irritation reactions. At 0.25%, 5 irritant reactions were observed; 9 irritant reactions were observed at 0.50% and 10 irritant reactions were observed at 4% (RIFM, 1975c).

4.2.2. Animal studies

4.2.2.1. No irritation was observed during the associated dermal LD_{50} study (see Section 4.1.2.1), when 4 rabbits received a dermal application of neat allyl cinnamate at 5.0 g/kg/bodyweight (RIFM, 1975a).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 4% (2760 μg/cm^2) allyl cinnamate in petrolatum on 22 healthy, male volunteers. Applications were made under occlusion to the same sites on the forearms of all subjects for five alternate-day 48-h periods. Following a 10–14 day rest period, a challenge patch was applied to a fresh site on the right side of the back for 48 h under occlusion. Reactions to challenge were read 48 and 72 h after patch removal. No sensitization reactions were observed; however, irritation was observed in 21/22 volunteers (RIFM, 1975b).

4.5. Phototoxicity and photoallergy

UV spectra revealed that allyl cinnamate peaked within the 245–278 nm range and showed minor absorption in the 290–320 nm region.

4.6. Absorption, distribution and metabolism

4.6.1. Metabolism studies

4.6.1.1. In vivo studies in animals

4.6.1.1.1. The hydrolysis of allyl cinnamate by nonspecific esterases was investigated in male Holtzman rats weighing between 130 and 340 g. After 18-h of fasting, the animals were dosed via gavage with allyl cinnamate in corn oil at dose levels of 250, 400 or 600 mg/kg/bodyweight. The rats were sacrificed 24 h after dosing. Plasma was collected and assayed for alanine-α-ketoglutarate transaminase (AKT) activity. Increased plasma AKT activ-

Table 4
Summary of mutagenicity and genotoxicity studies

Test method	Strain	Dose	Results	References
Ames with and without S9 activation	*Salmonella typhimurium* TA1535, TA100, TA1537, TA1538 and TA98	Doses up to 3.6 mg/plate in dimethyl sulfoxide	Negative	Wild et al. (1983)
Basc test	*Drosophila melanogaster* Berlin K (wild type) and Basc strains	1 mM in 5% saccarose (and/or 2% ethanol and 2% Tween 80)	Negative	Wild et al. (1983)
Bone marrow micronucleus test	NMRI mice	94, 188 and 282 mg/kg in olive oil	Negative	Wild et al. (1983)

ity was observed at all three dose levels. Triorthotolyl phosphate (TOTP) antagonized the hepatotoxic effects of 250–600 mg/kg oral allyl cinnamate in rats (Silver and Murphy, 1978).

4.6.1.2. In vitro studies in animals

4.6.1.2.1. The hydrolysis of allyl cinnamate by liver homogenates obtained from control or TOTP treated male Holtzman rats was investigated. Four rats were sacrificed; the livers were then removed and homogenized in preparation for a manometric carboxylesterase assay. Allyl cinnamate (16 mM) was then added to a flask containing 5 mg of liver homogenate in a bicarbonate buffer. The flasks were equilibrated for 5 min at 37 °C, with shaking. The amount of CO_2 evolved during a 20-min period was used to assess hydrolytic activity. It was observed that the preparation evolved 59 ± 18 μl of CO_2 over the 20-min period. Allyl cinnamate at 16 mM was hydrolysed by liver homogenates of control rats. The animals pretreated with TOTP, which is an inhibitor of nonspecific esterases, inhibited this hydrolysis by 96.6% (Silver and Murphy, 1978).

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity (Table 4)

4.9.1. Bacterial studies

4.9.1.1. In an Ames test (Ames et al., 1975) using *Salmonella typhimurium* strains TA1535, TA100, TA1537, TA1538 and TA98 with and without rat liver S9 metabolic activation, doses of allyl cinnamate up to 3600 μg/plate [3.6 mg/plate] in dimethyl sulfoxide were not mutagenic. The positive controls were 0.5 μg/plate sodium azide (strains TA1535 and TA100) and 5 μg/plate benzo[a]pyrene (strains TA100, TA1537, TA1538 and TA98) (Wild et al., 1983).

4.9.2. Insect studies

4.9.2.1. A Basc test using Berlin K (wild type) and Basc strains was performed on *Drosophila melanogaster*. Allyl cinnamate was added to the diet at the dose of 1 mM in 5% saccarose (with the possible addition of 2% ethanol and 2% Tween 80). No significant increases in sex-linked recessive lethal (SRL) mutations were observed (Wild et al., 1983).

4.9.3. Mammalian studies

4.9.3.1. Allyl cinnamate was determined to be non genotoxic in a micronucleus test. Groups of four male and female NMRI mice were treated usually once with 94, 188 or 282 mg/kg doses of allyl cinnamate in olive oil. The mice were sacrificed and bone marrow smears were prepared 30 h after the treatment. Polychromatic and normochromatic erythrocytes were then scored for the presence of micronuclei. The mean numbers of micronucleated polychromatic erythrocytes (PE) were 1.5, 2.0 and 1.5 PE/1000 which were similar to the control PE of 2.0 PE/1000 (Wild et al., 1983).

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on pentyl cinnamate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

A toxicologic and dermatologic review of pentyl cinnamate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Pentyl cinnamate

Contents

In 2006, a complete literature search was conducted on pentyl cinnamate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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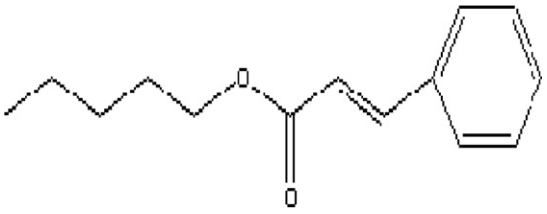

Fig. 1. Pentyl cinnamate.

1. Identification (Fig. 1)

1.1 Synonyms: Amyl cinnamate; Pentyl 3-phenyl-2-propenoate; 2-Propenoic acid, 3-phenyl-, pentyl ester.
1.2 CAS registry number: 3487-99-8.
1.3 EINECS number: 222-478-1.
1.4 Formula: $C_{14}H_{18}O_2$.
1.5 Molecular weight: 218.3.
1.6 Council of Europe: Pentyl cinnamate was included by the Council of Europe in the list of substances granted B – information required – 28 day oral study; hydrolysis study (COE No. 328) (Council of Europe, 2000).

2. Physical properties

2.1 Henry's law (calculated): 0.0000129 atm m^3/mol 25 °C.
2.2 Log K_{ow} (calculated): 4.32.
2.3 Vapor pressure (calculated): 0.000874 mm Hg 25 °C.
2.4 Water solubility (calculated): 7.167 mg/l @ 25 °C.

3. Usage (Table 1)

Pentyl cinnamate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of less than 0.1 metric tonnes per annum.

The maximum skin level that results from the use of pentyl cinnamate in formulae that go into fine fragrances has been reported to be 0.10% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.5% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0127 mg/kg for high end users of these products.

4. Toxicological data

4.1. Acute toxicity

No data available on this material.

4.2. Skin irritation

No data available on this material.

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

No data available on this material.

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing pentyl cinnamate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product %	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.5	0.0019
Face cream	0.80	2.00	1.000	0.003	0.5	0.0004
Eau de toilette	0.75	1.00	1.000	0.080	0.5	0.0050
Fragrance cream	5.00	0.29	1.000	0.040	0.5	0.0048
Antiperspirant	0.50	1.00	1.000	0.010	0.5	0.0004
Shampoo	8.00	1.00	0.010	0.005	0.5	0.0000
Bath products	17.00	0.29	0.001	0.020	0.5	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.5	0.0001
Toilet soap	0.80	6.00	0.010	0.015	0.5	0.0001
Hair spray	5.00	2.00	0.010	0.005	0.5	0.0000
Total						0.0127

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on *alpha*-amylcinnamyl alcohol

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc. 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

A toxicologic and dermatologic review of *alpha*-amylcinnamyl alcohol when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; *alpha*-amylcinnamyl alcohol

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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In 2006, a complete literature search was conducted on *alpha*-amylcinnamyl alcohol. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: *alpha*-Amylcinnamic alcohol; Amylcinnamyl alcohol; 2-Amyl-3-phenyl-2-propen-1-ol; 2-Benzylideneheptanol; 1-Heptanol, 2-(phenylmethylene)-; *alpha*-Penylcinnamyl alcohol.
1.2 CAS Registry Number: 101-85-9.
1.3 EINECS Number: 202-982-8.
1.4 Formula: $C_{14}H_{20}O$.
1.5 Molecular Weight: 204.31.
1.6 Council of Europe: *alpha*-Amylcinnamyl alcohol was included by the Council of Europe in the list of substances granted A – may be used in foodstuffs (COE No. 79) (Council of Europe, 2000).
1.7 FDA: *alpha*-Amylcinnamyl alcohol was approved by the FDA as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient – GRAS 3. (2065) (FEMA, 1965).
1.9 Joint Expert Committee on Food Additives (JECFA): The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 674) concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent (JECFA, 2000).
1.10 IFRA: *alpha*-Amylcinnamyl alcohol has an International Fragrance Association Standard (IFRA, 2007) – see Section 4.4.1. for details.

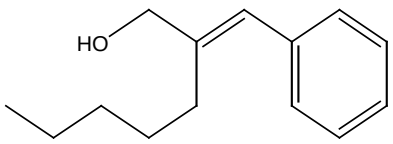

Fig. 1. *alpha*-Amylcinnamyl alcohol.

2. Physical properties

2.1 Vapor pressure (calculated): <0.001 mm Hg at 20 °C.
2.2 Flash point: >200 °F;CC.
2.3 Boiling point: >200 °C.
2.4 Log K_{OW} (calculated): 4.35.
2.5 Water Solubility (calculated): 25.72 mg/l @ 25 °C.
2.6 Specific Gravity: 0.958.
2.7 Henry's Law (calculated): 0.000000771 atm m^3/mol 25C.

3. Usage (Table 1)

alpha-Amylcinnamyl alcohol is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of less than 0.1 – 1 metric tonnes per annum.

The maximum skin level that results from the use of *alpha*-amylcinnamyl alcohol in formulae that go into fine fragrances has been reported to be 0.04% (IFRA, 2004), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.15% (IFRA, 2004), which would result in a conservative calculated maximum daily exposure on the skin of 0.0038 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Rats (10/dose) were orally administered *alpha*-amylcinnamyl alcohol at doses levels of 2.1, 2.6, 3.7, 5.0 and 6.5 g/kg. Observations were made over a 7-day period. At 2.1 g/kg, 1/10 deaths occurred; 3/10 deaths occurred at 2.6 g/kg; 5/10 deaths occurred at 3.7 g/kg; 5/10 deaths occurred at 5.0 g/kg; 10/10 deaths occurred at 6.5 g/kg. Deaths occurred from days 1 to 7, with the most deaths occurring within the first 48 h. Ataxia, hemorrhagic and mucoid enteritis were the systemic effects observed. The calculated LD_{50} was reported to be 4.0 g/kg (95% CI 3.08–5.20 g/kg) (RIFM, 1973a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg based on no (0/6) deaths at that dose. Six rabbits received a

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing *alpha*-amylcinnamyl alcohol

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product %	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.15	0.0006
Face cream	0.80	2.00	1.000	0.003	0.15	0.0001
Eau de toilette	0.75	1.00	1.000	0.080	0.15	0.0015
Fragrance cream	5.00	0.29	1.000	0.040	0.15	0.0015
Antiperspirant	0.50	1.00	1.000	0.010	0.15	0.0001
Shampoo	8.00	1.00	0.010	0.005	0.15	0.0000
Bath products	17.00	0.29	0.001	0.020	0.15	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.15	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.15	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.15	0.0000
Total						0.0038

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

24-h occluded application of *alpha*-amylcinnamyl alcohol at a dose level of 5.0 g/kg/bodyweight. Observations were made over a 14-day period. No clinical signs were observed (RIFM, 1973a).

4.2. Skin irritation

4.2.1. Human studies (Table 3)

4.2.1.1. Irritation was evaluated during the induction phase of a HRIPT (human repeated insult patch test). A 0.3 ml aliquot of the 3% *alpha*-amylcinnamyl alcohol in 3:1 diethyl phthalate: ethanol (DEP: EtOH) was applied for 24-h under occlusion, using a 25 mm webril/adhesive patch (Hill Top Chamber System) on 105 volunteers (31 male/74 female). A total of 9 induction applications were made, based on a Monday, Wednesday and Friday schedule. No irritation was observed (RIFM, 2004c).

4.2.1.2. In a pre-test for a human maximization study, no irritation was observed after a 48-h closed patch test with 8% *alpha*-amylcinnamyl alcohol in petrolatum on the backs of 5 healthy, male volunteers (RIFM, 1973b).

Table 2
Summary of acute toxicity data

Route	Species	No. animals/ dose group	LD_{50}	References
Oral	Rat	10	4.0 g/kg (95% CI 3.08–5.20 g/kg)	RIFM (1973a)
Dermal	Rabbit	6	>5.0 g/kg	RIFM (1973a)

4.3. Mucous membrane (eye) irritation

4.3.1

An eye irritation test was conducted in 3 healthy albino rabbits. A 0.1 ml aliquot of 1.25% *alpha*-amylcinnamyl alcohol in an unspecified vehicle was instilled into the right eye of each rabbit with no further treatment. The left eye remained untreated and served as a control. The eyes were examined every 24-h for 4 days and then again on day 7. Reactions were scored using the Draize scale. Mild conjunctival irritation was observed in 3/3 rabbits, which cleared on day 7 (RIFM, 1964).

4.4. Skin sensitization

4.4.1. Dermal sensitization quantitative risk assessment (QRA)

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This new methodology represents a significant change over current risk assessment practices because it specifically addresses the elements of exposure-based risk assessment that are unique to the induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment.

Full details of this risk assessment approach can be found in the "QRA Expert Group, Dermal Sensitization Quantitative Risk Assessment (QRA) for Fragrance Ingredients, Technical Dossier, revised June 22, 2006", and "IFRA/RIFM Quantitative Risk Assessment (QRA) for Fragrance Ingredients Booklet, May 11, 2006", at http://www.rifm/org/pub/publications.asp and http://www.ifra-org.org/News.asp.

Table 3
Summary of human skin irritation studies

Method	Dose (%)	Results	Reference
Induction phase – HRIPT	3% in 3:1 in diethyl phthalate: ethanol	No irritation was observed (0/105)	RIFM (2004c)
Maximization pre-test	8% in petrolatum	No irritation was observed (0/5)	RIFM (1973b)

An exposure-based Quantitative Risk Assessment (QRA) methodology has been used to determine acceptable exposure limits for *alpha*-amylcinnamyl alcohol and a new IFRA Standard (IFRA, 2007) has been issued (see Tables 4 and 5).

4.4.2. Human studies (Table 6)

4.4.2.1. Predictive studies

4.4.2.1.1. A Human repeated insult patch test (HRIPT) was conducted on 105 (31 male/74 female) volunteers. During the induction phase, a 0.3 ml aliquot of 3% (3543 μg/cm^2) *alpha*-amylcinnamyl alcohol in 3:1 DEP:EtOH was applied to a webril/adhesive patch (25 mm Hilltop® Chamber System), and then applied to the back of each volunteer for 24 h under occlusion. A total of 9 induction applications were made over a 3 week period. After a 2 week rest period, challenge patches with 3% (3543 μg/cm^2) *alpha*-amylcinnamyl alcohol in 3:1 DEP:EtOH were applied to a virgin site on the right side of the back and kept in place for 24 h under occlusion. The test sites were scored at 48, 72 and 96 h. No sensitization was observed (RIFM, 2004c).

4.4.2.1.2. A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 8% (5520 μg/cm^2) *alpha*-amylcinnamyl alcohol in petrolatum on 25 healthy, male volunteers. Application was made under occlusion to the same site on the forearms of all volunteers for five alternate-day 48-h periods. Patch sites were pre-treated for 24 h with 5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a 10-day rest period, challenge patches were applied under occlusion to fresh sites for 48 h. The challenge applications were preceded by 1 h application of 10% aqueous SLS under occlusion. The challenge sites were read on removal of the patch and 24 h thereafter. No sensitization was observed (RIFM, 1973b).

4.4.2.1.3. A modified Draize test was conducted with, 8% *alpha*-amylcinnamyl alcohol in petrolatum and in ethanol. A total of 78 volunteers received ten induction applications of 8% *alpha*-amylcinnamyl alcohol in petrolatum or ethanol on the arm or the upper back for 48–72 h under occlusion. Patches were applied using square occlusive Band-Aid without perforations. Following a 2-week rest period, a 72 h occluded challenge application was made to a new site. No reactions were observed with 8% *alpha*-amylcinnamyl alcohol in petrolatum; while reactions were observed in 1/78 volunteers with 8% *alpha*-amylcinnamyl alcohol in ethanol (Marzulli and Maiback, 1980).

4.4.2.2. Diagnostic studies (Table 7)

4.4.2.2.1. Patch tests using Silver Patch Testers were conducted on 179 patients with suspected cosmetic allergies. Reactions were evaluated after 48 and 72 h. Reactions were scored according to internationally accepted criteria. Seven reactions were observed to 20% *alpha*-amylcinnamyl alcohol in petrolatum (DeGroot et al., 1985).

4.4.2.2.2. In 1987, 162 patients who had reacted to a fragrance mix were tested with the individual ingredients of

Table 4
IFRA Standard based on the QRA

Limits in the finished product: For a description of the categories, refer to the QRA information booklet			
Category 1 – see Note box (1)	0.1%	Category 7	0.3%
Category 2	0.1%	Category 8	2.0%
Category 3	0.5%	Category 9	5.0%
Category 4	1.6%	Category 10	2.5%
Category 5	0.8%	Category 11 – see Note box (2)	
Category 6 – see Note box (1)	2.5%		

Note box:
(1) IFRA would recommend that any material used to impart perfume or flavour in products intended for human ingestion should consist of ingredients that are in compliance with appropriate regulations for foods and food flavourings in the countries of planned distribution and, where these are lacking, with the recommendations laid down in the Code of Practice of IOFI (International Organisation of the Flavor Industry). Further information about IOFI can be found on its website (www.iofiorg.org).
(2) Category 11 includes all non-skin contact or incidental skin contact products. Due to negligible skin contact the concentration of a fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product. For example, hypothetically if the usual concentration of a fragrance compound in the final product, for example a candle, is at 5%, then any individual fragrance ingredient (in this case *alpha*-amyl cinnamic alcohol) must not exceed 5% in the candle.

Table 5
Summary of the relevant sensitization data for the implementation of the QRA

LLNA weighted mean EC3 values (μg/cm^2) (no. studies)	Human data			Potency classification[b]	WoE NESIL (μg/cm^2)[c]
	NOEL – HRIPT (induction) (μg/cm^2)	Experimental NOEL – MAX (induction) (μg/cm^2)	LOEL[a] (induction) (μg/cm^2)		
>6250 [1]	3543	NA	NA	Weak	3500

[a] Data derived from HRIPT or HMT.
[b] Gerberick et al. (2001).
[c] WoE NESIL limited to two significant figures.

Table 6
Summary of predictive studies

Test method	Test concentration	Results	References
HRIPT	3% in 3:1 DEP:EtOH (3543 μg/cm^2)	0/105	RIFM (2004c)
Maximization	8% in petrolatum (5520 μg/cm^2)	0/25	RIFM (1973b)
Modified Draize test	8% in petrolatum 8% in ethanol	No reactions with 8% in petrolatum 1/78 reactions with 8% in ethanol	Marzulli and Maiback (1980)

Table 7
Summary of diagnostic studies

Method	Test concentration	Results	References
Patch test	20% in petrolatum	7/179	DeGroot et al. (1985)
Patch test	1% in petrolatum	2/162	Enders et al. (1989)
Patch test	2% in petrolatum	5/172	Calnan et al. (1980)
Patch-test	Not reported	2/50	Goodfield and Saihan (1988)
Patch test	2% and 5% (vehicle not specified)	0/99	Ishihara (1978) Ishihara et al. (1981)
Patch test	Not reported	50/1452	Becker et al. (1994)
Open application	5% in petrolatum	Reactions observed in 2/15 eczematous patients and 1/19 control volunteers	Emmons and Marks (1985)

the mix. Two patients reacted to 1% *alpha*-amylcinnamyl alcohol in petrolatum (Enders et al., 1989).

4.4.2.2.3. When patch tests with a fragrance mix were conducted at St. John's Hospital on 2461 eczematous patients from 1979 to 1980, reactions were observed in 172 patients. Most of the 172 patients who reacted to the perfume mix were tested with the individual components of the mix. Reactions to 2% *alpha*-amylcinnamyl alcohol in petrolatum were observed in 5 patients (Calnan et al., 1980).

4.4.2.2.4. The incidence of fragrance sensitivity in Nottinghamshire [United Kingdom] coal miners with eczematous skin problems was examined. Thirty-five miners and 55 male and 30 female non-miners were patch tested over a period of 18 months with the ICDRG standard series with fragrance mix and the components of the fragrance mix. Patch tests were performed when the eczema was quiescent. Reactions were read at 48 and 96 h. Reactions to *alpha*-amylcinnamyl alcohol (dose and vehicle not reported) were seen in 4% of the male non-miners. No effects in the miners or women were observed (Goodfield and Saihan, 1988).

4.4.2.2.5. Ninety-nine patients were patch tested with 2% and 5% *alpha*-amylcinnamyl alcohol (vehicle not specified). No reactions were observed. In addition, 4 patients who previously reacted to 2% cinnamic aldehyde were tested for cross sensitization with *alpha*-amylcinnamyl alcohol (vehicle not specified). No cross sensitization reactions were observed (Ishihara, 1978; Ishihara et al., 1981).

4.4.2.2.6. A total of 1452 patients were patch tested with the Epipharm Hungarian standard series. Fifty patients who reacted to the fragrance mix were further tested with the constituents of the fragrance mix. Reactions to *alpha*-amylcinnamyl alcohol (dose and vehicle not reported) were observed in 2 patients (Becker et al., 1994).

4.4.2.2.7. An open application of 5% *alpha*-amylcinnamyl alcohol in petrolatum was made to forearms of 50 male and female volunteers. The 50 volunteers consisted of 15 eczematous dermatitis patients, 16 cosmetic sensitivity patients and 19 control volunteers. Several millimeters of the *alpha*-amylcinnamyl alcohol-petrolatum mixture were smeared on the skin of the ventral forearm of all the volunteers in a 1 cm circle. Reactions were read 45 minutes after application. Reactions were observed in 2/15 eczematous patients, and in 1/19 control volunteers (Emmons and Marks, 1985).

4.4.3. Animal studies

4.4.3.1. Two separate open epicutaneous tests were conducted with 8% *alpha*-amylcinnamyl alcohol (vehicle not specified by material) in guinea pigs. Induction consisted of 21 daily open applications to the shaved flank of 6–8 guinea pigs/group. Open challenge applications were made on days 21 and 35. No reactions were observed (Klecak, 1979 and 1985).

4.4.4. Local lymph node assay (LLNA) (Table 8)

4.4.4.1. Sensitization to *alpha*-amylcinnamyl alcohol was evaluated in a local lymph node assay (LLNA) which was conducted in 4 female CBA strain mice at concentrations 1%, 2.5%, 5%, 10% or 25% w/v in (1:3) ethanol: diethyphthalate. A 25 μl aliquot of *alpha*-amylcinnamyl alcohol was applied to the dorsum of each ear for 3 consecutive days. The control group was treated with 1:3 EtOH:DEP alone. After the third application all mice were injected in the tail vein with 250 μl of PBS (phosphate buffered saline) containing approximately 20μCi of a 2.0 Ci/mmol specific activity 3H-methyl thymidine 3H(Tdr). After 5 h all animals were sacrificed. The draining auricular lymph nodes were removed from each

animal and placed in a PBS container. Single cell suspensions were prepared, washed with PBS and suspended in trichloroacetic acid (TCA). After overnight precipitation at 4 °C the suspensions were pelleted by centrifugation and then resuspended in 1 ml of TCA. The lymph node suspensions were transferred to scintillation vials and 10 ml of scintillant was added prior to β-scintillation counting. A test material was regarded as a sensitizer if one or more concentrations of the test material elicited a 3-fold or greater increase in isotope incorporation relative to the vehicle control group. The (EC3) value was calculated to be 25% w/v (6250 μg/cm^2). Under the conditions of the study, *alpha*-amylcinnamyl alcohol was not considered to be a sensitizer. This study was conducted in compliance with the GLP guidelines and according to the OECD Testing Guidelines 429 and OPPTS Guidelines 870.2600 (RIFM, 2004a).

4.4.4.2. Using the above method, another LLNA was conducted on 25 CBA/J female mice (5/group). The animals were treated on the dorsal surface of each ear with *alpha*-amylcinnamyl alcohol at 7.5%, 15% or 30% concentration (w/v) in 1:3 EtOH:DEP, once a day for 3 consecutive days. On day 6, mice were injected, i.v., with 20 μCi 3 H-methyl thymidine 3H(Tdr) in saline. After five hours all animals were sacrificed; the draining auricular lymph nodes were removed and the incorporation of H-thymidine was determined. The test material at 7.5, 15 and 30% (w/v) resulted in SI of 0.80, 0.95, and 1.53, respectively. All the animals appeared normal throughout the study. No statistical differences in ear measurements or the SI of the test material and the vehicle control group were observed. Under the conditions of the study, *alpha*-amylcinnamyl alcohol was not considered to be a sensitizer. This study was conducted in compliance with the GLP guidelines and according to the OECD Testing Guidelines 429 and OPPTS Guidelines 870.2600 (RIFM, 2004b).

4.5. Phototoxicity and photoallergy

UV spectra revealed that *alpha*-amylcinnamyl alcohol peaked within 245–278 nm range and showed minor absorption in the 290–320 nm region.

4.6. Absorption, distribution and metabolism

4.6.1. Percutaneous absorption

4.6.1.1. In vitro studies in humans

4.6.1.1.1. Penetration of *alpha*-amylcinnamyl alcohol through human epidermis was studied using a glass chamber. Human lower abdominal skin was excised from a cadaver; the subcutaneous tissue was removed and the epidermis separated from dermis. The upper surface of the epidermis was fixed to a glass tube which was then placed inside one arm of a U-shaped glass chamber. A 0.5 ml aliquot of saline was added to the chamber and was in complete contact with the bottom of the epidermis. A 0.2 ml aliquot of *alpha*-amylcinnamyl alcohol was applied to the top of the epidermis. To avoid evaporation, parafilm was placed over the mouth of the glass tube. The chamber was kept at 21 °C and 55% relative humidity for 72 h. The glass tube was removed from the glass chamber at 72 h and the saline was poured into a test tube. The U-shaped chamber and the bottom of the epidermis attached to the glass tube were both washed 3 times with saline which was also poured into the same test tube. The final volume in the tube of both original saline and the saline used for washing was approximately 10 ml. Saturated salt water and ether were added to the flask and mixed vigorously. The compound was extracted in ether then dehydrated, filtered and condensed. A 2 μl aliquot of the condensed sample was injected into a Shimazu GC-6A gas chromatograph. The experiment was repeated 6 times. The amount of *alpha*-amylcinnamyl alcohol that penetrated human skin was minimal; the percent penetration ± S.E. through excised human skin was 0.012% ± 0.002% (Jimbo, 1983).

Table 8
Summary of LLNA studies

Concentration (%)	Specie	Results	Reference
1%, 2.5%, 5%, 10% or 25% in (1:3) EtOH: DEP	Female CBA strain mice	Negative $EC_3 > 25\%$ (6250 μg/cm^2)	RIFM (2004a)
7.5%, 15% or 30% in (1:3) EtOH:DEP	CBA/J female mice	Negative $EC_3 > 30\%$ (7500 μg/cm^2)	RIFM (2004b)

Table 9
Summary of mutagenicity and genotoxicity studies

Test method	Strain	Dose	Results	References
Ames with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535, TA1537, 1538	Doses up to 3600 μg/plate	Negative	Wild et al. (1983)
Basc test	*Drosophila melanogaster.* Berlin K (wild type) and Basc	45 mM	Negative	Wild et al. (1983)
Micronucleus test	NMRI mice	204, 357, 510 mg/kg in olive oil	Negative	Wild et al. (1983)

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity (Table 9)

4.9.1. Bacterial studies

4.9.1.1. In an Ames test (Ames et al., 1975) using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 with and without rat liver S9 metabolic activation, doses up to 3600 μg/plate in dimethyl sulfoxide were not mutagenic. The positive controls were 0.5 μg/plate sodium azide (strains TA1535 and TA100) and 5 μg/plate benzo[a]-pyrene (strains TA100, TA1537, TA1538 and TA98). No effects were observed (Wild et al., 1983).

4.9.2. Insect studies

4.9.2.1. A Basc test using Berlin K (wild type) and Basc strains was performed on *Drosophila melanogaster*. *alpha*-Amylcinnamyl alcohol was added to the diet at a dose level of 45 mM in 5% saccarose (with the possible addition of 2% ethanol and 2% Tween 80). No significant increases in sex-linked recessive lethal (SRL) mutations were observed (Wild et al., 1983), (see Table 9).

4.9.3. Mammalian studies

4.9.3.1. In a micronucleus test, groups of male and female NMRI mice (4/dose) were given a single intraperitoneal dose of *alpha*-amylcinnamyl alcohol at dose levels of 204, 357 and 510 mg/kg in olive oil. Animals were sacrificed and bone marrow smears were prepared 30 h after treatment. There was no evidence of a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in animals when compared to the vehicle control. *alpha*-Amylcinnamyl alcohol was determined to be non-genotoxic (Wild et al., 1983) (see Table 9).

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on benzyl cinnamate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

A toxicologic and dermatologic review of benzyl cinnamate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Benzyl cinnamate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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In 2006, a complete literature search was conducted on benzyl cinnamate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: γ-Phenylacrylate; Benzyl 3-phenylpropenoate; Phenylmethyl 3-phenyl-2-propenoate; Cinnamein; 2-Propenoic acid, 3-phenyl-, phenylmethyl ester; Cinnamic acid, benzyl ester.
1.2 CAS Registry No.: 103-41-3.
1.3 EINECS No.: 203-109-3.
1.4 Formula: $C_{16}H_{14}O_2$.
1.5 Molecular weight: 238.29.
1.6 Council of Europe: Benzyl cinnamate was included by the Council of Europe in the list of substances granted A – may be used in foodstuffs (COE No. 331) (Council of Europe, 2000).
1.7 FDA: Benzyl cinnamate was approved by the FDA as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and Extract Manufacturers' Association States: Generally Recognized as Safe as a flavor ingredient – GRAS 3. (2142) (FEMA, 1965).
1.9 Joint Expert Committee on Food Additives: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 670) concluded that benzyl cinnamate does not present a safety concern at current levels of intake when used as a flavoring agent (JECFA, 2000).

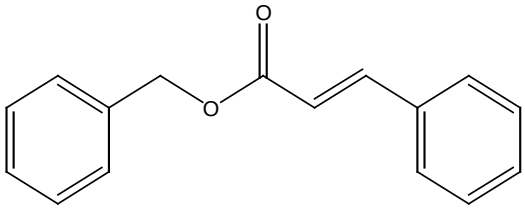

Fig. 1. Benzyl cinnamate.

2. Physical properties

2.1 Physical form: A white to pale yellow fused solid or crystal melting at very warm room temperature to a yellow liquid. It has a sweet balsamic odor.
2.2 Acid value (XV.B): 1.0 Max. 5.0 g.
2.3 Boiling point: 350 °C.
2.4 Congealing point (IE): 33.0–34.5 °C.
2.5 Flash point: >212 °F; CC.
2.6 Henry's Law (calculated): 0.000000334 atm m^3/mol 25 °C.
2.7 Log K_{OW} (calculated): 4.06.
2.8 Saponification value (XV.B): 1.0 Max 5.0 g.
2.9 Vapor pressure (calculated): <0.001 mm Hg at 20 °C.
2.10 Water solubility (calculated): 9.269 mg/l at 25 °C.

3. Usage (Table 1)

Benzyl cinnamate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 10–100 metric tonnes per annum.

The maximum skin level that results from the use of benzyl cinnamate in formulae that go into fine fragrances has been reported to be 0.89% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.0854% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0022 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Benzyl cinnamate dissolved in corn oil was administered via gavage to 10 Charles River rats (5/sex/dose) at dose levels of 2.0, 2.25, 3.0 and 5.0 g/kg/bodyweight. Observations were made for 14-days. At 2.0 and 2.25 g/kg, 2/10 deaths occurred; 4/10 deaths occurred at 3.0 g/kg. At 5.0 g/kg, 8/10 deaths occurred within 24 h post dosing, CNS effects were observed in these eight animals. No effects were observed at the lower doses. The LD_{50} was calculated to be 3.28 g/kg (19/20 CI 2.62–4.10 g/kg) (RIFM, 1972a).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing benzyl cinnamate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product (%)	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.0854	0.0003
Face cream	0.80	2.00	1.000	0.003	0.0854	0.0001
Eau de toilette	0.75	1.00	1.000	0.080	0.0854	0.0009
Fragrance cream	5.00	0.29	1.000	0.040	0.0854	0.0008
Antiperspirant	0.50	1.00	1.000	0.010	0.0854	0.0001
Shampoo	8.00	1.00	0.010	0.005	0.0854	0.0000
Bath products	17.00	0.29	0.001	0.020	0.0854	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.0854	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.0854	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.0854	0.0000
Total						0.0022

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rat	10 (5/sex)	3.28 g/kg (19/20 CI 2.62–4.10 g/kg)	RIFM (1972a)
Dermal	Rabbit	12 (4/dose)	~3 g/kg	RIFM (1972a)

4.1.1.2. Ten Osborne-Mendel rats (5/sex) were dosed orally with benzyl cinnamate. The rats were observed for 14 days. Deaths occurred between 4 h and 5 days. Depression and coma were the major clinical signs and they persisted for 24-h in some animals. The LD_{50} was calculated to be 5.53 g/kg (95% CI 3.10–7.74 g/kg) (Jenner et al., 1964; Bar and Griepentrog, 1967).

4.1.1.3. Using the above method, benzyl cinnamate was tested for toxicity in guinea pigs (number of animals not reported). Deaths occurred between 4 h and 6 days. Major clinical signs observed were depression, gastro-intestinal tract irritation and rectal bleeding. The oral LD_{50} was calculated to be 3.76 g/kg (95% CI 2.34–6.05 g/kg) (Jenner et al., 1964).

4.1.2. Dermal studies

4.1.2.1. Neat benzyl cinnamate was applied to healthy albino rabbits (4/dose) weighing 2–3 kg for 24-h under occlusion at dose levels of 1.0, 2.0 and 3.0 g/kg/bodyweight. Applications were made to clipped intact and abraded skin areas, which were later covered with a rubber sleeve or a dam. The animals were observed for 14-days. Initial and final hematogram values were compared. No toxic effects or deaths occurred. The LD_{50} is greater than 3.0 g/kg (RIFM, 1972a).

4.2. Skin irritation

4.2.1. Human studies (Table 3)

4.2.1.1. Irritation was evaluated during the induction phase of an human repeated insult patch test (HRIPT) conducted on 101 (25 male/76 female) volunteers. A 0.3 ml aliquot of 4% benzyl cinnamate in 3:1 diethyl phthalate: ethanol was applied for 24-h under occlusion, using a 25 mm webril/adhesive patch (Hill Top Chamber System®) on the backs of all the volunteers. A total of nine induction applications were made on a Monday, Wednesday and Friday schedule. No irritation was observed (RIFM, 2005a).

4.2.1.2. In a pre-test for a maximization test, no irritation was observed when 8% benzyl cinnamate in petrolatum was applied for 48-h under occlusion on the backs of five healthy, male volunteers (RIFM, 1972b).

4.2.1.3. Using the same procedure as above, another maximization pre-test was conducted with 8% benzyl cinnamate on five male and female volunteers. No irritation was observed (RIFM, 1975).

4.2.2. Animal studies (Table 4)

4.2.2.1. Prior to the induction phase of an open epicutaneous test, a preliminary irritation test with benzyl cinnamate at a range of concentrations was conducted. A 0.025 ml aliquot of the benzyl cinnamate (vehicle not specified) was applied to a 2 cm^2 area on the clipped flank of outbred

Table 3
Summary of irritation studies in humans

Method	Dose (%)	Results	Reference
HRIPT Induction phase	4% in 1:3 ethanol:diethyl phthalate	0/101	RIFM (2005a)
Maximization pre-test	8% in petrolatum	0/5	RIFM (1972b)
Maximization pre-test	8% in petrolatum	0/5	RIFM (1975)

Table 4
Summary of irritation studies in animals

Method	Dose (%)	Species	Results	Reference
Pre-test open epicutaneous test (24-h primary irritation)	A range of concentrations (vehicle not specified)	Himalayan white-spotted guinea pigs (6-8/sex/group)	3% = minimal irritating concentration (defined as lowest concentration producing mild erythema in at least 25% of animals)	Klecak et al. (1977)
Induction open epicutaneous test	A range of concentrations (vehicle not specified)	Himalayan white-spotted guinea pigs (6-8/sex/group)	3% = minimal irritating concentration	Klecak (1979)
Irritation evaluated as part of LD_{50} study	100%	Rabbit	Irritation was observed	RIFM (1972a)

Himalayan white-spotted guinea pigs (6–8/group). A single application was made. The application site was uncovered and reactions were read after 24 h. Benzyl cinnamate at 3% (vehicle not specified) was the lowest concentration to induce mild erythema in at least 25% of the animals and this concentration was selected as the minimal irritating concentration (Klecak et al., 1977).

4.2.2.2. As a part of an open epicutaneous test, irritation was evaluated during the 21-day induction period. A 0.1 ml aliquot of benzyl cinnamate at a range of concentrations (vehicle not specified) was applied to an area measuring 8 cm^2 on the clipped flank of 6–8 male and female outbred Himalayan white-spotted guinea pigs. The application sites were left uncovered and reactions were read after 24 h. The minimal irritating concentration after 21 daily applications was 3% (vehicle not specified) (Klecak et al., 1977).

4.2.2.3. As part of an associated dermal LD_{50} study (see Section 4.1.2.1), benzyl cinnamate was evaluated for irritation in 12 rabbits (4/dose). Moderate erythema was observed on day 1. The degree of erythema decreased after 24-h. After 48-h the treated areas returned to normal (RIFM, 1972a).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Dermal sensitization quantitative risk assessment (QRA)

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This new methodology represents a significant change over current risk assessment practices because it specifically addresses the elements of exposure-based risk assessment that are unique to the induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment.

Full details of this risk assessment approach can be found in the "QRA Expert Group, dermal sensitization Quantitative Risk Assessment (QRA) for Fragrance Ingredients, Technical Dossier, revised June 22, 2006", and "IFRA/RIFM Quantitative Risk Assessment (QRA) for Fragrance Ingredients Booklet, May 11, 2006", at http://www.rifm/org/pub/publications.asp and http://www.ifra-org.org/News.asp.

An exposure-based Quantitative Risk Assessment (QRA) methodology has been used to determine acceptable exposure limits for benzyl cinnamate and a new IFRA Standard (IFRA, 2007) has been issued (see Tables 5 and 6).

4.4.2. Human studies

4.4.2.1. Predictive studies (Table 7)

4.4.2.1.1. A human repeated insult patch test (HRIPT) was conducted on 101 (25 male/76 female) volunteers. During the induction phase, a 0.3 ml aliquot of 4% (4720 μg/cm^2) benzyl cinnamate in (1:3) EtOH:DEP was applied to a 25 mm Hill Top Chamber patch® which was then applied to the back for 24 h under occlusion. A series of nine induction applications were made during three successive weeks on a Monday, Wednesday and Friday schedule. After a 10–14 day rest day period, challenge patches with 4% (4720 μg/cm^2) benzyl cinnamate in (1:3) EtOH:DEP were applied to a previously untested site. After 24-h, the patches were removed and reactions were assessed 24, 48 and 72-h after application. No sensitization reactions (0/101) were observed (RIFM, 2005a).

4.4.2.1.2. A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 8% benzyl cinnamate (5520 μg/cm^2) concentration in petrolatum, on 25 healthy (11 male and 14 female) volunteers. Applications were made under occlusion to the same site on the forearms of all the volunteers for five alternate-day 48-h periods. Patch sites were pretreated for 24 h under occlusion with 5% aqueous sodium lauryl sulfate (SLS). Following a 10-day rest period, a challenge patch was applied to a different site for 48 h under occlusion. The challenge site was pretreated with SLS under occlusion. The challenge site was read on removal of patch and 24 h thereafter. No sensitization was observed (RIFM, 1975).

4.4.2.1.3. Using the same method as above, 8% benzyl cinnamate was tested in another maximization test that was conducted on 25 male volunteers. The challenge site was pretreated for 1 h with 10% SLS under occlusion. No sensitization was observed (RIFM, 1972b).

Table 5
IFRA standard based on the QRA

For a description of the categories, refer to the QRA information booklet			
Limits in the finished product			
Category 1 – see note (1)	0.1%	Category 7	0.4%
Category 2	0.2%	Category 8	2.0%
Category 3	0.7%	Category 9	5.0%
Category 4	2.1%	Category 10	2.5%
Category 5	1.1%	Category 11 – see note (2)	
Category 6 – see note (1)	3.4%		

Note. (1) IFRA would recommend that any material used to impart perfume or flavour in products intended for human ingestion should consist of ingredients that are in compliance with appropriate regulations for foods and food flavourings in the countries of planned distribution and, where these are lacking, with the recommendations laid down in the Code of Practice of IOFI (International Organisation of the Flavor Industry). Further information about IOFI can be found on its website (www.iofiorg.org). (2) Category 11 includes all non-skin contact or incidental skin contact products. Due to negligible skin contact the concentration of a fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product. For example, hypothetically if the usual concentration of a fragrance compound in the final product, for example a candle, is at 5%, then any individual fragrance ingredient (in this case benzyl cinnamate) must not exceed 5% in the candle.

Table 6
Summary of the relevant sensitization data for the implementation of the QRA

LLNA weighted mean EC3 values (μg/cm^2) [no. studies]	Human data			Potency classification[b]	WoE NESIL (μg/cm^2)[c]
	NOEL – HRIPT (induction) (μg/cm^2)	Experimental NOEL – MAX (induction) (μg/cm^2)	LOEL[a] (induction) (μg/cm^2)		
4600 [1]	4720	5517	NA	Weak	4700

[a] Data derived from HRIPT or HMT.
[b] Gerberick et al. (2001).
[c] WoE NESIL limited to two significant figures.

Table 7
Summary of predictive studies

Method	Test concentration	Results	References
HRIPT	4% in 1:3 EtOH:DEP (4720 μg/cm^2)	0/101	RIFM, 2005a
Maximization	8% in petrolatum (5520 μg/cm^2)	0/25	RIFM, 1975
Maximization	8% in petrolatum (5520 μg/cm^2)	0/25	RIFM, 1972b

4.4.2.2. Diagnostic studies (Table 8)

4.4.2.2.1. One hundred and 10 eczema patients, who had previously exhibited a reaction to Peru balsam when tested in a series of routine patch tests, were tested with 5% benzyl cinnamate in petrolatum in a 48-h closed patch test. The test material was applied using a Lysaplast Special® patch. The patch was applied to the anterior and lateral surfaces of the thigh and then covered with adhesive tape. Patches were removed after 48 h. Reactions were read 10–20 min after patch removal and again 72 or 96 h after patch removal. In addition, reactions were read again in 50% of the patients after 7–8 days. Reactions were observed in 21/110 patients to benzyl cinnamate (Hjorth, 1961a). Further, Hjorth (1961b) reported results from patch tests conducted on 103 subjects with 5% benzyl cinnamate in petrolatum. Reactions were observed in 19/103 patients.

4.4.2.2.2. One hundred and eighty-two patients who were suspected of having contact sensitization to cosmetics were patch tested with the standard ICDRG series and a fragrance series containing 22 fragrance raw materials. Reactions to 8% benzyl cinnamate in petrolatum were observed in 3.2% of the patients (Malten et al., 1983, 1984).

4.4.3. Animal studies (Table 9)

4.4.3.1. A guinea pig maximization test (Magnusson and Kligman, 1969) was conducted using outbred white-spotted Himalayan male and female guinea pigs weighing 400–500 g. The induction phase consisted of: intradermal injections on day 0, followed by a 48-h occluded patch on day 8. Intradermal injections consisted (each injection given twice) of 0.1 ml of 5% benzyl cinnamate; 0.1 ml of a 5% emulsion of benzyl cinnamate in FCA and 0.1 ml of FCA alone. On day 8, topical induction was conducted with 25% benzyl cinnmate in petrolatum for 48-h under occlusion. On day 21, the challenge was conducted via a 24-h closed patch with benzyl cinnamate at a subirritant concentration in petrolatum and was applied to the clipped flank. Reactions were read 24 and 48 h after patch removal. Sensitization was observed (Klecak et al., 1977).

Table 8
Summary of diagnostic studies

Method	Concentration	Results	References
Patch test	5% in petrolatum	21/110	Hjorth, 1961a
Patch test	5% in petrolatum	19/103	Hjorth, 1961b
Patch test	8% in petrolatum	6/182	Malten et al. (1983, 1984)

Table 9
Summary of guinea pig sensitization studies

Method	Concentration	Results	Reference
Guinea-pig maximization test	Intradermal induction 5% and 5% in FCA Topical Induction 25% in petrolatum challenge sub-irritant concentration	Sensitization was observed	Klecak et al. (1977)
Open epicutaneous test	3% (vehicle not specified)	No reactions	Klecak, 1979
Open epicutaneous test	8% (vehicle not specified)	No reactions	Klecak et al. (1977)
Open epicutaneous test	0.03–100% (vehicle not specified)	0.3%: minimum eliciting concentration 3%: minimum sensitization concentration	Klecak et al. (1977)
FCAT (Freund's complete adjuvant test)	Induction 50% in FCA challenge <10%	Sensitization was observed	Klecak et al. (1977)
Modified FCAT	Induction 1, 3 and 10% in FCA challenge 3% and 10% in acetone	Weak allergen 3% in acetone: 2/10 reactions 10% in acetone: 4/10 reactions	Hausen and Wollenweber (1988)
Modified FCAT	3% and 10% in acetone	Weak sensitization was observed	Hausen et al. (1995)
Modified Draize test	0.1% in isotonic saline	Sensitization was observed	Klecak et al. (1977)

4.4.3.2. Benzyl cinnamate at 3% and 8% did not produce any sensitization reactions when evaluated in an open epicutaneous test conducted on Himalayan male and female guinea pigs (Klecak, 1979 and 1985).

4.4.3.3. Reactions were observed to benzyl cinnamate at 0.3% and 3% concentrations (vehicle not reported) when tested for sensitization in an open epicutaneous test. Benzyl cinnamate at 0.3% was the minimum eliciting concentration while, 3% benzyl cinnamate was the minimum sensitizing concentration (Klecak et al., 1977).

4.4.3.4. Benzyl cinnamate was tested in a Freund's Complete Adjuvant Test (FCAT) in male and female outbred Himalayan guinea pigs weighing 400–500 g. The guinea pigs received five intradermal injections of a 0.1 ml aliquot of neat benzyl cinnamate and FCA as a 50:50 mixture, on days 0, 2, 4, 7 and 9. On days 21 and 35, a 24-h closed challenge patch was applied to the flanks at a sub-irritant concentration of benzyl cinnamate in petrolatum. Sensitization was observed (no further details reported) (Klecak et al., 1977).

4.4.3.5. A modified FCAT was conducted in female Pirbright white guinea pigs (10/dose). Six intradermal injections of benzyl cinnamate in physiologic saline containing FCA were made into the clipped and shaved shoulder area on days 1, 5 and 9 (for a total of 4.5 mg of the test material). The animals were challenged 11 days after induction with open applications of 0.05 ml aliquot of benzyl cinnamate at a sub-irritant dose in acetone. Reactions were read at 24, 48 and 72 h. Two (2/10) reactions were observed with 3% benzyl cinnamate in acetone; 4/10 reactions were observed with 10% benzyl cinnamate in acetone (Hausen and Wollenweber, 1988).

4.4.3.6. Weak sensitization effects were observed with both 3% and 10% benzyl cinnamate in acetone when tested in a modified FCAT (no further details reported) (Hausen et al., 1995).

4.4.3.7. Benzyl cinnamate was tested in another guinea pig sensitization study using a modified Draize procedure in male and female outbred Himalayan guinea pigs weighing 400–500 g. Induction consisted of 10 intradermal injections on alternate days (starting on day 0), with a 0.05 ml aliquot of 0.1% solution of benzyl cinnamate in isotonic saline. The animals were challenged on days 35 and 49 with an intradermal injection of 0.05 ml of a 0.1% solution of benzyl cinnamate in saline. Sensitization was observed (no further details reported) (Klecak et al., 1977).

4.4.4. Local lymph node assay (LLNA) (Table 10)

4.4.4.1. Sensitization was evaluated in a local lymph node assay (LLNA). Groups of four female CBA strain mice were tested with benzyl cinnamate at concentrations of 2.5, 5, 10, 25 or 50% w/v in (1:3) ethanol: diethyphthalate. A 25 μl aliquot of benzyl cinnamate was applied to the dorsum of each ear for three consecutive days. The control group was treated with 1:3 EtOH: DEP. After the third application, all mice were injected in the tail vein with 250 μl of phosphate buffered saline containing approximately 20 μCi of a 2.0 Ci/mmol specific activity 3H-methyl thymidine 3H(Tdr). After 5 h all animals were sacrificed. The draining auricular lymph nodes were removed from each animal and placed in a PBS container. Single cell suspensions were prepared, washed with PBS and suspended in trichloroacetic acid (TCA). After overnight precipitation at 4 °C the suspensions were pelleted by centrifugation and then resuspended in 1 ml of TCA. The lymph node suspensions were transferred to scintillation vials and 10 ml of scintillant was added prior to β-scintillation counting. A

Table 10
Summary of LLNA studies

Concentration (%)	Species	Results	Reference
2.5%, 5%, 10%, 25% or 50% w/v in (1:3) ethanol: diethyphthalate	Female CBA strain mice	$EC_3 = 18.4\%$ (4600 μg/cm^2)	RIFM, 2005a

test material was regarded as a sensitizer if one or more concentrations of the test material elicited a 3-fold or greater increase in isotope incorporation relative to the vehicle control group. The estimated benzyl cinnamate concentration giving rise to a 3-fold increase in lymphocyte (EC_3) proliferation was calculated to be 18.4% w/v (4600 μg/cm^2). Under the conditions of the study, benzyl cinnmate was considered to be a sensitizer (RIFM, 2005b).

4.5. Phototoxicity and photoallergy

UV spectra revealed that benzyl cinnamate peaked within 245–278 nm range and showed minor absorption in the 290–320 nm region.

4.6. Absorption, distribution and metabolism

4.6.1. Percutaneous absorption (Table 11)

4.6.1.2. In vivo studies in animals

4.6.1.2.1. Meyer and Meyer (1959) studied the skin absorption of benzyl cinnamate in five male mice. An area of 2.2 cm^2 on the shaved abdominal skin was used. Eserine (0.23%) was used as an indicator and the test material was used as a carrier for the eserine. The latency period between application to the skin and the appearance of the eserine effect in the periodically stimulated masticatory muscles was used as a measure of the absorption rate. There was no evidence that benzyl cinnamate was absorbed.

4.6.1.3. In vitro studies in animals

4.6.1.3.1. Benzyl cinnamate was evaluated as a skin-penetrating agent in excised guinea pig skin by assessing the depth to which Rhodamine B, as the active principle, penetrated the skin in the presence of 50% benzyl cinnamate (vehicle may have been ethylene glycol). Histological findings were evaluated in the epithelium, hair follicles, corium and subcutis. The authors defined genuine penetration as detection of the active principle in the corium or subcutis. After 2 h, Rhodamine B was slightly detectable in the epithelium. It was concluded that benzyl cinnamate did not enhance skin penetration of Rhodamine B (Meyer, 1965).

4.6.2. Metabolism

4.6.2.1. In vitro studies in animals

4.6.2.1.1. The relative rates of enzymatic hydrolysis of 26 esters of organic alcohols and acids were investigated using a preparation of pancreatin. Incubations were made in 0.5 M PO_4 buffer at pH 7.5 and 37 °C. The extent of hydrolysis was determined after 2 h by GC of the ester. Eighty percent (80%) hydrolysis was measured when 18 mg/l benzyl cinnamate was incubated with simulated intestinal fluid (Grundschober, 1977; RIFM, 1974).

4.7. Subchronic toxicity (Table 12)

4.7.1. Oral studies

4.7.1.1. A 19-week oral toxicity study was conducted in rats with benzyl cinnamate. Ten male and female weanling Osborne-Mendel rats (5/sex/dose) received benzyl cinnamate by dietary admixture at doses of 0, 1000 and 10,000 ppm [∼equivalent to 0, 50 and 500 mg/kg/bodyweight/day] for 19 weeks. Weight, food intake and general condition were recorded weekly. Hematological examinations that included white cell counts, red cell counts, hemoglobin and hematocrits were conducted at the termination of the study. On completion of the study all surviving animals were sacrificed and examined macroscopically. Organ weights were recorded and tissues were preserved for histopathologic examination. Detailed microscopic examinations were done on six or eight animals, evenly divided by sex, in the high dose group only. There were no mortalities or adverse clinical signs. There were no effects on growth or hematology and no macroscopic or microscopic changes in the tissues were observed. The no-effect-level (NOEL) was concluded to be 10,000 ppm (∼equivalent to 500 mg/kg/bodyweight/day) (Hagan et al., 1967; Bar and Griepentrog, 1967).

4.7.1.2. Groups of 10 rats (5/sex) were fed benzyl cinnamate at dose levels of 0, 1000 and 10,000 ppm [∼equivalent to 0, 50 and 500 mg/kg/bodyweight/day] (vehicle not reported) for 19 weeks. A group of 20 rats served as controls. Gross and microscopic examinations were performed

Table 11
Summary of in-vivo and in-vitro skin absorption studies

Test Method	Dose	Species	Results	References
In vivo skin absorption	Not reported	Mice area = 2.2 cm^2	No absorption	Meyer and Meyer (1959)
In vitro skin absorption Benzyl cinnamate was evaluated as a skin-penetrating agent in excised guinea pig skin by assessing the depth to which Rhodamine B, as the active principle	50% (vehicle not reported)	Guinea pig	Benzyl cinnamate did not enhance skin penetration of Rhodamine B	Meyer (1965)

Table 12
Summary of subchronic toxicity studies

Test Method	Dose	Species	Results	References
19-week study	50 and 500 mg/kg/day	Rats (5/sex/dose)	NOEL – 500 mg/kg/bodyweight/day	Hagan et al. (1967)
19-week study	50 and 500 mg/kg/day	Rats (5/sex)	NOEL – 500 mg/kg/bodyweight/day	RIFM (1954)

Table 13
Summary of mutagenicity studies

Test Method	Strain	Dose	Results	References
Modified Ames	*Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537	715 μg/plate in ethanol	No effects Sample precipitated	Florin et al. (1980)
Rec assay	*Bacillus subtilis* strains H17 (rec^+) and M45 (rec^-)	1000 μg/disk in dimethyl sulfoxide	Negative	Yoo (1986)

on all animals. No effects were observed. The NOEL was concluded to be 10,000 ppm (~equivalent to 500 mg/kg/bodyweight/day) (RIFM, 1954).

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity (Table 13)

4.9.1. Bacterial Studies

4.9.1.1. In a spot test for mutagenicity, based on the Ames test (Ames et al., 1975) using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 with and without S9 from aroclor-induced rats, a dose of 3 μmol/plate [~equivalent to 715 μg/plate] benzyl cinnamate in ethanol precipitated (Florin et al., 1980).

4.9.1.2. In a rec assay using *Bacillus subtilis* strains H17 (rec^+) and M45 (rec^-), Yoo (1986) reported that a dose of 1000 μg /disk benzyl cinnamate in dimethyl sulfoxide produced no effects.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on butyl cinnamate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc. 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

A toxicologic and dermatologic review of butyl cinnamate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Butyl cinnamate

Contents

In 2006, a complete literature search was conducted on butyl cinnamate. On-line databases that were surveyed included Chemical Abstract Services and the National

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: *n*-Butyl cinnamate; Butyl β-phenylacrylate; *n*-Butyl phenylacrylate; Butyl 3-phenylpropenoate; 2-Propenoic acid, 3-phenyl-, butyl ester.
1.2 CAS Registry Number: 538-65-8.
1.3 EINECS Number: 208-699-6.
1.4 Formula: $C_{13}H_{16}O_2$.
1.5 Molecular weight: 204.27.
1.6 Council of Europe: Butyl cinnamate was included by the Council of Europe in the list of substances granted B – information required – none listed (COE No. 326) (Council of Europe, 2000).
1.7 FDA: Butyl cinnamate was approved by the FDA as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient – GRAS 3. (2192) (FEMA, 1965).
1.9 Joint Expert Committee on Food Additives (JECFA): The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 663) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent (JECFA, 2000).

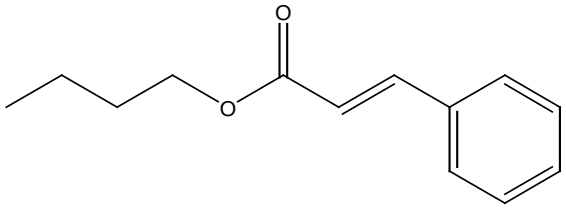

Fig. 1. Butyl cinnamate.

2. Physical properties

2.1 Physical form: A colorless oily liquid.
2.2 Boiling point: 271 °C.
2.3 Flash point: >200 °F; CC.
2.4 Log K_{ow} (calculated): 3.83.
2.5 Specific gravity: 1.01.
2.6 Vapor pressure (calculated): 0.549 mm Hg 25 °C.
2.7 Water solubility (calculated): 22.29 mg/l @ 25 °C.

3. Usage (Table 1)

Butyl cinnamate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of less than 0.1 metric tonnes per annum.

The maximum skin level that results from the use of butyl cinnamate in formulae that go into fine fragrances has been reported to be 0.10% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.5% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0127 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} in rats exceeded 5.0 g/kg, based on 0/10 deaths at that dose. Ten rats were dosed orally with butyl cinnamate at 5.0 g/kg/bodyweight.

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing butyl cinnamate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product%	Ingredient/ mixture[a]	Ingredient (mg/kg/day[b])
Body lotion	8.00	0.71	1.000	0.004	0.5	0.0019
Face cream	0.80	2.00	1.000	0.003	0.5	0.0004
Eau de toilette	0.75	1.00	1.000	0.080	0.5	0.0050
Fragrance cream	5.00	0.29	1.000	0.040	0.5	0.0048
Antiperspirant	0.50	1.00	1.000	0.010	0.5	0.0004
Shampoo	8.00	1.00	0.010	0.005	0.5	0.0000
Bath products	17.00	0.29	0.001	0.020	0.5	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.5	0.0001
Toilet soap	0.80	6.00	0.010	0.015	0.5	0.0001
Hair spray	5.00	2.00	0.010	0.005	0.5	0.0000
Total						0.0127

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Clinical signs observed during the study included piloerection and diarrhea. Gross necropsy was carried out on all animals. Necropsy revealed dark lungs in one animal and mottled and pale kidney in two animals (RIFM, 1977a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} exceeded 5.0 g/kg based on 0/10 deaths at that dose. Ten rabbits received a single dermal application of neat butyl cinnamate at 5.0 g/kg/bodyweight which was applied for 24 h under occlusion. The animals were observed over a 14 day period. Gross necropsy was conducted on all animals. No clinical signs were observed during the study. Necropsy revealed anogenital exudate in four, dark lungs in two, and dark liver in seven rabbits; kidneys were dark in one rabbit and mottled in another and there were yellow areas in the intestines of two rabbits (RIFM, 1977a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed following a 48-h closed patch test with 4% butyl cinnamate in petrolatum on the backs or volar forearms of 25 healthy, male and female volunteers (RIFM, 1977b).

4.2.2. Animal studies

4.2.2.1. In an associated dermal LD_{50} study, (see Section 4.1.2.1), irritant reactions to neat butyl cinnamate consisted of mild (5/10 rabbits) to moderate erythema (4/10 rabbits) and mild (4/10 rabbits) to moderate edema (6/10 rabbits) (RIFM, 1977a).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 4% (2760 μg/cm^2) butyl cinnamate in petrolatum on 25 healthy (17 male/8 female) volunteers. Applications were made under occlusion to the same site on the forearm or back of each subject for five alternate-day 48-h periods. Patch sites were pretreated for 24 h with 2.5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a 10-day rest period, a challenge patch was applied to a different site on the back for 48 h under occlusion. The challenge sites were pretreated for 1 h with 5–10% aqueous SLS under occlusion. Reactions were read at patch removal and 24 h after patch removal. No sensitization was observed (RIFM, 1977b).

Table 2
Summary of acute toxicity data

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rat	10	>5.0 g/kg	RIFM (1977a)
Dermal	Rabbit	10	>5.0 g/kg	RIFM (1977a)

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

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Review

Fragrance material review on cinnamyl acetate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc. 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

A toxicologic and dermatologic review of cinnamyl acetate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Cinnamyl acetate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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In 2006, a complete the literature search was conducted on cinnamyl acetate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: 3-Phenylallyl acetate; 3-Phenyl-2-Propen-1-yl acetate; 2-Propen-1-ol, 3-Phenyl-, acetate.
1.2 CAS Registry Number: 103-54-8.
1.3 EINECS Number: 203-121-9.
1.4 Formula: $C_{11}H_{12}O_2$.
1.5 Molecular Weight: 176.22.
1.6 Council of Europe: Cinnamyl acetate was included by the Council of Europe in the list of substances granted B – information required – hydrolysis study (COE No. 208) (Council of Europe, 2000).
1.7 FDA: Cinnamyl acetate was approved by the FDA as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and extract manufacturers' association states: Generally Recognized as Safe as a flavor ingredient – GRAS 3. (2293) (FEMA, 1965).
1.9 Joint Expert Committee on Food Additives (JECFA): The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 650) concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent (JECFA, 2000).

2. Physical properties

2.1 Physical form: Colorless to slightly yellow, oily liquid with sweet, balsamic, floral odor.
2.2 Boiling point: 113 °C.
2.3 Flash point: >200 °F; CC.
2.4 Henry's Law (calculated): 0.0000103 atm m^3/mol 25C.
2.5 Log K_{ow} (calculated): 2.85.
2.6 Specific gravity: 1.05.

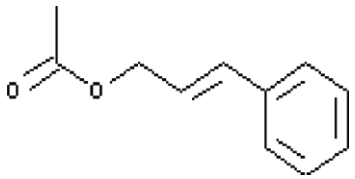

Fig. 1. Cinnamyl acetate.

2.7 Vapor pressure (calculated): 0.008 mm Hg at 20 °C.
2.8 Water solubility (calculated): 212.3 mg/l @ 25 °C.

3. Usage (Table 1)

Cinnamyl acetate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 10 to 100 metric tonnes per annum.

The maximum skin level that results from the use of cinnamyl acetate in formulae that go into fine fragrances has been reported to be 0.62% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.453% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0115 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Groups of rats (10/dose) were dosed orally with cinnamyl acetate at dose levels of 1.46, 2.22, 3.33 or 5.0 g/kg/bodyweight. Observations were made for 14 days. No deaths occurred at the 1.46 g/kg; 1/10 deaths occurred at 2.22 g/kg; 6/10 deaths at 3.33 g/kg and 10/10 at 5.0 g/kg. All deaths occurred within the first 48 h. The LD_{50} was calculated to be 3.3 g/kg (95% C.I. 2.9–3.7 g/kg). Clinical signs observed during the study included slow respiration, lethargy, depression and coarse tremors in high doses (RIFM, 1972a).

4.1.1.2. Groups of white rats, white mice and guinea pigs were dosed orally with a 20–45% solution of cinnamyl acetate in sunflower oil. For each species, the animals were tested 3/sex/dose and were observed for 15 days. The LD_{50} for all three species was reported to be 4.75 g/kg (no further details reported) (Zaitsev and Rakhmanina, 1974).

4.1.2. Dermal studies

4.1.2.1. The dermal LD_{50} in rabbits exceeded 5.0 g/kg based on 0/10 deaths at that dose. Ten rabbits received a single dermal application of neat cinnamyl acetate at a dose level of 5.0 g/kg/bodyweight which was applied for 24 h under occlusion. Observations were made over a 14-day period. No effects were observed during the study (RIFM, 1972a).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing cinnamyl acetate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product%	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.453	0.0017
Face cream	0.80	2.00	1.000	0.003	0.453	0.0004
Eau de toilette	0.75	1.00	1.000	0.080	0.453	0.0045
Fragrance cream	5.00	0.29	1.000	0.040	0.453	0.0044
Antiperspirant	0.50	1.00	1.000	0.010	0.453	0.0004
Shampoo	8.00	1.00	0.010	0.005	0.453	0.0000
Bath products	17.00	0.29	0.001	0.020	0.453	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.453	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.453	0.0001
Hair spray	5.00	2.00	0.010	0.005	0.453	0.0000
Total						0.0115

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity data

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rat	10	3.3 g/kg (95% C.I. 2.9–3.7 g/kg)	RIFM, 1972a
Dermal	Rabbit	10	>5.0 g/kg	RIFM, 1972a
I.P	Mouse	not specified	1.2 g/kg	Powers et al., 1961

Table 3
Summary of irritation studies in humans

Method	Dose (%)	Results	References
Maximization pre-test	5% in petrolatum	No irritation was observed	RIFM, 1972b
48-h semi-occluded patch test	32% in acetone	Irritation observed in 10–40% of subjects	Motoyoshi et al., 1979

4.1.3. Intraperitoneal studies

4.1.3.1. The intraperitoneal LD_{50} in male Swiss albino mice was calculated to be 1.2 g/kg. The number of mice tested was not reported (Powers et al., 1961).

4.2. Skin irritation

4.2.1. Human studies (Table 3)

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed after a 48-h closed patch test with 5% cinnamyl acetate in petrolatum on the backs of 5 healthy, male volunteers (RIFM, 1972b).

4.2.1.2. A 48-h semi-occluded patch test with 32% cinnamyl acetate in acetone was conducted on 50 male volunteers. A 0.05 ml aliquot of cinnamyl acetate was applied to a 15 mm patch which was then applied to the back of all the volunteers. After 48 h, the patches were removed and the residual test materials were swabbed with dry gauze. Reactions were read 30 min later and if needed, subsequent readings were performed at 72, 96 and 120 h. Cinnamyl acetate at 32% in acetone was considered to be mildly irritating as irritation was observed in 10–40% of the subjects (Motoyoshi et al., 1979).

4.2.2. Animal studies (Table 4)

4.2.2.1. Six Pitman–Moore Improved strain miniature swine received a single dermal application of 0.05 g of neat cinnamyl acetate on the clipped dorsal skin for 48 h under occlusion. The patches were secured in place with an adhesive tape, and the trunk was wrapped with rubberized cloth. After the 48-h exposure period, the patches were removed, and the reactions were then evaluated. No irritation was observed (Motoyoshi et al., 1979).

4.2.2.2. Cinnamyl acetate was evaluated for irritation in 6 male Hartley guinea pigs weighing 350–500 g. Prior to application, hair on 2 areas measuring 3×3 cm in the dorsal mid-lumbar region of each animal was clipped. Approximately 24 h later, a 0.1 ml aliquot of neat cinnamyl acetate was applied directly to the skin. Reactions were assessed after 24-h, after which the sites were again clipped free of hair and the test material was applied 30 min later. A second set of readings and applications were made 48 h later (72-h reading). Following the 72-h reading, all the hair on the dorsal surface of each animal was clipped and 40 mg/kg of Evans blue dissolved in physiological saline was injected intravenously into each animal. Mild irritation was observed (Motoyoshi et al., 1979).

Table 4
Summary of irritation studies in animals

Method	Dose (%)	Species	Results	References
A 48-h closed patch test	100	Miniature swine	No irritation	Motoyoshi et al., 1979
Open application	100	Guinea pig	Mild irritation	Motoyoshi et al., 1979
Open application	100	Rabbit	Moderate irritation	Motoyoshi et al., 1979

4.2.2.3. A dermal irritation study on rabbits was conducted, using neat cinnamyl acetate. Groups of 6 albino Angora rabbits received a dermal application of 0.1 ml of neat cinnamyl acetate on the clipped dorsal skin. The animals were then wrapped with a plastic collar around the neck for a 24-h period. The collar was then removed, and reactions were read. A second set of readings and applications were made using the same method as the above irritation test in guinea pigs (see Section 4.2.2.2). Neat cinnamyl acetate produced moderate irritation (Motoyoshi et al., 1979).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 5% (3450 μg/cm^2) cinnamyl acetate in petrolatum on 25 healthy, male volunteers. Application was under occlusion to the same site on the forearms of all subjects for five alternate-day 48-h periods. Patch sites were pretreated for 24 h with 5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a 10-day rest period, challenge patches were applied under occlusion to fresh sites for 48 h. Challenge applications were preceded by 1-h applications of 10% SLS under occlusion. The challenge sites were read on removal of patch and 24 h thereafter. No sensitization reactions were observed (RIFM, 1972b).

4.5. Phototoxicity and photoallergy

UV spectra revealed that cinnamyl acetate peaked within 245–278 nm range and showed minor absorption in the 290–320 nm region.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity (Table 5)

4.9.1. Bacterial studies

4.9.1.1. In an Ames test (Ames et al., 1975), cinnamyl acetate was tested in triplicates at concentrations up to 5000 μg/plate, in S*almonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537, in the presence and absence of the S9 mix. The vehicle used was dimethyl sulfoxide. No mutagenic effects were observed. This study was conducted according to the GLP (Good Laboratory Practice) guidelines and OECD Guideline 471; EEC Directive 67/548/EEC, Part B (RIFM, 2003).

4.9.1.2. Prior to conducting a mutagenicity assay, a dose range finding test was conducted with S. *typhimurium* strains TA100 and TA98 both with and without S9 mix. In strain TA98, toxicity was observed at 3330 and 5000 μg/plate in the absence of S9-mix and at 5000 μg/plate in the presence of S9-mix. In strain TA100, toxicity was observed at 3330 and 5000 μg/plate in the absence and presence of S9 mix (RIFM, 2003).

4.9.2. Mammalian studies

4.9.2.1. An *in vitro* cytogenetic assay in Chinese hamster ovary cells was conducted using cinnamyl acetate at concentrations 1.0–100 μM. CHO–K_1 cells were cultured in the presence or absence of cinnamyl acetate for a cell cycle. For the analysis of sister-chromatid exchanges (SCEs), bromodeoxyuridine (final concentration 5 μM) was added two cell cycles before fixation. After addition of bromodeoxyuridine, the cultures were incubated in total darkness. Cells were then treated with colchicine for 2 h at a final concentration of 50 μg/ml. Preparations were processed using a modified Giemsa procedure and harlequin-

Table 5
Summary of mutagenicity and genotoxicity studies

Test method	Strain	Dose	Results	References
Ames test	S. *typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537	up to 5000 μg/plate	Negative	RIFM, 2003
Sister-chromatid exchange	Chinese hamster ovary cells (CHO–K_1)	1.0–100 μM	Negative (highest dose tested was toxic)	Sasaki et al., 1989

stained chromosomes in 50 metaphases per culture were analyzed for SCEs. No significant increases in the mean SCE frequency were observed. The highest dose tested (100 μM) was toxic (Sasaki et al., 1989).

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on cinnamyl benzoate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, United States*
[b] *Manheimer Fragrances, Teterboro NJ, United States*

Abstract

A toxicologic and dermatologic review of cinnamyl benzoate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Cinnamyl benzoate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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In 2006, a complete literature search was conducted on cinnamyl benzoate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: 3-Phenyl-2-propen-1-yl benzoate; 2-Propen-1-ol, 3-phenyl-, benzoate.
1.2 CAS Registry Number: 5320-75-2.
1.3 EINECS Number: 226-180-2.
1.4 Formula: $C_{16}H_{14}O_2$.
1.5 Molecular Weight: 238.29.
1.6 Council of Europe: Cinnamyl benzoate was included by the Council of Europe in the list of substances granted B – information required – hydrolysis study (COE No. 743) (Council of Europe, 2000).
1.7 FDA: Cinnamyl benzoate was approved by the FDA as a flavor (21 CFR 172.515).
1.8 Joint Expert Committee on Food Additives (JECFA): The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 760) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent (JECFA, 2000).

2. Physical properties

2.1 Physical form: A white crystalline powder with an aromatic, spicy, balsamic odor.
2.2 Flash point: >200 °F;CC.

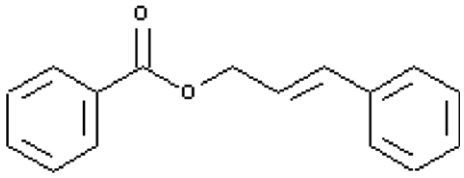

Fig. 1. Cinnamyl benzoate.

2.3 Henry's Law (calculated): 0.00000204 atm m^3/mol at 25 °C.
2.4 Log K_{ow} (calculated): 4.3.
2.5 Vapor Pressure (calculated): 0.000039 mm Hg at 25 °C.
2.6 Water Solubility (calculated): 5.8 mg/l at 25 °C.

3. Usage (Table 1)

Cinnamyl benzoate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of less than 0.1 metric tonnes per annum.

The maximum skin level that results from the use of cinnamyl benzoate in formulae that go into fine fragrances has been reported to be 0.10% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.5% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0127 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Groups of rats (10/dose) were dosed orally with cinnamyl benzoate at doses of 2.56, 3.2, 4.0 or 5.0 g/kg/bodyweight. Observations were made for 14 days. No

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing cinnamyl benzoate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product (%)	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.5	0.0019
Face cream	0.80	2.00	1.000	0.003	0.5	0.0004
Eau de toilette	0.75	1.00	1.000	0.080	0.5	0.0050
Fragrance cream	5.00	0.29	1.000	0.040	0.5	0.0048
Antiperspirant	0.50	1.00	1.000	0.010	0.5	0.0004
Shampoo	8.00	1.00	0.010	0.005	0.5	0.0000
Bath products	17.00	0.29	0.001	0.020	0.5	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.5	0.0001
Toilet soap	0.80	6.00	0.010	0.015	0.5	0.0001
Hair spray	5.00	2.00	0.010	0.005	0.5	0.0000
Total						0.0127

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

deaths occurred at the 2 lowest dose levels; 5/10 deaths occurred at 4.0 g/kg and 9/10 deaths occurred at 5.0 g/kg. All deaths occurred within the first 3 days. Slight lethargy was observed among the survivors in the 4.0 and 5.0 g/kg dose groups. No symptoms were observed at 2.56 and 3.2 g/kg dose levels. The LD_{50} was calculated to be 4.0 g/kg (95% C.I. 3.56–4.44 g/kg) (RIFM, 1975a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg, based on 0/10 deaths at that dose. Ten rabbits received a single dermal application of neat cinnamyl benzoate which was applied for 24 h under occlusion. Observations were made for 14 days. No effects were observed (RIFM, 1975a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed after a 48-h closed patch test with 5% cinnamyl benzoate in petrolatum on the forearms of 5 healthy, male and female volunteers (RIFM, 1975b).

4.2.2. Animal studies

4.2.2.1. Irritation was evaluated as part of an associated dermal LD_{50} study (see Section 4.1.2.1.). Slight (1/10 rabbits) to moderate erythema (1/10 rabbits) and slight (1/10 rabbits) to moderate edema (2/10 rabbits) were observed (RIFM, 1975a).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human Studies

4.4.1.1. A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 5% (3450 μg/cm^2) cinnamyl benzoate in petrolatum on the forearms of 25 healthy, (10 male and 15 female) volunteers. Application was made under occlusion to the same site on the forearms of all subjects for five alternate-day 48-h periods. Patch sites were pretreated for 24 h with 5% aqueous sodium lauryl sulfate (SLS) under occlusion for the initial patch only. Following a 10-day rest period, challenge applications were applied to fresh sites on all the volunteers. Challenge applications were preceded by applications of SLS under occlusion. The challenge sites were read upon patch removal and 24 h thereafter. No (0/25) sensitization reactions were observed (RIFM, 1975b).

Table 2
Summary of acute toxicity data

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rat	10	4.0 g/kg (95% C.I. 3.56–4.44 g/kg)	RIFM, 1975a
Dermal	Rabbit	10	>5.0 g/kg	RIFM, 1975a

4.5. Phototoxicity and photoallergy

UV spectra revealed that cinnamyl benzoate peaked within 245–278 nm range and showed minor absorption in the 290–320 nm region.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

4.7.1. Oral studies

4.7.1.1. Cinnamyl benzoate was tested in a 14-day feeding study in male albino rats. Twenty-four male albino rats (6/dose), weighing 40–60 g received cinnamyl benzoate by dietary admixture at concentrations of 0, 0.5, 1.0 or 2.0% [0, ~750, 1500 and 3000 mg/kg-bw/day]. Cinnamyl benzoate was incorporated into basal ration (Purina Laboratory Chow plus vitamins). The control group received the basal diet alone. All animals were fed ad libitum. Daily inspections were made for appearance and behavior. Body weight and food intake was recorded semi-weekly, and the efficiency of food utilization (EFU) was calculated at the conclusion of the study. No deaths occurred and behavior and appearance were normal for all animals. Statistically significant growth depression was observed in treated animals in all 3 dose groups. Food intake and food utilization were also statistically significantly depressed in low- and high-dose animals. Decreased food intake was noted in mid-dose animals but was not statistically significant. The depressed food intake was attributed to poor palatability. Gross observations at necropsy were normal for all animals (RIFM, 1958).

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl

Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on cinnamyl butyrate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

A toxicologic and dermatologic review of cinnamyl butyrate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Cinnamyl butyrate

Contents

In 2006, a complete literature search was conducted on cinnamyl butyrate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Butanoic acid, 3-phenyl-2-propenyl ester; 3-Phenylallyl butyrate; 3-Phenyl-2-propen-1-yl butanoate.
1.2 CAS Registry No.: 103-61-7.
1.3 EINECS No.: 203-128-7.
1.4 Formula: $C_{13}H_{16}O_2$.
1.5 Molecular weight: 204.27.
1.6 Council of Europe: Cinnamyl butyrate was included by the Council of Europe in the list of substances granted B – information required – hydrolysis study (COE No. 279) (Council of Europe, 2000).
1.7 FDA: Cinnamyl butyrate was approved by the FDA as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient – GRAS 3. (2296) (FEMA, 1965).
1.9 Joint Expert Committee on Food Additives: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 652) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent (JECFA, 2000).

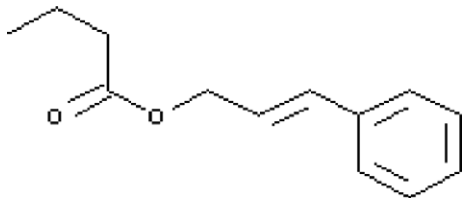

Fig. 1. Cinnamyl butyrate.

2. Physical properties

2.1 Physical form: Colorless to yellowish liquid with fruity, slightly floral odor.
2.2 Flash point: >200 °F;CC.
2.3 Henry's Law: 0.0000182 atm m^3/mol 25 C.
2.4 Log K_{ow} (calculated): 3.83.
2.5 Vapor pressure (calculated): 0.001 mm Hg at 20 °C.
2.6 Water solubility (calculated): 22.29 mg/l @ 25 °C.

3. Usage (Table 1)

Cinnamyl butyrate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of less than 0.1 metric tonnes per annum.

The maximum skin level that results from the use of cinnamyl butyrate in formulae that go into fine fragrances has been reported to be 0.020% (IFRA, 2001), assuming use of the fragrance oil at levels up to 2% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.1% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0025 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} in rats exceeded 5.0 g/kg, based on 0/10 deaths at that dose. Ten rats were dosed orally with cinnamyl butyrate at a dose of 5.0 g/kg/bodyweight.

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing cinnamyl butyrate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product (%)	Ingredient/mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.1	0.0004
Face cream	0.80	2.00	1.000	0.003	0.1	0.0001
Eau de toilette	0.75	1.00	1.000	0.080	0.1	0.0010
Fragrance cream	5.00	0.29	1.000	0.040	0.1	0.0010
Antiperspirant	0.50	1.00	1.000	0.010	0.1	0.0001
Shampoo	8.00	1.00	0.010	0.005	0.1	0.0000
Bath products	17.00	0.29	0.001	0.020	0.1	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.1	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.1	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.1	0.0000
Total						0.0025

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Observations were made for 14 days. No effects were observed (RIFM, 1976a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg, based on 0/4 deaths at that dose. Four rabbits received a single dermal application of neat cinnamyl butyrate which was applied for 24 h under occlusion. Observations were made for 14 days. No effects were observed (RIFM, 1976a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed after a 48-h closed patch test with 4% cinnamyl butyrate in petrolatum on the backs of 29 healthy, male volunteers (RIFM, 1976b).

4.2.2. Animal studies

4.2.2.1. Irritation was evaluated as part of the associated dermal LD_{50} study (see Section 4.1.2.1). Erythema that lasted for 24-h, was the only dermal reaction observed (RIFM, 1976a).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (Modified after Kligman, 1966; Kligman and Epstein, 1975) was carried out with 4% (2760 μg/cm^2) cinnamyl butyrate in petrolatum on 29 healthy, male volunteers. Application was made under occlusion to the same site on the forearms of all volunteers for five alternate-day 48-h periods. Patch sites were pre-treated for 24 h under occlusion with 5% aqueous sodium lauryl sulfate (SLS) for the initial patch only. Following a 10–14 day rest period, challenge patches were applied under occlusion to fresh sites for 48 h. Challenge applications were preceded by 30-min applications of 5% aqueous SLS under occlusion on the left side of the back, whereas the test material without SLS treatment was applied on the right side. A fifth site was challenged with SLS on the left and petrolatum controls on the right. No (0/29) sensitization was observed (RIFM, 1976b).

Table 2
Summary of acute toxicity data

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rat	10	>5.0 g/kg	RIFM (1976a)
Dermal	Rabbit	4	>5.0 g/kg	RIFM (1976a)

4.5. Phototoxicity and photoallergy

UV spectra revealed that cinnamyl butyrate peaked within 245–278 nm range and showed minor absorption in the 290–320 nm region.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufactures of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufactures of fragrances and consumer products containing fragrances.

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RIFM (Research Institute for Fragrance Materials, Inc.), 1976b. Report on human maximization studies. RIFM report number 1796, April 26 (RIFM, Woodcliff Lake, NJ, USA).

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Review

Fragrance material review on cinnamyl cinnamate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

A toxicologic and dermatologic review of Cinnamyl cinnamate when used as a fragrance ingredient is presented.

Keywords: Fragrance; Review; Cinnamyl cinnamate

Contents

In 2006, a complete literature search was conducted on cinnamyl cinnamate. On-line databases that were surveyed

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Phenylallyl cinnamate; 3-Phenylallyl cinnamate; 3-Phenyl-2-propen-1-yl 3-phenylpropenoate; 2-Propenoic acid, 3-phenyl-, 3-phenyl-2-propenyl ester.
1.2 CAS Registry Number: 122-69-0.
1.3 EINECS Number: 204–566-1.
1.4 Formula: $C_{18}H_{16}O_2$.
1.5 Molecular Weight: 264.33.
1.6 Council of Europe: Cinnamyl cinnamate was included by the Council of Europe in the list of substances granted B - information required - 28 day oral study (COE No. 332) (Council of Europe, 2000).
1.7 FDA: Cinnamyl cinnamate was approved by the FDA as a flavor (21 CFR 172.515).

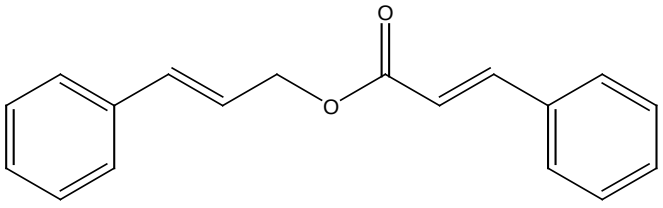

Fig. 1. Cinnamyl cinnamate.

1.8 FEMA: Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient - GRAS 3. (2298) (FEMA, 1965).
1.9 Joint Expert Committee on Food Additives (JECFA): The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 673) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent (JECFA, 2000).

2. Physical properties

2.1 Physical form: White or colorless crystals.
2.2 Flash point: >200°F;CC.
2.3 Henry's Law (calculated): 0.000000243 atm m^3/mol 25 °C.
2.4 Log K_{ow} (calculated): 4.83.
2.5 Vapor pressure: <0.001 mm Hg at 20 °C.
2.6 Water solubility (calculated): 3.116 mg/l @ 25 °C.

3. Usage (Table 1)

Cinnamyl cinnamate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 0.1–1 metric tonnes per annum.

The maximum skin level that results from the use of Cinnamyl cinnamate in formulae that go into fine fragrances has been reported to be 0.24% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.238% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0061 mg/kg for high end users of these products.

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing cinnamyl cinnamate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product(%)	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.238	0.0009
Face cream	0.80	2.00	1.000	0.003	0.238	0.0002
Eau de toilette	0.75	1.00	1.000	0.080	0.238	0.0024
Fragrance cream	5.00	0.29	1.000	0.040	0.238	0.0023
Antiperspirant	0.50	1.00	1.000	0.010	0.238	0.0002
Shampoo	8.00	1.00	0.010	0.005	0.238	0.0000
Bath products	17.00	0.29	0.001	0.020	0.238	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.238	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.238	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.238	0.0000
Total						0.0061

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} in rats was reported to be 4.2 g/kg, based on 5/10 deaths at that dose. Ten rats were dosed orally with Cinnamyl cinnamate at a dose of 5.0 g/kg/bodyweight. Observations were made for 14 days. All deaths occurred within 24–72-h. The only clinical effect observed was enteritis (RIFM, 1974a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg, based on 0/10 deaths at that dose. Ten rabbits received a single dermal application of neat Cinnamyl cinnamate which was applied for 24 h under occlusion. Observations were made for 14 days. No effects were observed (RIFM, 1974a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed after a 48-h closed patch test with 4% Cinnamyl cinnamate in petrolatum on the backs of 5 healthy, male and female volunteers (RIFM, 1974b).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 4% (2760 μg/cm^2) Cinnamyl cinnamate in petrolatum on 25 healthy, (13 male/12 female) volunteers. Application was under occlusion to the same site on the forearms of all subjects for five alternate-day 48-h periods. Patch sites were pretreated for 24 h with 5% aqueous sodium lauryl sulfate (SLS) under occlusion. Challenge applications were preceded by applications of SLS under occlusion. Following a 10 day rest period, a challenge patch was applied to a fresh site for 48 h under occlusion. Challenge applications were preceded by applications of SLS under occlusion. Reactions to the challenge were read at patch removal and 24 h thereafter. No sensitization reactions were observed (RIFM, 1974b).

Table 2
Summary of acute toxicity data

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rat	10	4.2 g/kg	RIFM (1974a)
Dermal	Rabbit	10	>5.0 g/kg	RIFM (1974a)

4.4.2. Animal studies

4.4.2.1. Weak sensitization effects were observed with both 3% and 10% Cinnamyl cinnamate in acetone, when tested in a modified Freund's Complete Adjuvant Test (FCAT) (Hausen et al., 1992, 1995).

4.5. Phototoxicity and photoallergy

UV spectra revealed that Cinnamyl cinnamate peaked within 245–278 nm range and showed minor absorption in the 290–320 nm region.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

4.7.1. Oral studies

4.7.1.1. A mixture of flavorings containing 897 ppm cinnamaldehyde and 25 ppm each of methyl cinnamate, ethyl cinnamate, Cinnamyl cinnamate, and α-methyl-cinnamaldehyde was added to the diet of rats for 12 weeks, resulting in the approximate daily intake of 110 mg/kg/bodyweight (male) and 119 mg/kg/bodyweight (female) (roughly equivalent to 103 mg/kg/bodyweight of cinnamaldehyde and 3 mg/kg/bodyweight of the other components). Each diet was fed ad libitum to a group of 24 rats (12/sex) with initial body weights of 50 to 70 g. Weekly observations were made of growth and food intake. Records were made of physical appearance and behavior. After 12 weeks, urinalysis was conducted on 6 animals (3/sex) and blood hemoglobin levels were determined. Respiratory infections were observed in rats in both test and control groups; one male in the control group died due to pulmonary pathology. Gross necropsy was conducted on all animals. Blood hemoglobin, urinalysis, liver and kidney weights, food intake, behavior and appearance were normal in both sexes. Depressed growth was observed in the male rats but was not considered statistically significant. Efficiency of food utilization (EFU) was significantly depressed in both sexes (RIFM, 1958).

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual fragrance material review is not intended as a stand alone document. Please refer to the Toxicologic

and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol when Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on cinnamyl formate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, United States*
[b] *Manheimer Fragrances, Teterboro NJ, United States*

Abstract

A toxicologic and dermatologic review of cinnamyl formate when used as a fragrance ingredient is presented.

Keyword: Cinnamyl formate

Contents

In 2006, a complete literature search was conducted on cinnamyl formate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

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* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: 3-Phenylallyl formate; 3-Phenyl-2-propen-1-yl formate; 2-Propen-1-ol, 3-phenyl-, formate.
1.2 CAS Registry No.: 104-65-4.
1.3 EINECS No.: 203-223-3.
1.4 Formula: $C_{10}H_{10}O_2$.
1.5 Molecular weight: 162.19.
1.6 Council of Europe: Cinnamyl formate was included by the Council of Europe in the list of substances granted B – information required – hydrolysis study (COE No. 352) (Council of Europe, 2000).
1.7 FDA: Cinnamyl formate was approved by the FDA as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient – GRAS 3. (2299) (FEMA, 1965).
1.9 Joint Expert Committee on Food Additives: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 649) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent (JECFA, 2000).

2. Physical properties

2.1 Physical form: Colorless to slightly yellow liquid, possessing a balsamic odor with a cinnamon background.
2.2 Flash point: >200 °F; CC.
2.3 Flash point: 212 °F; CC.
2.4 Henry's law (calculated): 0.0000141 atm m^3/mol, 25 °C.
2.5 Log K_{ow} (calculated): 2.3.
2.6 Refractive index: 1.5500–1.5560 (20 °C).
2.7 Specific gravity: 1.08.
2.8 Specific gravity 25 °C: 1.074–1.079.
2.9 Vapor pressure (calculated): 0.02 mm Hg at 20 °C.
2.10 Water solubility (calculated): 725.1 mg/l @ 25 °C.

3. Usage (Table 1)

Cinnamyl formate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 0.1–1 metric tonnes per annum.

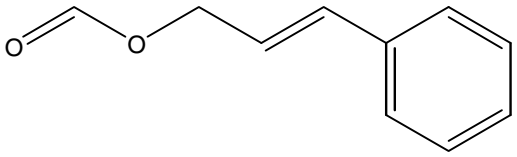

Fig. 1. Cinnamyl formate.

The maximum skin level that results from the use of cinnamyl formate in formulae that go into fine fragrances has been reported to be 0.01% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.04% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0010 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Rats (10/dose) were dosed orally with cinnamyl formate at dose levels of 2.0, 2.5, 3.2, 4.0 or 5.0 g/kg/bodyweight. Observations were made for 7 days. At 2.0 g/kg, 1/10 deaths occurred; 4/10 deaths occurred at 2.5 g/kg; 7/10 deaths at 3.2 g/kg; 9/10 deaths at 4.0 g/kg and 9/10 deaths were observed at 5.0 g/kg. A majority of the deaths occurred between days 1 and 2. The LD_{50} was calculated to be 2.9 g/kg (95% C.I. 2.38–3.54 g/kg). Ataxia and mucoid enteritis were observed (no further details reported) (RIFM, 1973a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg, based on 0/6 deaths at that dose. Six rabbits received a single dermal application of neat cinnamyl formate which was applied for 24 h under occlusion. Observations were made for 14 days. No clinical effects were observed (RIFM, 1973a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed after a 48-h closed patch test with 4% cinnamyl formate in petrolatum on the backs of 5 healthy, male volunteers (RIFM, 1973b).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 4% (2760 μg/cm^2) cinnamyl formate in petrolatum on 25 healthy, male volunteers. Application was made under occlusion to the same site on the forearms of all subjects for five alternate-day 48-h periods. Patch sites were pre-treated for 24 h with

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing cinnamyl formate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product (%)	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.04	0.0002
Face cream	0.80	2.00	1.000	0.003	0.04	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.04	0.0004
Fragrance cream	5.00	0.29	1.000	0.040	0.04	0.0004
Antiperspirant	0.50	1.00	1.000	0.010	0.04	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.04	0.0000
Bath products	17.00	0.29	0.001	0.020	0.04	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.04	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.04	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.04	0.0000
Total						0.0010

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity data

Route	Species	No. animals/ dose group	LD_{50}	References
Oral	Rat	10	2.9 g/kg (95% C.I. 2.38–3.54 g/kg)	RIFM (1973a)
Dermal	Rabbit	6	>5.0 g/kg	RIFM (1973a)

5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a 10-day rest period, challenge patches were applied under occlusion to fresh sites for 48 h. The challenge applications were preceded by a 1-h application of 10% aqueous SLS under occlusion. The challenge sites were read on removal of the patch and 24 h thereafter. No sensitization was observed (RIFM, 1973b).

4.5. Phototoxicity and photoallergy

UV spectra revealed that cinnamyl formate peaked within 245–278 nm range and showed minor absorption in the 290–320 nm region.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and development toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on cinnamyl isobutyrate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc. 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

A toxicologic and dermatologic review of cinnamyl isobutyrate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Cinnamyl isobutyrate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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In 2006, a complete literature search was conducted on cinnamyl isobutyrate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Cinnamyl 2-methylpropanoate; 3-Phenyl-2-propen-1-yl isobutyrate; 3-Phenyl-2-propen-1-yl 2-methylpropanoate; Propanoic acid, 2-methyl-, 3-phenyl-2-propenyl ester.
1.2 CAS Registry number: 103-59-3.
1.3 EINECS number: 203-126-6.
1.4 Formula: $C_{13}H_{16}O_2$.
1.5 Molecular weight: 204.27.
1.6 Council of Europe: Cinnamyl isobutyrate (COE No. 496) was included by the Council of Europe in the list of substances granted B – information required – hydrolysis study on one of the related cinnamyl compounds: cinnamyl acetate (No. 208), cinnamyl butyrate (No. 279), cinnamyl formate (No. 352), cinnamyl propionate (No. 414), and cinnamyl isovalerate (454) (Council of Europe, 2000).
1.7 FDA: Cinnamyl isobutyrate was approved by the FDA, as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and Extract Manufacturers' Association states: Generally recognized as safe as a flavor ingredient – GRAS 3 (2297) (FEMA, 1965).
1.9 Joint Expert Committee on Food Additives (JECFA): The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 653) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent (JECFA, 2000).

2. Physical properties

2.1 Physical form: colorless to slightly yellow liquid, with a sweet balsamic, fruity character.
2.2 Flash point: >200 °F; CC (FMA).
2.3 Flash point: >212 °F; CC (Givaudan index, 1961).
2.4 Henry's law (calculated): 0.0000182 atm m^3/mol 25 °C.
2.5 Log K_{ow} (calculated): 3.76.
2.6 Refractive index: 1.5230–1.5280 (20 °C).
2.7 Specific gravity: 1.01.
2.8 Specific gravity: 1.008–1.014 (25 °C).

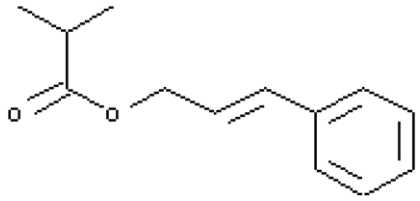

Fig. 1. Cinnamyl isobutyrate.

2.9 Vapor pressure (calculated): 0.002 mm Hg at 20 °C.
2.10 Water solubility (calculated): 25.75 mg/l @ 25 °C.

3. Usage (Table 1)

Cinnamyl isobutyrate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 0.1–1 metric tonnes per annum.

The maximum skin level that results from the use of cinnamyl isobutyrate in formulae that go into fine fragrances has been reported to be 0.02% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.019% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0005 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} exceeded 5.0 g/kg, based on 0/10 deaths at that dose. Ten rats were dosed orally with cinnamyl isobutyrate at a dose of 5.0 g/kg/bodyweight. Observations were made for 14 days. Gross necropsy was conducted on all animals. Clinical signs observed during the study included lethargy and diarrhea. Coma was observed in one animal. Gross observations at necropsy included enlarged spleen in two animals, and dried fecal matter anogenitally in one animal. Gross observations at necropsy were normal for all other animals (RIFM, 1977a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg, based on 0/10 deaths at that dose. Ten rabbits received a single dermal application of neat cinnamyl isobutyrate which was applied for 24 h under occlusion. Observations were made for 14 days. Gross necropsy was conducted on all animals. Gross observations at necropsy included dark spots in the lungs of three animals, dark liver in three

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing cinnamyl isobutyrate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product%	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.019	0.0001
Face cream	0.80	2.00	1.000	0.003	0.019	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.019	0.0002
Fragrance cream	5.00	0.29	1.000	0.040	0.019	0.0002
Antiperspirant	0.50	1.00	1.000	0.010	0.019	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.019	0.0000
Bath products	17.00	0.29	0.001	0.020	0.019	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.019	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.019	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.019	0.0000
Total						0.0005

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity data

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rat	10	>5.0 g/kg	RIFM, 1977a
Dermal	Rabbit	10	>5.0 g/kg	RIFM, 1977a

animals and red intestines in one animal. Gross observations at necropsy were normal for other animals (RIFM, 1977a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed after a 48-h closed patch test with 4% cinnamyl isobutyrate in petrolatum, on the backs of 31 healthy, male volunteers (RIFM, 1977b).

4.2.2. Animal studies

4.2.2.1. Irritation was evaluated as part of the associated acute dermal LD_{50} (see Section 4.1.2.1). Slight (9/10 rabbits) to moderate erythema (1/10 rabbits) and slight edema (10/10 rabbits) was observed (RIFM, 1977a).

4.3. Mucous membrane (eye) irritation

No data available on this test material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (modified after Kligman, 1966; Kligman and Epstein, 1975) was carried out with 4% (2760 μg/cm^2) cinnamyl isobutyrate in petrolatum on 31 healthy, male volunteers. Application was under occlusion to the same site on the forearms of all subjects for five alternate day 48-h periods. Patch sites were pretreated for 24 h with 5% aqueous SLS (sodium lauryl sulfate) under occlusion for the initial patch only. Following a 10–14 day rest period, challenge patches were applied under occlusion to fresh sites for 48 h. Challenge applications were preceded by 30-min applications of 5% aqueous SLS under occlusion on the left side of the back, whereas test material without SLS treatment was applied on the right side. A fifth site was challenged with SLS on the left and petrolatum control on the right. No sensitization was observed (RIFM, 1977b).

4.5. Phototoxicity and photoallergy

UV spectra revealed that cinnamyl isobutyrate peaked within 245–278 nm range and showed minor absorption in the 290–320 nm region.

4.6. Absorption, distribution and metabolism

No data available on this test material.

4.7. Subchronic toxicity

No data available on this test material.

4.8. Reproductive and developmental toxicity

No data available on this test material.

4.9. Mutagenicity and genotoxicity

No data available on this test material.

4.10. Carcinogenicity

No data available on this test material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related

Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on cinnamyl isovalerate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

A toxicologic and dermatologic review of cinnamyl isovalerate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Cinnamyl isovalerate

Contents

In 2006, a complete literature search was conducted on cinnamyl isovalerate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Butanoic acid, 3-methyl-, 3-phenyl-2-propenyl ester; Cinnamyl 3-methylbutanoate; 3-Phenylallyl isovalerate; 3-Phenylallyl 3-methylbutanoate; 3-Phenyl-2-propen-1-yl 3-methylbutanoate.
1.2 CAS Registry No.: 140-27-2.
1.3 EINECS No.: 205-407-9.
1.4 Formula: $C_{14}H_{18}O_2$.
1.5 Molecular weight: 218.39.
1.6 Council of Europe: Cinnamyl isovalerate (COE No. 454) was included by the Council of Europe in the list of substances granted B – information required – 28 day oral study on cinnamyl alcohol (COE No. 65); hydrolysis study on one of the related cinnamyl compounds: cinnamyl alcohol (No. 65), cinnamyl acetate (No. 208), cinnamyl butyrate (No. 279), cinnamyl formate (No. 352), cinnamyl propionate (No. 414) and cinnamyl isobutyrate (No. 496); (COE No. 454) (Council of Europe, 2000).
1.7 FDA: Cinnamyl isovalerate was approved by the FDA as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient – GRAS 3. (2302) (FEMA, 1965).
1.9 Joint Expert Committee on Food Additives (JECFA): The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 654) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent (JECFA, 2000).

2. Physical properties

2.1 Physical form: Colorless to slightly yellow liquid, with a pungent spicy-fruity odor.
2.2 Flash point: >200 °F; CC; >212 °F; CC.
2.3 Henry's Law (calculated): 0.0000241 atm m^3/mol 25 °C; CC.
2.4 Log K_{ow} (calculated): 4.25.

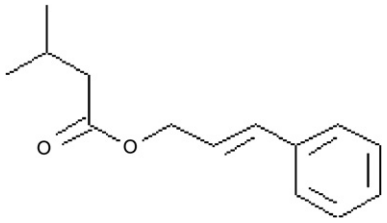

Fig. 1. Cinnamyl isovalerate.

2.5 Refractive index: 1.5180–1.5240 (20 °C).
2.6 Specific gravity: 0.991–0.995 (25 °C).
2.7 Vapor pressure: <0.001 mm Hg at 20 °C.
2.8 Water solubility (calculated): 8.282 mg/l @ 25 °C.

3. Usage (Table 1)

Cinnamyl isovalerate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of less than 0.1 metric tonnes per annum.

The maximum skin level that results from the use of cinnamyl isovalerate in formulae that go into fine fragrances has been reported to be 0.002% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.03% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0008 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} in rats was reported to be 5.0 g/kg, based on 5/10 deaths at that dose. Ten rats were dosed orally with cinnamyl isovalerate at a dose of 5.0 g/kg/bodyweight. Observations were made for 14 days. Deaths occurred on days 2, 6 and 12. The only clinical sign observed during the study was the loss of the righting reflex in 4/10 animals (RIFM, 1973a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg, based on 0/10 deaths at that dose. Ten rabbits received a single dermal application of neat cinnamyl isovalerate which was applied for 24 h under occlusion. Observations were made for 14 days. No clinical effects were observed (RIFM, 1973a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed after a 48-h closed patch test with 2% cinnamyl isovalerate in petrolatum on the backs of five healthy, male volunteers (RIFM, 1973b).

4.3. Mucous membrane (eye) irritation

No data available on this material.

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing cinnamyl isovalerate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product (%)	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.03	0.0001
Face cream	0.80	2.00	1.000	0.003	0.03	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.03	0.0003
Fragrance cream	5.00	0.29	1.000	0.040	0.03	0.0003
Antiperspirant	0.50	1.00	1.000	0.010	0.03	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.03	0.0000
Bath products	17.00	0.29	0.001	0.020	0.03	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.03	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.03	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.03	0.0000
Total						0.0008

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity data

Route	Species	No. animals/ dose group	LD_{50}	References
Oral	Rat	10	$\geqslant$5.0 g/kg	RIFM (1973a)
Dermal	Rabbit	10	>5.0 g/kg	RIFM (1973a)

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 2% (1380 μg/cm^2) cinnamyl isovalerate in petrolatum on 25 healthy, male volunteers. Application was made under occlusion to the same site on the forearms of all subjects for five alternative 48-h periods. Patch sites were pretreated for 24 h under occlusion with 5% aqueous SLS (sodium lauryl sulfate). Following a 10-day rest period, challenge patches were applied under occlusion to fresh sites for 48 h. Challenge applications were preceded by one hour applications of 10% aqueous SLS under occlusion. The challenge sites were read on removal of the patch and 24 h thereafter. No sensitization reactions were observed (RIFM, 1973b).

4.5. Phototoxicity and photoallergy

UV spectra revealed that cinnamyl isovalerate peaked within 245–278 nm range and showed minor absorption in the 290–320 nm region.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on cinnamyl propionate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

A toxicologic and dermatologic review of cinnamyl propionate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Cinnamyl propionate

Contents

In 2006, a complete literature search was conducted on cinnamyl propionate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: 3-Phenylallyl propionate; 3-Phenyl-2-propenyl propanoate; 3-Phenyl-2-propen-1-yl propionate; 2-Propen-1-ol, 3-phenyl-, propanoate.
1.2 CAS Registry Number: 103-56-0.
1.3 EINECS Number: 203-124-5.
1.4 Formula: $C_{12}H_{14}O_2$.
1.5 Molecular weight: 190.24.
1.6 Council of Europe: Cinnamyl propionate was included by the Council of Europe in the list of substances granted B – information required – hydrolysis study (COE No. 414) (Council of Europe, 2000).
1.7 FDA: Cinnamyl propionate was approved by the FDA as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and Extract Manufacturers' Association states: Generally recognized as safe as a flavor ingredient – GRAS 3. (2301) (FEMA, 1965).
1.9 Joint Expert Committee on Food Additives (JECFA): The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 651) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent (JECFA, 2000).

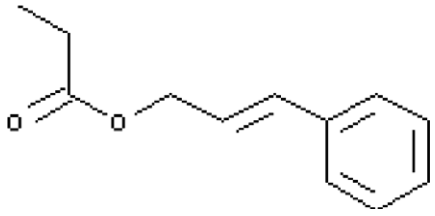

Fig. 1. Cinnamyl propionate.

2. Physical properties

2.1 Physical form: A colorless to slightly yellow liquid, with a fruity-floral odor.
2.2 Flash point: >200 °F; CC.
2.3 Henry's Law (calculated): 0.0000137 atm m^3/mol 25 C.
2.4 Log K_{ow} (calculated): 3.34.
2.5 Refractive index: 1.5320–1.5370 (20 °C).
2.6 Specific gravity: 1.03; 1.029–1.033 (25 °C).
2.7 Vapor pressure (calculated): 0.003 mm Hg at 20 °C.
2.8 Water Solubility (calculated): 68.97 mg/l @ 25 °C.

3. Usage (Table 1)

Cinnamyl propionate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 0.1–1 metric tonnes per annum.

The maximum skin level that results from the use of cinnamyl propionate in formulae that go into fine fragrances has been reported to be 0.02% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.092% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0023 mg/kg for high end users of these products.

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing cinnamyl propionate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product (%)	Ingredient/mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.092	0.0003
Face cream	0.80	2.00	1.000	0.003	0.092	0.0001
Eau de toilette	0.75	1.00	1.000	0.080	0.092	0.0009
Fragrance cream	5.00	0.29	1.000	0.040	0.092	0.0009
Antiperspirant	0.50	1.00	1.000	0.010	0.092	0.0001
Shampoo	8.00	1.00	0.010	0.005	0.092	0.0000
Bath products	17.00	0.29	0.001	0.020	0.092	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.092	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.092	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.092	0.0000
Total						0.0023

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Rats (10/dose) were dosed orally with cinnamyl propionate at dose levels of 2.5, 3.2, 4.0 and 5.0 g/kg/bodyweight. Observations were made for 14 days. At 2.56 g/kg, 1/10 deaths occurred; 2/10 deaths occurred at 3.2 g/kg; 5/10 deaths occurred at 4.0 g/kg and 9/10 deaths occurred at 5.0 g/kg. All deaths occurred on days 1 and 2. Clinical signs observed during the study included lethargy and coma which were observed at dose levels of 4.0 and 5.0 g/kg. The LD_{50} was calculated to be 3.4 g/kg (95% C.I. 3.2–3.6 g/kg) (RIFM, 1973a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg based on 1/10 deaths at that dose. Ten rabbits received a single dermal application of neat cinnamyl propionate which was applied for 24 h under occlusion. Observations were made for 14 days. One animal died on day 11. Anorexia and diarrhea were observed in the 1 animal that died (RIFM, 1973a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed after a 48-h closed patch test with 4% cinnamyl propionate in petrolatum on the backs of 5 healthy, male volunteers (RIFM, 1973b).

4.2.2. Animal studies

4.2.2.1. Irritation was evaluated as part of the associated acute dermal LD_{50} study (see Section 4.1.2.1.). Moderate (4/10 rabbits) to slight erythema (5/10 rabbits) and slight edema (1/10 rabbits) were observed (RIFM, 1973a).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 4% (2760 μg/cm^2) cinnamyl propionate in petrolatum on 25 healthy, male volunteers. Application was under occlusion to the same site on the forearms of all subjects for five alternate-day 48-h periods. Patch sites were pretreated for 24 h with 5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a 10-day rest period, challenge patches were applied under occlusion to fresh sites for 48 h. Challenge applications were preceded by 1-hour applications of 10% aqueous SLS under occlusion. Challenge sites were read on removal of the patch and 24 h thereafter. No sensitization reactions were observed (RIFM, 1973b).

Table 2
Summary of acute toxicity data

Route	Species	No. animals/ dose group	LD_{50}	References
Oral	Rat	10	3.4 g/kg (95% C.I. 3.2–3.6 g/kg)	RIFM (1973a)
Dermal	Rabbit	10	>5.0 g/kg	RIFM (1973a)

4.5. Phototoxicity and photoallergy

UV spectra revealed that cinnamyl propionate peaked within 245–278 nm range and showed minor absorption in the 290–320 nm region.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letiziza and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on cinnamyl tiglate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

A toxicologic and dermatologic review of cinnamyl tiglate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Cinnamyl tiglate

Contents

In 2006, a complete literature search was conducted on cinnamyl tiglate. On-line databases that were surveyed included chemical abstract services and the National Library of Medicine. In addition, fragrance companies

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: 2-Butenoic acid, 2-methyl-, 3-phenyl-2-propenyl ester, (E)-; Cinnamyl *trans*-2-methyl-2-butenoate; Cinnamyl α-methylcrotonate; Cinnamyl 2-methylcrotonate.
1.2 CAS registry number: 61792-12-9.
1.3 EINECS number: 263-215-0.
1.4 Formula: $C_{14}H_{16}O_2$.
1.5 Molecular weight: 216.28.

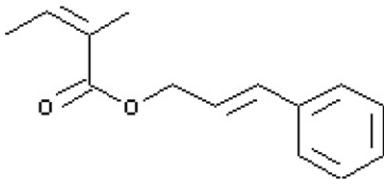

Fig. 1. Cinnamyl tiglate.

2. Physical properties

2.1 Physical form: A colorless oily liquid.
2.2 Acid value: 0.30.
2.3 Ester value after Acetylation: 97.24%.
2.4 Henry's Law (calculated): 0.0000134 atm m^3/mol 25 °C.
2.5 Log K_{ow} (calculated): 4.16.
2.6 Refractive index: 1.5510 (20 °C).
2.7 Specific gravity: 1.0342 (25 °C).
2.8 Vapor pressure (calculated): 0.00132 mm Hg 25 °C.
2.9 Water solubility (calculated): 10.04 mg/l @ 25 °C.

3. Usage (Table 1)

Cinnamyl tiglate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of less than 0.1 metric tonnes per annum.

The maximum skin level that results from the use of cinnamyl tiglate in formulae that go into fine fragrances has been reported to be 0.002% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.01% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0003 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} in rats exceeded 5.0 g/kg, based on 0/10 deaths at that dose. Ten rats were orally administered cinnamyl tiglate at a dose of 5.0 g/kg/bodyweight. Observations were made for 14 days. No effects were observed (RIFM, 1975a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg, based on 0/4 deaths at that dose. Four rabbits received a single dermal application of neat cinnamyl tiglate which was applied for 24 h under occlusion.

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing cinnamyl tiglate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product (%)	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.01	0.0000
Face cream	0.80	2.00	1.000	0.003	0.01	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.01	0.0001
Fragrance cream	5.00	0.29	1.000	0.040	0.01	0.0001
Antiperspirant	0.50	1.00	1.000	0.010	0.01	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.01	0.0000
Bath products	17.00	0.29	0.001	0.020	0.01	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.01	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.01	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.01	0.0000
Total						0.0003

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity data

Route	Species	No. animals/ dose group	LD_{50}	References
Oral	Rat	10	> 5.0 g/kg	RIFM (1975a)
Dermal	Rabbit	4	> 5.0 g/kg	RIFM (1975a)

Observations were made for 14 days. No clinical signs were observed (RIFM, 1975a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed after a 48-h closed patch test with 4% cinnamyl tiglate in petrolatum on the backs of 24 healthy, male volunteers (RIFM, 1975b).

4.2.2. Animal studies

4.2.2.1. Irritation was evaluated in four rabbits, as part of an associated dermal LD_{50} study (see Section 4.1.2.1). No irritation was observed (RIFM, 1975a).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (Modified after Kligman, 1966; Kligman and Epstein, 1975) was carried out with 4% (2760 μg/cm^2) cinnamyl tiglate in petrolatum on 24 healthy, male volunteers. Application was made under occlusion to the same sites on the forearms of all volunteers for five alternate-day 48-h periods. The patch sites were pretreated for 24 h with 5% aqueous sodium lauryl sulfate (SLS) under occlusion for the initial patch only. After a 10–14 day rest period, challenge patches were applied under occlusion to fresh sites for 48 h. Challenge applications were preceded by 30-min applications of 2% aqueous SLS under occlusion on the left side of the back, whereas the test material without SLS treatment was applied on the right side. A fifth site challenged with SLS on the left, and petrolatum on the right served as the control. One subject reacted to all four materials that were tested in this group (RIFM, 1975b). Upon re-testing, this subject did not react to cinnamyl tiglate and it was concluded that the original reaction was non-specific in nature. Under the conditions of the study, cinnamyl tiglate was considered to be non-sensitizing (RIFM, 1975c).

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

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Review

Fragrance material review on ethyl cinnamate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

A toxicologic and dermatologic review of ethyl cinnamate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Ethyl cinnamate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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In 2006, a complete literature search was conducted on ethyl cinnamate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Ethyl phenylacrylate; Ethyl 3-phenyl-propenoate; 2-Propenoic acid, 3-phenyl-, ethyl ester; Ethyl benzylideneacetate; Ethyl 3-phenyl-2-propenoate; 3-Phenyl-2-propenoic acid, ethyl ester.
1.2 CAS Registry Number: 103-36-6.
1.3 EINECS Number: 203-104-6.
1.4 Formula: $C_{11}H_{12}O_2$.
1.5 Molecular weight: 176.22.
1.6 Council of Europe: Ethyl cinnamate was included by the Council of Europe in the list of substances granted B – information required – 28 day oral study; hydrolysis study (COE No. 323) (Council of Europe, 2000).
1.7 FDA: Ethyl cinnamate was approved by the FDA as a flavor (21 CFR 172.515).

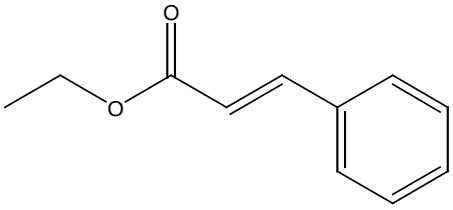

Fig. 1. Ethyl cinnamate.

1.8 FEMA: Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient – GRAS 3 (2430) (FEMA, 1965).
1.9 Joint Expert Committee on Food Additives (JECFA): The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 659) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent (JECFA, 2000).

2. Physical properties

2.1 Physical form: A colorless liquid with a sweet balsamic-honey note.
2.2 Boiling point: 271 °C.
2.3 Flash point: 200 F; CC.
2.4 Henry's law (calculated): 0.0000055 atom m^3/mol 25 °C.
2.5 Log K_{ow} (calculated): 2.85.
2.6 Refractive index: 1.5590–1.5610 (20 °C).
2.7 Refractive index: 1.5596.
2.8 Specific gravity: 1.0469.
2.9 Specific gravity: 1.047.
2.10 Specific gravity: 1.045–1.048 (25 °C).
2.11 Vapor pressure (calculated): 0.01 mm Hg at 20 °C.

3. Usage (Table 1)

Ethyl cinnamate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 1–10 metric tonnes per annum.

The maximum skin level that results from the use of ethyl cinnamate in formulae that go into fine fragrances has been reported to be 0.13% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing ethyl cinnamate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product (%)	Ingredient/mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.013	0.0000
Face cream	0.80	2.00	1.000	0.003	0.013	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.013	0.0001
Fragrance cream	5.00	0.29	1.000	0.040	0.013	0.0001
Antiperspirant	0.50	1.00	1.000	0.010	0.013	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.013	0.0000
Bath products	17.00	0.29	0.001	0.020	0.013	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.013	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.013	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.013	0.0000
Total						0.0003

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

cosmetics in general has been reported to be 0.013% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0003 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Groups of white rats, white mice and guinea pigs were dosed perorally with a 20–45% solution of ethyl cinnamate in sunflower oil (0.2–0.5 ml/100 g bodyweight). For each species, the animals were tested 3/sex/dose and observed over a 15-day period. The fructose diphosphate aldolase in blood serum and cholinesteranse levels in the blood increased. The LD_{50} for all three species was reported to be 4.0 g/kg/bodyweight (22.6 mM/kg/bodyweight) (Zaitsev and Rakhmanina, 1974).

4.1.1.2. The acute oral LD_{50} in rats was reported to be 1.52 g/kg. An unspecified number of rats were dosed via gavage with ethyl cinnamate (Bar and Griepentrog, 1967).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5 g/kg based on 0/10 deaths at that dose. Ten rabbits received a single dermal application of neat ethyl cinnamate which was applied for 24 h under occlusion. Observations were made over a 14-day period. No clinical effects were observed (RIFM, 1973a).

4.1.3. Intramuscular studies

4.1.3.1. Male and female Wistar rats (2/sex/dose) with average initial body weights of 150 g received intramuscular injections of 10% ethyl cinnamate dissolved in ethyl oleate at dose volumes of 0.1–0.5 cc. The total length of treatment was 57 days (injections into leg muscles were given daily; except for the last nine, which were given every other day). To measure cataractogenic activity, the eyes were examined with a slit lamp and an ophthalmoscope at regular intervals every 5–10 days. Ethyl cinnamate produced no effects (Moro et al., 1969).

4.2. Skin irritation

4.2.1. Human studies (Table 3)

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed after a 48-h closed patch test with 4% ethyl cinnamate in petrolatum on the forearms of 5 healthy, male volunteers (RIFM, 1973b).

4.2.1.2. Neat ethyl cinnamate was applied to a 1 cm area on the inner arm of 22 male and female volunteers. Immediately following application, the area was covered with an adhesive bandage for 24 h. Reactions were read daily for 5 days. Irritation was observed in 1 subject (Katz, 1946).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 4% (2740 μg/cm^2) ethyl cinnamate in petrolatum on 25 healthy, male volunteers. Application was under occlusion to the same sites on the forearms of all subjects for five alternate-day 48-h periods. The patch sites were pretreated for 24 h with 5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a ten-day rest period, challenge patches were applied to fresh sites for 48 h under occlusion. Challenge applications were pretreated for 1-h with 10% SLS under occlusion. The challenge sites were read on removal of patch and 24 h thereafter. No sensitization reactions were produced (RIFM, 1973b).

4.4.2. Animal studies

4.4.2.1. An open epicutaneous test (OET) was conducted on groups of 6 - 8 male and female outbred guinea pigs. For induction, a open application of a 0.1 ml aliquot of 4% ethyl cinnamate was applied to a 8 cm^2 area on the flank. A total of 21 daily open induction applications were made over a 3-week period. At challenge, an open application of a 0.025 ml aliquot of 4% ethyl cinnamate was made on the contralateral flank on days 21 and 35. Sensitization was not observed (Klecak, 1979; Klecak, 1985).

4.5. Phototoxicity and photoallergy

UV spectra revealed that ethyl cinnamate peaked within 245–278 nm range and showed minor absorption in the 290–320 nm region.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Reproductive and developmental toxicity

No data available on this material.

4.8. Subchronic toxicity (Table 4)

4.8.1. Oral studies

4.8.1.1. A mixture of flavorings containing 897 ppm cinnamaldehyde and 25 ppm each of methyl cinnamate, ethyl cinnamate, cinnamyl cinnamate, and α-methyl-cinnamaldehyde was added to the diet of rats for 12 weeks, resulting in the approximate daily intake of 110 mg/kg/bodyweight

(male) and 119 mg/kg/bodyweight (female) (roughly equivalent to 103 mg/kg/bodyweight of cinnamaldehyde and 3 mg/kg/bodyweight of the other components). Each diet was fed ad libitum to a group of 24 rats (12/sex) with initial body weights of 50–70 g. Weekly observations were made of growth and food intake. Records were made of physical appearance and behavior. After 12 weeks, urinalysis was conducted on 6 animals (3/sex) and blood hemoglobin levels were determined. Respiratory infections were observed in rats in both test and control groups; one male in the control group died due to pulmonary pathology. Gross necropsy was conducted on all animals. Blood hemoglobin, urinalysis, liver and kidney weights, food intake, behavior and appearance were normal in both sexes. Depressed growth was observed in the male rats but was not considered statistically significant. Efficiency of food utilization (EFU) was significantly depressed in both sexes (RIFM, 1958).

4.8.1.2. Groups of 12 male white rats received ethyl cinnamate perorally for 4 months at $0.02 \times LD_{50}$ at a dosing volume of 0.2 ml per 100 g of bodyweight. Blood work and liver function tests were carried out twice on days 40 and 140. A dose of 80 mg/kg/bodyweight ethyl cinnamate resulted in a 26% decrease in the fructose diphosphate aldolase activity in the blood serum by day 140. No pronounced pathological changes were observed (Zaitsev and Rakhmanina, 1974).

Table 2
Summary of acute toxicity data

Route	Species	No. animals/ dose group	LD_{50}	References
Dermal	Rabbit	10	>5 g/kg	RIFM (1973a)
Oral	Guinea pigs	3/sex	4.0 g/kg	Zaitsev and Rakhmanina (1974)
Oral	White rats	3/sex	4.0 g/kg	Zaitsev and Rakhmanina (1974)
Oral	White mice	3/sex	4.0 g/kg	Zaitsev and Rakhmanina (1974)

Table 3
Summary of human irritation studies

Method	Concentration	Results	References
Maximization pre-test	4% in petrolatum	No irritation observed (0/5)	RIFM (1973b)
24-h occluded patch	100%	1/22 irritant reactions	Katz (1946)

4.9. Mutagenicity and genotoxicity (Table 5)

4.9.1. Bacterial studies

4.9.1.1. In an Ames test (Ames et al., 1975) using *Salmonella typhimurium* strains TA92, TA94, TA98, TA100, TA1535 and TA1537 with and without S9 activation, doses up to 5000 μg/plate in dimethyl sulfoxide were not mutagenic (Ishidate et al., 1984).

4.9.1.2. In a rec assay using *Bacillus subtilis* strains H17 (rec^+) and M45 (rec^-), a dose of 20 μg/disk ethyl cinnamate in dimethyl sulfoxide had no effect (Oda et al., 1978).

4.9.2. Mammalian studies

4.9.2.1. An *in vitro* cytogenetic assay in Chinese hamster ovary ($CHO\text{-}K_1$) cells was conducted at concentrations of ethyl cinnamate ranging from 1.0, 3.3 and 10 μM. $CHO\text{-}K_1$ cells were cultured in the presence or absence of ethyl cinnamate for one cell cycle. For the analysis of sister-chromatid exchanges (SCEs), bromodeoxyuridine (final concentration 5 μM) was added two cell cycles before fixation. After addition of bromodeoxyuridine, the cultures were incubated in total darkness. Cells were then treated with colchicine for 2 h at a final concentration of 50 μg/ml. Preparations were processed using a modified Giemsa procedure and harlequin-stained chromosomes in 50 metaphases per culture were analyzed for SCEs. No significant increases in the mean SCE frequency were observed; the highest dose tested (33.3 μM) was toxic (Sasaki et al., 1989).

4.9.2.2. Ishidate et al. (1984) studied chromosome aberrations without metabolic activation in a Chinese hamster fibroblast cell line using multiple harvest times (24 and 48 h after the initiation of treatment). The assay was conducted at three different doses but only the maximum dose, 0.063 mg/ml ethyl cinnamate in dimethyl sulfoxide, was reported. The cells were exposed for a total of 24 or 48 h; colcemid was added 2 h prior to cell harvesting. Preparations were processed with Giemsa and 100 well spread metaphases were analyzed. Untreated cells and solvent treated cells served as negative controls. Equivocal increases in structural or numerical chromosome aberrations, as well as polyploidization effects were observed.

Table 4
Summary of subchronic toxicity studies

Study	Dose	Results	Reference
Oral 12-weeks	~3 mg/kg/bodyweight ethyl cinnamate (tested in a mixture containing 5 cinnamic flavoring agents)	Depressed growth; food utilization significantly decreased in both sexes	RIFM (1958)
Oral 16-weeks	80 mg/kg	Slight effects were observed	Zaitsev and Rakhmanina (1974)

Table 5
Summary of mutagenicity and genotoxicity studies

Test method	Strain	Dose	Results	References
Ames with and without S9 activation	S.*typhimurium* strains TA92, TA94, TA98, TA100, TA1535, TA1537	up to 5000 μg/plate	Negative	Ishidate et al. (1984)
Rec assay	*Bacillus subtilis* strains H17 (rec^+) and M45 (rec^-)	20 μg/disk	Negative	Oda et al. (1978)
Sister chromatid exchange	Chinese hamster ovary cells (CHO-K1)	1.0, 3.3 and 10 μM	Negative (highest dose tested was toxic)	Sasaki et al. (1989)
Chromosome aberrations test	Chinese hamster fibroblast cell line	0.063 mg/ml in dimethyl sulfoxide	Equivocal increases in chromosome aberrations and polyploidization effects were observed	Ishidate et al. (1984)

4.10. Carcinogenicity

No data available on this material.

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Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on *cis*-3-hexenyl cinnamate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

A toxicologic and dermatologic review of *cis*-3-hexenyl cinnamate when used as a fragrance ingredient is presented.

Keyword: *cis*-3-Hexenyl cinnamate

Contents

In 2006, a complete literature search was conducted on *cis*-3-hexenyl cinnamate. On-line databases that were surveyed included chemical abstract services and the national library of medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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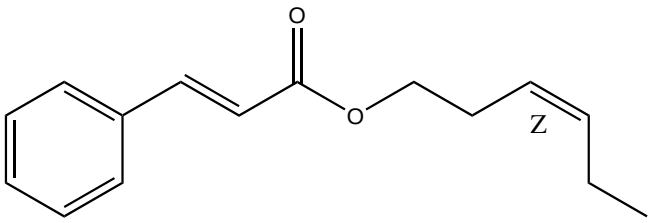

Fig. 1. *cis*-3-Hexenyl cinnamate.

1. Identification (Fig. 1)

1.1 Synonyms: (*Z*)-3-Hexenyl cinnamate; *cis*-3-Hexenyl cinnamate; 2-Propenoic acid, 3-phenyl-, (3*Z*)-3-hexenyl ester; 2-Propenoic acid, 3-phenyl-, 3-hexenyl ester, (?,*Z*)-.
1.2 CAS registry number: 68133-75-5.
1.3 EINECS number: 268-702-1.
1.4 Formula: $C_{15}H_{18}O_2$.
1.5 Molecular weight: 230.07.

2. Physical properties

2.1 Log K_{ow} (calculated): 4.6.
2.2 Henry's law (calculated): 0.000015 atm m^3/mol.
2.3 Vapor pressure (calculated): 0.000327 mm Hg 25 °C.
2.4 Water solubility (calculated): 3.592 mg/l @ 25 °C.

3. Usage (Table 1)

cis-3-Hexenyl cinnamate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of less than 0.1 metric tonnes per annum.

The maximum skin level that results from the use of *cis*-3-hexenyl cinnamate in formulae that go into fine fragrances has been reported to be 0.08% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.7% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0178 mg/kg for high end users of these products.

4. Toxicological data

4.1. Acute toxicity

No data available on this material.

4.2. Skin irritation

No data available on this material.

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

No data available on this material.

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing *cis*-3-hexenyl cinnamate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product (%)	Ingredient/mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.7	0.0027
Face cream	0.80	2.00	1.000	0.003	0.7	0.0006
Eau de toilette	0.75	1.00	1.000	0.080	0.7	0.0070
Fragrance cream	5.00	0.29	1.000	0.040	0.7	0.0068
Antiperspirant	0.50	1.00	1.000	0.010	0.7	0.0006
Shampoo	8.00	1.00	0.010	0.005	0.7	0.0000
Bath products	17.00	0.29	0.001	0.020	0.7	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.7	0.0001
Toilet soap	0.80	6.00	0.010	0.015	0.7	0.0001
Hair spray	5.00	2.00	0.010	0.005	0.7	0.0001
Total						0.0178

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

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References

Belsito, D., Bickers, D., Bruze, M., Calow, P., Greim, H., Hanifin, J.H., Rogers, A.E., Saurat, J.H., Sipes, I.G., Tagami, H., 2007. A toxicologic and dermatologic assessment of related esters and alcohols of cinnamic acid and cinnamyl alcohol when used as fragrance ingredients. Food and Chemical Toxicology 45 (1S1), S1–S23.

IFRA (International Fragrance Association), 2001. Use Level Survey, July 2001.

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Review

Fragrance material review on isoamyl cinnamate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, United States*
[b] *Manheimer Fragrances, Teterboro NJ, United States*

Abstract

A toxicologic and dermatologic review of isoamyl cinnamate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Isoamyl cinnamate

Contents

In 2006, a complete literature search was conducted on isoamyl cinnamate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Amyl(iso) cinnamate; Isoamyl β-phenyl acrylate; Isopentyl cinnamate; Isopentyl β-phenylacrylate; Isopentyl 3-phenylpropenoate; 2-Propenoic acid, 3-phenyl-, 3-methylbutyl ester.
1.2 CAS Registry Number: 7779-65-9.
1.3 EINECS Number: 231-931-2.
1.4 Formula: $C_{14}H_{18}O_2$.
1.5 Molecular weight: 218.3.
1.6 Council of Europe: Isoamyl cinnamate was included by the Council of Europe in the list of substances granted B – information required – hydrolysis study (COE No. 335) (Council of Europe, 2000).
1.7 FDA: Isoamyl cinnamate was approved by the FDA as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient – GRAS 3 (2063) (FEMA, 1965).
1.9 Joint Expert Committee on Food Additives (JECFA): The Joint FAO/WHO Expert Additives (JECFA No. 665) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent (JECFA, 2000).

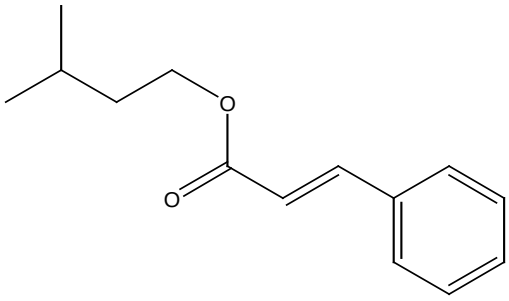

Fig. 1. Isoamyl cinnamate.

2. Physical properties

2.1 Physical form: A colorless to pale yellow liquid with a balsamic odor.
2.2 Boiling point: 310 °C.
2.3 Flash point: >200 °F; CC.
2.4 Log K_{ow} (calculated): 4.25.
2.5 Refractive index: 1.5350–1.5390 (20 °C).
2.6 Specific gravity: 0.995.
2.7 Specific gravity: 0.992–0.997 (25 °C).
2.8 Vapor pressure (calculated): <0.001 mm Hg at 20 °C.
2.9 Water solubility (calculated): 8.282 mg/l @ 25 °C.
2.10 Henry's Law (calculated): 0.0000129 atm m^3/mol 25 °C.

3. Usage (Table 1)

Isoamyl cinnamate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 0.1–1 metric tonnes per annum.

The maximum skin level that results from the use of isoamyl cinnamate in formulae that go into fine fragrances has been reported to be 0.05% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.113% (IFRA, 2001), which would result in a conservative calculated

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing isoamyl cinnamate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product (%)	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.113	0.0004
Face cream	0.80	2.00	1.000	0.003	0.113	0.0001
Eau de toilette	0.75	1.00	1.000	0.080	0.113	0.0011
Fragrance cream	5.00	0.29	1.000	0.040	0.113	0.0011
Antiperspirant	0.50	1.00	1.000	0.010	0.113	0.0001
Shampoo	8.00	1.00	0.010	0.005	0.113	0.0000
Bath products	17.00	0.29	0.001	0.020	0.113	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.113	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.113	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.113	0.0000
Total						0.0029

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

maximum daily exposure on the skin of 0.0029 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} in rats exceeded 5.0 g/kg based on 0/10 deaths at that dose. Ten rats were dosed orally with isoamyl cinnamate at 5.0 g/kg/bodyweight. The rats were observed for 14 days. Clinical signs that were observed included lethargy and piloerection (RIFM, 1974a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg based on 1/7 death at that dose. Seven rabbits received a single dermal application of neat isoamyl cinnamate which was applied for 24 h under occlusion. The animals were observed for 14 days. Death occurred in one animal on day 11. No clinical effects were observed (RIFM, 1974a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed following 48-h closed patch tests with 8% isoamyl cinnamate in petrolatum on the backs of five male and female volunteers (RIFM, 1974b).

4.2.2. Animal studies

4.2.2.1. Irritation was evaluated as part of an associated dermal LD_{50} study (see Section 4.1.2.1). Slight erythema was observed in 1/7 rabbits (RIFM, 1974a).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 8% (5520 μg/cm^2) isoamyl cinnamate in petrolatum on 25 healthy, male and female volunteers. Application was made under occlusion to the same site on the forearm of each subject for five alternate-day 48-h periods. Patch sites were pretreated with 5% aqueous sodium lauryl sulfate for 24 h under occlusion for the initial patch only. Following a 10-day rest period, challenge applications were made to fresh sites for 48-h under occlusion. Challenge applications were preceded by pretreatment with SLS. Reactions were at the removal of the challenge patch and 24 h thereafter. No sensitization reactions were observed (RIFM, 1974b).

4.5. Phototoxicity and photoallergy

UV spectra revealed that isoamyl cinnamate peaked within 245–278 nm range and showed minor absorption in the 290–320 nm region.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

4.9.1.

Isoamyl cinnamate was evaluated for mutagenicity using a preincubation modification of the Ames test (Ames et al., 1975). Isoamyl cinnamate was tested in *Salmonella typhimurium* strains TA97, TA98, TA100 and TA1535 with and without metabolic activation. No mutagenic effects were observed (Zeiger and Margolin, 2000).

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research

Table 2
Summary of acute toxicity data

Route	Species	No. animals/dose group	LD_{50} (g/kg)	References
Oral	Rat	10	>5.0	RIFM, 1974a
Dermal	Rabbit	7	> 5.0	RIFM, 1974a

institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on isobutyl cinnamate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

A toxicologic and dermatologic review of isobutyl cinnamate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Isobutyl cinnamate

Contents

In 2006, a complete literature search was conducted on isobutyl cinnamate. On-line databases that were surveyed included chemical abstract services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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1. Identification (Fig. 1)

1.1 Synonyms: Isobutyl β-phenylacrylate; Isobutyl 3-phenylpropenoate; Labdanol; 2-Methylpropyl cinnamate; 2-Methylpropyl β-phenylacrylate; 2-Methylpropyl 3-phenylpropenoate; 2-Propenoic acid, 3-phenyl-, 2-methylpropyl ester.
1.2 CAS Registry No.: 122-67-8.
1.3 EINECS No.: 204-564-0.
1.4 Formula: $C_{13}H_{16}O_2$.
1.5 Molecular weight: 204.27.
1.6 Council of Europe: Isobutyl cinnamate was included by the Council of Europe in the list of substances granted B – information required – hydrolysis study (COE No. 327) (Council of Europe, 2000).
1.7 FDA: Isobutyl cinnamate was approved by the FDA as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and Extract Manufacturers' Association states: Generally recognized as safe as a flavor ingredient – GRAS 3. (2193) (FEMA, 1965).
1.9 Joint Expert Committee on Food Additives (JECFA): The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 664) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent (JECFA, 2000).

2. Physical properties

2.1 Physical form: A colorless liquid with a sweet, fruity balsamic odor.
2.2 Flash point: >200 °F; CC.
2.3 Henry's law (calculated): 0.00000969 atm m^3/mol 25 °C.
2.4 Log K_{ow} (calculated): 3.76.
2.5 Refractive index: 1.5390–1.5410 (20 °C).
2.6 Specific gravity: 1.001–1.004 (25 °C).
2.7 Specific gravity: 1.004.

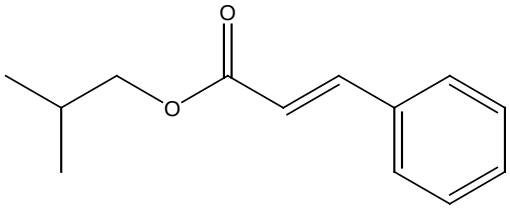

Fig. 1. Isobutyl cinnamate.

2.8 Vapor pressure (calculated): 0.002 mm Hg at 20 °C.
2.9 Water solubility (calculated): 25.75 mg/l at 25 °C.

3. Usage (Table 1)

Isobutyl cinnamate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 0.1–1 metric tonnes per annum.

The maximum skin level that results from the use of isobutyl cinnamate in formulae that go into fine fragrances has been reported to be 0.10% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.5% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0127 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} in rats exceeded 5.0 g/kg, based on 2/10 deaths at that dose. Ten rats were dosed orally with isobutyl cinnamate at a dose of 5.0 g/kg/bodyweight. Observations were made for 14 days. Deaths occurred on days 2 and 4. No clinical effects were observed (RIFM, 1975a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg, based on 0/4 deaths at that dose. Four rabbits received a single dermal application of neat isobutyl cinnamate which was applied for 24 h under occlusion. Observations were made for 14 days. No clinical effects were observed (RIFM, 1975a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed after a 48-h closed patch test with 8% isobutyl cinnamate in petrolatum on the backs of 24 healthy, male volunteers (RIFM, 1975b).

4.2.2. Animal studies

4.2.2.1. Irritation was evaluated during an associated acute dermal LD_{50} study (see Section 4.1.2.1). Mild erythema that lasted 24 h was the only dermal reaction observed (RIFM, 1975a).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing isobutyl cinnamate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product (%)	Ingredient/ mixture[a]	Ingredient (mg/kg/ day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.5	0.0019
Face cream	0.80	2.00	1.000	0.003	0.5	0.0004
Eau de toilette	0.75	1.00	1.000	0.080	0.5	0.0050
Fragrance cream	5.00	0.29	1.000	0.040	0.5	0.0048
Antiperspirant	0.50	1.00	1.000	0.010	0.5	0.0004
Shampoo	8.00	1.00	0.010	0.005	0.5	0.0000
Bath products	17.00	0.29	0.001	0.020	0.5	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.5	0.0001
Toilet soap	0.80	6.00	0.010	0.015	0.5	0.0001
Hair spray	5.00	2.00	0.010	0.005	0.5	0.0000
Total						0.0127

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity data

Route	Species	No. animals/ dose group	LD_{50}	References
Oral	Rat	10	>5.0 g/kg	RIFM, 1975a
Dermal	Rabbit	4	>5.0 g/kg	RIFM, 1975a

4.3. Mucous membrane (Eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (Modified after Kligman, 1966; Kligman and Epstein, 1975) was carried out with 8% (5520 μg/cm^2) isobutyl cinnamate in petrolatum on 24 healthy, male volunteers. Application was made under occlusion to the same site on the forearms of all subjects for five alternate-day 48 h periods. Patch sites were pretreated for 24 h with 5% aqueous sodium lauryl sulfate (SLS) under occlusion for the initial patch only. Following a 10–14 days rest period, challenge patches were applied under occlusion to fresh sites for 48 h. Challenge applications were preceded by 30-min applications of 5% aqueous SLS under occlusion on the left side of the back, whereas test material without SLS treatment was applied on the right side. A fifth site challenged with SLS on the left and petrolatum on the right served as controls. No sensitization reactions were observed (RIFM, 1975b).

4.5. Phototoxicity and photoallergy

UV spectra revealed that isobutyl cinnamate peaked within 245–278 nm range and showed minor absorption in the 290–320 nm region.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on isopropyl cinnamate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro, NJ, USA*

Abstract

A toxicologic and dermatologic review of isopropyl cinnamate when used as a fragrance ingredient is presented.

Contents

In 2006, a complete literature search was conducted on isopropyl cinnamate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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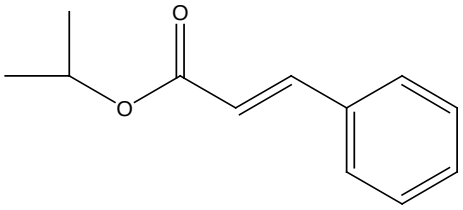

Fig. 1. Isopropyl cinnamate.

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1. Identification (Fig. 1)

1.1 Synonyms: Isopropyl 3-phenylpropenoate; 1-Methylethyl 3- phenylpropenoate; 2-Propenoic acid, 3-phenyl-, 1-methylethyl ester.
1.2 CAS Registry Number: 7780-06-5.
1.3 EINECS Number: 231-949-0.
1.4 Formula: $C_{12}H_{14}O_2$.
1.5 Molecular Weight: 190.24.
1.6 Council of Europe: Isopropyl cinnamate was included by the Council of Europe in the list of substances granted B – information required – hydrolysis study (COE No. 325) (Council of Europe, 2000).
1.7 FDA: Isopropyl cinnamate was approved by the FDA as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient - GRAS 3. (2939) (FEMA, 1965).
1.9 Joint Expert Committee on Food Additives (JECFA): The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 661) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent (JECFA, 2000).

2. Physical properties

2.1 Physical Description: A colorless liquid.
2.2 Henry's Law (calculated): 0.0000073 atm m3/mol 25 °C.
2.3 Log K_{ow} (calculated): 3.27.
2.4 Vapor Pressure (calculated): 0.0197 mm Hg 25 °C.
2.5 Water Solubility (calculated): 79.69 mg/l @ 25 °C.

3. Usage (Table 1)

Isopropyl cinnamate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 0.1–1 metric tonnes per annum.

The maximum skin level that results from the use of isopropyl cinnamate in formulae that go into fine fragrances has been reported to be 0.01% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.03% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0008 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Guinea pigs (10/dose) were dosed orally with isopropyl cinnamate. The animals were observed for 6 days following dosage. The LD_{50} was calculated to be 2.7 ml/kg [~2.7 g/kg] (Draize et al., 1948).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing isopropyl cinnamate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product%	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.03	0.0001
Face cream	0.80	2.00	1.000	0.003	0.03	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.03	0.0003
Fragrance cream	5.00	0.29	1.000	0.040	0.03	0.0003
Antiperspirant	0.50	1.00	1.000	0.010	0.03	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.03	0.0000
Bath products	17.00	0.29	0.001	0.020	0.03	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.03	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.03	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.03	0.0000
Total						0.0008

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity data

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Guinea pig	10	~2.7 g/kg	Draize et al. (1948)
Oral	Rat	10	>5.0 g/kg	RIFM (1982a)
Dermal	Rabbit	10	>5.0 g/kg	RIFM (1982a)
Dermal	Rabbit	Not specified	~10 g/kg	Draize et al. (1948)

4.1.1.2. The acute oral LD_{50} in rats exceeded 5.0 g/kg based on 0/10 deaths at that dose. Ten healthy male rats with initial bodyweights of 215–298 g were dosed orally with isopropyl cinnamate at 5.0 g/kg/bodyweight. The animals were observed 3–4 h post-dosing and daily thereafter for 14 days. Clinical signs observed during the study included diarrhea which was observed in five animals and chromodacryorrhea, chromorhinorrhea, lethargy, piloerection ptosis and brown staining in the anogenital area, all of which were observed in one or two animals. Gross necropsy was conducted on all animals. Gross observations at necropsy were normal for all animals (RIFM, 1982a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg based on 0/10 deaths at that dose. Ten healthy albino rabbits, weighing 2.1–2.9 kgs, received a single dermal application of neat isopropyl cinnamate which was applied to clipped, intact and abraded skin for 24 h under occlusion. The animals were observed daily for 14 days. Gross necropsy was conducted on all animals. Clinical signs observed during the study included diarrhea, alopecia, yellow nasal discharge, few feces and flaking skin, each seen in at least two rabbits. Gross observations at necropsy were normal for all animals (RIFM, 1982a).

4.1.2.2. Draize et al. (1948) reported that the acute dermal LD_{50} in rabbits exceeded 10.0 ml/kg [~10 g/kg].

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In the pre-test for a maximization study, no irritation was observed after a 48 h closed patch test on the backs of 28 healthy, male and female volunteers with 6% isopropyl cinnamate in petrolatum (RIFM, 1982b).

4.2.2. Animal studies

4.2.2.1. Irritation was evaluated as part of the associated acute dermal LD_{50} study (see Section 4.1.2.1). Very slight (2/10 rabbits) to well-defined erythema (8/10 rabbits) and very slight (6/10 rabbits) to slight edema (4/10 rabbits) were observed (RIFM, 1982a).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was conducted with 6% (4140 μg/cm^2) isopropyl cinnamate in petrolatum on 28 healthy, male and female volunteers. Application was made under occlusion to the same site on the forearm of each subject for five alternate-day 48 h periods. Patch sites were pretreated with 7.5% aqueous sodium lauryl sulfate (SLS) for 24 h under occlusion, for the initial patch only. After 10–14 day rest period, challenge patches were applied under occlusion to fresh sites for 48 h. Challenge applications were preceded by 30 min applications of 7.5% aqueous SLS under occlusion on the left side whereas the test material without SLS was applied on the right side. Additional SLS controls and petrolatum were placed on the left and right sides, respectively on a site labeled 5. No sensitization reactions were observed (RIFM, 1982b).

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

4.7.1

A 90-day dermal toxicity study was conducted in rabbits according to the method described by Draize et al. (1944). Isopropyl cinnamate, at dose levels of 0.5, 1.0, 2.0 and 4.0 ml/kg [~equivalent to 500, 1000, 2000 and 4000 mg/kg], was gently rubbed into intact, clipped, dorsal skin (over approximately 10% of the entire body surface) with a glass rod once daily for 90 consecutive days. Local skin reactions were recorded and urine and blood were examined. Histopathology was routinely conducted on all animals that died and all animals in the higher dose groups; gross and microscopic examinations were also conducted on most animals that survived the first 72 h of the test. Moderate chronic dermatitis was observed. At the two highest dose levels, atrophy of the testes, hyperplasia of the bone marrow, slight inanition and severe skin irritation were also observed. The 90-day LD_{50} was reported to exceed 4 ml/kg [~4000 mg/kg body

weight]. The No-Observed-Adverse-Effect Level (NOAEL) was concluded to be 1 ml/kg [~1000 mg/kg body weight] (Draize et al., 1948).

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Addendum to Fragrance material review on linalyl cinnamate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc. 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

Addendum to Fragrance Material Review on Linalyl Cinnamate.

Keywords: Addendum; Fragrance; Linalyl cinnamate; Review

Contents

A Fragrance Material Review on Linalyl Cinnamate was published by Letizia et al. in Food and Chemical Toxicology 41 (2003) 989–993. This addendum to that earlier publication will only report studies that were conducted after the fragrance material review was published.

1. Identification (Fig. 1)

1.1 Synonyms: Cinnamic acid, linalyl ester; 3,7-Dimethyl-1,6-octadien-3-yl β-phenylacrylate; 3,7-Dimethyl-1,6-octadien-3-yl cinnamate; 3,7-Dimethyl-1,6-octadien-3-yl 3-phenylpropenoate; Linalyl 3-phenylpropenoate; 2-Propenoic acid, 3-phenyl-, 1-ethenyl-1,5-dimethyl-4-hexenyl ester.
1.2 CAS Registry Number: 78-37-5.
1.3 EINECS Number: 201-110-3.

2. Usage (Table 1)

Linalyl cinnamate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 0.1–1 metric tonnes per annum.

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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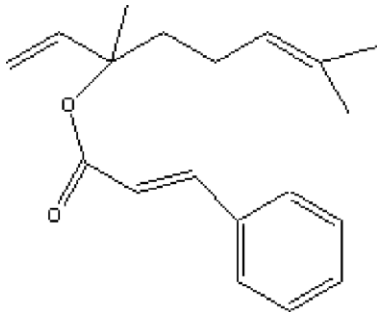

Fig. 1. Linalyl cinnamate.

The maximum skin level that results from the use of linalyl cinnamate in formulae that go into fine fragrances has been reported to be 0.42% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 1.05% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.0268 mg/kg for high end users of these products.

3. Mutagenicity and genotoxicity

3.1. Bacterial studies

3.1.1

Using the Ames test (Ames et al., 1975) linalyl cinnamate was evaluated for mutagenicity using *Salmonella typhimurium* strains TA98, TA100, TA102 and TA1535, with and without S9 activation. Three replicates were used at each test point. The test material was administered at doses of 313–5000 μg/plate in dimethylsulphoxide. Precipitation of the test material was observed at the highest dose level with all tester strains. No increases in revertant numbers were observed at any concentration. Positive controls were within expected ranges. It was concluded that linalyl cinnamate was not mutagenic. This was a GLP study and was conducted according to EEC Council Directive 2000/32, Annex 4D and OECD Guideline for the testing of chemicals No. 471; ICH S2A Genotoxicity: Specific Aspects of Regulatory Tests, Step 5 (RIFM, 2003).

3.1.2

Prior to conducting the Ames test (see Section 3.1.1), a preliminary toxicity test was conducted using the plate incorporation method to select the concentrations of the test material. The dose range was 39.1–5000 μg/plate. Toxicity as indicated by thinning of the background lawn and reduction in revertant numbers was observed at higher dose levels with all tester strains with and without S9 mix. No increases in revertant numbers were observed at any concentration assayed. Positive controls were within expected ranges. Under the conditions of the study, linalyl cinnamate does not induce reverse mutation in *S. typhimurium* in any of the strains tested (RIFM, 2003).

Please see the published Fragrance Material Review on Linalyl Cinnamate (Letizia et al., 2003) for more information on this material. Also, Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) and the Toxicologic and Dermatologic Assessment of Linalool and Related Esters (Bickers et al., 2003) for an overall assessment of this material.

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing linalyl cinnamate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product%	Ingredient/mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	1.05	0.0040
Face cream	0.80	2.00	1.000	0.003	1.05	0.0008
Eau de toilette	0.75	1.00	1.000	0.080	1.05	0.0105
Fragrance cream	5.00	0.29	1.000	0.040	1.05	0.0102
Antiperspirant	0.50	1.00	1.000	0.010	1.05	0.0009
Shampoo	8.00	1.00	0.010	0.005	1.05	0.0001
Bath products	17.00	0.29	0.001	0.020	1.05	0.0000
Shower gel	5.00	1.07	0.010	0.012	1.05	0.0001
Toilet soap	0.80	6.00	0.010	0.015	1.05	0.0001
Hair spray	5.00	2.00	0.010	0.005	1.05	0.0001
Total						0.0268

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Bickers, D., Greim, H., Hanifin, J.H., Rogers, A.E., Saurat, J.H., Sipes, I.G., Smith, R.L., Tagami, H., 2003. A toxicologic and dermatologic assessment of linalool and related esters when used as fragrance ingredients. Food Chem. Toxicol. 41, 919–942.

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Review

Fragrance material review on methyl cinnamate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc. 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

A toxicologic and dermatologic review of methyl cinnamate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Methyl cinnamate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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In 2006, a complete literature search was conducted on methyl cinnamate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Methyl 3-phenylpropenoate; 2-Propenoic acid, 3-phenyl-, methyl ester.
1.2 CAS Registry Number: 103-26-4.
1.3 EINECS Number: 203-093-8.
1.4 Formula: $C_{10}H_{10}O_2$.
1.5 Molecular weight: 162.19.
1.6 Council of Europe: Methyl cinnamate was included by the Council of Europe in the list of substances granted A – may be used in foodstuffs (COE No.333) (Council of Europe, 2000).
1.7 FDA: Methyl cinnamate was approved by the FDA as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and Extract Manufacturers' Association states: Generally recognized as safe as a flavor ingredient – GRAS 3. (2698) (FEMA, 1965).
1.9 The Joint Expert Committee on Food Additives (JECFA): The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 658) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent (JECFA, 2000).

2. Physical properties

2.1 Physical form: A white to slightly yellow solid with a fruity balsamic odor.

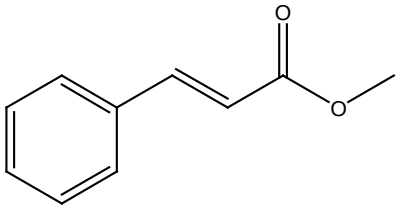

Fig. 1. Methyl cinnamate.

2.2 Boiling point: 262 °C.
2.3 Flash point: >200 °F;CC.
2.4 Henry's law (calculated): 0.00000414 atm m^3/mol 25 °C.
2.5 Log K_{ow} (measured) (OECD 117) : 2.6 at 30 °C.
2.6 Log K_{ow} (calculated): 2.36.
2.7 Vapor pressure (calculated): 0.02 mm Hg at 20 °C.
2.8 Melting point: 33 °C.
2.9 Water solubility (calculated): 387.1 mg/l @ 25 °C.

3. Usage (Table 1)

Methyl cinnamate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 10–100 metric tonnes per annum.

The maximum skin level that results from the use of methyl cinnamate in formulae that go into fine fragrances has been reported to be 0.31% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.21% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0054 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Male and female Sprague-Dawley albino rats (5/dose) with initial body weights of 150–250 g were dosed via gavage with methyl cinnamate at dose levels up to 6.0 g/kg/bodyweight. Methyl cinnamate was administered as a 50% solution or in corn oil. Observations were made 1–4 h post dose and once daily thereafter for 14 days. Gross necropsy was conducted on all animals. Clinical signs observed during the study included a decrease in respiration at a dose of 3.16 g/kg and higher. Necropsy revealed fluid filled stomachs of the animals who died 24 h after dosing. Gross observations at necropsy were normal for all other animals. The LD_{50} was calculated to be 2.61 g/kg (95% C.I. 2.00 – 3.41 g/kg (RIFM, 1971a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg based on 0/4 deaths at that dose. Methyl cinnamate was

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing methyl cinnamate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product (%)	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.21	0.0008
Face cream	0.80	2.00	1.000	0.003	0.21	0.0002
Eau de toilette	0.75	1.00	1.000	0.080	0.21	0.0021
Fragrance cream	5.00	0.29	1.000	0.040	0.21	0.0020
Antiperspirant	0.50	1.00	1.000	0.010	0.21	0.0002
Shampoo	8.00	1.00	0.010	0.005	0.21	0.0000
Bath products	17.00	0.29	0.001	0.020	0.21	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.21	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.21	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.21	0.0000
Total						0.0054

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity data

Route	Species	No. animals/ dose group	LD_{50}	References
Oral	Rat	5	2.61 g/kg (95% C.I. 2.00 – 3.41 g/kg)	RIFM (1971a)
Dermal	Rabbit	4	>5.0 g/kg	RIFM (1971a)

administered as a 50% solution or the test material was suspended in corn oil. Four male and female New Zealand white rabbits weighing 2.5–3.0 kg, received a single dermal application of methyl cinnamate at a dose of 5.0 g/kg/ bodyweight. The dose site, approximately 240 cm^2 (about 10% of the body surface) was clipped and the skin was abraded in one-half of the animals and intact in the other half of the animals. The test area was covered for 24 h with a non-absorbent binder. Observations were made for 14 days. Gross necropsy was conducted on all animals. No clinical effects were observed during the study. Gross observations at necropsy were normal for all animals (RIFM, 1971a).

4.2. Skin irritation

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg based on 0/4 deaths at that dose. Methyl cinnamate was administered as a 50% solution or the test material was suspended in corn oil. Four male and female New Zealand white rabbits weighing 2.5–3.0 kg, received a single dermal application of methyl cinnamate at a dose of 5.0 g/kg/ bodyweight. The dose site, approximately 240 cm^2 (about 10% of the body surface) was clipped and the skin was abraded in one-half of the animals and intact in the other half of the animals. The test area was covered for 24 h with a non-absorbent binder. Observations were made for 14 days. Gross necropsy was conducted on all animals. No clinical effects were observed during the study. Gross observations at necropsy were normal for all animals (RIFM, 1971a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed after a 48-h closed patch test with 10% methyl cinnamate in petrolatum on the forearms of five healthy, male and female volunteers (RIFM, 1975).

4.2.2. Animal studies (*Table 3*)

4.2.2.1. Prior to an open epicutaneous test, methyl cinnamate, at a range of concentrations, was evaluated for irritation in 6–8 male and female outbred Himalayan white-spotted guinea pigs. A 0.025 ml aliquot was applied

Table 3
Summary of irritation studies in animals

Method	Dose (%)	Species	Results	References
Preliminary irritation screen for an open epicutaneous test	A range of concentrations (vehicle not specified)	Guinea pigs	30% = minimal irritating concentration	Klecak et al. (1977)
Induction phase for an open epicutaneous test	A range of concentrations (vehicle not specified)	Guinea pigs	3% = minimal irritating concentration	Klecak et al. (1977)
Irritation evaluated as part of an LD_{50} study	100	Rabbits	No irritation was observed	RIFM (1971a)

with a pipette to an area measuring 2 cm^2 on the clipped flank. The application site was left uncovered and reactions were read after 24 h. Methyl cinnamate at 30% (vehicle not specified) was the lowest concentration to produce mild erythema in at least 25% of the animals and this dose was selected as the minimal irritating concentration (Klecak et al., 1977).

4.2.2.2. Methyl cinnamate was evaluated for irritation, at several dose levels, during the induction phase of an open epicutaneous test. A 0.1 ml aliquot of methyl cinnamate applied to an area measuring 8 cm^2 on the clipped flank of 6–8 male and female outbred Himalayan white-spotted guinea pigs. The application site was left uncovered and reactions were read after 24 h. A total of 21 daily applications were made. The minimal irritating concentration was 3% (vehicle not specified) (Klecak et al., 1977).

4.2.2.3. As part of an associated dermal LD_{50} study (see Section 4.1.2.1), irritation was evaluated in four rabbits using the Draize scoring method. The test material was administered to clipped intact and abraded areas for 24 h under occlusion. No irritation was observed (RIFM, 1971a).

4.3. Mucous membrane irritation

4.3.1. Eye irritation (Table 4)

4.3.1.1. In an eye irritation test, a 0.1 ml aliquot of neat methyl cinnamate was instilled into one eye of each of the six New Zealand white rabbits with no further treatment. The untreated eyes served as controls. Observations were made at 1, 4, 24, 48, 72 and 96 h and daily thereafter for a total of 7 days. Conjunctival irritation was observed in 1/6 rabbits for 24-h. Under the conditions of this test, methyl cinnamate was considered to be non-irritating (RIFM, 1971a).

4.3.1.2. An eye irritation test was conducted in rabbits (number not specified). A 0.1 ml aliquot of methyl cinnamate at 15% (vehicle not reported) and 100% was instilled into the lower eye of all animals. The eye was held shut for 1 second. No irritation was produced by methyl cinnamate at 15% or at 100% (RIFM, 1971b).

Table 4
Summary of eye irritation studies

Dose (%)	Vehicle	Results	References
100	NA	No irritation observed	RIFM (1971a)
15 and 100	NA	Non-irritating	RIFM (1971b)

4.3.2. Vaginal irritation

4.3.2.1. A 0.5 ml aliquot of 15% methyl cinnamate in 1.25% Tween 20 in distilled water was applied to the vaginal mucous membrane of six female New Zealand white rabbits. The material was released near the top of the vaginal vault. The animals were continuously observed for 4.5 h. The membrane was examined at 4.5, 24, 48 and 72 h. All vaginal examinations were negative. Methyl cinnamate at 15% was considered to be non-irritating to the vaginal membrane of rabbits (RIFM, 1971b).

4.4. Skin sensitization

4.4.1. Human studies (Table 5)

4.4.1.1. Predictive studies

4.4.1.1.1. A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 2% (1380 μg/cm^2) methyl cinnamate in petrolatum on 25 healthy, male volunteers. Application was under occlusion to the same site on the forearms of all subjects for five alternate-day 48-h periods. Patch sites were pretreated with 5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a ten-day rest period, challenge patches were applied to fresh sites on the back for 48 h under occlusion. The challenge site was pretreated for 1 h with 10% SLS. The challenge site was read at 48 and 72 h. No sensitization reactions were observed (RIFM, 1970).

4.4.1.1.2. Using the same above method, another maximization test was carried out with 10% (6900 μg/cm^2) methyl cinnamate in petrolatum on 25 healthy, male and female volunteers. No sensitization reactions were observed (RIFM, 1975).

4.4.1.2. Diagnostic studies

4.4.1.2.1. Patch tests with some components of Peru balsam were carried out at 8 worldwide centers in 142 patients who had previously reacted to 25% Peru balsam. Reactions to methyl cinnamate (dose and vehicle not reported) were observed in 6/142 patients (no further details reported) (Mitchell, 1975; Mitchell et al., 1976).

4.4.2. Animal studies (Table 6)

4.4.2.1. In a guinea pig sensitization test (Magnusson and Kligman, 1969), it was reported by Klecak et al. (1977) that a subirritant concentration of methyl cinnamate produced sensitization (number of reactions not reported). Induction

Table 5
Summary of skin sensitization studies in humans

Test Method	Concentration	Results	References
Maximization	2% in petrolatum (1380 μg/cm^2)	0/25	RIFM (1970)
Maximization	10% in petrolatum (6900 μg/cm^2)	0/25	RIFM (1975)

Table 6
Summary of guinea pig sensitization studies

Method	Induction concentration	Challenge concentration	Results	References
Maximization	5% in FCA (intradermal) 25% in petrolatum (topical)	Subirritant	Sensitization observed	Klecak et al. (1977)
Intradermal sensitization	0.1% suspension in 5% ethyl alcohol in distilled water	0.1% suspension in 5% ethyl alcohol in distilled water	No sensitization	RIFM (1971b)
FCAT	50% in FCA	subirritant	Sensitization observed	Klecak et al. (1977)
FCAT	10% in acetone	10% in acetone	Weak sensitization	Hausen et al. (1992)
FCAT	10% in acetone	10% in acetone	No sensitization	Hausen et al. (1995)
Open epicutaneous test	30% in unspecified vehicle	3% in unspecified vehicle	Sensitization observed	Klecak et al. (1977)
Open epicutaneous test	2% in unspecified vehicle	2% in unspecified vehicle	No sensitization	Klecak (1979)
Open epicutaneous test	10% in unspecified vehicle	10% in unspecified vehicle	No sensitization	Klecak (1985)
Draize test	0.1% in isotonic saline	0.1% in saline	Sensitization observed	Klecak et al. (1977)

consisted of two stages; intradermal injection followed eight days later by a 48-h occluded patch application. Male and female outbred Himalayan guinea pigs weighing 400–500 g were used. The intradermal injections consisted of two injections of 0.1 ml of 5% methyl cinnamate; two injections of 0.1 ml of a 5% emulsion of methyl cinnamate in FCA; two injections of FCA alone. The topical induction concentration was 25% in petrolatum. On day 21, an occlusive patch with a sub-irritant concentration of methyl cinnamate in petrolatum was applied to the flank for 24 h. Reactions were read 24 and 48 h after patch removal.

4.4.2.2. A guinea pig sensitization test was conducted on white male guinea pigs, weighing approximately 311–397 g. Methyl cinnamate was tested as a 0.1% suspension in 5% ethyl alcohol in distilled water. Induction consisted of ten intradermal injections made over a period of three and a half weeks. A 0.05 ml aliquot of methyl cinnamate was used for the first intradermal induction injection and a 0.1 ml aliquot of methyl cinnamate was used for the second - tenth intradermal injections. Following a ten-day rest period, an intradermal challenge injection with a 0.05 ml aliquot of a 0.1% suspension of methyl cinnamate in 5% ethyl alcohol in distilled water was administered. Reactions were read 24 h later. No sensitization reactions were produced (RIFM, 1971b).

4.4.2.3. Methyl cinnamate was tested in a Freund's Complete Adjuvant Test (FCAT) in male and female outbred Himalayan guinea pigs weighing 400–500 g. Guinea pigs received five intradermal injections of 0.1 ml aliquot of methyl cinnamate in FCA, as a 50:50 mixture on days 0, 2, 4, 7 and 9. Challenge was by a 24-h occluded patch with a subirritant concentration of methyl cinnamate in petrolatum that was applied to the flank on days 21 and 35. Sensitization was observed (no further details reported) (Klecak et al., 1977).

4.4.2.4. Two separate modified FCATs were conducted in guinea pigs to evaluate sensitization to 10% methyl cinnamate in acetone. Weak sensitization effects were observed (Hausen et al., 1992). In another study conducted using the same method and test material concentration no sensitization effects were observed (Hausen et al., 1995; Hausen and Wollenweber, 1988).

4.4.2.5. Methyl cinnamate was tested in an open epicutaneous test (OET) in male and female outbred Himalayan guinea pigs (6–8/group) weighing 400–500 g. Guinea pigs received 21 daily open applications of 0.1 ml of 30% methyl cinnamate (vehicle not specified) that was applied to an 8 cm^2 area on the clipped flank. Reactions were read 24 h after each application. Guinea pigs were challenged by an open application with 0.025 ml of 3% methyl cinnamate (vehicle not specified) that was applied to a skin area measuring 2 cm^2 on the contralateral flank on days 21 and 35. Reactions were read 24, 48 and/or 72 h after application. Six to eight untreated controls were also treated with methyl cinnamate on days 21 and 35. Sensitization was observed (Klecak et al., 1977).

4.4.2.6. Two separate OET were conducted with 2% and 10% methyl cinnamate (vehicle not specified by material) in guinea pigs. Induction consisted of 21 daily open applications to the shaved flank of 6–8 guinea pigs/group. Open challenge applications were made on days 21 and 35. No reactions were observed (Klecak, 1979; Klecak, 1985).

4.4.2.7. Methyl cinnamate was tested in another guinea pig sensitization study using a modified Draize procedure in male and female outbred Himalayan guinea pigs weighing 400–500 g. Induction consisted of ten intradermal injections on alternate days with 0.05 ml of a 0.1% solution of methyl cinnamate in isotonic saline starting on day 0. The animals were challenged on days 35 and 49 with an intradermal injection of 0.05 ml of a 0.1% solution of

methyl cinnamate in saline. Sensitization effects were observed (Klecak et al., 1977).

4.5. Phototoxicity and photoallergy

UV spectra revealed that methyl cinnamate peaked within the 245–278 nm range and showed minor absorption in the 290–320 nm region.

4.6. Absorption, distribution and metabolism

4.6.1. Percutaneous absorption

No data available on this material.

4.6.2. Metabolism

4.6.2.1. In vivo studies in animals

4.6.2.1.1. Female white New Zealand rabbits, weighing 3–4 kg, received a single oral dose of 500 mg methyl cinnamate as a suspension in warm water. Urine was collected for 24 h. Metabolites were isolated and examined by paper, TLC (thin layer chromatography) and GLC (gas–liquid chromatography). The following metabolites were identified as a percentage of the dose: hippuric acid (56.0%) and glucosiduronic acid (8%). Using the same method, Wistar rats were dosed with 50 mg of methyl cinnamate. The following metabolites were identified as a percentage of the dose: hippuric acid (67.0%) and glucosiduronic acid (3%) (Fahelbum and James, 1977).

4.7. Subchronic toxicity

4.7.1. Oral studies

4.7.1.1. A mixture of flavorings containing 897 ppm cinnamaldehyde and 25 ppm each of methyl cinnamate, ethyl cinnamate, cinnamyl cinnamate, and α-methyl-cinnamaldehyde was added to the diet of rats for 12 weeks, resulting in the approximate daily intake of 110 mg/kg/bodyweight (male) and 119 mg/kg/bodyweight (female) (roughly equivalent to 103 mg/kg/bodyweight of cinnamaldehyde and 3 mg/kg/bodyweight of the other components). Each diet was fed ad libitum to a group of 24 rats (12 /sex) with initial body weights of 50–70 g. Weekly observations were made of growth and food intake. Records were made of physical appearance and behavior. After 12 weeks, urinalysis was conducted on six animals (3/sex) and blood hemoglobin levels were determined. Respiratory infections were observed in rats in both test and control groups; one male in the control group died due to pulmonary pathology. Gross necropsy was conducted on all animals. Blood hemoglobin, urinalysis, liver and kidney weights, food intake, behavior and appearance were normal in both sexes. Depressed growth was observed in the male rats but was not considered statistically significant. Efficiency of food utilization (EFU) was significantly depressed in both sexes (RIFM, 1958).

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity (*Table 7*)

4.9.1. Bacterial studies

4.9.1.1. In a rec assay using *Bacillus subtilis* strains H17 (rec^+) and M45 (rec^-), a dose of 20 μg/disk methyl cinnamate in dimethyl sulfoxide produced no genotoxic effects (Oda et al., 1978, 1979).

4.9.2. Mammalian studies

4.9.2.1. An *in vitro* cytogenetic assay in Chinese hamster ovary cells (CHO-K_1) was conducted at concentrations of methyl cinnamate ranging from 1.0–100 μM. CHO-K_1 cells were cultured in the presence or absence of methyl cinnamate for one cell cycle. For the analysis of sister-chromatid exchanges (SCEs), bromodeoxyuridine (final concentration 5 μM) was added two cell cycles before fixation. After addition of bromodeoxyuridine, the cultures were incubated in total darkness. Cells were then treated with colchicine for 2 h at a final concentration of 50 μg/ml. Preparations were processed using a modified Giemsa procedure and harlequin-stained chromosomes in 50 metaphases per culture were analyzed for SCEs. No significant increases in the mean SCE frequency were observed; the highest dose tested (100 μM) was toxic (Sasaki et al., 1989).

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Table 7
Summary of mutagenicity and genotoxicity studies

Test method	Strain	Dose	Results	References
Rec assay	*Bacillus subtilis* strains H17 (rec^+) and M45 (rec^-)	20 μg/disk	No effects	Oda et al. (1978, 1979)
Sister chromatid exchange	Chinese hamster ovary cells (CHO-K_1)	1.0, 3.3, 10 and 33.3 μM	Negative (highest dose tested was toxic)	Sasaki et al. (1989)

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on α-methylcinnamic alcohol

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

A toxicologic and dermatologic review of α-methylcinnamic alcohol when used as a fragrance ingredient is presented.

Keywords: Fragrance; Review; α-Methylcinnamic alcohol

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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In 2006, a complete literature search was conducted on α-methylcinnamic alcohol. On-line databases that were surveyed included Chemical abstract services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Cinnamyl alcohol, α-methyl; Methylcinnamic alcohol; α-Methylcinnamyl alcohol; 3-Phenyl-2-methyl-2-propen-1-ol.
1.2 CAS Registry No.: 1504-55-8.
1.3 EINECS No.: 216-128-7.
1.4 Formula: $C_{10}H_{12}O$.
1.5 Molecular weight: 148.21.

2. Physical properties

2.1 Physical form: A clear, colorless to pale yellow liquid having a characteristic balsam odour.
2.2 Aldehydes: 1.0% Max.
2.3 Congealing point (I.E): 18.0 °C.
2.4 Flash point: >200 °F; CC.
2.5 Henry's law (calculated): 0.000000248 atm m3/mol 25 °C.
2.6 Log K^{ow} (calculated): 2.39.
2.7 Purity (X.A.1.): 97.0 Min.
2.8 Refractive index (I.B.): 1.571–1.574 (20 °C).
2.9 Specific gravity (I.A.): 1.026–1.032 (20 °C).
2.10 Specific gravity (I.A.): 1.024–1.030 (25 °C).
2.11 Vapor pressure (calculated): 0.00158 mm Hg 25 °C.
2.12 Water solubility (calculated): 2274 mg/l at 25 °C.

3. Usage (Table 1)

α-Methylcinnamic alcohol is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 0.1–1 metric tonnes per annum.

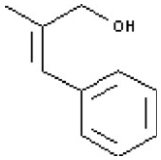

Fig. 1. α-Methylcinnamic alcohol.

The maximum skin level that results from the use of α-methylcinnamic alcohol in formulae that go into fine fragrances has been reported to be 0.01% (IFRA, 2004), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.2% (IFRA, 2004), which would result in a conservative calculated maximum daily exposure on the skin of 0.0051 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Groups of rats (10/dose) were dosed orally with α-methylcinnamic alcohol at dose levels of 2.0, 2.5, 3.5 and 4.0 ml/kg/bodyweight [~2.0, 2.5, 3.5 and 4.0 g/kg]. Observations for mortality and/or systemic effects were made. There were 3/10 deaths at 2.0 ml/kg, 6/10 deaths at 2.5 ml/kg, 8/10 deaths at 3.5 ml/kg and 8/10 deaths at 4.0 ml/kg. The LD_{50} was calculated to be 2.4 ml/kg [~2.4 g/kg] (95% C.I. 1.9–3.0 ml/kg) [95% C.I. 1.9–3.0 g/kg]. No effects were observed (RIFM, 1974a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg based on 0/4 deaths at that dose. Four rabbits received a single dermal application of neat α-methylcinnamic alcohol for 24 h under occlusion at 5.0 g/kg/bodyweight. No effects were observed (RIFM, 1974a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed following a 48-h closed patch test with 2% α-methylcinnamic alcohol in petrolatum on the backs of 5 healthy, male and female volunteers (RIFM, 1974b).

4.2.2. Animal studies (Table 3)

4.2.2.1. Prior to conducting a sensitization test, a prescreen test was conducted to determine the intradermal induction concentration. A 0.1 ml aliquot of α-methylcinnamic alcohol at 1%, 5%, 10% and 25% concentration w/v in arachis oil BP was applied intradermally to four guinea pigs, with each animal receiving four injections of only one concentration of α-methylcinnamic alcohol. Reactions were assessed approximately 24, 48 and 72 h and 7 days after the injections according to the Draize scale. α-Methylcinnamic alcohol at 5% was selected as the intradermal induction concentration, because it was the highest

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing α-methylcinnamic alcohol

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product (%)	Ingredient/mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.2	0.0008
Face cream	0.80	2.00	1.000	0.003	0.2	0.0002
Eau de toilette	0.75	1.00	1.000	0.080	0.2	0.0020
Fragrance cream	5.00	0.29	1.000	0.040	0.2	0.0019
Antiperspirant	0.50	1.00	1.000	0.010	0.2	0.0002
Shampoo	8.00	1.00	0.010	0.005	0.2	0.0000
Bath products	17.00	0.29	0.001	0.020	0.2	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.2	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.2	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.2	0.0000
Total						0.0051

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity data

Route	Species	No. animals/dose group	LD_{50}	Reference
Oral	Rat	10	~2.4 g/kg (95% C.I. ~1.9–3.0 g/kg)	RIFM (1974a)
Dermal	Rabbit	4	>5.0 g/kg	RIFM (1974a)

concentration that caused a mild to moderate dermal irritation (RIFM, 1997a).

4.2.2.2. A 48 h occluded patch test was conducted to determine the topical induction concentration for an associated guinea pig maximization test (see Section 4.4.2.1). α-Methylcinnamic alcohol at 100% (neat), 75%, 50%, and 25% v/v in arachis oil BP was applied to the clipped flanks of two guinea pigs (intradermally injected with Freund's Complete Adjuvant 17 days earlier) for 48 h under occlusion. Reactions were assessed at 1, 24, and 48 h after patch removal. The neat material was selected as the topical induction concentration because it was the highest concentration producing only mild to moderate dermal irritation (RIFM, 1997a).

4.2.2.3. Using the above method, a 24-h occluded patch test was conducted to determine the topical challenge concentration, for a guinea pig maximization test (see Section 4.4.2.1). α-Methylcinnamic alcohol at 100% (neat), 75%, 50%, and 25% v/v in arachis oil BP was applied to the clipped flanks of two guinea pigs (intradermally injected with Freund's Complete Adjuvant 17 days earlier) for 24-h under occlusion. Reactions were assessed at 1, 24, and 48 h after removal of the patch. α-Methylcinnamic alcohol at 100% (neat) and 75% v/v in arachis oil BP were selected for the topical challenge (RIFM, 1997a).

4.2.2.4. As part of an associated acute dermal LD_{50} study (see Section 4.1.2.1), irritation was evaluated in 4 rabbits. Mild erythema with drying of the skin was observed (no further details reported) (RIFM, 1974a).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 2% (1380 μg/cm^2) α-methylcinnamic alcohol in petrolatum on 25 (16 male/9 female) healthy volunteers. Application was under occlusion to the same site on the forearm of each subject for five alternate-day 48 h periods. Patch sites were pretreated with

Table 3
Summary of animal skin irritation studies

Method	Concentration	Species	Results	Reference
Intradermal pre-screen test for a maximization test	25%, 10%, 5%, and 1% w/v in arachis oil BP	Guinea pigs	5% = highest concentration that caused a mild to moderate skin irritation	RIFM (1997a)
Topical pre-screen for a maximization test	100%, 50%, 75% and 25% v/v in arachis oil BP	Guinea pigs	Very slight erythema was observed in 1/2 at 25%, 75% and 100%	RIFM (1997a)
Topical pre-screen for a maximization test	100%, 50%, 75% and 25% v/v in arachis oil BP	Guinea pigs	No irritation observed at any dose level	RIFM (1997a)
Irritation evaluated during an associated LD_{50} study	100%	Rabbits	Mild irritation	RIFM (1974a)

5% aqueous sodium lauryl sulfate for 24 h under occlusion. Following a ten-day rest period, a challenge patch was applied to a fresh site for 48 h under occlusion. The challenge sites were pretreated with aqueous SLS under occlusion. Reactions were read at patch removal and 24 h thereafter. No sensitization was observed (RIFM, 1974b).

4.4.2. Animal studies

4.4.2.1. α-Methylcinnamic alcohol was tested for sensitization using a Magnusson and Kligman guinea pig maximization test. This study was conducted in compliance with GLP and according to OECD guidelines No. 45 (OECD/GD (92) 32). Twenty male Dunkin Hartley albino guinea pigs weighing 300–390 g were used. The experiment consisted of two phases: induction phase (intradermal and topical) and the challenge phase. Intradermal induction consisted of three injections of (1) 0.1 ml Freund's Complete Adjuvant (FCA) diluted 1:1 with distilled water, (2) 0.1 ml of 5% (w/v) α-methylcinnamic alcohol in arachis oil BP, and (3) 0.1 ml of a 5% (w/v) α-methylcinnamic alcohol in 1:1 FCA plus distilled water, which were applied intradermally on a 40 × 60 mm clipped area of the shoulder region. On day 7, a 40 × 20 mm Whatman No. 4 filter paper saturated with neat α-methylcinnamic alcohol was applied topically on the previously tested area for 48 h under occlusion. Challenge applications were made on day 21. A Whatman No. 4 filter paper saturated with neat α-methylcinnamic alcohol and a separate patch saturated with 75% α-methylcinnamic alcohol v/v in arachis oil BP were applied. Reactions were assessed 24 and 48 h after patch removal. No sensitization (0/20) was observed (RIFM, 1997a).

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity (Table 4)

4.9.1. Bacterial studies

4.9.1.1. In an Ames assay (Ames et al., 1975) using *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 with and without metabolic activation, α-methylcinnamic alcohol was tested for mutagenicity in triplicates at doses up to 5000 μg/plate in dimethyl sulfoxide (DMSO). Visible reduction in growth of the bacterial background lawn and a decrease in the frequency of revertant colonies was observed. The test material was toxic at 5000 μg/plate to *S. typhimurium* strain TA100. Statistically significant and dose related increases in the frequency of revertant colonies were observed in the tester strains TA100, TA1535 and TA1537 at the doses of 1000–2500 μg/plate and at 5000 μg/plate without metabolic activation. Under the conditions of this study, the test material was classified as weakly mutagenic. This study was conducted in compliance with GLP (1997 (S) 1997/654) and according to the OECD (471) guidelines (RIFM, 1997a).

4.9.2. Mammalian studies

4.9.2.1. α-Methylcinnamic alcohol was evaluated for its potential to induce forward mutation in the L5178Y TK+/− mouse lymphoma cell line. α-Methylcinnamic alcohol was assayed for mutagenicity at dose levels upto 600 nl/ml in DMSO with and without S9 activation. α-Methylcinnamic alcohol did not induce dose-dependent increases in the mutant frequency at the TK locus in L5278Y mouse lymphoma cells. α-Methylcinnamic alcohol was considered to be inactive in the mouse-lymphoma assay. This study was conducted according to GLP and OECD (476) and EEC Commission Directive 87/302/EEC guidelines (RIFM, 1998).

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research

Table 4
Summary of mutagenicity and genotoxicity studies

Test Method	Strain	Dose	Results	Reference
Ames assay	*Salmonella typhimurium* strains TA98,TA100, TA102, TA1535 and TA1537	Up to 5000 μg/plate	Weakly mutagenic	RIFM (1997b)
Mouse lymphoma assay	L5178Y TK+/− mouse lymphoma cell line	600 nl/ml	No effects	RIFM (1998)

was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on phenethyl cinnamate

S.P. Bhatia [a,*], G.A. Wellington [a], J. Cocchiara [b], J. Lalko [a], C.S. Letizia [a], A.M. Api [a]

[a] *Research Institute for Fragrance Materials Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA*
[b] *Manheimer Fragrances, Teterboro NJ, USA*

Abstract

A toxicologic and dermatologic review of phenethyl cinnamate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Phenethyl cinnamate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8070.
E-mail address: sbhatia@rifm.org (S.P. Bhatia).

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In 2006, a complete literature search was conducted on phenethyl cinnamate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document. More details have been provided for unpublished data.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Benzylcarbinyl cinnamate; β-Phenethyl β-phenylacrylate; Phenylethyl cinnamate; 2-Phenylethyl cinnamate; 2-Phenylethyl 3-phenylpropenoate; 2-Propenoic acid, 3-phenyl-, 2-phenylethyl ester.
1.2 CAS Registry number: 103-53-7.
1.3 EINECS number: 203-120-3.
1.4 Formula: $C_{17}H_{16}O_2$.
1.5 Molecular weight: 252.32.
1.6 Council of Europe: Phenethyl cinnamate was included by the Council of Europe in the list of substances granted B – information required – hydrolysis study (COE No. 336) (Council of Europe, 2000).

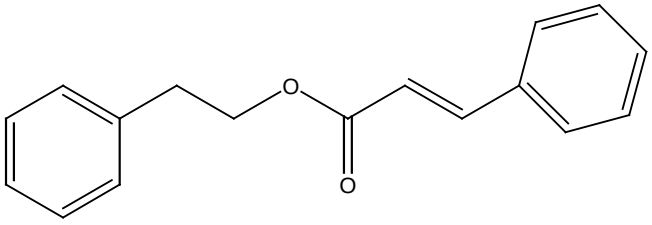

Fig. 1. Phenethyl cinnamate.

1.7 FDA: Phenethyl cinnamate was approved by the FDA as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and Extract Manufacturers' Association states: generally recognized as safe as a flavor ingredient – GRAS 3. (2863) (FEMA, 1965).
1.9 Joint Expert Committee on Food Additives (JECFA): The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 671) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent (JECFA, 2000).

2. Physical properties

2.1 Physical form: A white crystalline powder with a heavy balsamic-like rose note.
2.2 Flash point: >200 °F; CC.
2.3 Henry's law (calculated): 0.000000444 atm m^3/mol 25 °C.
2.4 Melting point: 54 °C.
2.5 Log K_{ow} (calculated): 4.56.
2.6 Vapor pressure (calculated): <0.001 mm Hg at 20 °C.
2.7 Water solubility (calculated): 2.954 mg/l @ 25 °C.

3. Usage (Table 1)

Phenethyl cinnamate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 1–10 metric tonnes per annum.

The maximum skin level that results from the use of phenethyl cinnamate in formulae that go into fine fragrances has been reported to be 0.22% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing phenethyl cinnamate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product%	Ingredient/mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.768	0.0029
Face cream	0.80	2.00	1.000	0.003	0.768	0.0006
Eau de toilette	0.75	1.00	1.000	0.080	0.768	0.0077
Fragrance cream	5.00	0.29	1.000	0.040	0.768	0.0074
Antiperspirant	0.50	1.00	1.000	0.010	0.768	0.0006
Shampoo	8.00	1.00	0.010	0.005	0.768	0.0001
Bath products	17.00	0.29	0.001	0.020	0.768	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.768	0.0001
Toilet soap	0.80	6.00	0.010	0.015	0.768	0.0001
Hair spray	5.00	2.00	0.010	0.005	0.768	0.0001
Total						0.0196

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

0.768% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0196 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Ten rats were orally administered phenethyl cinnamate at a dose of 5.0 g/kg/bodyweight. Observations were made for 14 days. Death occurred in 5/10 animals on day 1. The acute oral LD_{50} was determined to be approximately 5.0 g/kg based on 5/10 deaths at that dose. The only effect observed was lethargy (RIFM, 1975a).

4.1.1.2. Groups of white rats, white mice and guinea pigs were dosed perorally with a 20–45% solution of phenethyl cinnamate in sunflower oil. For each species, 3 animals/sex/dose were tested and observed over a 15-day period. The LD_{50} for all three species was reported to be 4.5 g/kg/bodyweight (no further details reported) (Zaitsev and Rakhmanina, 1974).

4.1.1.3. The acute oral LD_{50} in mice exceeded 5.0 g/kg, based on 0/10 deaths at that dose. Ten mice were orally administered phenethyl cinnamate at 5.0 g/kg/bodyweight. Observations were made for 14 days. No clinical signs were observed (RIFM, 1975b).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg based on 0/10 deaths at that dose. Ten rabbits received a dermal application of neat phenethyl cinnamate at a dose of 5.0 g/kg/bodyweight. Observations were made for 14 days. Diarrhea in 2/10 animals on day 1 was the only effect observed (RIFM, 1975a).

4.1.2.2. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg based on 0/4 deaths at that dose. Four rabbits received a dermal application of neat phenethyl cinnamate at a dose of 5.0 g/kg/bodyweight. Observations were made for 14 days. No clinical effects were observed (RIFM, 1975b).

4.1.3. Intraperitoneal studies

4.1.3.1. Prior to an antimicrobial study, the toxicity of phenethyl cinnamate was evaluated by intraperitoneal injection in 2 rabbits. A suspension of phenethyl cinnamate (0.5%) was prepared in 0.5% Tween 80 in water for injection in each rabbit. Observations were made for 7 days. No toxicity was observed during the test period (Gupta and Rao, 1978).

4.2. Skin irritation

4.2.1. Human studies (Table 3)

4.2.1.1. Two separate maximization pre-tests were carried out with 2% phenethyl cinnamate in petrolatum. Phenethyl cinnamate was applied to normal sites on the backs and/or forearms of 10 male and female volunteers for 48-h under occlusion. No irritation was observed (RIFM, 1975c, 1975d).

4.2.2. Animal studies (Table 4)

4.2.2.1. Irritation was evaluated during the acute dermal LD_{50} study described above (see Section 4.1.2.1). The dermal reactions consisted of slight (2/10 rabbits) erythema and slight (1/10 rabbits) edema (RIFM, 1975a).

4.2.2.2. Irritation was evaluated during the acute dermal LD_{50} study described above (see Section 4.1.2.2). No irritation was observed (RIFM, 1975b).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies (Table 5)

4.4.1.1. Two maximization tests (Kligman, 1966; Kligman and Epstein, 1975) were carried out with 2% phenethyl cinnamate (1380 μg/cm^2) in petrolatum on a total of 50 (17 male/33 females) volunteers. Each panel consisted of 25 subjects. Application was under occlusion to the same site on the forearms of all the volunteers for five alternate-day

Table 2
Summary of acute toxicity data

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rat	10	~5.0 g/kg	RIFM (1975a)
Oral	Mouse	10	>5.0 g/kg	RIFM (1975b)
Dermal	Rabbit	10	>5.0 g/kg	RIFM (1975a)
Dermal	Rabbit	4	>5.0 g/kg	RIFM (1975b)

Table 3
Summary of irritation studies in humans

Test method	Test concentration	Results	References
Maximization pre-test	2% in petrolatum	No irritation (0/5)	RIFM (1975c)
Maximization pre-test	2% in petrolatum	No irritation (0/5)	RIFM (1975d)

Table 4
Summary of irritation studies in animals

Method	Dose (%)	Species	Results	Reference
Irritation evaluated during a dermal LD_{50} study	100	Rabbits	Slight irritation	RIFM (1975a)
Irritation evaluated during a dermal LD_{50} study	100	Rabbits	No irritation	RIFM (1975b)

Table 5
Summary of sensitization studies in humans

Test method	Test concentration	Results	References
Maximization	2% (1380 μg/cm^2) in petrolatum	No sensitization (0/25)	RIFM (1975c)
Maximization	2% (1380 μg/cm^2) in petrolatum	No sensitization (0/25)	RIFM (1975d)

48-hour periods. The patch sites were pre-treated for 24 h with 5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a 10-day rest period, challenge applications were made to fresh sites on all the volunteers. Challenge applications were preceded by pretreatment with SLS. The challenge sites were read on removal of the patch and 24 h thereafter. No sensitization reactions were observed in either study (RIFM, 1975c, 1975d).

4.4.2. Animal studies

4.4.2.1 A guinea pig open epicutaneous test (OET) was conducted on groups of 6–8 male and female guinea pigs weighting 300–450 grams. Daily open applications of phenethyl cinnamate were made for 3 weeks to an 8 cm^2 clipped area on the flank of each guinea pig. The test sites were uncovered and the reactions were read 24 h after each application. A total of 21 applications of 0.1 ml phenethyl cinnamate in an unspecified vehicle were made for 21 days. The 10 controls were either left untreated or treated with a 0.1 ml sample of the vehicle for 21 days. At the challenge phase, both the test and control animals were treated on days 21 and 35 on the contralateral flank with 2% phenethyl cinnamate. No sensitization was observed (Klecak, 1985).

4.5. Phototoxicity and photoallergy

UV spectra revealed that phenethyl cinnamate peaked within 245–278 nm range and showed minor absorption in the 290–320 nm region.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Related Esters and Alcohols of Cinnamic Acid and Cinnamyl Alcohol When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

S.P. Bhatia, G.A. Wellington, J. Cocchiara, J. Lalko, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

A toxicologic and dermatologic assessment of ionones when used as fragrance ingredients ☆

The RIFM Expert Panel
D. Belsito [a], D. Bickers [b], M. Bruze [c], P. Calow [d], H. Greim [e], J.M. Hanifin [f], A.E. Rogers [g], J.H. Saurat [h], I.G. Sipes [i], H. Tagami [j]

[a] *University of Missouri (Kansas City), clo American Dermatology Associates, LLC, 6333 Long Avenue, Third Floor, Shawnee, KS 66216, USA*
[b] *Columbia University Medical Center, Department of Dermatology, 161 Fort Washington Avenue, New York, NY 10032, USA*
[c] *Lund University, Malmo University Hospital, Department of Occupational and Environmental Dermatology, Sodra Forstadsgatan 101, Entrance 47, Malmo SE-20502, Sweden*
[d] *Institut for Miliovurdering, Environmental Assessment Institute, Linnésgade 18, First Floor, Copenhagen 1361 K, Denmark*
[e] *Technical University of Munich, Institute for Toxicology and Environmental Hygiene, Hohenbachernstrasse 15-17, Freising-Weihenstephan D-85354, Germany*
[f] *Oregon Health Sciences University, Department of Dermatology L468, 3181 SW Sam Jackson Park Road, Portland, OR 97201-3098, USA*
[g] *Boston University School of Medicine, Department of Pathology and Laboratory Medicine, 715 Albany Street, L-804, Boston, MA 02118-2526, USA*
[h] *Hospital Cantonal Universitaire, Clinique et Policlinique de Dermatologie, 24, Rue Micheli-du-Crest, Geneve 14 1211, Switzerland*
[i] *Department of Pharmacology, University of Arizona, College of Medicine, 1501 North Campbell Avenue, P.O. Box 245050, Tucson, AZ 85724-5050, USA*
[j] *3-27-1 Kaigamori, Aoba-ku, Sendai 981-0942, Japan*

Abstract

An evaluation and review of a structurally related group of fragrance materials.

Keywords: Safety; Review; Fragrance; Ionones

Contents

☆ All correspondence should be addressed to: A. M. Api, 50 Tice Boulevard, Woodcliff Lake, NJ07677, USA. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: amapi@rifm.org

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1. Chemical identity, regulatory status and exposure

This report summarizes and synthesizes scientific data relevant to the risk assessment for the group of ionones used as fragrance ingredients (see Tables 1 and 2). The ionones fall into two major groups – ionones and rose ketones, with one compound common to both groups (1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-butane-1,3-dione – RIFM # 6347). A total of 30 compounds in the 2 groups were included in this summary. Most of these substances are used as fragrance and flavor ingredients. Included in this report are animal and human data by various routes of exposure, and a brief overview of environmental data. The scientific evaluation focuses on dermal exposure, which is considered to be the primary route for fragrance materials. Where relevant, toxicity, metabolism and biological fate data from other exposures have been considered.

The current format for these RIFM publications includes a summary evaluation paper of the chemical group and individual Fragrance Material Reviews on the individual chemicals. The group summary is an evaluation of relevant data selected from the large bibliography of studies and reports on the individual chemicals. The selected data were deemed to be relevant based on the nature of the protocols, quality of the data, statistical significance, and appropriate exposure. These data are presented in tabular form in the group summary. The Fragrance Material Reviews on each individual ionone contain a comprehensive summary of published and unpublished reports and comprehensive bibliographies.

Ionones are ingredients used in many fragrances. They may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Rose ketones have been defined as fragrance ingredients with the general formula, ''1-(trimethylcyclohexenyl/hexadienyl)-2-buten-1-one''. There are numerous possible isomers with this general formula. The cyclohexenyl derivatives are called damascones, and the cyclohexadienyl derivatives are called damascenones. The three methyl groups on the cyclohexenyl ring are all in the 2,6,6 positions except for isodamascone which is 2,4,4, and for the γ-structures where the 2 methyl group is converted to a double bond methylene group. All of the materials contain the 2-buten-1-one structure. This structure can have *cis–trans*-isomers around the double bond.

Several of the ionones in this report have been evaluated and approved for use as flavor ingredients in foodstuffs. In the United States, 7 ionones (allyl α-ionone, α-ionone, β-ionone, α-irone, methyl-α-ionone, α-*iso*-methylionone, methyl-β-ionone), have been approved for use as flavors by the Food and Drug Administration (FDA) in accordance with (21 CFR 172.515). In addition, 15 of these com-

Table 1
Material identity

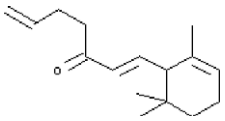
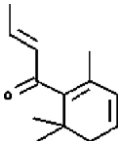
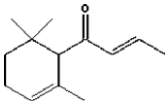
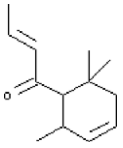
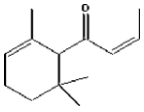
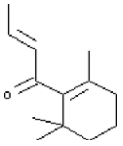
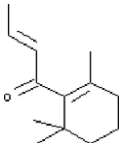
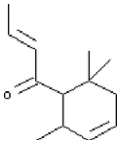
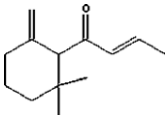
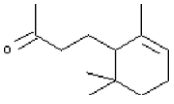
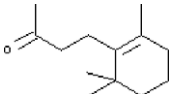
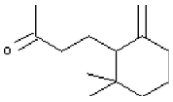
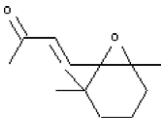
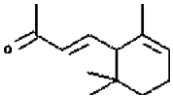
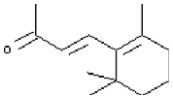
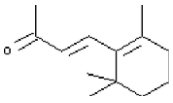
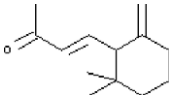
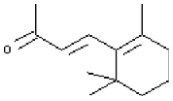
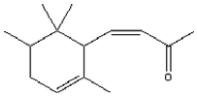
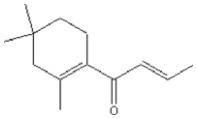
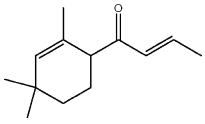
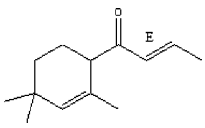
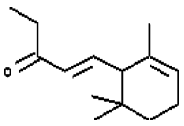
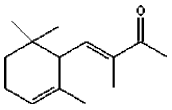
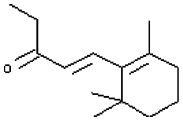
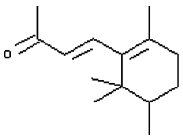
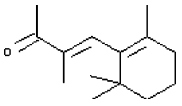
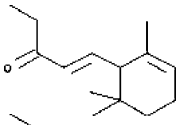
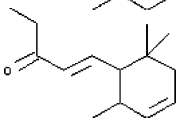
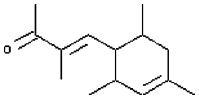
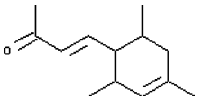
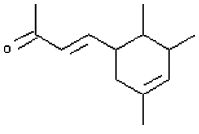
Compound	Structure	Synonyms
Allyl α-ionone CAS# 79-78-7 Molecular weight 232.37 log K_{ow} (calculated) 5.63		Allyl cyclocitrylideneacetone α-Allylionone α-Cyclocitrylidenemethyl butenyl ketone Cetone V 1,6-Heptadien-3-one 1-(2,6,6-trimethyl-2-cyclohexen-1-yl)- 1-(2,6,6-Trimethyl-2-cyclohexene-1-yl)-1,6-heptadien-3-one
Damascenone CAS# 23696-85-7 Molecular weight 190.28 log K_{ow} (calculated) 4.21		2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)- Floriffone
α-Damascone CAS# 43052-87-5; 24720-09-0 Molecular weight 192.3 log K_{ow} (calculated) 3.9		2-Butene-1-one, 1-(2,6,6-trimethyl-2-cyclohexen-1-yl)- α-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one (*E*)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one *trans*-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)but-2-en-1-one 2-Buten-1-one, 1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-(2*E*)- Dihydrofloriffone A *trans*-α-Damascone
δ-Damascone CAS# 57378-68-4 Molecular weight 192.3 log K_{ow} (calculated) 4.16		2-Buten-1-one, 1-(2,6,6-trimethyl-3-cyclohexen-1-yl)- δ-1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one Dihydrofloriffone TD 1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one
cis-α-Damascone CAS# 23726-94-5 Molecular weight 192.02 log K_{ow} (calculated) 4.29		2-Buten-1-one, 1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-(*Z*)- *cis*-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one (*Z*)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one
cis-β-Damascone CAS# 23726-92-3 Molecular weight 192.3 log K_{ow} (calculated) 4.42		2-Buten-1-one,1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-(2*Z*)- Damasione (*Z*)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one (*Z*)-β-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one
trans-β-Damascone CAS# 23726-91-2 Molecular weight 192.02 log K_{ow} (calculated) 4.42		Dihydrofloriffone B (*E*)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one (2*E*)-1-(2,6,6-Trimethyl-1-cyclocehexene-1-yl)-2-buten-1-one
trans,*trans*-δ-Damascone CAS# 71048-82-3 Molecular weight 192.02 log K_{ow} (calculated) 4.16		[1.α.(*E*),2.β.]-1-(2,6,6-Trimethylcyclohex-3-en-1-yl)but-2-en-1-one
γ-Damascone CAS# 35087-49-1 Molecular weight N/A log K_{ow} (calculated) N/A		1-(2,2-Dimethyl-6-methylenecyclohexyl)but-2-en-1-one
Dihydro-α-ionone CAS# 31499-72-6 Molecular weight 194.32 log K_{ow} (calculated) 4.22		2-Butanone, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)- Dihydro-α-ionone 4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-butan-2-one
Dihydro-β-ionone CAS# 17283-81-7 Molecular weight 194.32 log K_{ow} (calculated) 4.35		2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- Dihydro-β-ionone 4-(2,6,6-Trimethyl-1-cyclohexenyl)-butan-2-one

Table 1 (*continued*)

Compound	Structure	Synonyms
Dihydro-γ-ionone CAS# 13720-12-2 Molecular weight 194.18 log K_{ow} (calculated) 4.3		2-Butanone, 4-(2,2-dimethyl-6-methylenecyclohexyl)- 4-(2,2-Dimethyl-6-methylenecyclohexyl)-butan-2-one
4-(1,2-Epoxy-2,6,6-trimethylcyclohexyl)-3-buten-2-one CAS# 23267-57-4 Molecular weight 196.29 log K_{ow} (calculated) 2.93		3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)- 5,6-Epoxy-β-ionone Ionone epoxide, β β-Ionone-5,6-epoxide 4-(2,2,6-Trimethyl-7-oxabicyclo(4.1.0)hept-1-yl)-3-buten-2-one
α-Ionone CAS# 127-41-3 Molecular weight 192.3 log K_{ow} (calculated) 4.29		3-Buten-2-one,4-(2,6,6-trimethyl-2-cyclohexen-1-yl)- α-Cyclocitrylideneacetone α-Irisone 4-(2,2,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one
β-Ionone CAS# 14901-07-6 Molecular weight 192.3 log K_{ow} (calculated) 4.42		3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- β-Cyclocitrylideneacetone β-Irisone 4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one
(*E*)-β-Ionone CAS# 79-77-6 Molecular weight 192.02 log K_{ow} (calculated) 4.42		3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- (*E*)-β-Ionone *trans*-β-Ionone (*E*)-4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one
γ-Ionone CAS# 79-76-5 Molecular weight 192.3 log K_{ow} (calculated) 4.37		4-(2-Methylene-6,6-dimethylcyclohexyl)-3-buten-2-one 4-(2,2-Dimethyl-6-methylene-cyclohexyl)-3-buten-2-one 3-Buten-2-one, 4-(2,2-dimethyl-6-methylenecyclohexyl)-
Ionone (mixed isomers) CAS# 8013-90-9 Molecular weight 192.3 log K_{ow} (calculated) 4.42		Cyclocitrylidenacetone α- and β-isomers α- and β-Ionone Ionone (mixed isomers)
α-Irone CAS# 79-69-6 Molecular weight 206.33 log K_{ow} (calculated) 4.71		3-Buten-2-one, 4-(2,5,6,6-tetramethyl-2-cyclohexen-1-yl)- *cis*- *cis*-(2,6)-*cis*-(2(1),2(2))-α-Irone 6-Methylionone 6-Methyl-α-ionone 4-(2,5,6,6-Tetramethyl-2-cyclohexen-1-yl)-3-buten-2-one
Isodamascone (standard quality) CAS# 70266-48-7 Molecular weight 192.02 log K_{ow} (calculated) 4.42		2-Buten-1-one, 1-(2,4,4-trimethyl-2-cyclohexen-1-yl) 1-(2,4,4-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one 1-(2,4,4-Trimethyl-1-cyclohexene-1-yl)-2-buten-1-one
Isodamascone (isomer unspecified) CAS# 33673-71-1 Molecular weight 192.3 log K_{ow} (calculated) 4.29		2-Buten-1-one, 1-(2,4,4-trimethyl-2-cyclohexen-1-yl)- 1-(2,4,4-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one
α-Isodamascone CAS# 39872-57-6 Molecular weight 192.02 log K_{ow} (calculated) 4.29		2-Buten-1-one, 1-(2,4,4-trimethyl-2-cyclohexen-1-yl)- (2*E*)- 2-Buten-1-one, 1-(2,4,4-Trimethyl-2-cyclohexen-1-yl)- (*E*)-Isodamascone (*E*)-1-(2,4,4-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one α-Cetone
Methyl-α-ionone CAS# 127-42-4 Molecular weight 206.33 log K_{ow} (calculated) 4.78		α-Cyclocitrylidenebutanone α-Cyclocitrylidenemethyl ethyl ketone α-Methylionone 1-Penten-3-one 1-(2,6,6-Trimethyl-2-cyclohexen-1-yl),[*R*-(*E*)]- (*R*-(*E*))-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-pent-1-en-3-one

(*continued on next page*)

Table 1 (*continued*)

Compound	Structure	Synonyms
α-*iso*-Methylionone CAS# 127-51-5 Molecular weight 206.33 log K_{ow} (calculated) 4.84		3-Buten-2-one, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)- Iraldeine γ Isoraldeine 95 γ-Methylionone α-Methyl ionone Methyl-γ-ionone 3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one Raldeine γ
Methyl-β-ionone CAS# 127-43-5 Molecular weight 206.33 log K_{ow} (calculated) 4.91		β-Cetone β-Cyclocitrylidenebutanone β-Iraldeine β-Methylionone 1-Penten-3-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)- 5-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-4-penten-3-one 1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-pent-1-en-3-one
6-Methyl-β-ionone CAS# 79-70-9 Molecular weight 206.29 log K_{ow} (calculated) 4.84		3-Buten-2-one, 4-(2,5,6,6-tetramethyl-1-cyclohexen-1-yl)- β-Ionone, 6-methyl- β-Irone 4-(2,5,6,6-Tetramethyl-1-cyclohexen-1-yl)-3-buten-2-one
iso-Methyl-β-ionone CAS# 79-89-0 Molecular weight 206.33 log K_{ow} (calculated) 4.97		3-Buten-2-one, 3-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- δ-Iraldeine Isomethyl-β-ionone 3-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)-but-3-en-2-one
Methyl ionone (mixture of isomers) CAS# 1335-46-2 Molecular weight 206.33 log K_{ow} (calculated) 4.84		Ionone, methyl- Isoraldeine Iralia
Methyl-δ-ionone CAS# 7784-98-7 Molecular weight 206.33 log K_{ow} (calculated) 4.66		5-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-4-penten-3-one 1-Penten-3-one, 1-(2,6,6-trimethyl-3-cyclohexen-1-yl)-
3-Methyl-4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one CAS# 67801-29-0 Molecular weight 206.29 log K_{ow} (calculated) 4.81		3-Buten-2-one, 3-methyl-4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-
4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one CAS# 67801-38-1 Molecular weight 192.3 log K_{ow} (calculated) 4.26		3-Buten-2-one, 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)- Iritone
4-(3,5,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one CAS# 67801-39-2 Molecular weight 192.02 log K_{ow} (calculated) 4.26		3-Buten-2-one, 4-(3,5,6-trimethyl-3-cyclohexen-1-yl)-

pounds have been granted Generally Recognized as Safe (GRAS) status by the Flavor and Extract Manufacturers' Association.

Some of these materials, namely α-ionone, α-irone, α-*iso*-methylionone, and methyl-β-ionone were also included in the Council of Europe list of substances (Nos. 141, 145, 169, 144) that may be used in foodstuffs. Allyl-α-ionone (COE No. 2040) and *iso*-methyl-β-ionone (COE No. 650) were included by the Council of Europe in the list of substances granted B status (information required – 28-day oral toxicity study) while dihydro-α-ionone (COE No. 11059), and dihydro-β-ionone (COE No. 11060) were

Table 2
Volume of use and dermal exposure

Material	RIFM number	Annual worldwide metric tons	Dermal systemic exposure in cosmetic products (mg/kg/day)	Maximum skin level (%)
Allyl α-ionone	240	10–100	0.0176	0.32
Damascenone	1297	1–10	0.002	0.02
α-Damascone	1298	1–10	0.0031	0.07
cis-α-Damascone	5472	1–10	0.0025	0.02
cis-β-Damascone	1299	1–10	0.0018	0.02
trans-β-Damascone	5471	1–10	0.0018	0.02
δ-Damascone	1300	100–1000	0.0024	0.02
trans,*trans*-δ-Damascone	5960	0.1–1.0	0.002	0.02
γ-Damascone	6402	<0.1	0.0005[a]	0.0
Dihydro-α-ionone	788	<0.1	0.0005[a]	0.02
Dihydro-β-ionone	5026	10–100	0.1085	1.34
Dihydro-γ-ionone	5409	<0.1	0.0002	0.001
4-(1,2-Epoxy-2,6,6-trimethylcyclohexyl)-3-buten-2-one	5126	<0.1	0.0006	0.003
α-Ionone	6132	100–1000	0.0512	1.0
β-Ionone	5022	100–1000	0.1106	2.34
(*E*)-β-Ionone	6067	10–100	0.0792	1.46
Ionone (mixed isomers)	135	100–1000	0.0764	1.57
Isodamascone	6429	<0.1	0.0005[a]	0.02
Isodamascone (isomer unspecified)	6305	<0.1	0.0005[a]	0.02
α-Isodamascone	1215	0.1–1.0	0.001	0.014
6-Methyl-α-ionone (α-Irone)	336	1–10	0.0056	0.29
Methyl-α-ionone	6250	10–100	0.0004	0.001
α-*iso*-Methylionone (methyl-γ-ionone [so-called])	6273	100–1000	0.3312	3.69
Methyl-β-ionone	6272	10–100	0.0025	0.02
6-Methyl-β-ionone (β-Irone)	6066	<0.1	0.0025	0.02
iso-Methyl-β-ionone	6083	10–100	0.2375	1.18
Methyl ionone (mixture of isomers)	140	100–1000	0.2502	5.64
3-Methyl-4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one	5847	<0.1	0.013	0.02
4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one (iritone)	1037	<0.1	0.001	0.007
4-(3,5,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one	5854	<0.1	0.0005[a]	0.02

[a] A default value of 0.02 was used to calculate the dermal systemic exposure.

included by the Council of Europe in the list of substances granted "Waiting" status.

Finally, the International Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1999) evaluated 15 of the 30 ionones/rose ketones assessed in this report. The Committee concluded that use of these substances as flavoring agents would not present a safety concern at the current estimated intake levels (JECFA, 1999). An Acceptable Daily Intake (ADI) of 0–0.1 mg/kg for α- and β-ionone singly, or in combination, was established (JECFA, 1999). The International Fragrance Association (IFRA) has established Standards for rose ketones and methyl ionones (please see the individual Fragrance Material Reviews on these materials for more information on the IFRA Standards).

Methyl ionone (mixture of isomers) and α-*iso*-methylionone are High Production Volume (HPV) materials and, as such, have been included in a robust summary and test plan for "Ionone Derivatives" which has been prepared by the Flavor and Fragrance High Production Volume Consortium.

Ionone derivatives occur mainly in plants containing β-carotene. α- and β-Ionone and related substances have been detected in a variety of foods including raspberries, carrots, roasted almonds, fruits and herbs (Maarse et al., 1994; CIVO-TNO, 1999).

Data from a survey conducted in the year 2000 indicate that the annual worldwide use of the individual ionones varies greatly and ranges from <0.1 to 1000 metric tonnes per annum (Table 2).

1.1. Estimated consumer exposure

The availability of fragrance ingredients for potential consumer exposure is estimated in two ways (see Table 2). One estimates potential percutaneous absorption (systemic exposure) from the entire body surface due to the use of many different fragranced products. The other estimates potential dermal exposure due to the use of products, such as fine fragrances, that usually contain higher concentrations and are used on smaller localized skin sites. Potential systemic exposure to ionones is estimated based on the concentrations in 10 types of cosmetic products (body lotion, face cream, eau de toilette, fragrance cream, anti-perspirant, shampoo, bath products, shower gel, toilet soap, and hair spray). The maximum skin exposure levels that result from ionones in formulae that go into fine fragrances vary widely and have been reported to range from 0.001%

to 5.64%. For consideration of potential sensitization, the exposure is calculated as the percent concentration applied to the skin. Exposure to ionones used in fine fragrance products is calculated based on the use of 20% of the fragrance mixture (the maximum used) in the fine fragrance consumer product (IFRA, 2001). The calculated exposures for the ionones used in cosmetic products are listed in Table 2. Maximum daily exposures on the skin range from 0.0002–0.331 mg/kg/day for the individual ionones for high end users of cosmetic products containing these materials (see Table 2). Exposure data were provided by the fragrance industry. Explanations of how the data were obtained and of how exposures were determined have been previously reported by Cadby et al. (2002) and Ford et al. (2000).

2. Absorption, distribution and metabolism, and potential for enzyme induction

2.1. Absorption

In the scientific literature there are no definitive data from which to quantify the *in vivo* absorption of ionones and/or rose ketones following dermal exposure. By analogy with fragrance ketones and aldehydes for which *in vivo* absorption data are available, dermal absorption of ionones/rose ketones is likely to be significant. All are lipophilic substances with oil/water partition coefficient ($\log K_{ow}$) values in the range of 3.85–5.20. In light of these data, and the lack of specific information on any of the individual ionones/rose ketones, a dermal absorption rate of 100% was conservatively assumed for the purposes of human health risk assessment. The assumption of 100% dermal bioavailability is considered especially conservative given that in an *in vitro* dermal penetration/permeability study, only 0.7% or undetectable amounts of methyl ionone (mixture of isomers) were recovered in the fluid beneath the skin preparations of rats and pigs, respectively, 6 h after application of a 3000 μg dose (600 μg/cm^2 over 5 cm^2 of skin) (RIFM, 1984a). In this study, approximately 50% (rat) and 10% (pig) of methyl ionone ^{14}C penetrated into, but not through the epidermis and dermis, while another 30% was lost to evaporation.

There also are no oral pharmacokinetic studies available from which the bioavailability of this class of compounds can be quantitatively determined. Based on metabolic studies on α-ionone (Prelog et al., 1951) and β-ionone (Bielig and Hayasida, 1940; Ide and Toki, 1970) in which ionone-specific metabolites were recovered in the urine of treated rabbits, and in the urine of dogs treated orally with β-ionone (Prelog and Meier, 1950), oral absorption of these compounds does occur to some extent. These studies, however, were not designed as pharmacokinetic investigations suitable to determine oral absorption. Given that a certain, but unquantifiable amount, of orally ingested α- and β-ionone is absorbed, it is prudent to assume that the other 34 structurally related ionones and rose ketones assessed in this report would also be bioavailable *via* the oral route. As a result, rose ketones were assumed to be 100% bioavailable for the purposes of human health risk assessment. Given the *in vitro* skin penetration data (RIFM, 1984a) on methyl ionone (mixed isomers), bioavailability by the oral route is likely to be considerably greater than by the dermal route. However, the magnitude of this potential difference cannot be quantified or extrapolated to all chemicals included in this assessment.

2.2. Distribution and pharmacokinetics

Data available describing the distribution and pharmacokinetics of ionones/rose ketones following absorption are limited to a single study in mice reporting the presence of β-ionone at trace levels (<0.1 ng/ml) in the blood 30–90 min following a 1-h inhalation exposure to 0.00001 ppm (Buchbauer et al., 1993).

2.3. Metabolism

All the compounds discussed in this group are simple molecular modifications of the basic ionone and damascene structures, which are in essence cyclohexene derivatives carrying a butanone side chain. Therefore ionone and damascone can be regarded as being archetypal for the group as a whole. Furthermore, it is anticipated that compounds in this group will show a high degree of metabolic homology, bearing in mind that, in general, the same functional groups will be involved in biotransformation reactions. The α- and β-ionones are structural positional isomers as are also the α- and β-damascones. The only structural differences between the ionones and the rose ketones are the position of the allylic double bond and of the ketone in the butanone side chain (see Table 1 for structure and CAS numbering system).

The ionones and rose ketones, because of their highly lipophilic nature would be expected to be extensively metabolized *in vivo* and eliminated as transformation products. This appears to be the case as in several studies involving the administration of α- or β-ionone to rabbits and dogs. Little unchanged compound was recovered from the urine compared to the relatively large amounts of transformation products that could be isolated (Bielig and Hayasida, 1940; Prelog and Meier, 1950; Ide and Toki, 1970). Based upon the molecular structures of the ionones and rose ketones several metabolic options might be predicted:

1. hydroxylation/oxygenation of the cyclohexene ring;
2. reduction of the buteneone group to a secondary alcohol;
3. oxidation of the angular methyl groups;
4. reduction of the double bond in the exocyclic alkenyl side chain to form dihydro derivatives;
5. conjugation of the hydroxylated metabolites with glucuronic acid;
6. conjugation with glutathione.

Finally there could be various combinations of these pathways to produce an array of metabolites.

Overall, while the empirical metabolic data are limited to studies primarily on β-ionone, it should be noted that the ionones and rose ketones are close structural analogues, both having a cyclohexa(e)ne ring with an allylic side chain containing a ketone moiety. Differences in the structures are related to the presence of an additional ketone group [e.g., 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-butane-1,3-dione], unsaturation of the cyclohexene ring (e.g., dihydro-γ-ionone), unsaturation of the allylic side chain, differences in the points of methylation of the cyclohexene ring, the position of the double bond in the allylic side chain (i.e., the ionones *versus* the rose ketones), and various combinations of the above. While these differences would be expected to lead to the production of compound-specific metabolites without a common terminal metabolite, some generalizations can be made. As reported by JECFA (1999), α-ionone, dihydro-α-ionone, methyl-α-ionone, α-irone, α-*iso*-methylionone, and allyl-α-ionone would likely share a common metabolic pathway, with differences in rates of metabolism only. Likewise, β-ionone, dihydro-β-ionone, and methyl-β-ionone, could be expected to be metabolized in a very similar manner. For the other compounds, while common pathways cannot be clearly established, similar metabolic processes would be expected to occur and could include various combinations of hydroxylation/oxygenation of the cyclohexene ring, reduction of the butenone group to a secondary alcohol, oxidation of the angular methyl groups, reduction of the double bond in the exocyclic alkenyl side chain to form dihydro derivatives, and conjugation of the hydroxylated metabolites with glucuronic acid.

Other metabolic routes such as epoxidation may potentially be available to certain ionones and rose ketones, but no metabolites indicative of this pathway have been reported. It should be noted that the rose ketones, which are more likely to undergo epoxidation have not been subjected to metabolic study. For most ionones and rose ketones, the endocyclic unsaturated bond is structurally hindered by methyl substituents which likely impede epoxidation reactions at this site. Similarly, based on *in vitro* studies with two archetypal α,β-unsaturated ketones included in the chemicals under assessment, namely 4-(2,6,6-trimethylcyclohex-1-enyl)-2-buten-4-one and 1-(2,6,6-trimethylcyclohexa-1,3-dienyl)-2-buten-1-one, reactivity with glutathione, and hence the potential for electrophilic reactions with biological molecules, was concluded to be minimal (Portoghese et al., 1989). The authors concluded that these compounds exhibit low reactivity towards glutathione because the electrophilic centers are sterically hindered by directly attached substituents (methyl groups) and neighboring groups. Reactivity with other nucleophilic centers (e.g., guanine components of nucleotides) would be expected to be dramatically less than with glutathione. As a result, the metabolism of the α,β-unsaturated ketone in the side chain of the rose ketones is not expected to produce reactive intermediates of greater toxicity than similar metabolism of the more sterically hindered α,β-unsaturated ketone side chain of the ''ionone'' series.

Three rose ketones (*trans*,*trans*-δ-damascones; δ-damascone; damascone) as well as dehydrodihydroionone, have an additional and unhindered double bond in the cyclic ring structure that could provide a potential site for epoxidation to occur. Similarly, for methyl-δ-ionone, the cyclohexene ring contains a point of unsaturation less hindered by the presence of methyl groups, possibly increasing the likelihood of epoxidation. Epoxidation of these specific chemicals could produce products with higher reactivities/toxicities than other members of this class.

In summary, empirical metabolic data on ionone isomers demonstrate the activity of various metabolic pathways leading to polar metabolites, both in free and conjugated forms. The primary differences in the chemical structure of members of this class of compounds that could affect metabolism, and potentially the toxicity of metabolites, are the position of the double bond in the allylic side chain (ionones *versus* rose ketones) and the potential for epoxidation depending upon the number and position of the double bonds in the cyclohexene ring. Since the allylic side chain of the rose ketones does not appear to have strong electrophilic activity, based on *in vitro* data (Portoghese et al., 1989), the damascone metabolites are unlikely to be of greater toxicity than those of the ionones. However, based on metabolic considerations, unique epoxide metabolites could be generated for each of *trans*,*trans*-δ-damascones; δ-damascone; 1-(2,6,6-trimethyl-3-cyclohexa-1-e-dienyl)-2-buten-1-one); dehydrodihydroionone, and methyl-δ-ionone. Thus, these compounds may have greater toxic potential than other members of this class.

The most complete *in vivo* metabolic data are from animal studies; there are no human data for these compounds. The most extensive data are for β-ionone with a limited amount of data for the α isomer; the metabolic data available can be viewed as being representative for the class as a whole. Following administration of β-ionone to a male rabbit (oral gavage, 1 g/day for seven days), Ide and Toki (1970) isolated from the urine and characterized the following transformation products (numbered on the CAS system): 3-oxo-β-ionone, 3-oxo-β-ionol, dihydro-3-oxo-β-ionol and 3-hydroxy-β-ionol together with the glucuronides of 3-oxo-β-ionol and dihydro-3-oxo-β-ionol. Only a small amount of unchanged β-ionone (circa 1% of dose) was recovered from the urine of the dosed animal.

In an earlier study, Bielig and Hayasida (1940) isolated β-ionol and dihydro-β-ionol as reduction products from the urine of dogs fed β-ionone; three additional hydroxylated metabolites were detected but not characterized. Prelog and Meier (1950) confirmed these findings and identified 3-oxo-β-ionol and 3-hydroxy-β-ionol or 3-hydroxy-β-ionone. In the single metabolic study of α-ionone in mammals, Prelog et al. (1951) isolated a trans-

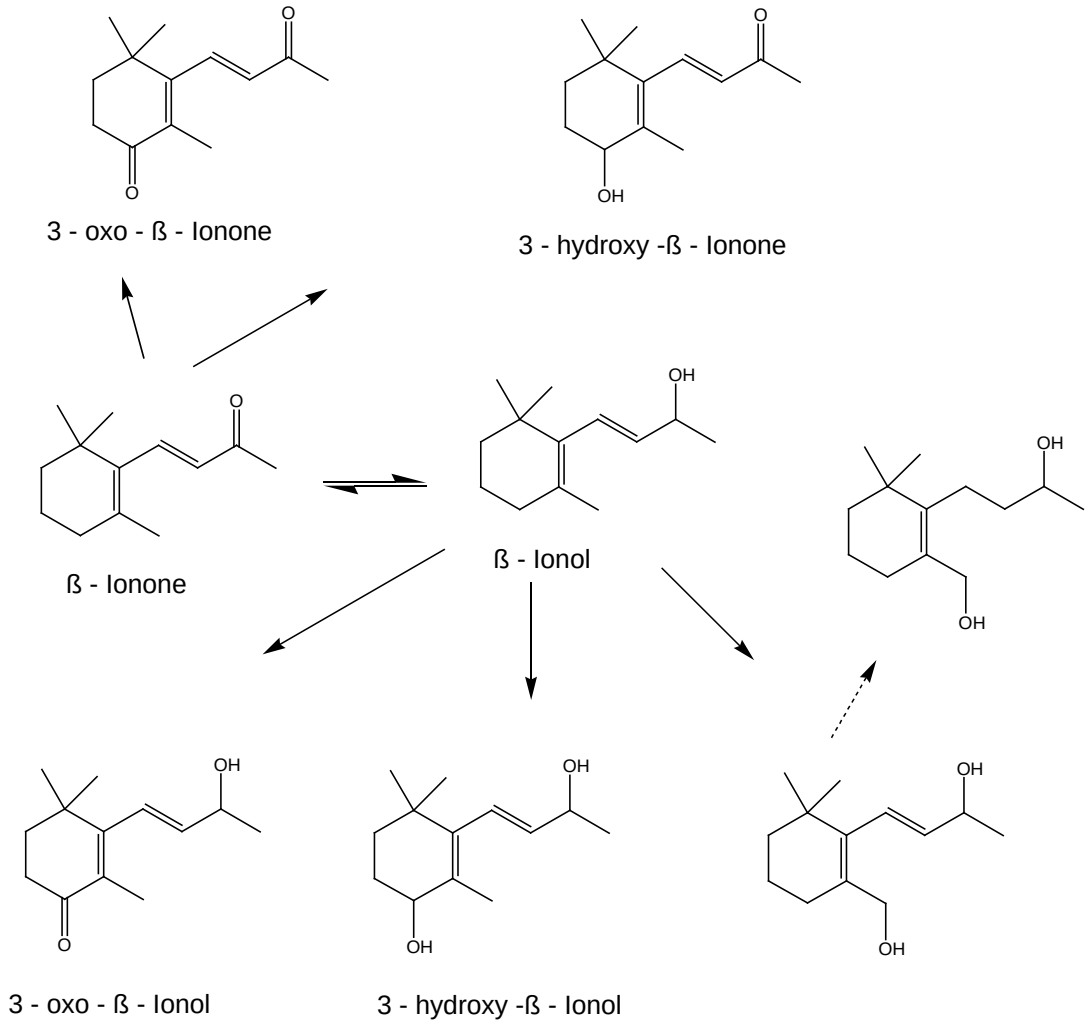


Fig. 1. Major pathways of metabolism of β-ionone in mammals.

formation product in urine of rabbits which appeared to be an oxidation product, tentatively identified as 4-oxo-tetrahydro-ionone (Fig. 1).

There is no available information on the metabolic fate of the ionones and rose ketones in humans, but one might reasonably presume that it would be similar to that seen in mammals such as the rabbit and dog, i.e., oxidative and reductive transformation followed by conjugation. Support for this view comes from the pattern of metabolism of other compounds containing the ionone structure. For example, the retinoids, such as 13-*cis*-retinoic acid (isotretinoid) contain the ionone ring structure. 13-*cis*-Retinoic acid undergoes extensive metabolism in humans by oxidation and conjugation, including oxidation of the ionone nucleus to give the 4-oxo-13-*cis*-retinoic acid metabolite (Vane et al., 1990; Kraft et al., 1991). This position of oxidation is analogous to the 3-oxo metabolites of β-ionone as numbered using the CAS system of nomenclature. In summary, the available evidence indicates that the ionones and rose ketones are extensively metabolized *in vivo* by pathways involving oxidation, reduction and conjugation. These metabolites do not raise issues of toxicological concern.

3. Toxicological studies

3.1. Acute toxicity

Overall, the acute oral and dermal toxicity of ionones is low to moderate based on the lowest reported oral LD_{50} of 1500–1800 mg/kg body weight for α-1-(2,6,6,-trimethyl-3-cyclohexen-1-yl)-2-buten-1-one (RIFM, 1979a; Piccirillo et al., 1979). Many of the ionones have oral LD_{50} values of >2000 mg/kg body weight, the normal limit dose in this assay. Acute dermal LD_{50} values exceeded 2000 mg/kg. Parenteral administrations of β-ionone and ionone (60% α- and 40% β-isomers) yielded LD_{50} values of 700 mg/kg

and 2277 mg/kg, respectively. The subcutaneous LD_{50} of ionone (60% α- and 40% β-isomers) in mice was 2605 mg/kg. Further data on the acute toxicity of ionones and rose ketones are presented in Tables 3a (oral), 3b (dermal), and 3c (other routes of exposure).

3.2. Subchronic toxicity

The results of subchronic studies with ionones are summarized in Table 4 and described below.

3.2.1. Dermal studies

Of the 30 ionones/rose ketones assessed, only α-*iso*-methyl ionone (RIFM, 1980a, 1981a) has been subjected to 90-day subchronic dermal toxicity testing (2 rat studies).

In the first study (RIFM, 1980a), Sprague–Dawley rats (15/sex/dose) were administered 50, 170, 580, or 2000 mg/kg body weight/day of neat α-*iso*-methyl ionone (no dosing vehicle) *via* clipped skin for a period of 90 days. Clinical, laboratory and gross and histopathological evaluations were conducted.

On the skin at the application site there was a dose-dependent increase in the severity of erythema, and eschar formation. Since erythema and eschar formation occurred in all treatment groups, a NOAEL for this effect could not be established.

Body weight gains were significantly reduced in females in the highest dose group and in males treated at 580 and 2000 mg/kg body weight/day. Total food consumption throughout the study was significantly increased in females treated at the 2 highest dose levels and there was a significant decrease in food efficiency and food intake in both sexes in the 2 highest dose groups. The body weight changes may not represent a direct, test-material-related effect since many of these animals manifested severe skin lesions.

There were hematological changes in the 2 highest dose groups and reduced serum glucose in the high-dose animals, all largely attributable to the inflammation and infection at the site of application.

Table 3a
Acute oral toxicity studies

Material	Species	No. of animals/ dose/group	LD_{50}[a] (mg/kg)	Reference
Allyl-α-ionone	Mice	5–10	9500	RIFM (1955)
Dihydro-α-ionone	Rats	10	>5000	RIFM (1976d)
Damascenone	Rats	5	>2000	RIFM (1986a)
α-Damascone	Rats	10 (5/sex)	1800 for males; 1500 for females; 1670 combined	RIFM (1979a)
δ-Damascone	Mice	10 (5/sex)	1821 (95% C.I. 1354–2414)	RIFM (1978f), Moran et al. (1980)
γ-Damascone	Rats	10 (5/sex)	>2000	RIFM (1987a)
trans-β-Damascone	Rats	10 (5/sex)	>2000	RIFM (1986b)
trans-β-Damascone	Rats	10 (5/sex)	2920 (95% C.I. 2655–3212)	RIFM (1969), Posternak and Vodoz (1975)
Dihydro-β-ionone	Rats	6 (3/sex)	>2000	RIFM (1999a)
Dihydromethyl-α-ionone[b]	Rats	10	>5000	RIFM (1976f)
1,3-Dimethyl-α-ionone[b]	Rats	10 (5/sex)	>5000	RIFM (1984g)
1,3-Dimethyl-α-ionone[b]	Rats	10	>5000	RIFM (1976e)
α-Ionone	Mice	10	6657 ± 652	RIFM (1967a)
α-Ionone	Mice	10	7000	RIFM, 1980i
β-Ionone	Mice	5	5331 ± 755	RIFM (1967a)
β-Ionone	Mice	10	2000	RIFM (1980i)
β-Ionone	Rats	10	3290	RIFM (1980i)
Ionone	Mice	10 (5/sex)	10,000	RIFM (1980j)
Ionone	Rats	10 (5/sex)	4590 (95% C.I. 3880–5400)	Jenner et al. (1964), Bár and Griepentrog (1967)
α-Irone	Rats	10 (5/sex)	>5000	RIFM (1972c)
α-Irone	Mice	10	7410 ± 519	RIFM (1967b)
Isodamascone	Rats	10 (5/sex)	6300	RIFM (1979s)
Iso-β-ionone[b]	Rats	10	>5000	RIFM (1980e)
α-*iso*-Methylionone	Mice	10	8714 ± 252	RIFM (1967a)
α-*iso*-Methylionone	Mice	10 (5/sex)	~10,000	RIFM (1980k)
α-*iso*-Methylionone	Rats	10	>5000	RIFM (1973)
Methyl ionone	Rats	10	>5000	RIFM (1973)
Methyl ionone	Mice	10 (5/sex)	Between 5000 and 1000	RIFM (1980l)
4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one	Rats	10	5200 (95% C.I. 3800–7200)	RIFM (1978e)

[a] Units have been converted to make easier comparisons; original units are in the Fragrance Material Reviews.
[b] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

Table 3b
Acute dermal toxicity studies

Material	Species	No. of animals/dose/group	LD_{50}[a] (mg/kg)	Reference
Allyl α-ionone	Rabbits	6	>5000	RIFM (1971c)
Damascenone	Rabbits	6 (3/sex)	>2000	RIFM (1979aa)
α-Damascone	Rabbits	6 (3/sex)	>2000	RIFM (1979z)
α-Damascone	Rat	10 (5/sex)	2900 (95% C.I. 2164–3886)	RIFM (1979cc)
γ-Damascone	Rabbits	10 (5/sex)	>2000	RIFM (1987b)
trans-β-Damascone	Rabbits	6 (3/sex)	>2000	RIFM (1979t)
Dihydro-α-ionone	Rabbits	10	>5000	RIFM (1976d)
Dihydromethyl-α-ionone[b]	Rabbits	10	>5000	RIFM (1976f)
1,3-Dimethyl-α-ionone[b]	Rabbits	10 (5/sex)	>2000	RIFM (1984b)
1,3-Dimethyl-α-ionone[b]	Rabbits	10	>5000	RIFM (1976e)
α-Irone	Rabbits	3	>5000	RIFM (1972c)
Iso-β-ionone[b]	Rabbits	10	>5000	RIFM (1980e)
α-*iso*-Methylionone	Rabbits	8	>5000	RIFM (1973)
Methyl ionone	Rabbits	8	>5000	RIFM (1973)
4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one	Rabbits	10	>5000	RIFM (1978e)

[a] Units have been converted to make easier comparisons; original units are in the Fragrance Material Reviews.
[b] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

Table 3c
Acute miscellaneous toxicity studies

Material	Dose route	Species	No. of animals/dose group	LD_{50}[a] (mg/kg)	Reference
Ionone	s.c. injection	Mice	10	2605 (95% C.I. 2113–3198)	Wenzel and Ross (1957)
Ionone	i.p. injection	Mice	620	2277	Sporn et al. (1963)
β-Ionone	i.p. injection	Mice	10	700	RIFM (1980i)

[a] Units have been converted to make easier comparisons; original units are in the Fragrance Material Reviews.

A significant increase in serum BUN was reported in males in the top 2 dose groups. Urinalysis showed a significant increase in the incidence of albuminuria in males in the 3 highest dose groups. In the high-dose males, abundant eosinophilic globules were observed in the kidney epithelium at necropsy.

At necropsy there was a significant increase in the absolute and relative liver weights in both sexes at all dose levels. Increases, most of which attained statistical significance, in the absolute and relative weights of the kidneys were reported in all but the lowest dose groups of each sex. The absolute adrenal weights were significantly increased in the 2 highest dose groups of both sexes.

The interpretation of the data is complicated by the severe skin damage at the application site, especially in the 2 highest dose groups. Depressed body weight gains and increased neutrophil count are probably attributable to infection and inflammation. Azotemia and proteinuria likely are a result of chronic severe tissue damage and infection. The liver weight increase probably resulted from induction of microsomal mixed-function oxidases. Increased adrenal weights probably reflect the response to stress caused by tissue damage and infection.

Severe tissue destruction and infection in the skin may have combined to elicit increased kidney weight at higher doses and epithelial eosinophilic globules in the convoluted tubules of the outer cortex. To determine if these effects were specific to male rat nephropathy, a review of the histopathology of kidneys from rats in this study was conducted. This lesion occurred in a dose-responsive fashion in males only and was seen also in male control rats. It was accompanied by interstitial nephritis in control and treated rats. The findings suggest an endogenous disease process which was exacerbated by the application of the irritating test material and marked skin necrosis. On the basis of the review of the kidney histopathology data and considering the dermal inflammation and infection in these animals, the results of this study are concluded to show a systemic NOAEL of topical α-*iso*-methyl ionone of 50 mg/kg (RIFM, 1980a).

In a subsequent 90-day study (RIFM, 1981a), α-*iso*-methyl ionone was applied dermally daily to groups of 5 male and 5 female Sprague–Dawley rats at a daily dose of 10 mg/kg as a 1% solution in phenethyl alcohol (PEA) (RIFM, 1981a). No dermal reaction to treatment was noted at any time during the study. The hematology, clinical chemistry, and urinalysis parameters evaluated were comparable to the controls. A slight, but significant increase in serum alkaline phosphatase activity was reported in males. The relationship of this finding to treatment was considered questionable. There was no evidence of a treatment-related effect on body weight gain, necropsy observations, organ weights, or on the results of the microscopic examination. As a result, the NOAEL for the skin appeared to be 10 mg/kg, the only dose tested, but the inclusion of only 5 animals per sex and of only one dose precludes statistical analyses of the data. The lack of dermal reactions in this study (RIFM, 1981a) contrasts to

Table 4
Subchronic toxicity studies

Material	Method	Dose[a]	Species	Results	Reference
trans-β-Damascone	Oral (diet) 90-day toxicity study	2.26 mg/kg	16 CF/Gif rats sex/dose	No adverse toxic effects	RIFM (1969), Posternak and Vodoz (1975)
α-Ionone	Oral (diet) 90-day study	10 and 100 mg/kg/day	Sprague–Dawley rats (15/sex/dose)	10 mg/kg: Increase liver weight, decrease erythrocyte and packed cell volume 100 mg/kg: Reduced weight gain, food consumption and serum glucose concentration, increased water intake, mild renal changes; increase in neutrophil and decrease lymphocytes Increased hepatic p450 content and activity of drug metabolizing enzymes. Increased liver weight most likely resulted from enzyme induction	RIFM (1983a)
α-Ionone	Oral (diet) 90-day study	Males 11.8 mg/kg, females 11.1 mg/kg	15 FDRL rats sex/dose	No adverse toxic effects	Oser et al. (1965)
α-Ionone	Oral (diet) 90-day study	10.6 mg/kg	Unspecified number of rats	No adverse toxic effects	Bár and Griepentrog (1967)
β-Ionone	Oral (diet) 90-day study	10 and 100 mg/kg	60 Sprague–Dawley rats (15/sex/dose)	Higher relative liver weights in male; relative brain, liver, kidney and serum weights were also significantly higher in females. Males exhibited a significant decrease in serum alkaline phosphate activity and females exhibited a significant increase and decrease in serum urea and glucose concentration, respectively	RIFM (1983a)
β-Ionone	Oral (diet) 90-day study	Males 11.6 mg/kg, females 13.1 mg/kg	15 FDRL rats sex/dose	No adverse toxic effects	Oser et al. (1965)
β-Ionone	Oral (gavage) 12 weeks study	11.4 mg/kg	Rats	No adverse toxic effects	Bár and Griepentrog (1967)
Ionone (mixed isomers)	Oral (diet) 8 weeks study	10 mg on alternate days	32 young white rats/8/group	No adverse toxic effects	Sporn and Dinu (1964)
Ionone (mixture of 60% α, 40% β)	Oral (diet) 17 weeks study	50, 125 and 500 mg/kg/day	Osborne–Mendel rats 10/sex	1000 ppm: Very slight swelling of parenchymal cells 2500 ppm: Slight swelling of parenchymal cells 10,000 ppm: Moderate swelling of parenchymal cells	Hagan et al. (1967), Bár and Griepentrog (1967)
Ionone (mixed isomers)	Oral (diet) 8 weeks study	10 mg on alternate days	56 white rats	Significant increase of deoxyribonucleic acid content and a significant decrease of aspartate-glutamic transaminase	Sporn and Dinu (1964)
Ionone (mixed isomers)	Oral (diet) 7 weeks study	3 mg in oil every 2nd day for 7 weeks	72 young white rats. 10 rats/group	No adverse toxic effects	Sporn et al. (1963)
α-Irone	Oral (diet) 90-day study	5.2 and 5.9 mg/kg/day	FDRL strain rats (15/sex)	No adverse toxic effects in males Females exhibited increase: food consumption, hematocirt hemoglobin, lymphocytes	Oser et al. (1965)
α-*iso*-Methylionone	Oral (gavage) 90-day study	5, 30 and 500 mg/kg/day	Crl:CD (SD) IGS BR rats (10/sex/dose)	NOAEL of 30 mg/kg	RIFM (2006a)
α-*iso*-Methylionone	Oral (gavage) 90-day study	3.4 mg/kg	Rats	No adverse toxic effects	Bár and Griepentrog (1967), Oser et al. (1965)
α-*iso*-Methylionone	Dermal 90-day toxicity study	10 mg/kg (1%) in phenethyl alcohol	10 Sprague–Dawley albino rats (5/sex)	A slight increase in alkaline phosphatase values in males, but the relationship to treatment was questionable. Small group sizes limit the interpretation of the study	RIFM (1981a)

(*continued on next page*)

Table 4 (*continued*)

Material	Method	Dose[a]	Species	Results	Reference
α-*iso*-Methylionone	Dermal 90-day toxicity study	50, 170, 580 and 2000 mg/kg	15 Sprague–Dawley rats/sex/dose	50 mg/kg: Dose related increase in liver weight and changes in urinalysis parameters at this dose 170 mg/kg: Changes in hematology parameters in both sexes. BUN levels increased with dose in males. Urine albumin levels were significantly increased in male groups at termination. Increases in the absolute and relative weights in the liver and kidneys in both sexes 580 and 2000 mg/kg: Reduced body weight gain in females and in males. Food consumption elevations in females, lower efficiency food utilization in both male and females. Serum glucose levels were depressed in males at week 7 and in both sexes at termination. BUN levels increased with dose in males. Urine albumin levels were significantly increased in male groups at termination. Increases in the absolute and relative weights in the liver and kidneys in both sexes Moderate to severe erythema and eschar formation was observed in all test groups and increased with increasing levels of test material. Severe tissue destruction and infection on doses above 50 mg/kg may have combined to elicit increased kidney weight at higher doses	RIFM (1980a)

[a] Units have been converted to make easier comparisons; original units are in the Fragrance Material Reviews.

the severe skin effects in the earlier study (RIFM, 1980a) as expected with the application of a far lower and less concentrated solution (1% in PEA).

It is concluded that there are currently no entirely adequate studies available to assess the subchronic dermal toxicity of any individual ionones/rose ketones or the group as a whole. Of those dermal studies available, a systemic NOAEL from the RIFM (1980a) study was established at 50 mg/kg body weight/day.

3.2.2. Oral studies

A 90-day repeated dose oral subchronic toxicity study was conducted with α-*iso*-methylionone. Ten rats/sex/dose were gavaged once daily for 90 days with α-*iso*-methylionone at dosages of 0, 5, 30, and 500 mg/kg/day. There were no unscheduled deaths, treatment-related changes in behavioral or functional performance parameters or in sensory reactivity. There were no adverse effects on bodyweight, food consumption, or water consumption. There were no treatment-related ocular or hematological changes observed. No macroscopic abnormalities were detected at necropsy. At the high dose only, increased salivation, red/brown stained fur, episodes of noisy respiration and hunched posture were evident in a number of animals throughout the treatment period. Blood chemistry analyses showed statistically significant increases in total protein, albumin and cholesterol. Increase in absolute and relative liver and kidney weights and histopathological changes in liver, kidneys, thyroid and bone marrow was observed. Due to the histopathological observations in the high-dose rats, examination of sections of liver, kidneys, thyroid, and bone marrow from all animals in the low and intermediate dose groups was performed and showed liver hepatocyte enlargement in animals treated with 500 mg/kg/day, globular accumulation of eosinophilic material in kidney tubular epithelium of males treated with 30 and 500 mg/kg/day, higher incidence of follicular cell hypertrophy in thyroid and adipose infiltration of the bone marrow in males pretreated with 500 mg/kg/day. The NOEL was established to be 30 mg/kg/day for females and 5 mg/kg/day for males. The kidney changes identified histopathologically were consistent with well documented changes that are peculiar to the male rat in response to treatment with some hydrocarbons, therefore, for the purposes of hazard evaluation the NOAEL for males, was established as 30 mg/kg/day (RIFM, 2006a).

Subchronic oral toxicity studies have been conducted on α- and β-ionone (Oser et al., 1965; RIFM, 1983a; Bár and Griepentrog, 1967), ionones (mixed isomers) (Sporn et al., 1963; Sporn and Dinu, 1964; Hagan et al., 1967; Bár and Griepentrog, 1967), α-*iso*-methylionone (Oser et al., 1965; Bár and Griepentrog, 1967), α-irone (Oser et al., 1965), and β-damascone (RIFM, 1969). Oser et al. (1965) tested

a number of chemicals with relatively few details reported of the methods used and results obtained. However, this study is useful since several ionones were tested allowing for a comparison, albeit limited in detail, of the toxicity of various members of this class of compounds. The oral studies reported in RIFM (1983a) on α- and β-ionone and in RIFM (1969) on β-damascone are considered to have utilized the appropriate study design and protocols and to have reported the results to an extent to allow independent evaluation of the data. There are no oral subchronic studies available on the 3 rose ketones [*trans*, *trans*-δ-damascone, δ-damascone; damascone and 2 ionones (dehydrodihydroionone and methyl-δ-ionone)].

In the Oser et al. (1965) study, toxicological tests were conducted on several ionones. Rats of the FDRL strain were fed diets containing, in cottonseed oil, α-ionone (11.8 and 11.1 mg/kg/day in males and females, respectively), β-ionone (11.6 and 13.1 mg/kg body weight/day in males and females, respectively), α-*iso*-methylionone (3.6 and 4.1 mg/kg body weight/day in males and females, respectively), or α-irone (5.2 and 5.9 mg/kg body weight/day in males and females, respectively) for a period of 90 days. There were no adverse effects on body weight gain and food consumption for any of the 4 ionones tested. For α-ionone and β-ionone, no effects were observed in measured hematology and blood chemistry parameters. Male rats receiving α-*iso*-methylionone had a slightly reduced (more than 2 standard deviations, no other statistical data cited) hemoglobin level. However, the hematocrit and erythrocyte counts were within the control ranges. This group also had a mean BUN level below (more than 2 standard deviations, no other statistical data cited) that of the controls. No evidence of adverse toxic effects was observed in the males treated with α-irone. Females treated with α-irone exhibited an increased efficiency of food utilization (13.7 g body weight gain/100 g food eaten) as compared to controls (13.0). Females were also reported to have a slightly increased (more than 2 standard deviations, no other statistical data cited) hematocrit, hemoglobin, and lymphocyte count. Liver and kidney weights were not affected by any of the 4 ionones tested, and there was no adverse effect on the gross or microscopic appearance of major organs at necropsy. The NOAEL values for this study were identified as 11.1 mg/kg α-ionone, 11.6 mg/kg β-ionone, 3.6 mg/kg α-*iso*-methylionone, and 5.2 mg/kg α-irone body weight/day. In all cases, the NOAEL values represent the only dose tested and are the lower of the doses reported (for males or females). These data, while limited in scope, indicate that at these low oral doses, the ionones are non-toxic and well tolerated and that, at least within the ionone series, major differences in toxicity are not expected.

Hagan et al. (1967) administered ionone (60% α- and 40% β-isomers) to groups of 10 male and 10 female Osborne–Mendel rats at dietary concentrations of 1000, 2500, or 10,000 ppm for 17 weeks, equivalent to approximately 50, 125, and 500 mg/kg body weight/day. There were no reported effects on body weight gain, clinical signs, or on any of the measured hematological parameters. At necropsy, no macroscopic changes were observed. Histopathology examination of the liver, the only organ analyzed revealed slight to moderate swelling of hepatocytes in high-dose animals, slight swelling in mid-dose animals, and very slight swelling of hepatocytes in the low-dose group. Due to the limited reporting of the methodology and the results, the study is difficult to assess in terms of establishing a NOAEL value. The hepatocellular swelling is presumably related to microsomal enzyme induction and not an "adverse" effect.

α-Ionone and β-ionone were tested separately in groups of 15 male and female Sprague–Dawley rats *via* dietary administration to provide daily doses of 10 or 100 mg/kg body weight for a period of 90 days (RIFM, 1983a). There were no mortalities, and no abnormal clinical signs in rats treated with either α- or β-ionone. No significant effects on mean body weights occurred in treated males. Sporadic, statistically significant, lower body weights were reported at various times in the last 6 weeks of the study in females treated with α-ionone, 100 mg/kg body weight/day. Food intake of both sexes treated with either α- or β-ionone, 100 mg/kg body weight/day, was significantly reduced, with the effect greater in females. It is possible that the lower mean body weights and reduced food consumption at the high dose may have been related to an unpalatable diet.

At 6 weeks, erythrocyte counts and packed cell volumes showed a significant decrease in males treated with 100 mg β-ionone/kg body weight/day, and decreased erythrocyte counts were reported in 10 mg/kg body weight/day α-ionone treated males. Males given 100 mg α-ionone/kg body weight/day showed a slight, but significant, increase in neutrophil counts and a decrease in lymphocytes. No effects of α- or β-ionone, at either 10 or 100 mg/kg body weight/day, were reported on hematological parameters in females. No hematological changes were observed in either sex after 13 weeks of treatment. The earlier abnormalities were considered to be of no toxicological significance.

The serum chemistry analyses were normal except for significantly lower alkaline phosphatase values in males and lower glucose concentrations in females dosed with 100 mg of either α- or β-ionone/kg body weight/day. Given the small magnitude of these changes, they were considered to be of no biological significance.

In high-dose animals treated with either α- or β-ionone, mild changes in urinary parameters were considered not to be of biological significance. Relative kidney weights were significantly increased in males but not females treated with the high dose of α-ionone. Males treated with the β-isomer (100 mg/kg body weight/day) showed significantly increased absolute and relative liver weights and females showed increased relative liver and brain weights at the high dose of α- and β-ionone. Relative liver weights were significantly increased also in females given 10 mg/kg body weight/day α-ionone.

Necropsy revealed no effect of treatment on gross or microscopic pathology except for statistically significant "desquamation" of the thyroid epithelium in 3 females treated with 100 mg/kg body weight/day of α-ionone. There were no gross or histopathological correlates to increased absolute and/or relative kidney and/or liver weights. As a result, the only biologically significant finding at the low dose of either ionone was an increase in relative liver weight in females treated with α-ionone, a finding likely associated with enzyme induction. Thus, 10 mg/kg body weight/day is considered to be a NOAEL value for both α- and β-ionone.

One of the rose ketones, β-damascone, has been tested in a study in which groups of 16 male and female CF/Gif rats were administered the compound in feed at a dose of 2.26 mg/kg body weight/day for a period of 13 weeks (RIFM, 1969). There were no mortalities and no abnormal clinical signs during the course of the study. Feed consumption increased for both males (+5.8%) and females (+9.5%), with the increase statistically significant in females. There was, therefore, a moderate decrease in feed efficiency in both sexes (−9.04% in the males and −9.60% in the females). The study authors ascribed no toxicological significance to the feed consumption and utilization data.

The absolute weights of the liver and kidneys in females were increased (7.8%, respectively), and relative weights of the liver and kidneys were reported to be significantly increased in both sexes. There was no effect of treatment on gross or histopathological appearance of tissues including the liver and kidney. The authors considered the test article to be well tolerated and not to produce any changes of toxicological significance. Given the lack of histopathological changes in the liver and kidneys and the relatively minor increases in relative weights of these organs and decreases in food utilization efficiency, the single dose of 2.26 mg/kg body weight/day in this 13 week study is considered to approximate a NOAEL value.

3.2.3. Summary of subchronic toxicity studies

Dermal subchronic toxicity studies have been conducted on α-*iso*-methyl ionone (RIFM, 1980a, 1981a), and several subchronic oral toxicity studies have been conducted on certain of the ionones (Sporn et al., 1963; Sporn and Dinu, 1964; Oser et al., 1965; Bár and Griepentrog, 1967; RIFM, 1983a) and one of the rose ketones (RIFM, 1969). Not one material has been subject to both subchronic oral and subchronic dermal toxicity testing. The 90-day dermal toxicity study (the most appropriate route) of α-*iso*-methyl ionone (RIFM, 1980a) was significantly compromised by severe effects of the test chemical on the skin. A systemic NOAEL of 50 mg/kg body weight/day was identified (given the likelihood that many of the systemic "effects" observed were secondary to infection/inflammation associated with the severe necrosis and ulceration of the skin). In the second, limited dermal study, of α-*iso*-methyl ionone (RIFM, 1981a), the NOAEL was 10 mg/kg, the only dose tested. It is concluded that there are currently no adequate studies available to assess the subchronic dermal toxicity of any individual ionones/rose ketones or the group as a whole; the available data do not indicate a high order of toxicity in the absence of severe effects on the skin.

The oral subchronic toxicity studies, of which one performed on α- and β-ionone (RIFM, 1983a) and another on the β-damascone (RIFM, 1969) and a more recent one on α-*iso*-methylionone (RIFM, 2006a), are considered the most useful to characterize the toxicity of this group of chemicals. They demonstrate a low order of toxicity. In each of these studies, the most notable findings were of modestly, but significantly, increased absolute and/or relative liver weights. Since this group of chemicals is known to induce microsomal enzymes, an effect well established to be associated with generalized increases in liver weight, and noting the absence of histological effects on the liver in these studies, these findings are likely of minimal toxicological significance. In the absence of other evidence of overt toxicity, 10 mg/kg body weight/day is considered to represent the NOAEL for α- and β-ionone (RIFM, 1983a), and 2.26 mg/kg body weight/day (the only dose tested) is considered a provisional NOAEL value for β-damascone (RIFM, 1969) and 30 mg/kg/body weight/day is the NOAEL for α-*iso*-methylionone (RIFM, 2006a). Similar results were obtained by Oser et al. (1965) for α- and β-ionone. No toxicity was also observed for α-*iso*-methylionone at 3.6 mg//kg body weight and for α-irone at 5.2 mg/kg body weight (Oser et al., 1965).

From the available data, there do not appear to be large differences in the toxicity of the ionones by the oral route of exposure. Comparison with dermal exposure is hindered by the lack of appropriate comparative studies and by direct dermal toxicity. There are no dermal or subchronic oral toxicity studies available on the ionones and rose ketones that may be subject to metabolism by epoxidation and, hence, have a higher potential for the generation of toxic metabolites. In conclusion, keeping in mind the inadequacies of the studies available, a dermal NOAEL value of 10 mg/kg body weight (RIFM, 1981a) and a systemic NOAEL of 50 mg/kg body weight/day associated with dermal exposure (RIFM, 1980a) and a systemic NOAEL of 30 mg/kg body weight/day associated with oral exposure can be used for quantitative human health risk assessment of the use of the ionones as fragrance compounds.

3.3. Chronic toxicity

No chronic toxicity data are available on any of the 36 ionones evaluated.

3.4. Mutagenicity and genotoxicity

Of the 30 ionones, several have been tested in various *in vitro* and *in vivo* test systems. In a number of studies, results for a large number of compounds have been insufficiently described so that interpretation of the data is

difficult. Studies that did not report the concentration/dose of the test material are not included in this safety assessment. Results of those studies that provide sufficient details for evaluation are summarized in Tables 5–7 and are described below.

3.4.1. Bacterial studies

In the Ames assay using *Salmonella typhimurium* with and without metabolic activation, allyl-α-ionone (Wild et al., 1983), dihydro-β-ionone (RIFM, 2000a), 4-(1,2-epoxy-2,6,6-trimethylcyclohexyl)-3-buten-2-one (RIFM, 1988a) α-ionone (Kasamaki et al., 1982), β-ionone (Florin et al., 1980; Mortelmans et al., 1986), ionone mixed isomers (RIFM, 1980b, 2004a), α-*iso*-methyl ionone (RIFM, 1980c), methyl ionone (RIFM, 1999b), methyl-α-ionone (Wild et al., 1983), methyl-δ-ionone (Wild et al., 1983), and methyl ionone mixed isomers (RIFM, 1980d) have all been reported to be without mutagenic activity. Similarly, the rose ketones damascone and α-damascone did not show any mutagenic activity in the bacterial reverse mutation test with *S. typhimurium* and *Escherichia coli* WP2 *uvrA* (RIFM, 2000b, 2003).

Only in a non-standard Ames test in which the TA1535 strain contained a plasmid carrying the fused gene *umuC'-lac2*, was one of the ionones (β-ionone) reported to have mutagenic activity (Ono et al., 1991). The utility of this assay for predicting mutagenic potential is questionable given that a number of chemicals considered "non-mutagenic" were also reportedly positive in this assay. Ionone was reported to be marginally genotoxic in the Rec assay (Yoo, 1986).

Overall, the ionones and rose ketones are concluded to be without mutagenic potential in bacteria in *in vitro* studies.

3.4.2. Mammalian studies

Clastogenic potential of methyl ionone was tested in a chromosome aberration assay using Chinese hamster ovary (CHO) cells. At the highest dose of 50 μg/ml, the cell growth inhibition was about 60–68% in all treatment groups. Methyl ionone did not produce any significant structural or numerical chromosome aberrations after 4-h treatment without S9, but in the presence of S9 there was an increase in structural chromosome aberrations at the highest dose tested of 50 μg/ml. However, since the increase in the percentage of structurally aberrant cells was within the range of historical control values, the chromosome aberrations observed in the presence of S9 after 4-h treatment with methyl ionone were not considered biologically significant. Structural chromosome aberrations were reported after 20-h exposure at concentrations of 12.5 and 25 μg/ml in the absence of S9. There were no increases in numerical chromosome aberrations in this group. Based on these results, it was concluded that methyl ionone was positive in the absence of S9 and negative in the presence of S9 for the induction of structural chromosome aberrations in CHO cells. Methyl ionone was negative in both the absence and presence of S9 for the induction of numerical chromosome aberrations in CHO cells (RIFM, 2000c).

In order to evaluate the biological significance of the positive *in vitro* chromosome aberration assay, an erythrocyte micronucleus test was performed with methyl ionone in mice. After a preceding toxicity test, groups of 5 male and female mice were dosed with 462.5, 925, or 1850 mg methyl ionone/kg body weight by a single intraperitoneal injection. In male mice, 24 h after treatment with 925 mg/kg, a significant increase in micronucleated polychromatic erythrocytes (0.8 ± 0.45/1000 polychromatic erythrocytes (PCE), mean ± SD) relative to the control values (0.1 ± 0.22/1000) was observed. No such effect was reported in females. No effects on the incidence of micronucleated PCE were reported in either sex at the highest dose tested of 1850 mg/kg body weight. The historical control value for micronucleated PCEs in the performing laboratory during 1996–1998 was 0.65 ± 0.76 in males and 0.7 ± 0.80 in females, with a range of between 0 and 7/1000 cells in both genders. Considering the historical controls, the lack of a dose–response of the effects, and the negative data after 48 h at the highest dose tested, it is concluded that methyl ionone is negative in the micronucleus test (RIFM, 2000d).

A mouse micronucleus test was also performed with α-ionone in order to evaluate the biological significance of the positive *in vitro* chromosome aberration assay. α-Ionone, at doses of 300, 600, or 1200 mg/kg in corn oil was administered by intraperitoneal injection to male and female ICR mice (5/sex/dose). Reductions (up to 21%) in the ratio of polychromatic erythrocytes to total erythrocytes were observed in some of the α-ionone treated groups relative to the respective vehicle controls. These reductions suggest the bioavailability of α-ionone to the bone marrow. There were no statistically significant increases in the incidence of micronucleated polychromatic erythrocytes in α-ionone treated groups relative to their respective vehicle control in either male or female mice, regardless of dose level or bone marrow collection time. α-Ionone was concluded to be negative in the mouse micronucleus assay (RIFM, 2006b).

In an older mouse micronucleus assay, Wild et al. (1983) tested allyl-α-ionone (2 doses of 464, 696, or 928 mg/kg by i.p. injection) and methyl-α-ionone (single dose of 825, 1444, or 2063 mg/kg by i.p. injection). These two ionones did not show evidence of genotoxic activity in this assay.

Based on the foregoing data, it is concluded that the ionones tested, including α-ionone (Kasamaki et al., 1982) and methyl ionone (RIFM, 2000c), may have weak clastogenic activity in mammalian cells *in vitro*. However, these responses do not appear to be translated to *in vivo* exposures, based on results from mouse micronucleus assays (Wild et al., 1983; RIFM, 2000d).

Table 5
Mutagenicity and genotoxicity: bacterial studies

Material	Test system	Species	Concentration	Results	Reference
Allyl α-ionone	Ames with and without S9 activation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3600 μg/plate	Negative	Wild et al. (1983)
Damascenone	Bacterial reverse mutation assay with and without S9 activation using plate incorporation method	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537 and *E. coli* WP2 uvrA	Up to 5000 μg/plate	Negative	RIFM (2000b)
α-Damascone	Ames with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535 and TA1537 and *E. coli* WP2uvrA	Up to 125 μg/plate for TA98 and TA1537, 250 μg/plate for TA1535 and TA100, and 5000 μg/plate for *E. coli*	Negative	RIFM (2003)
γ-Damascone	Ames with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	Up to 5000 μg/plate	Negative	RIFM (1986c)
Dihydro-β-ionone	Pre-incubation assay with S9 activation	*S. typhimurium* TA102	Up to 1000 μg/plate	Negative	RIFM (2000a)
Dihydro-β-ionone	Direct incorporation test with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	Up to 1000 μg/plate	Negative	RIFM (2000a)
4-(1,2-Epoxy-2,6, 6-trimethylcyclohexyl)-3-buten-2-one	Ames with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535, TA1537	5–500 μg/plate	Negative	RIFM (1988a)
α-Ionone	Rec-assay	*Bacillus subtilis* in strains H17 (rec+) and M45 (rec−)	19 μg/disk	Negative	Oda et al. (1978)
α-Ionone	Ames with and without S9 activation	*S. typhimurium* TA98 and TA100	0.01–50 μg/plate	Negative	Kasamaki et al. (1982)
β-Ionone	Ames with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 μM/plate	Negative	Florin et al. (1980)
β-Ionone	Ames test preincubation assay with and without S9 activation	*S. typhimurium* TA1535, TA98, TA100 and in either TA97 or TA1537	1–180 μg/plate	Negative	Mortelmans et al. (1986)
Ionone	Ames test with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0.001–1 μl/plate	Negative	RIFM (1980b)
Ionone	Reverse mutation assay	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	Up to 5000 μg/plate	Negative	RIFM (2004a)
Ionone	Umu-test	*S. typhimurium* TA1535/ pSK1002	100 μg/ml	Positive	Ono et al. (1991)
Ionone	Spore plate rec-assay	*B. subtilis* H17 & M45	20 μl/ plate	Positive	Yoo (1986)
Ionone	Antimutagenic test	*E. coli* WP2 uvrA (trp-)	10–40 mg/ml	Negative	Yoo (1986)
Ionone	Mutation test	*E. coli* WP2 uvrA (trp-)	2.5–20.0 mg/plate	Negative	Yoo (1986)
Methyl-α-ionone	Ames with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538,	3600 μg/plate	Negative	Wild et al. (1983)
Methyl-δ-ionone[a]	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Up to 3600 μg/plate	Negative	Wild et al. (1983)
α-*iso*-Methylionone	Ames test with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	9.3–9300 μl/plate	Negative	RIFM (1980c)
Methyl ionone	Bacterial reverse mutation assay with and without S9 using the plate incorporation method	*S. typhimurium* TA98, TA100, TA1535 and TA1537 and *E. coli* WP2uvrA	Up to 5000 μg/plate	Negative	RIFM (1999b)
Methyl ionone	Ames test with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	Up to 10 μg/plate	Negative	RIFM (1980d)

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

3.4.3. Summary of genotoxicity data

The mutagenicity and genotoxicity data are summarized in Tables 5 and 6. In the standard *Salmonella*/microsome assays, as well as in *E. coli* mutation assays, the different ionones tested were negative. Only in a non-standard Ames test in which the TA1535 was used, β-ionone reported to have mutagenic activity. The utility of this assay for predicting mutagenic potential is

Table 6
Mutagenicity and genotoxicity: mammalian studies

Material	Test system	Species	Dose or concentration	Results	Reference
Allyl α-ionone	*In vivo* micronucleus test	Male and female NMRI mice	464, 696, and 928 mg/kg	Negative	Wild et al. (1983)
α-Ionone	*In vitro* chromosome aberration assay	CH cell line B241	25 mM	Positive	Kasamaki et al. (1982)
α-Ionone	*In vivo* micronucleus test	Male and female ICR mice	300, 600, or 1200 mg/kg by i.p. injection	Negative	RIFM (2006b)
Methyl-α-ionone	*In vivo* micronucleus test	male and female NMRI mice	825, 1444, and 2063 mg/kg	Negative	Wild et al. (1983)
Methyl ionone	*In vitro* chromosome aberration assay with and with out S9 activation	Chinese Hamster Ovary (CHO)	12.5–175 μg/ ml	Positive in the absence of S9 and negative in the presence of S9 for the induction of structural chromosome aberrations; negative with and without S9 for induction of numerical chromosome aberrations	RIFM (2000c)
Methyl ionone	*In vivo* micronucleus test	Male and female ICR mice	462.5, 925, or 1850 mg/kg by i.p. injection	Negative	RIFM (2000d)

Table 6a
Mutagenicity and genotoxicity: insect studies

Material	Test system	Species	Dose or concentration (mM)	Results	Reference
Allyl-α-ionone	Basc test	*Drosophila melanogaster*	25	Negative	Wild et al. (1983)
Methyl-α-ionone	Basc test	*Drosophila melanogaster*	20	Negative	Wild et al. (1983)

Table 7
Carcinogenicity studies

Material	Method	Dose[a]	Species	Results	Reference
β-Ionone	Tumor inhibiting or tumor enhancing activity in a tumor promoting system of 7,12-dimethyl benz[*a*]anthracene (DMBA). Mice were initiated once with 0.125 mg DMBA in 0.25 ml acetone applied to their backs. Three weeks later the mice received application of 0.25 ml of 0.04% β-ionone in acetone or application of a mixture of β-ionone and 0.006% croton resin five times weekly for 18 weeks	~3 mg/kg body weight	30 ICR Swiss mice	No evidence of carcinogenic activity	Shamberger (1974)
β-Ionone	Chemo preventive activity of β-ionone against DMBA mammary tumor genesis was examined by administering a diet containing 36 mmol β-ionone/ kg to rats for 2 weeks prior to and following treatment with DMBA at 35 mg/kg body weight	~350 mg/kg body weight/day	27 female Sprague–Dawley rats	No evidence of carcinogenic activity	Yu et al. (1993, 1995)
β-Ionone	Following implantation of B16 melanoma cells, animals were palpated for the presence of tumors and initiated on dietary treatment with β-ionone following detection of tumors	~50 mg/kg body weight/day	12 C57 Bl female mice	No evidence of carcinogenic activity	He et al. (1997)
α-Irone	Mice received intraperitoneal injections of α-irone in re-distilled tricaprylin 3 times weekly for 8 weeks. Twenty-four weeks after the first injection, animals were sacrificed	1950 and 9600 mg/ kg bodyweight	A/He mice (15/sex/dose)	No evidence of carcinogenic activity	Stoner et al. (1973)

[a] Units have been converted to make easier comparisons; original units are in the Fragrance Material Reviews.

questionable given that a number of chemicals considered "non-mutagenic" were also reportedly positive in this assay. Ionone was reported to be marginally genotoxic in the Rec assay.

In *in vitro* chromosome aberration tests, methyl ionone increases structural aberrations when incubated with CHO cells in the absence of S9 for 20 h. After 4 h incubation, methyl ionone was negative with and without S9.

In the *in vivo* mouse micronucleus test single intraperitoneal doses of methyl ionone produced equivocal effects which, following careful evaluation, are not considered to represent genotoxic activity of methyl ionone. In all cases in this mouse micronucleus assay, there was no apparent dose–response relationship; absolute incidence rates for structural chromosome aberrations were within historical control values; the positive controls produced much higher frequencies of structural and numerical aberrations, and, at the highest dose tested, there were no differences in the incidence of either structural or numerical aberrations between treated groups (both sexes) and controls. In addition, a recent mouse micronucleus test with α-ionone at doses as high as 1200 mg/kg did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in either male or female ICR mice and it was concluded that α-ionone was negative in the mouse micronucleus assay. Also, another mouse micronucleus assay previously reported in the literature (Wild et al., 1983) failed to detect any indication of a genotoxic effect of either methyl-α-ionone or allyl-α-ionone. Given the foregoing, it is concluded that the ionones do not possess significant *in vivo* mutagenic or genotoxic potential under intended conditions of use as fragrance ingredients.

3.5. Carcinogenicity

No standard 2-year rodent bioassays investigating the carcinogenic potential of any of the 36 ionones are available. One dermal tumor promotion study of β-ionone is available (Shamberger, 1974). There are 2 oral studies conducted on β-ionone to assess its potential to inhibit tumor formation and/or growth (Yu et al., 1995; He et al., 1997). Details of these and other studies are provided in Table 7 and in the following sections.

3.5.1. Tumor initiation and promotion studies

β-Ionone has been tested in a tumor promoting system in ICR Swiss mice initiated once with 0.125 mg 7,12-dimethylbenz[*a*]anthracene (DMBA) (about 4 mg/kg body weight) in 0.25 ml acetone applied to their backs (Shamberger, 1974). After 3 weeks the mice received applications of 0.25 ml of 0.04% β-ionone in acetone (about 3 mg/kg body weight) or of a mixture of β-ionone and 0.006% croton resin five times weekly for 18 weeks. A group given DMBA and tumor promoter (croton resin) served as positive control. β-Ionone treatment did not have any effect on the incidence of tumors, but the number of papillomas per mouse was slightly but not statistically decreased (90% of control). No tumors were seen with DMBA or β-ionone alone (Shamberger, 1974). There was no evidence of tumor initiation or promotion activity of β-ionone.

In an intraperitoneal study (Stoner et al., 1973), α-irone, injected 3 times a week in tricaprylin for 8 weeks (to groups of 15 A/He mice of each sex to provide total doses of either 1950 or 9600 mg/kg body weight), was reported to produce a significant increase in lung tumors in comparison to controls. A repeat of this study was conducted due to concerns about the quality of the tricaprylin vehicle. Use of re-distilled tricaprylin and a similar dosing regimen resulted in no differences in the incidence of lung tumors between α-irone treated and control mice.

3.5.2. Anti-carcinogenic effects

The anti-tumor effect of β-ionone was examined by Yu et al. (1995) in female Sprague–Dawley rats fed diets containing 36 mmol/kg (approximately 6922 ppm in the diet, equivalent to a dietary dose of about 350 mg/kg body weight/day) of β-ionone dissolved in corn oil. At the end of 2 weeks feeding, tumors were induced by single gastric intubation of DMBA, 65 mg/kg body weight in sesame oil. The dietary regimen continued for 22 weeks. β-Ionone significantly reduced the number of animals with mammary tumors (adenocarcinomas, adenomas, fibroadenomas) which were seen in 91% of control rats and in 41% of β-ionone fed rats. Tumor latency was significantly increased in β-ionone fed rats, 74 days in control rats and >151 days in β-ionone fed rats. Tumor multiplicity was significantly reduced by β-ionone.

He et al. (1997) reported that following the injection of B 16 melanoma cells into weanling C57Bl female mice, addition of 2 mmol/kg β-ionone (diluted in vitamin E-stripped corn oil) (dose equivalent to approximately 385 ppm in the diet, or about 50 mg/kg body weight/day) to the diet increased the median duration of survival compared to controls (not administered β-ionone) by about 50%, and mean duration of survival by about 30%.

Overall, the Yu et al. (1995) study indicates that β-ionone may possess anti-carcinogenic activity in the face of exposure to a potent carcinogen. The model used by He et al. (1997) tests the potential to block establishment and growth of injected malignant cells and is not relevant to toxicity.

3.5.3. Summary of the carcinogenicity data

In summary, on the basis of the negative results obtained in a dermal initiation–promotion study (Shamberger, 1974), a study design of relevance to human exposure to ionones through their use as fragrance ingredients, there is no evidence to indicate that β-ionone has tumor initiating or promoting potential. The intraperitoneal study on α-irone by Stoner et al. (1973) that produced initial tumor inducing or promoting results was confounded by the quality of the dosing regimen vehicle. In the repeat study that used re-distilled tricaprylin as vehicle, no evidence of lung tumor induction or promotion was elicited (Stoner et al., 1973). It is therefore concluded that the ionones are unlikely to possess tumor initiation or promotion potential. This conclusion is supported by the result of a study demonstrating that β-ionone may actually possess anti-carcinogenic activity (Yu et al., 1995).

3.6. Reproductive and developmental toxicity

Several reproductive and/or developmental toxicity studies have been conducted on ionones, most notably on α- and β-ionone (Sporn et al., 1963; Willhite, 1986; Verrett et al., 1980; Gomes-Carneiro et al., 2003) and α-*iso*-methylionone (RIFM, 2005). There are no adequate reproductive/developmental toxicity studies available on any of the rose ketones. The developmental studies in rats reported by RIFM (2005) were conducted according to standardized protocols and represent the most appropriate studies from which to assess developmental toxicity. Also, Sporn et al. (1963) presented data on a reproductive toxicity study in which females were monitored through 3 consecutive reproductive cycles and in which reproductive performance of an F1 generation was also studied (this study was not conducted to modern standards). Willhite (1986) studied teratogenesis by administration of a single dose of β-ionone on day 8 of pregnancy in timed-pregnant hamsters (this study did not evaluate the entire period of organogenesis). The Sporn et al. (1963), Willhite (1986), and the RIFM (2005) studies provide the most useful data to assess reproductive/developmental toxicity and teratogenic potential of the ionones. Since the studies of Gomes-Carneiro et al. (2003) (evaluation of the inhibition of cyclophosphamide-induced teratogenesis in rats by β-ionone) and of Verrett et al. (1980) (teratogenicity in the White Leghorn chickens) utilized non-standard protocols, or protocols that have not been validated for the purposes of human risk assessment, they are not discussed further. In any case, no adverse effects were reported in either the Gomes-Carneiro et al. (2003) or the Verrett et al. (1980).

Prior to the 2005 RIFM developmental toxicity study, a preliminary dose-range finding study was conducted. Forty presumed pregnant female rats were dosed *via* gavage on days 7 through 17 of gestation with α-*iso*-methylionone at dosages of 1.25, 2.5, 5, or 10 mg/kg. All female rats were pregnant and survived to the scheduled sacrifice. No fetal effects were observed (RIFM, 2005).

Based on the above results, the developmental toxicity of α-*iso*-methylionone was investigated in 100 (25/group) presumed pregnant female rats dosed, *via* gavage, on days 7 through 17 of gestation with α-*iso*-methylionone at dosages of 0, 3, 10, or 30 mg/kg/day. All female rats survived to the scheduled sacrifice. Pregnancy occurred in 21–25 rats in each dosage group. There were no abnormal clinical observations or necropsy observations in the female rats that were determined to be test article related. No fetal effects were observed that were determined to be test article related. Based on these data, the maternal and developmental NOAEL of α-*iso*-methylionone is greater than 30 mg/kg/day (RIFM, 2005).

Sporn et al. (1963) studied the effects of ionone on reproduction in rats given 2 mg ionone in 0.1 ml oil solution on alternate days for 8 months (equivalent to a dose of approximately 8–10 mg/kg body weight/day). The female rats were studied through 3 reproduction cycles for number of pregnancies, weight, number of offspring, live pups, weight of pups at birth and after 7 and 21 days, and the viability of the pups after birth. The F1 generation (offspring) were allowed to reach maturity and treated with 15 mg/kg of ionone prior to being subject to reproductive toxicity testing. Their offspring, the F2 generation were evaluated for reproduction parameters. Ionone had no adverse effect on any of the parameters measured. Based on these data, no effects were observed for ionone at approximately 10 mg/kg body weight/day Sporn et al. (1963) study.

The teratogenic potency of β-ionone was evaluated in pregnant hamsters administered a single intubation dose of 48, 240, or 480 mg/kg body weight on day 8 of pregnancy (Willhite, 1986). The animals were sacrificed on day 14 of pregnancy. There was no significant effect of β-ionone on maternal weight gain, number of litters, incidence of abnormal litters, number of implantation sites and resorptions, number of dead or abnormal fetuses, or the types of malformations if present. A NOAEL of 480 mg/kg body weight was identified in this study; however, the utility of this study is limited by the use of a single dose only at day 8 of gestation.

In summary, a limited number of ionones (α- and β-ionone and α-*iso*-methylionone) have been subject to reproductive, developmental, and teratogenicity testing. No rose ketones have been evaluated. The reproductive toxicity studies on α-*iso*-methylionone (RIFM, 2005) indicate that these compounds are unlikely to be reproductive toxicants. Supporting data on ionone in the form of a one-dose teratogenicity study (Willhite, 1986) and a limited 2-generation, 3-reproductive cycle, study in rats (Sporn et al., 1963) also show no evidence of adverse effects on reproductive parameters. From the RIFM (2005) studies, a reproductive, maternal and fetal, NOAEL of at least 30 mg/kg body weight/day, the highest dose tested, can be established. This is in reasonable agreement with a 90-day oral subchronic toxicity study on each of α- and β-ionone in which the low dose of 10 mg/kg body weight/day approximated the NOAEL and no overt toxicity was reported even at the high dose of 100 mg/kg body weight/day (RIFM, 1983a).

3.7. Skin irritation

3.7.1. Human studies

Approximately 459 male and female volunteers were tested. Minimal irritation was observed with α-*iso*-methylionone at 60%, and moderate irritation was observed with α-ionone at a concentration of 32% which may be attributed to the use of acetone as a vehicle. No irritation was observed with any other ionone (allyl-α-ionone, dihydro-β-ionone, ionone, α-irone, α-iso methyl ionone and methyl ionone) when tested at concentrations ranging from 2% to 100%.

Mild to marked cumulative irritation was observed with *cis*-β-damascone at concentrations as low as 0.05%, α-isodamascone at 2% and 0.1% α-damascone. No other

Table 8
Skin irritation studies in humans

Material	Method	Concentration	Subjects	Results	Reference
Allyl α-ionone	HRIPT induction phase	2% in petrolatum	50 male and female volunteers	No irritation	RIFM (1971b)
Allyl α-ionone	Maximization pretest	10% in petrolatum	5 volunteers	No irritation	RIFM (1972a)
Damascenone	HRIPT induction phase	0.5% in specially denaturated alcohol (SDA 39C)	15 male and female volunteers	No irritation	RIFM (1978c)
Damascenone	HRIPT induction phase	0.05% in alcohol SDA 39C	29 male and female volunteers	No irritation	RIFM (1978c)
Damascenone	HRIPT induction phase	3% in triacteoin	50 male and female volunteers	No irritation	RIFM (1979b)
α-Damascone	HRIPT induction phase	1% in petrolatum	54 male and female volunteers	No irritation	RIFM (1979o)
α-Damascone	HRIPT induction phase	0.1% in isopropyl alcohol	51 volunteers	Irritation observed in 3/51	RIFM (1979d)
α-Damascone	HRIPT induction phase	0.5% in DEP	107 male and female volunteers	No irritation	RIFM (2001a)
α-Damascone	Maximization pretest	0.2% in petrolatum	25 male and female volunteers	No irritation	RIFM (1985c)
δ-Damascone	HRIPT induction phase	1% in ethanol	15 volunteers	No irritation	RIFM (1978b)
δ-Damascone	HRIPT induction phase	0.1% in ethanol	30 volunteers	No irritation	RIFM (1978b)
cis-β-Damascone	HRIPT induction phase	0.05% in alcohol SDA 39C	53 male and female volunteers	No irritation	RIFM (1980h)
cis-β-Damascone	HRIPT induction phase	0.5% in ethanol (Panel I) 0.05% in ethanol (Panel II)	18 volunteers (Panel I) and 32 volunteers (Panel II)	0.5%: Irritation observed in 3/18 0.05%: Irritation observed in 2/32	RIFM (1979c)
trans-β-Damascone	HRIPT induction phase	1% in white petrolatum	54 male and female volunteers	No irritation	RIFM (1979p)
trans-β-Damascone	HRIPT induction phase	0.5% in DEP	104 male and female volunteers	No irritation	RIFM (2000e)
trans-β-Damascone	Maximization pretest	0.2% in white petrolatum	23 male and female volunteers	No irritation	RIFM (1985b)
Dihydro-α-ionone	Maximization pretest	12% in petrolatum	25 male and female volunteers	No irritation	RIFM (1976a)
Dihydromethyl-α-ionone[a]	Maximization pretest	4% in petrolatum	25 male and female volunteers	No irritation	RIFM (1976b)
1,3-Dimethyl-α-ionone[a]	Maximization pretest	10% in petrolatum	27 male and female volunteers	No irritation	RIFM (1985a)
1,3-Dimethyl-α-ionone[a]	Maximization pretest	4% in petrolatum	26 volunteers	No irritation	RIFM (1976c)
α-Ionone	48-h closed patch test	32% in acetone	50 male volunteers	Moderate irritation (no further details reported)	Motoyoshi et al. (1979)
Ionone	24-h closed patch test	100% (vehicle not specified)	11 male and female volunteers	No irritation	Katz (1946)
α-Irone	Maximization pretest	10% in petrolatum	5 male volunteers	No irritation	RIFM (1972b)
Isodamascone	HRIPT induction phase	1% in DEP	65 male and female volunteers	No irritation	RIFM (1995b)
α-Isodamascone	HRIPT induction phase	0.2% in DEP	103 male and female volunteers	No irritation	RIFM (1995a)
α-Isodamascone	HRIPT induction phase	2% in DEP	22 female volunteers	Irritation observed in 1/22	RIFM (1994)
Iso-β-ionone[a]	Maximization pretest	12% in petrolatum	25 male and female volunteers	No irritation	RIFM (1980f)
α-*iso*-Methylionone	HRIPT Induction phase	10% in alcohol	28 volunteers	No irritation	RIFM (1962)
α-*iso*-Methylionone	HRIPT induction phase	2% in dimethyl phthalate (DEP)	8 volunteers	No irritation	RIFM (1968)
α-*iso*-Methylionone	HRIPT induction phase	60% in 3:1 DEP:Ethanol (EtOH)	12 male and female volunteers	No irritation	RIFM (2002c)

Table 8 (*continued*)

Material	Method	Concentration	Subjects	Results	Reference
α-*iso*-Methylionone	HRIPT induction phase	60% in 3:1 DEP:EtOH	106 male and female volunteers	Irritation observed in 1/106	RIFM (2004b)
α-*iso*-Methylionone	HRIPT induction phase	60% in 3:1 EtOH:DEP	12 male and female volunteers	No irritation	RIFM (2002c)
α-*iso*-Methylionone	HRIPT induction phase	60% in 3:1 EtOH:DEP	23 male and female volunteers	No irritation	RIFM (2004c)
Methyl ionone	24-h closed patch test	100%	16 male and female volunteers	No irritation	Katz (1946)
Methyl ionone	24-h closed patch test	5% in vaselinum aldum or unguentum hydrophilicum	19 male and female volunteers	No irritation	Fujii et al. (1972)
4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one	Maximization pretest	20% in petrolatum	28 healthy male volunteers	No irritation	RIFM (1978a)

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

irritation reactions were observed with rose ketones. The variability of results may be due to different concentrations being tested in different vehicle. See Table 8 for details of individual studies.

3.7.2. Animal studies

Mixed results were observed when ionones were evaluated for irritation. Dihydro-α-ionone, dihydro-β-ionone, 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one, α-ionone, β-ionone, ionone, α-*iso*-methyl ionone and methyl ionone were tested in guinea pigs, rabbits or rats.

No irritation or slight to well defined irritation reactions lasting 24 h were observed at a concentration of 5% with α- or β-ionone in rabbits. Irritant reactions were produced by most of the ionones when used at concentrations of 100%. No other concentrations were tested.

With guinea pigs, dihydro-β-ionone did not produce irritation reactions at 1%, but discrete irritation was observed at concentrations of 5% or higher. No irritation reactions were observed when α- or β-ionone and methyl ionone were used at concentrations up to 25%.

No irritation or very slight irritation reactions were observed with ionones at 10% in rats. Irritant reactions were observed at concentrations of 30% or higher, with severe cumulative irritation produced by neat α-*iso*-methylionone in 90-day study.

cis-β-Damascone and α-damascone produced irritation in guinea pigs at 1.5% and 1.8%, respectively, when tested as a part of a delayed contact hypersensitivity study. γ-Damascone produced irritation reactions in guinea pigs at 20% and 50%, when tested prior to a Buehler sensitization study. No other irritation reactions were observed with other rose ketones when tested at concentrations ranging from 0.0025% to 50%. For details of individual studies see Table 9.

3.8. Mucous membrane (eye) irritation

The potential for the ionones to induce eye irritation has been evaluated only in a limited manner. Irritation reactions were observed only with α-*iso*-methylionone at 12.5%.

Rose ketones tested (damascone, α-isodamascone, isodamascone, *cis*-β-damascone and α-damascone) have shown no evidence of eye irritation at concentrations of 0.5–100% (see Table 10).

3.9. Skin sensitization

3.9.1. Human studies

Both ionones and rose ketones were evaluated for the potential to induce sensitization. For details of individual studies, see Table 11 and the corresponding Fragrance Material Reviews.

Seven ionones (allyl-α-ionone, dihydro-α-ionone, ionone, α-irone, α-*iso*-methylionone, methyl ionone and 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one) were evaluated in maximization and human repeated insult patch tests (HRIPT) at concentrations ranging from 2% to 60% in 524 volunteers. No sensitization reactions were observed.

Patch tests conducted by Frosch et al. (1995) and deGroot (1985, 1988) on α-ionone, ionone (mixed isomers), α-irone and α-*iso*-methyl ionone did not produce sensitization reactions at concentrations up to 1%.

The rose ketones have been tested in 2 maximization tests, and 18 HRIPT studies in 992 volunteers. Sensitization reactions were observed when eight different isomers (damascone, *trans*-β-damascone, α-isodamascone, *cis*-β-damascone, α-damascone, isodamascone, and γ-damascone) were evaluated at concentrations ranging from 0.5% to 20%. No effects were observed at concentrations of 0.2% or lower.

α-Damascone and *cis*-β-damascone were also evaluated in patch tests. No sensitization was observed with α-damascone at 3% or *cis*-β-damascone at 2%.

There have been a few cases of positive patch testes on dermatological patients but with no consistent pattern.

3.9.2. Cross sensitization

Cross sensitization reactions have been reported in humans who were induced with 1% γ-damascone and

Table 9
Skin irritation studies in animals

Material	Method	Concentration	Species	Results	Reference
Damascenone	Primary irritation test (24-h closed patch test)	0.5% in alcohol SDA 39C	3 Albino rabbits	No irritation	RIFM (1978d)
Damascenone	Primary irritation test	50% in triacetoin	6 Albino rabbits	No irritation	RIFM (1979e)
Damascenone	Buehler pretest	10% in propylene glycol	11 male Hartley guinea pigs	No irritation	RIFM (1971a)
Damascenone	24-h closed patch test	0.0625%, 0.125%, 0.25%, and 0.5% in distilled water	4 Hartley–Dunkin guinea pigs	No irritation	RIFM (1979h)
Damascenone	24-h closed patch test	0.375%, 0.75%, 1.5%, and 3% in distilled water	4 Hartley–Dunkin guinea pigs	No irritation	RIFM (1979h)
α-Damascone	Primary irritation test (24-h closed patch test)	0.5% in alcohol SDA 39C	6 Albino rabbits	No irritation	RIFM, 1979
α-Damascone	Primary irritation test (24-h closed patch test)	50% in alcohol SDA 39C	6 Albino New Zealand rabbits	No irritation	RIFM (1979bb)
α-Damascone	Primary irritation test (24-h closed patch test)	100% (vehicle not specified)	6 Albino rabbits	No irritation	RIFM (1979g)
α-Damascone	Maximization pretest	1.25%, 2.5%, 5%, and 10% in distilled water	4 Hartley–Dunkin guinea pigs	No irritation	RIFM (1980g)
α-Damascone	Maximization pretest	0.125%, 0.25%, 0.5%, 1.0% in distilled water	4 Hartley–Dunkin guinea pigs	No irritation	RIFM (1980g)
α-Damascone	Buehler pretest	0.6% and 1.8% in 80% alcohol (ethanol)	Hartley guinea pigs	0.6%: No irritation 1.8%: Irritation observed in 4/4	RIFM (1983c)
α-Damascone	Buehler pretest	10% in propylene glycol	11 Hartley guinea pigs	No irritation	RIFM (1971a)
γ-Damascone	Irritation evaluated during an associated LD_{50} study	100%	10 Albino New Zealand rabbits (5/sex)	Irritation observed in 10/10	RIFM (1987b)
γ-Damascone	4-h semi-occlusive patch test	40%, 55%, 75% in ethanol or 100%	4 Albino New Zealand rabbits	40%, 55%, and 75%: No irritation 100%: Irritation observed in 4/4	RIFM (1986d) RIFM (1986e)
γ-Damascone	Buehler pretest	10%, 20%, 50% in ethanol or 100%	4 Dunkin–Hartley guinea pigs	10%: No irritation 20%, 50%, and 100%: Irritation observed in 2/4	RIFM (1986f)
cis-β-Damascone	Irritation evaluated as part of delayed contact hypersensitivity study	1.5% in 80% ethanol	20 Hartley guinea pigs (10/sex)	Irritation observed in 18/20	RIFM (1992b)
cis-β-Damascone	Primary irritation test	0.5% in alcohol SDA 39 C	6 Albino New Zealand rabbits	No irritation	RIFM (1979x)
cis-β-Damascone	Buehler pretest	1.5% in 80% ethanol	4 Hartley guinea pigs 2/sex	No irritation	RIFM (1983b)
trans-β-Damascone	Primary irritation test (24-h closed patch test)	50% in triethyl citrate	6 Albino rabbits	No irritation	RIFM (1979f)
trans-β-Damascone	Maximization pretest	0.625%, 1.25%, 2.5%, and 5% in distilled water	4 Hartley–Dunkin guinea pigs	No irritation	RIFM (1979i)
trans-β-Damascone	Maximization pretest	0.125%, 0.25%, 0.5%, and 1% in distilled water	4 Hartley–Dunkin guinea pigs	No irritation	RIFM (1979i)
Dihydro-α-ionone	Irritation evaluated during an associated LD_{50} study	100%	10 rabbits	Irritation observed in 10/10	RIFM (1976d)
Dihydro-β-ionone	Maximization pretest	25%, 50%, 75%, and 100%	2 male Himalayan guinea pigs	25%: Irritation observed in 2/2 50%: Irritation observed in 2/2 75%: Irritation observed in 2/2 100%: Irritation observed in 2/2	RIFM (1999c)

Table 9 (*continued*)

Material	Method	Concentration	Species	Results	Reference
Dihydro-β-ionone	Maximization pretest	1%, 5%, 10%, and 15% in PEG (polyethylene gylocol) 400	2 Himalayan spotted guinea pigs	1%: No irritation 5%: Irritation observed in 1/2 10%: Irritation observed in 2/2 15%: Irritation observed in 2/2	RIFM (1999c)
Dihydro-β-ionone	Maximization pretest	100%	10 Himalayan spotted guinea pigs	Irritation observed in 10/10	RIFM (1999c)
Dihydro-β-ionone	Primary skin irritation study 4-h semi-occluded patch test	100%	3 New Zealand white rabbits	No irritation	RIFM (1999d)
1,3-Dimethyl-α-ionone[a]	Primary skin irritation study 4-h semi-occluded patch test	100%	6 Albino rabbits	Irritation observed in 6/6	RIFM (1984c)
1,3-Dimethyl-α-ionone[a]	Irritation evaluated during an associated LD_{50} study	100%	10 Albino rabbits	Irritation observed in 10/10	RIFM (1984b)
1,3-Dimethyl-α-ionone[a]	Irritation evaluated during an associated LD_{50} study	100%	10 rabbits	Irritation observed in 10/10	RIFM (1976e)
1,3-Dimethyl-α-ionone[a]	Irritation evaluated during an associated maximization study	0.1% in peanut oil	10 Hartley guinea pigs	Irritation observed in 7/10	RIFM (1984f)
α-Ionone	Primary irritation test (24-h closed patch test)	100% and 5% in DEP	3 rabbits/dose	5%: Irritation observed in 1/3 100%: Irritation observed in 3/3	RIFM (1967a)
α-Ionone	Draize pretest	30% (vehicle not specified)	4 Hartley albino guinea pigs	No irritation	Sharp (1978)
α-Ionone	24-h closed patch test	100%	6 Albino Angora rabbits	Irritation observed (no further details reported)	Motoyoshi et al. (1979)
α-Ionone	24-h closed patch test	100%	6 male Hartley guinea pigs	Irritation observed (no further details reported)	Motoyoshi et al. (1979)
α-Ionone	48-h closed patch test	100%	6 Pitman–Moore miniature swine	No irritation	Motoyoshi et al. (1979)
β-Ionone	24-h closed patch test	5% and 100% in DEP	3 rabbits	5%: Irritation observed in 2/3 100%: Irritation observed in 3/3	RIFM (1967b)
β-Ionone	Irritation evaluated during an associated phototoxicity study	5%, 10%, 30%, and 50% in acetone	5 Hartley Albino guinea pigs	No irritation	RIFM (1999e)
Ionone	4-h semi-occluded patch test	100%	8 New Zealand white albino rabbits	Irritation observed in 8/8	RIFM (1979u)
Ionone	Irritation evaluated during an associated phototoxicity study	10%, 30%, 100% in ethanol	5 Albino Wistar rats	Irritation observed in 5/5	RIFM (1981b)
α-Irone	Irritation evaluated during an associated LD_{50} study	100%	6 Albino rabbits	Irritation observed (no further details reported)	RIFM (1972c)
Isodamascone	Maximization pretest	25%, 50%, and 100% in peanut oil	Pilbright white guinea pigs (2/dose)	No irritation observed at 25% and 50% 100%: Irritation observed in 2/2	RIFM (1991)
Iso-β-ionone[a]	Irritation evaluated during an associated LD_{50} study	100%	10 New Zealand white rabbits	Irritation observed in 10/10	RIFM (1980e)
α-*iso*-Methylionone	Irritation evaluated during an associated LD_{50} study	100%	8 rabbits	Irritation observed in 6/8	RIFM (1973)
α-*iso*-Methylionone	Primary irritation test	100%	3 New Zealand white Albino rabbits	Irritation observed in 3/3	RIFM (1984d)
α-*iso*-Methylionone	Primary irritation test	100%	4 New Zealand white Albino rabbits	No irritation	RIFM (1985d)
α-*iso*-Methylionone	Irritation was evaluated during associated 90 day study	1% in phenethyl alcohol	Sprague–Dawley Albino rats (5/sex)	No irritation	RIFM (1981a)
α-*iso*-Methylionone	Irritation was evaluated during associated 90 day study	100%	15 Sprague–Dawley rats	Irritation observed in 15/15	RIFM (1980a)

(*continued on next page*)

Table 9 (*continued*)

Material	Method	Concentration	Species	Results	Reference
α-*iso*-Methylionone	Primary irritation test (24-h closed patch test)	100% and 5% in DEP	3 rabbits/group	5%: No irritation 100%: Irritation observed in 2/3	RIFM (1967a)
α-*iso*-Methylionone	4-h semi-occluded patch test	100%	8 New Zealand white Albino rabbits	Irritation observed in 8/8	RIFM (1979v)
α-*iso*-Methylionone	Irritation evaluated during an associated phototoxicity study	10%, 30%, and 100% in ethanol	5 Albino Wistar rats	10%: No irritation 30%: Irritation observed in 5/5 100%: Irritation observed in 5/5	RIFM (1981c)
Methyl ionone	4-h semi-occlusive patch test	100%	8 New Zealand White rabbits	Irritation observed in 8/8	RIFM (1979w)
Methyl ionone	Irritation evaluated during an associated phototoxicity study	30% in ethanol	4 Albino Wistar rats Colworth colony	Irritation observed in 4/4	RIFM (1982e)
Methyl ionone	Irritation evaluated during an associated LD_{50} study	100%	8 rabbits	Irritation observed in 6/8	RIFM (1973)
Methyl-β-ionone	4-h semi-occlusive patch	100%	3 Albino rabbits	Irritation observed in 3/3	RIFM (1988b)
Methyl-β-ionone	Buehler pretest	100%, 50%, 25%, and 12.5% in ethanol	4 female Dunkin–Hartley Albino guinea pigs	12.5% and 25%: No irritation 50%: Irritation observed in 2/4 100%: Irritation observed in 4/4	RIFM (1989)
Methyl-β-ionone	Buehler pretest	50%, 20%, 10%, and 5% in light liquid paraffin	4 female Dunkin–Hartley Albino guinea pigs	No irritation	RIFM (1989)
4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one	Irritation evaluated during an associated LD_{50} study	100%	10 rabbits	Irritation observed in 10/10	RIFM (1978e)

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

Table 10
Mucous membrane (eye) irritation studies (in rabbits)

Material	Concentration	Results	Reference
1,3-Dimethyl-α-ionone[a]	100%	No irritation	RIFM (1984e)
Damascenone	0.5% in propylene glycol	No irritation	RIFM (1978d)
Damascenone	50% in triacetoin	No irritation	RIFM (1979j)
α-Damascone	100% (vehicle not specified)	No irritation	RIFM (1979y)
α-Damascone	0.5% in propylene glycol	No irritation	RIFM (1979n)
cis-β-Damascone	0.5% in propylene glycol	No irritation	RIFM (1979aa)
trans-β-Damascone	50% in triethyl citrate	No irritation	RIFM (1979k)
α-Ionone	5% and 100% in DEP	No irritation	RIFM (1967a)
β-Ionone	5% and 100% in DEP	No irritation	RIFM (1967b)
Isodamascone	1.5% in petrolatum	No irritation	RIFM (1979l)
α-*iso*-Methylionone	12.5% (vehicle not specified)	Intense conjunctival irritation observed in 3/3	RIFM (1963)

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

then cross challenged with 0.1% α-damascone or *cis*-β-damascone. When the mixture of α- and β-damascone was evaluated, sensitization reactions were observed at 0.2%.

3.9.3. Animal studies

Ionones and rose ketones were evaluated for sensitization in guinea pigs using various test methods including Magnusson–Kligman maximization test, Buehler delayed hypersensitivity test, Freund's Complete Adjuvant Test, Open Epicutaneous Test and modified Draize test.

Methyl-β-ionone at a concentration of 12.5% produced sensitization reactions in Buehler delayed hypersensitivity test, but when animals were rechallenged with 5% methyl-β-ionone, no sensitization was observed. No sensitization reactions were observed when other ionones (dihydro-α-ionone, dihydro-β-ionone, α-ionone, β-ionone, ionone, α-irone, and methyl ionone) were tested at concentrations ranging from 0.1% to 50%.

Sensitization reactions were observed when damascone, *trans*-β-damascone, *cis*-β-damascone and α-damascone were tested at concentrations ranging from 0.5% to 10%. No sensitization was observed with isodamascone at 50%.

Table 11
Skin sensitization studies in humans

Material	Method	Concentration	Subjects	Results	Reference
Allyl α-ionone	MAX[a]	10% in petrolatum	25 male volunteers	No reactions (0/25)	RIFM (1972a)
Allyl α-ionone	HRIPT[b]	2% in petrolatum	50 male and female volunteers	No reactions (0/50)	RIFM (1971a)
Damascenone	HRIPT	0.5% induction 0.05% challenge in alcohol	14 volunteers	2/14 reactions plus one questionable reaction	RIFM (1978c)
Damascenone	HRIPT	0.05% in alcohol SDA 39C	23 volunteers	No reactions (0/23)	RIFM (1978c)
Damascenone	HRIPT	3.0% in triacetoin	50 male and female volunteers	1/50 reactions	RIFM (1979b)
α-Damascone	HRIPT	1% in white petrolatum	54 volunteers	No reactions (0/54)	RIFM (1979o)
α-Damascone	MAX	0.2% in petrolatum	25 male and female volunteers	No reaction (0/25)	RIFM (1985c)
α-Damascone	HRIPT	10% in petrolatum	50 male and female volunteers	Study aborted because of strong reactions during induction	RIFM (1992a)
α-Damascone	HRIPT	0.1% in alcohol	51 volunteers	No reactions (0/51)	RIFM (1979d)
α-Damascone	HRIPT	0.5% in DEP	107 male and female volunteers	No reactions (0/107)	RIFM (2001a)
δ-Damascone	HRIPT	1% in SDA-39C alcohol	54 male and female volunteers	7/54 reactions	RIFM (1982a)
δ-Damascone	HRIPT	1% in ethanol	15 volunteers	2/15 reactions plus 2 questionable reactions	RIFM (1978b)
δ-Damascone	HRIPT	0.1% in ethanol	24 volunteers	No reactions (0/24)	RIFM (1978b)
cis-β-Damascone	HRIPT	0.05% in alcohol SDA 39C	53 male and female volunteers	No reactions (0/53)	RIFM (1980h)
cis-β-Damascone	HRIPT	0.5% induction 0.05% challenge in ethanol	17 volunteers	6/17 reactions	RIFM (1979c)
cis-β-Damascone	HRIPT	0.05% in ethanol	28 volunteers	0/28	RIFM (1979c)
cis-β-Damascone	HRIPT	5% in white petrolatum	50 male and female volunteers	Study aborted because of strong reactions during induction	RIFM (1992a)
trans-β-Damascone	HRIPT	0.5% in DEP	104 male and female volunteers	No reactions (0/104)	RIFM (2000e)
trans-β-Damascone	HRIPT	1% in white petrolatum	54 volunteers	No reactions (0/54)	RIFM (1979p)
trans-β-Damascone	MAX	0.2% in petrolatum	23 male and female volunteers	No reactions (0/23)	RIFM (1985b)
Dihydro-α-ionone	MAX	12% in petrolatum	25 male and female volunteers	No reactions (0/25)	RIFM (1976a)
Dihydromethyl-α-ionone[c]	MAX	4% in petrolatum	25 male and female volunteers	No reactions (0/25)	RIFM (1976b)
1,3-Dimethyl-α-ionone[c]	MAX	10% in petrolatum	27 male and female volunteers	No reactions (0/27)	RIFM (1985a)
1,3-Dimethyl-α-ionone[c]	MAX	4% in petrolatum	26 male and female volunteers	No reactions (0/26)	RIFM (1976c)
Ionone	MAX	8% (vehicle not specified)	25 male and female volunteers	No reactions (0/25)	Greif (1967)
α-Irone	MAX	10% in petrolatum	25 male volunteers	No reactions (0/25)	RIFM (1972b)
Isodamascone	HRIPT	1% in DEP	65 males and females volunteers	No reaction (0/65)	RIFM (1995b)
α-Isodamascone	HRIPT	0.2% in DEP	103 male and female volunteers	No reactions (0/103)	RIFM (1995a)
α-Isodamascone	HRIPT	2% in DEP	22 female volunteers	2/22 reactions	RIFM (1994)
Iso-β-ionone[c]	MAX	12% in petrolatum	25 male and female volunteers	No reactions (0/25)	RIFM (1980f)

(*continued on next page*)

Table 11 (*continued*)

Material	Method	Concentration	Subjects	Results	Reference
Methyl ionone	MAX	10% (vehicle not specified)	25 volunteers	No reactions (0/25)	Greif (1967)
α-*iso*-Methylionone	HRIPT	2% in dimethyl phthalate	52 male and female volunteers	No reactions (0/52)	RIFM (1968)
α-*iso*-Methylionone	HRIPT	10% in alcohol	28 volunteers	No reactions (0/28)	RIFM (1962)
α-*iso*-Methylionone	HRIPT	12.5% in unspecified vehicle	37 male and female volunteers	No reactions (0/37)	RIFM (1964)
α-*iso*-Methylionone	HRIPT	60% in 3:1 EtOH:DEP	23 male and female volunteers	No reactions (0/23)	RIFM (2004c)
α-*iso*-Methylionone	HRIPT	60% in 3:1 DEP:EtOH	106 male and female volunteers	No reactions (0/106)	RIFM (2004b)
4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one	MAX	20% in petrolatum	28 male volunteers	No reactions (0/28)	RIFM (1978a)

[a] Human maximization test (MAX).
[b] Human repeated insult patch test (HRIPT).
[c] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

Sensitization was also evaluated in mice using 8 Local Lymph Node Assays conducted with both ionones and rose ketones. With ionones, dihydro-γ-ionone was not considered a sensitizer at concentrations up to 30% (EC3 value not calculable), but α-*iso*-methylionone was considered a likely sensitizer at concentrations of 25% or higher (EC3 value 21.8%).

The following rose ketones: damascone, *trans*-β-damascone and γ-damascone produced evidence indicative of weak to moderate skin sensitization potential at concentrations ranging from 0.25% to 30% and EC3 values between 1.22% and 9.6%. For details of individual studies see Table 12.

3.10. Phototoxicity and photoallergenicity

UV spectra have been obtained for 10 materials (dihydro-α-ionone; β-ionone; ionone; α-*iso*-methylionone; methyl ionone; damascenone; 4-(2,6,-trimethyl-3-cyclohexene-1-yl)-3-buten-2-one; α-isodamascone; *cis*-β-damascone; γ-damascone) with the UVB light absorbed at a range of 220–400 nm. The results of phototoxicity–photoallergenicity are summarized in Tables 13–17 and described below.

3.10.1. Human studies

Phototoxicity and photoallergy (using exposure to 365 nm wavelength light at an intensity of 1680 μW/cm^2) have been investigated as a part of HRIPT tests. Sensitization, but not photosensitization, was reported in 1/20 subjects exposed to damascone (RIFM, 1979q). No evidence of photosensitization, skin sensitization or irritation, was reported with either of α-damascenone or *cis*-β-damascone (RIFM, 1992a).

3.10.2. Animal studies

Hartley guinea pigs treated with β-ionone at concentrations of 5%, 10%, 30%, and 50% in acetone showed no evidence of phototoxicity (RIFM, 1999e). In 20 Pirbright white guinea pigs no phototoxic or photoallergenic effects were observed with 1.5% isodamascone (RIFM, 1979r).

3.11. Environmental data

In addition to a human health assessment, environmental assessment of fragrance materials is performed according to a standard framework (Salvito et al., 2002). This screens chemicals in the RIFM/FEMA database for their potential to present a hazard to the aquatic environment by considering their removal in wastewater treatment, minimal dilution in the mixing zone, and the application of large uncertainty factors to ecotoxicological endpoints determined using quantitative structure–activity relationships. This screening, based on conservative assumptions, identifies priority materials that may require further study to quantitatively assess potential environmental risks. None of the materials in the ionone group were identified as priority material for risk assessment refinement.

However, there are environmental data in the RIFM/FEMA Database for materials within the ionone group. These include biodegradation, bioconcentration, acute *Daphnia* and fish studies, and algal population growth inhibition data. Due to the limited availability of data and the apparent consistency in the ecotoxicity data, the ionone and rose ketone groups are discussed collectively and not as two separate groups. Data are available for 7 materials. Overall, these materials appear to be readily biodegradable; the acute toxicities range from 1 to 20 mg/L. The one bioconcentration study indicates limited bioconcentration with a maximum BCF of 56 reported at 1 μg/L (RIFM, 1985e).

In addition, three papers describe the fate of some of the ionone compounds in the environment. In a study by Difrancesco et al. (2004), α-*iso*-methylionone was spiked into wastewater treatment plant sludge amended to soil in a

Table 12
Skin sensitization studies in animals

Material	Method	Concentration	Species	Results	Reference
Damascenone	Buehler test	10% in propylene glycol	11 Male Hartley guinea pigs	No reactions	RIFM (1971a)
Damascenone	Maximization test	1.5% in distilled water	10 Hartley–Dunkin guinea pigs	1/10 reactions	RIFM (1979h)
Damascenone	Maximization test	3% in distilled water	10 Hartley–Dunkin guinea pigs	2/10 reactions	RIFM (1979h)
Damascenone	Maximization test	0.25% in distilled water	10 Hartley–Dunkin guinea pigs	1/10 reactions	RIFM (1979h)
Damascenone	Maximization test	0.5% in distilled water	10 Hartley–Dunkin guinea pigs	1/10 reactions	RIFM (1979h)
Damascenone	LLNA	0.25%, 0.5%, 1.0%, 2.5%, 5.0% in 4:1 acetone/olive oil	CBA/J Hsd female mice (5/dose)	EC3 = 1.24%	RIFM (2001b)
Damascenone	LLNA	0.25%, 5%, 1.0%, 2.5%, 5.0% in 4:1 acetone/olive oil	CBA/J Hsd female mice (5/dose)	EC3 = 1.22%	RIFM (2002d)
α-Damascone	Maximization test	0.6% in 80% ethanol (primary challenge concentration) 1.8% in 80% ethanol (rechallenge concentration)	19 Hartley guinea pigs	Primary challenge 0.6%: 1/19 reactions Rechallenge 1.8%: 9/18 reactions	RIFM (1983c)
α-Damascone	Maximization test	5% and 10% in distilled water	10 Hartley–Dunkin guinea pigs	5%: No reactions 10%: 3/10 reactions	RIFM (1980g)
α-Damascone	Maximization test	0.5% and 1% in distilled water	10 Hartley–Dunkin guinea pigs	0.5%: No reactions 1%: 3/10 reactions	RIFM (1980g)
α-Damascone	Buehler test	10% in propylene glycol	11 Male Hartley guinea pigs	No reactions	RIFM (1971a)
α-Damascone	Maximization test	2%, 5%, and 10% in petrolatum	20 Hartley Guinea pigs	2% and 5%: No reactions 10%: 2/20 reactions	Kozuka et al. (1996)
α-Damascone	LLNA	0.1, 0.25, 0.5, 1.0, 2.5, 5.0 in 4:1 acetone/olive oil	CBA/J Hsd female mice (5/dose)	EC3 = 3.3%	RIFM (2001d)
δ-Damascone	LLNA	0.25%, 0.5%, 1%, 2.5%, or 5% in 4:1 acetone/olive oil	Female CBA/J mice	EC3 = 0.9%	RIFM (2002a)
δ-Damascone	LLNA	0.25%, 0.5%, 1%, 2.5%, or 5% in 4:1 acetone/olive oil	Female CBA/J mice	EC3 = 5.19%	RIFM (2002b)
δ-Damascone	LLNA	7.5%, 15% or 30% in 3:1 DEP: EtOH	Female CBA/J mice (5/dose)	EC3 = 9.6%	RIFM (2004)
γ-Damascone	Buehler test	5% or 10% in ethanol	20 Hartley guinea pigs	5%: 1/10 reaction 10%: 2/10 reactions	RIFM (1986f)
γ-Damascone	LLNA	0.25%, 0.5%, 1%, 2.5%, or 5% in 4:1 acetone/olive oil	Female CBA/J mice	EC3 = 4.6%	RIFM (2001e)
cis-β-Damascone	Delayed hypersensitivity test	1.5% in 80% ethanol	20 Hartley guinea pigs (10/sex)	1/20 reactions	RIFM (1992b)
cis-β-Damascone	Maximization test	2%, 5%, and 10% in petrolatum	19 Hartley female guinea pigs	2%: 17/19 reactions 5%: 18/19 reactions 10%: 18/19 reactions	Kozuka et al. (1996)
cis-β-Damascone	Buehler test	1.5% in 80% ethanol	20 Hartley guinea pigs	1/20 reactions	RIFM (1983b)
trans-β-Damascone	Maximization test	0.5% and 1.0% in distilled water	10 Hartley–Dunkin guinea pigs	0.5%: No reactions 5%: 1/10 reactions	RIFM (1979i)
trans-β-Damascone	Maximization test	2.5% and 5% in distilled water	10 Hartley–Dunkin guinea pigs	2.5%: 1/10 reactions 5%: 2/10 reactions	RIFM (1979i)
trans-β-Damascone	LLNA	0.1%, 0.25%, 0.5%, 1%, 2.5%, or 5% in 4:1 acetone/olive oil	6 female CBA/J Hsd mice	EC3 = 2.4%	RIFM (2001c)
Dihydro-α-ionone	Open epicutaneous test (OET)	12% (unspecified vehicle)	6–8 male and female guinea pigs	No reactions	Klecak (1985)
Dihydro-β-ionone	Maximization test	1% in PEG 400	10 male Himalayan spotted guinea pigs	No reactions (0/10)	RIFM (1999c)
Dihydro-γ-ionone	LLNA	7.5%, 15%, 30% in 3:1 DEP:Ethanol	25 female CBA/J mice	7.5%: SI = 1.39 15%: SI = 1.52 30%: SI = 1.76	RIFM, 2004e

(*continued on next page*)

Table 12 (*continued*)

Material	Method	Concentration	Species	Results	Reference
1,3-Dimethyl-α-ionone[a]	Maximization test	0.1% in peanut oil	10 Hartley Albino guinea pigs	3/10 reactions	RIFM (1984f)
α-Ionone	Modified Draize test	ACC (application challenge concentration) dose was 30% ICC (intradermal challenge concentration) dose was 0.1%	10 Hartley Albino guinea pigs	No reactions (0/10)	Sharp (1978)
β-Ionone	Maximization test	5%, 10%, 20%, and 40% in acetone	5 Hartley guinea pigs	No reactions (0/5)	RIFM (1999e)
Ionone	OET	100%	6–8 Himalayan white-spotted pigs	No reactions	Klecak et al. (1977)
Ionone	Draize test	0.1% in isotonic saline	6–8 Himalayan white-spotted pigs	No reactions	Klecak et al. (1977)
Ionone	Maximization test	5% in isotonic saline	6–8 Himalayan white-spotted pigs	No reactions	Klecak et al. (1977)
Ionone	Freund's complete adjuvant test (FCAT)	50% in Freund's Complete Adjuvant	6–8 Himalayan white-spotted pigs	No reactions	Klecak et al. (1977)
Ionone	OET	8% (vehicle not specified)	6–8 Himalayan white-spotted pigs	No reactions	Klecak (1979, 1985)
Ionone	Maximization test	10% (vehicle not specified)	Hartley guinea pigs	No reactions	Ishihara et al. (1986)
α-Irone	OET	10% (vehicle not specified)	6–8 guinea pigs per group	No reactions	Klecak (1979)
Isodamascone	Maximization test	50% in peanut oil	20 Pirbright white guinea pigs	No reactions	RIFM (1991)
α-*iso*-Methylionone	LLNA	2.5%, 5%, 10%, 25%, 50% in 3:1 DEP: EtOH	24 female CBA/Ca mice	EC3 = 21.8%	RIFM (2005)
Methyl ionone	OET	100%	6–8 Outbred Himalayan white-spotted male and female guinea pigs	No reactions	Klecak et al. (1977)
Methyl ionone	Maximization test	10% (vehicle not reported)	Guinea pigs	No reactions	Ishihara et al. (1986)
Methyl ionone	Draize test	0.1% in isotonic saline	6–8 Himalayan white-spotted guinea pigs	No reactions	Klecak et al. (1977)
Methyl ionone	Maximization test	25% in petrolatum	6–8 Himalayan white-spotted guinea pigs	No reactions	Klecak et al. (1977)
Methyl ionone	FCAT	50% in Freund's Complete Adjuvant	6–8 Himalayan white-spotted guinea pigs	No reactions	Klecak et al. (1977)
Methyl ionone	OET	10% (vehicle not reported)	6–8 Outbred Himalayan white-spotted male and female guinea pigs	No reactions	Klecak (1979, 1985)
Methyl-β-ionone	Delayed hypersensitivity test	12.5% and 25% in ethanol	20 female Dunkin–Hartley Albino guinea pigs	12.5%: 4/20 reactions 25%: 8/20 reactions	RIFM (1989)

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

Table 13
Phototoxicity studies in humans

Material	Concentration	Subjects	Results	Reference
Damascenone	3% in triacetoin	20 male and female volunteers	No phototoxicity was observed	RIFM (1979q)
α-Damascone	10% in petrolatum	20 male and female volunteers	No phototoxicity was observed	RIFM (1992a)
cis-β-Damascone	5% in petrolatum	20 male and female volunteers	No phototoxicity was observed	RIFM (1992a)

series of experiments to determine its dissipation in the soil compartment and potential to leach from the upper 10 cm of soil. α-*iso*-Methylionone was undetected after 3 months in the soil compartment and not detected in the leachate.

Simonich et al. (2002) reported that removal of γ-methyl ionone in a variety of wastewater treatment plants in Europe and the United States exceeded 87%. Final effluent concentrations in these plants were consistently below 0.5 μg/L. This confirmed earlier work reported in Simonich et al. (2000).

The ionones present a negligible environmental risk and would not be considered persistent, bioaccumulative or toxic chemicals as indicated by applying the RIFM framework (Salvito et al., 2002) and reviewing the limited environmental data.

4. Summary

1. In the scientific literature and in studies in the RIFM database, there are no definitive data from which to quantify the *in vivo* absorption of ionones and/or rose ketones following dermal exposure. Similarly, there are no oral pharmacokinetic studies available from which the bioavailability of this class of compounds can be quantitatively determined. By analogy with

Table 14
Phototoxicity studies in animals

Material	Concentration	Species	Results	Reference
β-Ionone	5%, 10%, 30%, and 50% in acetone	5 Hartley Albino guinea pigs	No phototoxicity observed	RIFM (1999e)
Ionone	100%	10 Wistar Albino rats	No phototoxicity observed	RIFM (1981b)
Isodamascone	1.5% in petrolatum	20 Pirbright White guinea pigs	No phototoxicity observed	RIFM (1979s)
α-*iso*-Methylionone	30% in ethanol	10 Wistar rats	No phototoxicity observed	RIFM (1981c)
Methyl ionone	0.1%, 0.25%, 0.5%, 1.0%, 2.5%, 5%, 10%, and 25% in 6% acetone/saline	4 guinea pigs	Phototoxic reactions observed at 25% Questionable reactions observed at lower doses (no further details reported)	RIFM (1982b)
Methyl ionone	1.5, 5, 17, 60, 200 and 660 mg/kg in olive oil	C57 BL Colworth mice	No phototoxicity observed	RIFM (1982d)
Methyl ionone	30% in ethanol	Rats	Phototoxicity was observed (no further details reported)	RIFM (1982c)

Table 15
Photoallergy studies in humans

Material	Method	Concentration	Subjects	Results	Reference
Damascenone	HRIPT procedure with UV irritation after the 1st, 4th, 7th and 9th induction applications and again after the challenge application	3% in triacetoin	20 male and female volunteers	No reactions (0/20)	RIFM (1979q)

Table 16
Photoallergy studies in animals

Material	Method	Concentration	Subjects	Results	Reference
Isodamascone	Nine induction applications (3 times a week for 3 weeks) followed by irradiation after each application; then a 3-week rest period, followed by a challenge application and irradiation	1.5% in petrolatum	19 Pirbright white guinea pigs	No reactions (0/19)	RIFM (1979s)

Table 17
Summary of UV spectra data for ionones

Material	UV spectra range of absorption (nm)
Dihydro-α-ionone	Peaked at 235–255 nm range minor absorption in 260–300 nm region
β-Ionone	Peaked at 285–295 nm range minor absorption in 300–340 nm region
Ionone (mixed isomers)	Peaked at 290–295 nm range minor absorption in 300–320 nm region
α-*iso*-Methylionone	Does not absorb UV light at wavelengths in range of 290–400 nm
Methyl ionone (mixture of isomers)	Peaked at 230–235 nm range minor absorption in 245–320 nm region
4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one (Iritone)	Does not absorb UV light at wavelengths in the range of 290–400 nm
Damascenone	Peaked within 238–280 nm range minor absorption in 290–340 nm
cis-β-Damascone	Peaked within 220–280 nm range minor absorption in 260–300 nm
δ-Damascone	Does not absorb UV light at wavelengths in range of 290–400 nm
α-Isodamascone	Does not absorb UV light at wavelengths in range of 290–400 nm

fragrance ketones and aldehydes for which *in vivo* absorption data are available, dermal or oral absorption of ionones/rose ketones is likely to be significant and is conservatively assumed for purposes of risk assessment to be 100%. Based on metabolic studies on α-ionone and β-ionone in which ionone-specific metabolites were recovered in the urine of treated rabbits and dogs, oral absorption of these compounds does occur; it is assumed to be 100%. Bioavailability by the oral route is likely to be considerably greater than by the dermal route, based on *in vitro* rat and pig skin absorption studies.

2. The primary differences in the chemical structure of members of this class of compounds that could affect metabolism, and potentially the toxicity of metabolites, are the position of the double bond in the allylic side chain (ionones *versus* rose ketones) and the potential for epoxidation depending upon the number and position of the double bonds in the cyclohexene ring. Since the allylic side chain of the rose ketones does not appear to have strong electrophilic activity, the rose ketone metabolites are unlikely to be of greater toxicity than those of the ionones. However, based on metabolic considerations, unique epoxide metabolites could be generated for each of *trans*,*trans*-δ-damascones, δ-damascone, damascone, and methyl-δ-ionone. Thus, these compounds may have greater toxic potential than other members of this class.
3. The limited metabolic data on α- and β-ionone obtained in animals demonstrate the activity of biotransformation pathways involving combinations of hydroxylation/oxygenation of the cyclohexene ring, reduction of the butenone group to a secondary alcohol, oxidation of the angular methyl groups, reduction of the double bond in the exocyclic alkenyl side chain to form dihydro derivatives, and conjugation of the hydroxylated metabolites with glucuronic acid. Although there are no data available on the metabolic fate of the ionones and rose ketones in humans, the animal metabolic data (i.e., showing oxidative and reductive transformation followed by conjugation) and the theoretical considerations discussed above, are likely to be applicable to humans.
4. The acute oral and dermal toxicity of ionones is low to moderate. Many of the ionones have oral LD_{50} values of >2 g/kg body weight, the normal limit dose in this assay.
5. There appear to be no clear differences in the toxicity of the ionones following dermal or oral routes of exposure. This conclusion is tentative because appropriate studies comparing the two routes of exposure or to assess the subchronic dermal toxicity of any individual ionone/rose ketones or the group as a whole have not been reported. The most appropriate 90-day dermal toxicity study was conducted on α-*iso*-methyl ionone; however, its interpretation related to systemic effects is significantly compromised by severe effects of the test chemical on the skin. The available data do not indicate systemic toxicity in the absence of severe effects on the skin. Tentatively, a systemic NOAEL of 50 mg/kg body weight/day associated with dermal exposure to α-*iso*-methylionone and an oral NOAEL of 30 mg/kg body weight/day can be used for quantitative human health risk assessment of the use of the ionones as fragrance compounds. There are no dermal or subchronic oral toxicity studies available on those ionones and rose ketones that may undergo epoxidation [i.e., *trans*,*trans*-δ-damascones; δ-damascone; and 1-(2,6,6-trimethyl-3-cyclohexa-1-e-dienyl)-2-buten-1-one)], and hence have a higher potential for the generation of toxic metabolites.
6. The ionones tested are non-mutagenic in standard bacterial reverse mutation assays. In *in vitro* chromosome aberration tests, increases in structural aberrations have been reported with methyl ionone and α-ionone at high concentrations. There is one negative and one equivocal *in vivo* mouse micronucleus test with methyl ionone and a negative mouse micronucleus test with α-ionone at doses as high as 1200 mg/kg.
7. There are no long-term studies that directly evaluated the carcinogenicity of ionones. Based on the lack of significant genotoxic potential, a lack of tumor promoting activity, and the reported anti-carcinogenic effects of one ionone, it appears that ionones have no significant carcinogenicity under the recommended current conditions of use as fragrance ingredients (some uncertainty remains for those rose ketones and ionones that may be subject to metabolism by epoxidation).
8. The reproductive/developmental toxicity studies of α-*iso*-methylionone and ionone, demonstrate that these materials do not cause reproductive/developmental effects at doses that approach the maternal NOAEL.
9. At concentrations likely to be encountered by humans through the use of the ionones and rose ketones as fragrance ingredients, these chemicals are considered to be non-irritating. Rose ketones could produce some skin irritation in sensitive individuals. As neat solutions (100% concentration), the ionones and rose ketones are irritants in laboratory animals.
10. The eye irritation data indicate weak eye irritation potential of certain ionones. The rose ketones tested have shown no evidence of eye irritation potential. Under the conditions of use, fragrance ingredients at low concentrations in cosmetic products, both ionones and rose ketones evaluated in this report are expected to be non-irritating to mucous membranes (eyes).
11. The ionones are without significant skin sensitization potential. The rose ketones can be sensitizers but not when present at concentrations of 0.2% or less (based on human data). IFRA (2007) has established Standards on the methyl ionones and the rose ketones using a Quantitative Risk Assessment (QRA) for dermal sensitization (see the individual fragrance material reviews on these materials for more information).
12. The ionones included in this summary are likely to have no phototoxic or photoallergic potential.

5. Conclusion

- For evaluation of the ionones and rose ketones 100% bioavailability should be assumed for the dermal and oral routes of exposure.

- The limited metabolic data on ionones demonstrate biotransformation pathways involving combinations of hydroxylation/oxygenation, reduction, oxidation, and conjugation. Metabolism of the majority of this class of compounds is not likely to increase the toxicity of parent compounds. Those that could undergo epoxidation have not been subjected to subchronic testing and are considered to be inadequately characterized for the purposes of human health safety assessment.
- Ionones have low to moderate oral toxicity (LD_{50} values of 1.5 g to >5 g/kg body weight). In acute dermal toxicity studies, LD_{50} values are greater than 2 or 5 g/kg body weight (the limit doses commonly used in LD_{50} assays).
- No systemic toxicity was observed in uncomplicated subchronic oral or dermal 90-day toxicity studies in rats. It is concluded that these materials administered by the dermal route have a systemic NOAEL value of 50 mg/kg/day. They have an oral NOAEL value of 10 mg/kg body weight.
- Under intended conditions of use the ionones and rose ketones do not have significant genotoxic, reproductive or developmental potential.
- The ionones at concentrations likely to be encountered by humans through their use as fragrance ingredients are non-irritating, and the rose ketones have limited irritation potential in sensitive subjects.
- The ionones are considered to be without significant skin sensitization potential, while the rose ketones are sensitizers when present at concentrations in excess of 0.2% (based on human data). IFRA (2007) has established Standards on the methyl ionones and the rose ketones using a Quantitative Risk Assessment (QRA) for dermal sensitization (see the individual fragrance material reviews on these materials for more information).
- Use of the ionones and rose ketones in fragrances produces low levels of exposure relative to doses that elicit adverse dermal or systemic effects in laboratory animals exposed *via* dermal or oral routes. The estimate for maximum systemic exposure of humans using cosmetic products containing ionones or rose ketones ranges from 0.0002 to 0.331 mg/kg/day. If the estimate of 100% absorption is used and using the NOAEL of 10 mg/kg body weight/day, a margin of safety for systemic exposure of humans to the individual ionones in cosmetic products can be calculated to range from 30 to 50,000 times the maximum daily exposure.

Conflict of interest statement

D. Belsito, D. Bickers, M. Bruze, P. Calow, H. Greim, J.M. Hanifin, A.E. Rogers and J.H. Saurat are members of the Expert Panel of the Research Institute for Fragrance Materials, an independent group of experts who evaluate the safety of fragrance materials that is supported by the manufacturers of fragrances and consumer products containing fragrances. I.G. Sipes and H. Tagami are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on allyl α-ionone

J. Lalko, A. Lapczynski *, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of allyl α-ionone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; α-ionone

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

In 2005, a complete literature search was conducted on allyl α-ionone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

1. Identification (Fig. 1)

1.1 Synonyms: Allyl cyclocitrylideneacetone; allyl α-ionone; α-allylionone; α-cyclocitrylidenemethyl butenyl ketone; Cetone V;1,6-heptadien-3-one, 1-(2,6,6-trimethyl-2-cyclohexen-1-yl); 1-(2,6,6-trimethyl-2-cyclohexene-1-yl)-1,6-heptadien-3-one.
1.2 CAS Registry No.: 79-78-7.
1.3 EINECS No.: 201-225-9.
1.4 Formula: $C_{16}H_{24}O$.
1.5 Molecular weight: 232.37.
1.6 COE: Allyl α-ionone was included by the Council of Europe in the list of substances granted B – information required – 28 day oral study (COE No. 2040).

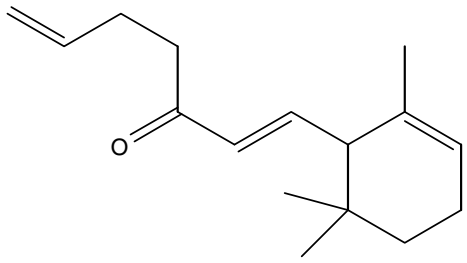

Fig. 1. Allyl α-ionone.

1.7 FDA: Allyl α-ionone was approved by the Food and Drug Administration as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and Extract Manufacturers' Association states: Generally recognized as safe as a flavor ingredient – GRAS 3 (2033).
1.9 JECFA: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 401) concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent.

2. Physical properties

2.1 Physical form: Yellow liquid with strong fruital aroma reminiscent of pineapple.
2.2 Flash point: >93.3 °C; CC.
2.3 Boiling point: 265 °C.
2.4 Log Kow (calculated): 5.63.
2.5 Vapor pressure (calculated): <0.001 mm Hg 20 °C.

3. Usage

Allyl α-ionone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 10–100 metric tonnes per annum.

The maximum skin level that results from the use of allyl α-ionone in formulae that go into fine fragrances has been reported to be 0.32% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.69% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.018 mg/kg for high end users of these products (see Table 1).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing allyl α-ionone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product (%)	Ingredient/mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.69	0.0026
Face cream	0.80	2.00	1.000	0.003	0.69	0.0006
Eau de toilette	0.75	1.00	1.000	0.080	0.69	0.0069
Fragrance cream	5.00	0.29	1.000	0.040	0.69	0.0067
Antiperspirant	0.50	1.00	1.000	0.010	0.69	0.0006
Shampoo	8.00	1.00	0.010	0.005	0.69	0.0000
Bath products	17.00	0.29	0.001	0.020	0.69	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.69	0.0001
Toilet soap	0.80	6.00	0.010	0.015	0.69	0.0001
Hair spray	5.00	2.00	0.010	0.005	0.69	0.0001
Total						0.0176

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1 The acute oral LD_{50} of allyl α-ionone was investigated in CF-1 mice. Groups of five or ten animals were administered a single oral dose via gavage of 5, 7 or 20 g/kg allyl α-ionone in distilled water plus Tween 20. In addition, ten animals were administered 8, 9, 10 or 15 g/kg allyl α-ionone in distilled water plus Tween 20. Observations for mortality or clinical signs were made over a 5-day period. No deaths occurred at 5 and 7 g/kg. One death (1/5) occurred at 8 g/kg, 4/10 deaths occurred at 9 g/kg; 7/10 deaths occurred at 10 g/kg; 9/10 deaths occurred at 15 g/kg and 5/5 animals died at 20 g/kg. The LD_{50} was calculated to be 9.5 g/kg (RIFM, 1955).

4.1.2. Dermal studies

4.1.2.1 The acute dermal toxicity of allyl α-ionone was investigated in six albino rabbits. Neat allyl α-ionone was applied to intact or abraded skin for 24 h under occlusion at a dose of 5.0 g/kg. Observations for mortality or systemic effects were made over a period of 14 days. No deaths occurred. Dry cracked skin was noted at the treatment sites. The acute dermal LD_{50} was calculated to be >5.0 g/kg (RIFM, 1971a).

Table 2
Summary of acute toxicity studies

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Mice	5–10	9.5 g/kg	RIFM (1955)
Dermal	Rabbit	6	>5.0 g/kg	RIFM (1971a)

Table 3
Human studies for skin sensitization

Test method	Test concentration	Results	References
Maximization test	10% in petrolatum	No reaction (0/25)	RIFM (1972)
HRIPT	2% in petrolatum	No reaction (0/50)	RIFM (1971b)

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1 In a pre-test for a human maximization study, 10% allyl α-ionone in petrolatum was tested in a 48-h closed patch test on the backs of five healthy, male volunteers. No irritation was observed (RIFM, 1972).

4.2.1.2 Irritation was evaluated during the induction phase of a human Repeated Insult Patch Test conducted on 50 male and female volunteers. Allyl α-ionone at 2% in petrolatum was applied to a 3 cm^2 patch, which was then applied to the upper arm of each volunteer for 24 h under occlusion. A total of fifteen 24-h applications were made on a Monday–Wednesday–Friday schedule. Reactions were read at patch removal. No irritation was observed (RIFM, 1971b).

4.2.2. Animal studies

No data available on this material.

4.3. Skin sensitization

See Table 3.

4.3.1. Human studies

4.3.1.1 A maximization test (Kligman, 1966) was carried out with 10% allyl α-ionone in petrolatum on 25 male volunteers. Application was under occlusion to the same site on the volar forearms of all subjects for five alternate-day 48-h periods. Patch test sites were pretreated for 24 h with 5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a 10-day rest period, a challenge patch with 10% test material in petrolatum was applied to a fresh site for 48 h under occlusion. The challenge sites were pretreated for 1 h with 10% aqueous SLS. Reactions to challenge were read at removal and 24 h after patch removal. No reactions were observed (RIFM, 1972).

4.3.1.2 A repeated insult patch test was conducted on 50 males and female volunteers. Allyl α-ionone at 2% in petrolatum was applied to a 3 cm^2 patch, which was then applied to the upper arm of each volunteer for 24 h under occlusion. A total of fifteen 24-h applications were made on a Monday–Wednesday–Friday schedule. After a 2-week rest period, a 24 h occluded challenge patch was applied to a virgin site. Reactions were read at patch removal, and 24 and 48 h thereafter. No reactions were produced with 2% allyl α-ionone (RIFM, 1971b).

4.3.2. Animal studies

No data available on this material.

4.4. Phototoxicity and photoallergy

No data available on this material.

4.5. Absorption, distribution and metabolism

No data available on this material.

4.6. Developmental toxicity

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Mutagenicity and genotoxicity

4.8.1. Bacterial studies

4.8.1.1 In an Ames test (Ames et al., 1975) using *Salmonella typhimurium* strains TA1535, TA100, TA1537, TA1538 and TA98 with and without rat liver S9 metabolic activation, doses up to 3.6 mg/plate allyl α-ionone in dimethyl sulfoxide were not mutagenic (Wild et al., 1983).

4.8.2. Mammalian studies

4.8.2.1 In a micronucleus test, groups of male and female NMRI mice (4/dose) were given two intraperitoneal injections of allyl α-ionone at dose levels of 464, 696 or 928 mg/kg in olive oil. Control animals were dosed with olive oil alone. Animals were sacrificed 30 h later and bone marrow was extracted and smear preparations were made and stained. Polychromatic and normochromatic erythrocytes were then scored for the presence of micronuclei. No deaths were observed during the study. There was no evidence of a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in animals when compared to the concurrent vehicle control. The mean number of micronucleated polychromatic erythrocytes per 1000 polychromatic erythrocytes was 2.5 at 464 mg/kg, 2.6 at 696 mg/kg, 2.5 at 928 mg/kg and 2.6 for the control group. Allyl α-ionone was considered to be non-genotoxic under the conditions of the test (Wild et al., 1983).

4.9. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

J. Lalko, A. Lapczynski, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute of Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on damascenone

A. Lapczynski *, J. Lalko, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of damascenone when used as a fragrance ingredient is presented.

Keywords: Fragrance; Review; Damascenone

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on damascenone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: 2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl; 1-(2,6,6-trimethylcyclohexa-1,3-dienyl)-2-buten-1-one; floriffone.
1.2 CAS Registry Number: 23696-85-7.
1.3 EINECS Number: 245-833-2.
1.4 Formula: $C_{13}H_{18}O$.
1.5 Molecular weight: 190.28.
1.6 FEMA: Flavor and Extract Manufacturers' Association states: generally recognized as safe as a flavor ingredient – GRAS 7. (3420).
1.7 JECFA: Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 387) concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent.
1.8 IFRA: Damascenone has an International Fragrance Association Standard (IFRA, 2007) – see Section 4.4.1 for details.

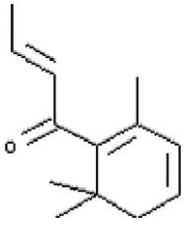

Fig. 1. Damascenone.

2. Physical properties

2.1 Physical form: A pale yellow to yellow liquid possessing a very powerful floral fruity note. It smells intensely natural, rose, plum, grape, raspberry and sugary
2.2 Log K_{ow} (calculated): 4.21.
2.3 Flash point: >200° F; CC.
2.4 Refractive index @ 20 °C : 1.508–1.514.
2.5 Specific gravity: 0.945.
2.6 Vapor pressure (calculated): 0.02 mm Hg 20 °C.

3. Usage

Damascenone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 1–10 metric tones per annum.

The maximum skin level that results from the use of damascenone in formulae that go into fine fragrances has been reported to be 0.02% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.077% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.002 mg/kg/day for a high end users of these products (see Table 1).

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1 The acute oral LD_{50} of damascenone was evaluated in Sprague Dawley rats. Ten rats (5/sex/dose) received a single oral dose (gavage) of 2.0 g/kg damascenone in a 0.25% aqueous solution of gum tragacanth. Observations for mortality, body weight changes and systemic effects were made over a 14-day period. Gross necropsy was

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing damascenone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.077	0.0003
Face cream	0.80	2.00	1.000	0.003	0.077	0.0001
Eau de toilette	0.75	1.00	1.000	0.080	0.077	0.0008
Fragrance cream	5.00	0.29	1.000	0.040	0.077	0.0007
Antiperspirant	0.50	1.00	1.000	0.010	0.077	0.0001
Shampoo	8.00	1.00	0.010	0.005	0.077	0.0000
Bath products	17.00	0.29	0.001	0.020	0.077	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.077	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.077	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.077	0.0000
Total						0.0020

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

conducted on all animals. No deaths or systemic effects were observed during the course of study. Bodyweights were observed to increase during the observation period in all animals. No abnormalities were observed during necropsy. The LD_{50} was reported to be greater than 2.0 g/kg (RIFM, 1986).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} of damascenone in rabbits was reported to be greater than 2.0 g/kg. Six rabbits (3/sex/dose) were administered a single dermal application of neat test material under occlusion to clipped, abraded skin for 24 h. Mortality and/or systemic effects were observed over a 14-day period. No deaths or systemic effects were observed during the course of the study. The average weight of all animals was reported to increase during the observation period (RIFM, 1979).

4.2. Skin irritation (Table 3)

4.2.1. Human studies

4.2.1.1 Irritation was evaluated in 15 (Panel I) and 29 (Panel II) male and female volunteers during the induction phase of a human repeated insult patch test (HRIPT). A 0.4 ml aliquot of damascenone was applied under occlusion (20×20 mm Webril® swatch affixed to 40×40 mm adhesive square) to the upper arm of each subject. A total of nine, 24 h applications were made. The reactions were scored 24 h after removal of the patch. Irritation was not observed with damascenone at 0.5% (Panel I) or at 0.05% (Panel II) in alcohol SDA 39C (RIFM, 1978).

4.2.1.2. Irritation was evaluated in 50 male and female volunteers during the induction phase of a HRIPT test. A 0.2 g portion of 3% damascenone in triacetin was applied to the upper arm of each volunteer. A total of nine, 24 h semi-occluded applications (gauze and loosely applied Dermical® tape) were made. No irritation was observed (RIFM, 1979a).

4.2.2. Animal studies

4.2.2.1. Damascenone was evaluated for primary irritation in 3 albino rabbits. A 0.5 ml aliquot of a 0.5% solution of damascenone in alcohol SDA 39C was applied under occlusion (2×2 Webril® patches and Blenderm® surgical tape) to clipped, intact and abraded skin for 24 h. Reactions were evaluated per Draize at patch removal and 48 h thereafter. Irritation was not observed (RIFM, 1978a).

Table 2
Summary of acute toxicity studies

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rat	5/sex/dose	>2.0 g/kg	RIFM (1986)
Dermal	Rabbit	3/sex/dose	>2.0 g/kg	RIFM (1979)

Table 3
Summary of human irritation studies

Method	Dose (%)	Vehicle	Results	Reference
Induction phase (HRIPT)	0.5	Alcohol SDA 39C	No irritation	RIFM (1978)
Induction phase (HRIPT)	0.05	Alcohol SDA 39C	No irritation	RIFM (1978)
Induction phase (HRIPT)	3.0	Triactoin	No irritation	RIFM (1979a)

4.2.2.2. Damascenone was evaluated for primary irritation in 6 albino rabbits. A 0.5 ml aliquot of a 50% solution of damascenone in triacetin was applied to clipped, intact and abraded skin under occlusion for 24 h. Reactions were evaluated per Draize at patch removal and 72 h thereafter. Irritation was not observed (RIFM, 1979b).

4.2.2.3. Irritation was evaluated during the induction phase of a Buehler guinea pig sensitization study in eleven Hartley guinea pigs weighing 300–414 g. Induction consisted of 24-h closed patch applications to the same clipped site on the dorsal surface with 0.1 ml aliquot of 10% damascenone in propylene glycol. Induction applications were made on alternate days for 3 weeks (10 applications in total). Reactions were scored at patch removal. No irritation was observed (RIFM, 1971).

4.2.2.4. A preliminary irritation screen was conducted on 4 Hartley-Dunkin guinea pigs prior to a maximization test. Damascenone at 0.375, 0.75, 1.5 or 3% in distilled water was applied to 2×2 cm Whatman® No. 3 filter paper and secured by two adhesive bandages on the clipped flanks for 24 h. Animals were pretreated with Freund's Complete Adjuvant (FCA). Observations were made at 24 and 48 h following patch removal. No irritation was observed (RIFM, 1979c).

4.2.2.5. Irritation was evaluated during a range finding study prior to a Magnusson– Kligman guinea pig maximization test using 4 Hartley guinea pigs that were pretreated with Freund's Complete Adjuvant. Damascenone was applied at concentrations of 0.0625, 0.125, 0.25 or 0.5% damascenone in distilled water to a 2 cm^2 Whatman® No. 3 patch which was then applied to the clipped flank for 24 h under occlusion. Observations were made at 24 and 48 h following patch removal. Irritation was not observed (RIFM, 1979c).

4.3. Mucous membrane (eye) irritation (Table 4)

4.3.1

A rabbit eye irritation test was conducted in 3 healthy, albino rabbits. A 0.1 ml aliquot of 0.5% damascenone in propylene glycol was instilled into the right eye of each

rabbit with no further treatment. The untreated left eye served as a control. Observations were made every 24 h for 4 days and then again on day 7. Scorings were recorded according to the Draize scale for ocular lesions. Irritation was not observed (RIFM, 1978a).

4.3.2

A rabbit eye irritation test was conducted in 6 healthy, albino rabbits. A 0.1 ml aliquot of a 50% solution of damascenone in triacetin was instilled into the right eye of each rabbit with no further treatment. The untreated left eye served as a control. The treated eyes were examined at 1, 2, 3, 5, and 7 days following the instillation. Scorings were recorded according to the "Illustrated Guide for Grading Eye Irritation by Hazardous Substances". Irritation was not observed (RIFM, 1979d).

4.4. Skin sensitization

4.4.1. Dermal sensitization quantitative risk assessment (QRA)

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This new methodology represents a significant change over current risk assessment practices because it specifically addresses the elements of exposure-based risk assessment that are unique to the induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment.

Full details of this risk assessment approach can be found in the "QRA Expert Group, Dermal Sensitization Quantitative Risk Assessment (QRA) for Fragrance Ingredients, Technical Dossier, revised June 22, 2006", and "IFRA/RIFM Quantitative Risk Assessment (QRA) for Fragrance Ingredients Booklet, May 11, 2006", at http://www.rifm/org/pub/publications.asp and http://www.ifraorg.org/News.asp.

An exposure-based quantitative risk assessment (QRA) methodology has been used to determine acceptable exposure limits for damascenone and a new IFRA Standard (IFRA, 2007) has been issued (see Tables 5–7).

4.4.2. Human studies

4.4.2.1. A human repeated insult patch test (HRIPT) was conducted in 14 volunteers. A 0.4 ml aliquot of 0.5% damascenone in alcohol SDA 39C was applied under occlusion to the upper arm of each volunteer. A series of nine, 24 h applications were made over a 3-week period on a Monday–Wednesday–Friday schedule. Following a 2-week rest period, subjects were challenged at a naive site using a 24 h occluded patch with 0.05% damascenone in alcohol SDA 39C. Reactions were read at patch removal and 24 and 48 h thereafter. Two sensitization reactions and one questionable reaction were observed (RIFM, 1978). Using the same method a HRIPT study was conducted in 23 volunteers using 0.05% damascenone in alcohol SDA 39C for both induction and challenge applications. No sensitization reactions were observed (RIFM, 1978).

4.4.2.2. A repeated insult patch test was conducted in 9 male and 41 female volunteers using 3% damascenone in triacetin. A 0.2 g portion of damascenone was applied to the upper arm of each subject for 24 h under semi-occlusion. This procedure was repeated 3 times a week for 3 weeks for a total of 9 applications. After a 14-day rest period, a challenge application of 3% damascenone in triacetin was applied to the same skin sites as well as to previously untreated skin sites on the same arm. The challenge applications were removed after 24 h and the sites were examined. The sites were re-examined after 48 and 72 h. One sensitization reaction was observed (RIFM, 1979a).

4.4.3. Animal studies

4.4.3.1. A Buehler sensitization test was conducted on 11 Hartley guinea pigs weighing 300–414 g. A 0.1 ml aliquot of 10% damascenone in propylene glycol was placed on a half inch square of surgical gauze, which was then applied

Table 4
Summary of eye irritation studies in rabbits

Dose (%)	Vehicle	Results	References
0.5	Propylene glycol	No reactions	RIFM (1978a)
50	Triactoin	No reactions	RIFM (1979d)

Table 5
IFRA standard based on the QRA

For a description of the categories, refer to the QRA information booklet	
Limits in the finished product	
Category 1 – see Note (1)	0.003%
Category 2	0.004%
Category 3	0.02%
Category 4	0.05%
Category 5	0.02%
Category 6 – see Note (1)	0.07%
Category 7	0.008%
Category 8	0.1%
Category 9	0.5%
Category10	0.8%
Category 11 – see Note (2)	

Notes: The above limits apply to rose ketones used individually or in combination.

(1) IFRA would recommend that any material used to impart perfume or flavour in products intended for human ingestion should consist of ingredients that are in compliance with appropriate regulations for foods and food flavourings in the countries of planned distribution and, where these are lacking, with the recommendations laid down in the Code of Practice of International Organisation of the Flavor Industry (IOFI). Further information about IOFI can be found on its website (www.iofiorg.org).

(2) Category 11 includes all non-skin contact or incidental skin contact products. Due to negligible skin contact the concentration of a fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product.

For example, hypothetically if the usual concentration of a fragrance compound in the final product, for example a candle, is at 5%, then any individual fragrance ingredient (in this case a 'rose ketone') must not exceed 5% in the candle.

Table 6
Summary of the relevant sensitization data for the implementation of the QRA

CAS no	LLNA weighted mean EC3 values (μg/cm^2) [no. studies]	Human data			Potency classification[b]	WoE NESIL (μg/cm^2)[c]
		NOEL – HRIPT (induction) (μg/cm^2)	Experimental NOEL – MAX (induction) (μg/cm^2)	LOEL[a] (induction) (μg/cm^2)		
57378-68-4	1579 [3]	NA	NA	1333	Moderate	100 [LLNA weighted mean for class = 1496 μg/cm^2]
43052-87-5	NA	133 (51)	0.2%	NA	Moderate	
24720-09-0	826 [1]	500 (DEP)	NA	NA	Moderate	
23696-85-7	308 [2]	100 (23)	NA	1000	Moderate	
23726-92-3	NA	67 (53)	NA	375	Moderate	
23726-91-2	600	1000 (pet/54)	NA	NA	Moderate	
23726-94-5	NA	NA	NA	NA	Moderate	
39872-57-6	NA	236 (DEP)	NA	2362	Moderate	
71048-82-3	NA	100 (24)	NA	1000	Moderate	
33673-71-1	NA	NA	NA	NA	Moderate	
70266-48-7	NA	1181 (DEP)	NA	NA	Moderate	

NOEL = no observed effect level; HRIPT = human repeat insult patch test; MAX = human maximization test; LOEL = lowest observed effect level; NA = not available.
[a] Data derived from HRIPT or HMT.
[b] Gerberick et al. (2001).
[c] WoE NESIL limited to two significant figures.

Table 7
Summary of human sensitization studies

Test method	Test concentration	Results	References
HRIPT	0.5% induction 0.05% Challenge	2/14 reactions plus 1 questionable reaction	RIFM (1978)
HRIPT	0.05%	No reactions (0/23)	RIFM (1978)
HRIPT	3.0%	1/50 reactions	RIFM (1979a)

to the clipped inter-scapular region of each animal for 24 h under occlusion. A total of ten induction applications were made on alternate days over a three week period. After a two week rest period, a challenge patch was applied for 24 h under occlusion. Reactions were read at patch removal. No sensitization reactions were observed (RIFM, 1971).

4.4.3.2. A Magnusson–Kligman guinea pig maximization test was conducted using 10 Hartley-Dunkin guinea pigs weighing 300–500 g. Induction consisted of two stages, intradermal injection followed one week later by a 48 h occluded patch application. A total of 6 intradermal injections were administered. They comprised: 2 injections of 0.1 ml of Freund's complete adjuvant (FCA); 2 injections of 0.1 ml of 3% damascenone in dipropylene glycol; 2 injections of 0.05 ml of 3% damascenone in dipropylene glycol and FCA (50:50). The topical induction consisted of 3% damascenone in dipropylene glycol. Fourteen days after the topical induction guinea pigs were challenged on the shaved flank by a 24 h occluded application of 1.5 and 3% damascenone in distilled water. The treatment sites were examined 24 and 48 h following patch removal. At 3%, sensitization was observed in two animals and sensitization was observed in one animal at 1.5%. Damascenone was classified as a mild sensitizer (RIFM, 1979c).

4.4.3.3. A Magnusson and Kligman guinea pig maximization test was conducted in the same manner as above (Section 4.4.2.2) using 0.5% damascenone in distilled water as the intradermal and topical induction concentrations. Guinea pigs were challenged with 0.25% and 0.5% damascenone in dipropylene glycol. Sensitization was observed in one guinea pig at 0.25% and in one guinea pig at 0.5% (RIFM, 1979c).

4.4.4. Local lymph node assay

4.4.4.1. A Local Lymph Node Assay (LLNA) was conducted on female CBA/J Hsd mice. A 25 μl aliquot of damascenone was applied to the dorsum of each ear at concentrations of 0.25, 0.5, 1.0, 2.5, or 5.0% damascenone in 4:1 acetone:olive oil stabilized with tocopherol. Control animals received 4:1 acetone:olive oil with a known sensitizer, isoeugenol at concentrations of 0.5, 1.0, and 5.0%. Dosing occurred for three consecutive days. Three days after the final auricular application, the animals were injected intravenously via the tail vain with ^{125}I-labled IuDR to label proliferating cells. Five hours later, the lymph nodes for each animal were taken, dissociated and placed into suspensions. ^{125}IuDR incorporation was measured with a gamma counter. The EC3 value was calculated to be 1.24%. Under the conditions of the test, damascenone was classified as a moderate sensitizer (RIFM, 2001). A second study, using the same methodology was conducted using 0.25, 0.5, 1.0 or 5% damascenone in 4:1 acetone:oil. The EC3 value was calculated to be 1.22%. Based on the results, damascenone was again classified as a moderate sensitizer (RIFM, 2002).

4.5. Phototoxicity and photoallergy

UV spectra revealed that damascenone peaked within 238–280 nm range and showed minor absorption in the 290–340 nm region.

4.5.1. Phototoxicity study

4.5.1.1. Phototoxicity was evaluated as a part of associated HRIPT study. A subset of 20 (4 males and 16 females) volunteers received a duplicate set of patch applications on the opposite arm with an additional 0.2 g sample of 3% damascenone in triacetin. The test site was then irradiated with UVA for 15 min using a Spectroline Model B-100 blacklight flood lamp (365 nm, 1680 microwatts/cm^2) at a distance of 15 inches. After the exposure period, the test sites were covered with a semi-occlusive covering of gauze and loosely applied Dermicel® tape. The patches remained in place for 24 h. The sites were scored at patch removal and again 24 h later. A total of nine applications were made over a 3-week period. The sites were irradiated at applications 1, 4, 7 and 9. No phototoxicity was observed (RIFM, 1979a).

4.5.2. Photoallergy study

4.5.2.1. Photoallergy was also evaluated as part of an associated HRIPT. A subset of 20 volunteers received duplicate patch applications on the opposite arm with 2 g of 3% damascenone in triacetin. The test site was then irradiated with UVA for 15 min using a Spectroline Model B-100 blacklight flood lamp (365 nm, 1680 microwatts/cm^2) at a distance of 15 inches. After UV exposure, the test sites were covered with a semi-occluded patch for 24 h. Nine induction applications were made over a 3-week period. After a 2-week rest period, a 24 h occluded challenge application was made to both the original and virgin sites on the arm. Test sites were irradiated at applications 1, 4, 7, 9 and the challenge application. Reactions to challenge were read at 24, 48 and 72 h after application. Photoallergy was not observed (RIFM, 1979a).

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

4.9.1.

A bacterial reverse mutation assay (Ames et al., 1975) using the plate incorporation was performed using *Salmonella typhimurium* tester strains, TA98, TA100, TA102, TA1535 and TA1537 and *E. coli* strain WP2 uvrA. Damascenone was tested in the presence and absence of Aroclor-induced rat liver S-9 at dose levels of 75, 200, 600, 1800 or 5000 μg/plate in dimethyl sulfoxide. Damscenone did not cause a positive response with any of the tester strains in the presence or absence of S9. No precipitate was observed but toxicity was generally observed at doses of 1800 or 5000 ug/plate. Under the conditions of the test, damascenone was not considered to be mutagenic (RIFM, 2000).

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, J. Lalko, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on α-damascone

A. Lapczynski *, J. Lalko, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of α-damascone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance: α-Damascone

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail addresses: alapczynski@rifm.org (A. Lapczynski), cletizia@rifm.org (C.S. Letizia).

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This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

In 2005, a complete literature search was conducted on α-damascone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

1. Identification (Fig. 1)

1.1 Synonyms: 2-Buten-1-one, 1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-; α-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one, dihydrofloriffone A; *trans*-α-damascone; (E)-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2-buten-1-one.
1.2 CAS registry numbers: 43052-87-5; 24720-09-0.
1.3 EINECS numbers: 245-845-8; 246-430-4.
1.4 Formula: $C_{13}H_{20}O$.
1.5 Molecular weight: 192.3.
1.6 FEMA: Flavor and Extract Manufactures' Association states: Generally Recognized as Safe as a flavor ingredient – Gras 13; GRAS 22 (3659; 4088).
1.7 JECFA: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 385) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent.
1.8 IFRA: Rose ketone has an International Fragrance Association Standard (IFRA, 2007) – see Section 4.4.1. for details.

2. Physical properties

2.1 Physical form: A colorless to pale yellow liquid possessing a very diffusive and distinctive floral fruity note.
2.2 Flash point: >100 °C; CC.
2.3 Refractive index @ 20 °C : 1.493–1.499.
2.4 Log K_{OW} (calculated) 3.9.

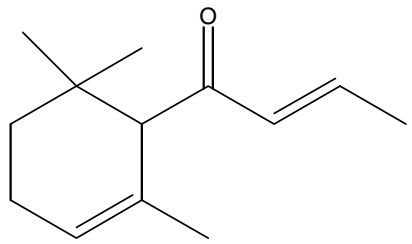

Fig. 1. α-Damascone.

3. Usage

α-Damascone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 1–10 metric tonnes per annum.

The maximum skin level that results from the use of α-damascone in formulae that go into fine fragrances has been reported to be 0.07% (IFRA, 2002, 2003), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.12% (IFRA, 2002, 2003), which would result in a conservative calculated maximum daily exposure on the skin of 0.0031 mg/kg for high end users of these products (see Table 1).

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. A preliminary range finding study was conducted to select dose levels for a subsequent LD_{50} study. Five groups of 2 male and 2 female CD® Sprague–Dawley out-bred albino rats weighing 135–219 g were orally administered single doses of α-damascone at 0.05, 0.16, 0.5, 1.6 or 5.0 g/kg in a corn oil vehicle by gavage, following an overnight fast. The animals were observed for mortality for three days. Necropsies were performed on all animals dying during the observation period and on survivors on day 4. Two males dosed with 1.6 g/kg and all four animals receiving 5.0 g/kg died within 3 days of dosing. Gross pathology revealed the presence of the test material in the stomach of all dead animals and generally the cecum contained soft stools. No toxic effects were observed at doses of 0.05–0.500 g/kg. Based on these results, dose levels of 1.0, 1.47, 2.15, 3.16 and 4.64 g/kg were selected for the LD_{50} study (RIFM, 1979a).

4.1.1.2. Groups of five male and five female CD® Sprague–Dawley out-bred albino rats, weighing 146–202 g, were orally administered α-damascone by gavage at a dose of 1.0, 1.47, 2.15, 3.16 or 4.64 g/kg in a corn oil vehicle, following an overnight fast. The animals were observed for signs of toxicity and for mortality at 1, 3, 6, and 24 h after dosing and daily thereafter for 14 days. Necropsies were performed on all animals at the time of death and on survivors sacrificed after 14 days. Soft mucoid feces were

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing *alpha*-damascone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.12	0.0005
Face cream	0.80	2.00	1.000	0.003	0.12	0.0001
Eau de toilette	0.75	1.00	1.000	0.080	0.12	0.0012
Fragrance cream	5.00	0.29	1.000	0.040	0.12	0.0012
Antiperspirant	0.50	1.00	1.000	0.010	0.12	0.0001
Shampoo	8.00	1.00	0.010	0.005	0.12	0.0000
Bath products	17.00	0.29	0.001	0.020	0.12	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.12	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.12	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.12	0.0000
Total						0.0031

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

observed in animals at all dose levels between 1 h and 4 days post dosing. A roughening of the coat was seen on days 2–4 in two females dosed with 2.15 g/kg and on day 2 in one female dosed with 3.16 g/kg. By day 6, all surviving animals were normal. Animals surviving to day 14 had increased body weights. Those dying prior to day 14 had decreased body weights. Gross pathology showed soft stools in the cecum, fecal stained anal region, and crust on the nose, mouth, and forepaws. The LD_{50} was determined to be 1.8 g/kg for male rats and 1.5 g/kg for female rats. The combined male and female rat LD_{50} was reported to be 1.67 g/kg (RIFM, 1979a).

4.1.2. Dermal studies

4.1.2.1. A preliminary range finding study was performed in CD Sprague–Dawley rats (2/sex/dose) to select dose levels for the main LD_{50} study. A single dermal dose of 0.05, 0.16, 0.5, 1.6 or 5 g/kg of α-damascone in alcohol was applied to the shaved dorsal area of each animal at a constant volume of 6 ml/mg of body weight. The treatment area was not occluded and the test material remained in contact for 24 h. All animals were observed daily for three days for clinical signs and mortality. No effects were observed at 0.05, 0.16 or 0.5 g/kg. With the exception of one female with soft feces at 3 and 6 h post-dosing, no effects were observed in animals receiving 1.6 g/kg. Two females and one male in the high-dose group died on or prior to Day 3 post-dosing. The surviving male appeared morbid on Day 3 post-dosing. Necropsy of the animals that died revealed red or darkened lungs in the male and in one of the females (RIFM, 1979c).

Table 2
Summary of acute toxicity studies

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rat	5/sex	1.67 g/kg	RIFM (1979a)
Dermal	Rabbit	3/sex	>2 g/kg	RIFM (1979b)
Dermal	Rabbit	5/sex	2.9 g/kg	RIFM (1979c)

4.1.2.2. Based on the results of the range-finding study, an acute LD_{50} study was conducted in CD Sprague–Dawley rats (5/sex/dose). α-Damascone was diluted in alcohol and administered dermally as a single dose to the previously shaved backs of animals at dose levels of 1.67, 2.15, 2.78, 3.6 or 4.64 g/kg. All animals were observed for signs of toxicity and for mortality at 1, 3 and 24 h after dosing and daily thereafter for 14 days. Gross necropsy was conducted on all animals. No deaths occurred at 1.67 and 2.15 g/kg. Three (3/10) animals died at 2.78 g/kg dose, 5/10 deaths occurred at 3.6 g/kg and 10/10 deaths occurred at 4.64 g/kg. Mean body weight gain was slightly lower in males at dose levels up to 3.6 g/kg and in females receiving 2.15 g/kg. Changes in mean body weights could not be calculated for higher doses due to the deaths in these groups. No gross tissue alterations were observed in any of the animals. The acute dermal LD_{50} was calculated to be 2.9 g/kg (95% CI of 2.164–3.886 g/kg) (RIFM, 1979c).

4.1.2.3. Six albino rabbits (3/sex) weighing 2–3 kg were clipped free of hair on the back, 24 h prior to testing. The backs of all animals were abraded and a single application of neat α-damascone was made at 2.0 g/kg. The treated areas were covered with gauze patches and an impervious material was wrapped around the trunk of each animal. The patches were removed after 24 h and the animals were observed for toxicity and mortality for 14 days. Gross necropsies were performed on all animals. No deaths occurred and no toxic signs were observed during the study. The acute dermal LD_{50} was reported to be greater than 2 g/kg. (RIFM, 1979b).

4.2. Skin irritation

4.2.1. Human studies (Table 3)

4.2.1.1. Irritation was evaluated during the induction phase of an associated human repeated insult patch test (HRIPT) with 0.5% α-damascone in DEP. A total of 107 volunteers received nine 24-h occluded induction applications of 0.5%

α-damascone in DEP over a 3-week period. Reactions were read 24 and 48 h after patch removal. No irritation was observed (RIFM, 2001).

4.2.1.2. Primary and cumulative irritation was evaluated during the induction phase of an associated HRIPT study conducted on 51 adult volunteers. A 0.2 ml aliquot of 0.1% α-damascone in alcohol was applied to 4 cm^2 Parke-Davis Readi-Bandages® which was then applied to the back of each subject for 24 h. This procedure was repeated three times per week on a Monday–Wednesday–Friday schedule until nine applications had been made over a three week period. Reactions were graded prior to each application. Mild to severe cumulative irritation was observed in three subjects (RIFM, 1979d).

4.2.1.3. As a part of a HRIPT, primary and cumulative irritation due to α-damascone was evaluated during the induction phase. Fifty-four healthy volunteers (17 male/37 female), ages 16–65 years participated in the study. Approximately, 0.2 g of 1% α-damascone in white petrolatum was applied to a 1 in. gauze pad which was applied to the upper back for 24 h under semi-occlusion. This procedure was repeated three times per week, on a Monday, Wednesday, Friday schedule, for a total of nine applications. Each site was evaluated prior to re-application. No irritation was observed (RIFM, 1979e).

4.2.1.4. Irritation was evaluated during an associated maximization study. Twenty-five healthy male and female volunteers received a single application of 0.2% α-damascone in petrolatum under occlusion to the same site on the upper arm for 5 alternate day 48-h periods. Patch sites were pretreated for 24-h with 5% aqueous sodium lauryl sulfate (SLS) under occlusion for the initial patch only. No irritation was observed (RIFM, 1985a).

4.2.2. Animal studies

4.2.2.1. A preliminary dose-range study was conducted as a part of guinea pig sensitization study to determine the maximum non-irritant concentration of α-damascone. A 24-h closed patch test was conducted using four Hartley-Dunkin guinea pigs weighing 300–500 g. The animals were pretreated with Freund's complete adjuvant. α-Damascone at 1.25%, 2.5%, 5% or 10% in distilled water was applied to a 2 cm^2 Whatman® no. 3 filter paper, which was then applied to the clipped flanks of each animal for 24 h under occlusion. The application sites were examined at 24 and 48 h after removal of the patches. No irritation was observed (RIFM, 1980).

4.2.2.2. As a part of a guinea pig sensitization study, a preliminary dose-range test was conducted using four Hartley–Dunkin guinea pigs weighing 300–500. The animals were pretreated with Freund's complete adjuvant. α-Damascone at 0.125%, 0.25%, 0.5%, or 1.0% in distilled water was applied to 2 cm^2 Whatman® no. 3 filter paper which was then applied to the clipped flanks for 24 h under occlusion. Reactions were read 24 and 48 h after patch removal. No irritation was observed (RIFM, 1980).

4.2.2.3. As a part of sensitization study, four Hartley guinea pigs per dose (2/sex) were used to evaluate primary irritation. α-Damascone at 0.6% or 1.8% in 80% ethanol was applied under occlusion for 6 h to the clipped skin on the dorsal left shoulder of each guinea pig. Reactions were read at 24 and 48 h after patch removal. Irritation was not observed with 0.6%; irritation was observed in 4/4 animals at 1.8% (RIFM, 1983).

4.2.2.4. Irritation was evaluated during the induction phase of a Buehler guinea pig sensitization study in 11 male Hartley guinea pigs weighing 300–414 g. A 0.1 ml aliquot of 10% α-damascone in propylene glycol was placed on a half-inch square of surgical gauze which was then applied to the clipped interscapular region of the guinea pigs for 24 h under occlusion. Ten such applications were made on alternate days during a three-week period. Reactions were read at patch removal. There was no evidence of irritation (RIFM, 1971).

4.2.2.5. Primary irritation due to α-damascone was evaluated in six albino rabbits. A 0.5 ml aliquot of neat α-damascone was applied to clipped, intact or abraded skin for 24 h under occlusion. Reactions were read according to Draize at patch removal and again at 72 h. Very slight erythema was observed in 4/6 rabbits; well-defined erythema in 1/6 rabbits; very slight edema in 2/6 rabbits, and moderate edema in 1/6 rabbits. The primary irritation score was reported to be 1.33. Under the conditions of the test, α-damascone was not considered to be a primary irritant (RIFM, 1979f).

4.2.2.6. A primary dermal irritation test was conducted in six New Zealand albino rabbits (3/sex). A 0.5 ml aliquot of 0.5% α-damascone in alcohol SDA 39C was applied to intact or abraded skin on the back of each animal for 24 h under occlusion. Reactions were scored according to Draize at 24 and 72 h after application. The primary irritation score was 0.041. Under the conditions of the study, α-damascone was classified as non-irritating (RIFM, 1979g).

Table 3
Summary of human irritation studies

Method	Dose (%)	Vehicle	Results	Reference
Induction phase (HRIPT)	0.5	DEP	0/107	RIFM (2001)
Induction phase (HRIPT)	0.1	Alcohol	3/51	RIFM (1979d)
Induction phase (HRIPT)	1	Petrolatum	0/54	RIFM (1979e)
Maximization study	0.2	Petrolatum	0/25	RIFM (1985)

4.3. Mucous membrane (eye) irritation

4.3.1. Animal studies (Table 4)

4.3.1.1. A primary eye irritation study was conducted in six New Zealand albino rabbits (3/sex). A 0.1 ml aliquot of 0.5% α-damascone in propylene glycol was instilled into the right eye of each rabbit while the untreated left eye served as a control. The eyes were examined at 24, 48 and 72 h and at 4 and 7 days. Roughening of the bulbar conjunctivae was observed in two rabbits; slight hyperemia and slight discharge were noted in all six rabbits and slight opacity was observed in one rabbit. By day 7 all eyes were clear in five rabbits; slight discharge was noted in the 6th rabbit. The primary ocular irritation score was 5.0 at 24 h and 0.3 at 72 h. Under the conditions of the test, α-damascone was classified as practically non-irritating to the eye (RIFM, 1979h).

4.3.1.2. A 0.1 ml aliquot of neat α-damascone was instilled into the conjunctival sac of the right eye of six healthy young adult albino rabbits without further treatment. The untreated left eyes served as controls. The eyes were examined at 1, 2, 3, 5, and 7 days following instillation. Slight conjunctival irritation, which cleared by day 2, was observed in 3/6 rabbits. Moderate conjunctival irritation, which cleared by day 5, was seen in 1/6 rabbits. The average ocular irritation score was 5.0 on day 1, 1.7 on day 2 and 1.0 on day 3 and 0 by day 5 (RIFM, 1979i).

4.4. Skin sensitization

4.4.1. Dermal Sensitization Quantitative Risk Assessment (QRA)

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This new methodology represents a significant change over current risk assessment practices because it specifically addresses the elements of exposure-based risk assessment that are unique to the induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment.

Full details of this risk assessment approach can be found in the "QRA Expert Group, Dermal Sensitization Quantitative Risk Assessment (QRA) for Fragrance Ingredients, Technical Dossier, revised June 22, 2006", and IFRA/RIFM Quantitative Risk Assessment (QRA) for Fragrance Ingredients Booklet, May 11, 2006", at http://www.rifm/org/pub/publications.asp and http://www.ifraorg.org/News.asp.

Table 4
Summary of eye irritation studies in rabbits

Dose (%)	Vehicle	Results	References
0.5	Propylene glycol	No irritation	RIFM (1979h)
100	N/A	No irritation	RIFM (1979i)

An exposure-based Quantitative Risk Assessment (QRA) methodology has been used to determine acceptable exposure limits for rose ketone and a new IFRA Standard (IFRA, 2007) has been issued (see Tables 5 and 6).

4.4.2. Human studies (Table 7)

4.4.2.1. A repeated insult patch test (HRIPT) was conducted on 107 male and female volunteers (24 males/87 females). A 0.2 ml aliquot of 0.5% α-damascone in diethyl phthalate was applied to a 2 cm^2 Webril® patch which was then applied to the back for 24 h under occlusion. The induction phase of the study consisted of nine, 24-h applications over a three week period. After a 10–15 day rest period, a 24-h occluded challenge application with 0.5% α-damascone in diethyl phthalate was applied to a virgin site. Reactions were read 24 and 48 h after patch removal. No sensitization was observed (RIFM, 2000).

4.4.2.2. A HRIPT study was conducted on 50 adult volunteers (9 males/41 females). A 0.2 g sample of 10% α-damascone in petrolatum was applied to the upper arm of each subject for 24 h under semi-occlusion. After a 24-h rest period, subjects were again patched at the same site. A total of nine induction applications were to be made over a three-week period, however, by the end of the eighth induction application there were reactions in 10 subjects. In addition,

Table 5
IFRA Standard based on the QRA

Limits in the finished product:	
For a description of the categories, refer to the QRA Information booklet	
Category 1 – see note (1)	0.003%
Category 2	0.004%
Category 3	0.02%
Category 4	0.05%
Category 5	0.02%
Category 6 – see note (1)	0.07%
Category 7	0.008%
Category 8	0.1%
Category 9	0.5%
Category 10	0.8%
Category 11 – see note (2)	

Note: The above limits apply to rose ketones used individually or in combination.

(1) IFRA would recommend that any material used to impart perfume or flavour in products intended for human ingestion should consist of ingredients that are in compliance with appropriate regulations for foods and food flavourings in the countries of planned distribution and, where these are lacking, with the recommendations laid down in the Code of Practice of IOFI (International Organisation of the Flavor Industry). Further information about IOFI can be found on its website (www.iofiorg.org).

(2) Category 11 includes all non-skin contact or incidental skin contact products. Due to negligible skin contact the concentration of a fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product.

For example, hypothetically if the usual concentration of a fragrance compound in the final product, for example a candle, is at 5%, then any individual fragrance ingredient (in this case a 'rose ketone') must not exceed 5% in the candle.

Table 6
Summary of the relevant sensitization data for the implementation of the QRA

CAS no.	LLNA weighted mean EC3 values (μg/cm^2) [no. studies]	Human data			Potency classification[b]	WoE NESIL (μg/cm^2)[c]
		NOEL – HRIPT (induction) (μg/cm^2)	Experimental NOEL – MAX (induction) (μg/cm^2)	LOEL[a] (induction) (μg/cm^2)		
57378-68-4	1579 [3]	NA	NA	1333	Moderate	100 [LLNA weighted mean for class = 1496 μg/cm^2]
43052-87-5	NA	133 (51)	0.2%	NA	Moderate	
24720-09-0	826 [1]	500 (DEP)	NA	NA	Moderate	
23696-85-7	308 [2]	100 (23)	NA	1000	Moderate	
23726-92-3	NA	67 (53)	NA	375	Moderate	
23726-91-2	600	1000 (pet/54)	NA	NA	Moderate	
23726-94-5	NA	NA	NA	NA	Moderate	
39872-57-6	NA	236 (DEP)	NA	2362	Moderate	
71048-82-3	NA	100 (24)	NA	1000	Moderate	
33673-71-1	NA	NA	NA	NA	Moderate	
70266-48-7	NA	1181 (DEP)	NA	NA	Moderate	

NOEL, no observed effect level; HRIPT, human repeat insult patch test; MAX, human maximization test; LOEL, lowest observed effect level; NA, not available.
[a] Data derived from HRIPT or HMT.
[b] Gerberick et al. (2001).
[c] WoE NESIL limited to two significant figures.

Table 7
Summary of human sensitization studies

Test Method	Test concentration (%)	Results	References
HRIPT	0.5	0/107	RIFM (2000)
HRIPT	10	10/50	RIFM (1992)
HRIPT	0.1	0/51	RIFM (1979d)
HRIPT	1	0/54	RIFM (1979e)
Maximization study	0.2	0/25	RIFM (1985)

another six subjects who had reacted strongly to α-damascone, some by the fifth induction application, were no longer being tested. Because of these reactions, the study was terminated at the end of the 8th induction application and it was concluded that α-damascone was a sensitizer at this concentration (RIFM, 1992).

4.4.2.3. Four subjects who had previously taken part in a HRIPT study (see 4.4.2.2) with α- and β-damascone (two of these subjects had reacted to α- and β-damascone) were rechallenged approximately 6 months later with α-damascone. In addition, two subjects who had not participated in the original HRIPT were also tested. Subjects were tested at concentrations of 0.25%, 0.5%, 1.0% and 10% α-damascone in petrolatum. The first patch application was followed 2.5 weeks later by a second patch application at the same concentrations. The two subjects who had previously reacted to α- and β-damascone reacted very strongly to α-damascone at the three lower dose levels and did not receive a second patch application and were not tested with 10% α-damascone. The two subjects who had not reacted to α- or β-damascone in the original HRIPT study reacted to 10% α-damascone but did not react at the three lower doses. The two subjects who were not previously patch tested also reacted to 10% α-damascone but did not react at the three lower doses (RIFM, 1992).

4.4.2.4. Three subjects who previously showed positive reactions (4.4.2.2) to α-damascone in a HRIPT and three subjects who did not react to α-damascone in a HRIPT were tested with a purified sample of α-damascone at 1% in petrolatum. Sensitization reactions were observed in the three subjects who had previously reacted to α-damascone. No reactions were observed in the three subjects who had not previously reacted to α-damascone (RIFM, 1992).

4.4.2.5. Cross sensitization was evaluated in three subjects who had previously reacted to 1% δ-damascone in alcohol SDA 39C when tested in a HRIPT study. A 24-h occluded application of 0.1% α-damascone in alcohol SDA 39C was made to a naive site approximately 3 weeks after the associated HRIPT study on δ-damascone. Reactions were graded at patch removal and again at 24, 48, and 72 h after patch removal. All three subjects cross-reacted to α-damascone (RIFM, 1982).

4.4.2.6. A HRIPT was conducted on fifty-one healthy volunteers. A 0.2 ml aliquot of 0.1% α-damascone in alcohol was applied to a 4 cm^2 Parke-Davis Readi-Bandage® which was then secured on the back of each subject for 24 h under occlusion. A series of nine alternate day 24-h induction applications were made over a three-week period. Seventeen days after application of the last induction patch, an occluded challenge patch was applied to virgin sites on the arm. The challenge patch was removed after 24 h. Reactions to challenge were evaluated at patch removal and at 24, 48, and 72 h after patch removal. No sensitization reactions were observed (RIFM, 1979d).

4.4.2.7. A HRIPT was conducted with 1% α-damascone on 54 healthy volunteers (17 male/37 female). A 0.2 g sample of 1% α-damascone in white petrolatum was applied to a

1 in. gauze pad which was then secured to the upper back of each subject for 24 h under semi-occlusion. This procedure was repeated three times a week on a Monday, Wednesday, Friday schedule for a total of nine applications. Following a 14-day rest period, a semi-occlusive challenge patch with 1% of α-damascone in petrolatum was applied to the original site and to a virgin site for 24 h. Reactions were evaluated 24 and 48 h after application. Sensitization was not observed (RIFM, 1979e).

4.4.2.8. A maximization test was carried out with 0.2% α-damascone in petrolatum on 25 healthy, male and female volunteers. Application was under occlusion to the same site on the upper aspect of the arm for 5 alternate day 48-h periods. Patch sites were pretreated for 24 h with 5% aqueous sodium lauryl sulfate (SLS) under occlusion for the initial patch only. Following a 10–14 day rest period, challenge patches were applied under occlusion to fresh sites for 48 h. Challenge applications were preceded by 30-min applications of 7.5% aqueous SLS under occlusion on the left side whereas 0.2% α-damascone in petrolatum was applied without SLS treatment on the right side. Reactions were read at 48 and 72 h after patch removal. No evidence of sensitization was observed (RIFM, 1985).

4.4.2.9. From November 1998 to May 2000, 1606 consecutive contact dermatitis patients from six European dermatology departments were patch tested with a series of fragrance materials. α-Damascone at 30% in petrolatum was applied to the back of each volunteer for 48 h using Finn Chambers® on Scanpor®, with the exception of 1 center that used Van der Bend chambers®. Reactions were read on days 2 and 4. Reactions were observed in eight patients; questionable reactions were observed in seven patients (Frosch et al., 2002).

4.4.2.10. A total of 202 patients with contact dermatitis were patch tested in Japan between September 1990 and April 1991. Patch tests were conducted with Finn Chambers® on Scanpor®. Reactions were assessed according to the guidelines of the International Contact Dermatitis Research Group. α-Damascone at 3% in petrolatum did not produce any reactions (Kozuka et al., 1996).

4.4.3. Animal studies

4.4.3.1. α-Damascone was evaluated in a skin sensitization study (Buehler, 1965) in male and female Hartley guinea pigs (10/sex) weighing 300–500 g. The dorsal left shoulder of each animal was clipped free of hair. A 0.4 ml aliquot of 0.6% α-damascone in 80% ethanol was applied under a 37 × 40 mm Parke-Davis bandage and covered with dental dam. The patches were removed after 6 h and the sites were examined and scored at 24 and 48 h. This procedure was performed once a week for 3 weeks, for a total of three 6-h inductions. Fourteen days after the last induction, all animals were challenged in the same manner on a naive site with 0.6% α-damascone in ethanol. The sites were scored at 24 and 48 h. Six days after the primary challenge, animals were re-challenged with 1.8% α-damascone. At primary challenge, sensitization reactions were observed in 1/19 at 24 h and in 0/19 at 48 h. At rechallenge with 1.8%, sensitization reactions were observed in 6/18 at 24 h and in 9/18 at 48 h (RIFM, 1983).

4.4.3.2. A guinea pig sensitization study (Buehler, 1965) was conducted in 11 male Hartley guinea pigs. Induction was carried out by applying a 0.1 ml aliquot of 10% α-damascone in propylene glycol to a half-inch square of surgical gauze, which was affixed to the clipped interscapular region of the guinea pigs for 24 h under occlusion. Ten such induction applications were made on alternate days during a three-week period. After a two-week rest period, a challenge patch was applied under occlusion for 24 h. Reactions were read at patch removal. No sensitization reactions were produced (RIFM, 1971).

4.4.3.3. A maximization test (Magnusson and Kligman, 1969) was conducted on 10 Hartley–Dunkin guinea pigs. Induction consisted of intradermal injections followed one week later by topical application. On either side of the clipped shoulder of the animal, three pairs of intradermal injections were made as follow: 0.1 ml of Freund's Complete Adjuvant (FCA), 0.1 ml of 10% α-damascone in distilled water, and 0.05 ml of α-damascone emulsified with 0.05 ml FCA. One week after injections, a 4 × 2 cm patch of Whatman® No. 3 filter paper was saturated with 10% α-damascone in distilled water and was applied to the injection site for 48 h under occlusion. A control group of four guinea pigs was similarly treated with sterile distilled water in place of α-damascone. Two weeks after topical induction, the animals were challenged on the shaved flank by a 24 h occluded patch with 5% and 10% α-damascone in distilled water. Reactions were read 24 and 48 h after patch removal. No sensitization reactions were observed with 5% α-damascone, but sensitization was produced in 3/10 animals with 10% (RIFM, 1980).

4.4.3.4. Using the same method as above (4.4.2.2), 10 Hartley guinea pigs were induced with 0.1% α-damascone and challenged with 0.5% and 1%. No sensitization reactions were observed with 0.5% but sensitization was produced in 3/10 animals with 1% (RIFM, 1980).

4.4.3.5. Kozuka et al. (1996) conducted another guinea pig maximization test using 20 female Hartley guinea pigs. Animals were induced with 10% α-damascone in liquid paraffin (intradermal induction) and 50% α-damascone in petrolatum (topical induction). Animals were challenge 14 days later with 2%, 5% and 10% test material in petrolatum. No sensitization was observed with 2% and 5% α-damascone. Sensitization reactions were observed in animals challenged with 10%.

4.4.4. Local Lymph Node Assay

4.4.4.1. A Local Lymph Node Assay was conducted using female CBA/J mice (6/dose) 6–8 weeks of age (15.5–20.9 g). A 25 μl aliquot of 0.1%, .25%, 0.5%, 1.0%, 2.5% or 5.0% α-damascone in acetone/olive oil (4:1) was applied to the dorsum of each ear, once daily for 3 consecutive days. The animals were allowed to rest on days 4 and 5. On day 6, mice were injected in the lateral tail vein with 0.25 mL containing 2 μCi of ^{125}I-labeled Iododeoxyuridine and 10^{-5} M FuDR in phosphate buffered saline (PBS). Five hours later, the mice were euthanized, lymph nodes were excised and dissociated using Hanks' Balanced Salt Solution (HBSS) and then with PBS, resuspended in 5% tricholoroacetic acid (TCA) and refrigerated at 4 °C. Seventeen hours later the cells were centrifuged and resuspended in fresh 5% TCA. Radioactivity was measured using a gamma counter. The EC3 value was calculated to be 3.3%. Under the conditions of the test, α-damascone was considered to be a sensitizer (RIFM, 2001a).

4.5. Phototoxicity and photoallergy

4.5.1. Phototoxicity

4.5.1.1. Phototoxicity was evaluated as a part of an accompanying HRIPT study. Twenty healthy subjects (5 males/15 females) participating in the sensitization study were also treated with an additional 0.2 g of 10% α-damascone in petrolatum on the opposite arm. This site was then irradiated with UV-A light using a Spectroline model B-100 Black light flood lamp (365 nm, 1680 microwatts/cm^2) for 15 min. The exposure distance was 15 in. from the lamp. After the exposure, the test site was covered with a semi-occlusive patch for 24 h. Reactions were read at patch removal and again 24 h after patch removal. No phototoxic effects were observed (RIFM, 1992).

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Repeated dose studies

4.7.1

α-Damascone was evaluated in a 14 day oral study using groups of five male and five female CD Sprague–Dawley rats. α-Damascone was mixed with Purina Rodent Chow MEal 5001 and administered in the diet at dose levels of 0.25, 0.5, 1.0 or 2.0 g/kg/day. Animals were observed daily for physical appearance, behavior, mortality and pharmacotoxic signs. In the high dose group, the average actual doses administered were far below the theoretical value of 2.0 g/kg due to extremely low food consumption seen in these animals; the average actual dosage was 804 mg/kg/day for males and 734 mg/kg/day for females. All animals from 2.0 g/kg group died on day 10 of the study (this more likely resulted from starvation). No other deaths occurred during the study. In the two highest dose groups (1.0 and 2.0 g/kg) decreased efficiency of food utilization, severely decreased food consumption and decreased liver and kidney weights (probably indicative of the starving state of the animals) were observed. Significantly lower body weights and food consumption were observed at 0.5 g/kg. No significant treatment related effects were observed at 0.25 g/kg (RIFM, 1979j).

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

4.9.1. Bacterial studies

4.9.1.1. Two bacteria reverse mutation assays using the direct plate incorporation method were performed using *Escherichia coli* strain WP2 uvrA in the presence and absence of S9. α-Damascone was tested in both assays at dose levels of 312.5, 625, 1250, 2500 and 5000 μg/plate in dimethyl sulfoxide (DMSO). No mutagenic activity was observed (RIFM, 2003).

4.9.1.2. Two bacterial reverse mutation assays using the direct plate incorporation method (Ames et al., 1975) were performed using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537. In the first assay, α-damascone was tested at dose levels of 7.8, 15.6, 31.3, 62.5 and 125 μg/plate in DMSO for TA98 and TA1537 with and without S9; and 31.2, 62.5, 125, 250 and 500 μg/plate in DMSO for TA100 and TA1535 with and without S9. In the second assay, α-damascone was tested at dose levels of 15.6, 31.2, 62.5, 125 and 250 μg/plate in DMSO for all strains without S9 and for TA98 and TA1537 with S9; and 31.2, 62.5, 125, 250 and 500 μg/plate in DMSO for TA100 and TA1535 with S9. No mutagenic effects were observed (RIFM, 2003).

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, J. Lalko, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for

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Review

Fragrance material review on *cis*-α-damascone

A. Lapczynski *, J. Lalko, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 51 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of *cis*-α-damascone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; *cis*-α-Damascone

Contents

In 2006, a complete literature search was conducted on *cis*-α-damascone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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1. Identification (Fig. 1)

1.1 Synonyms: 2-Buten-1-one, 1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-, (Z)-;(Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one; *cis*-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2-Buten-1-one.
1.2 CAS Registry Number: 23726-94-5.
1.3 EINECS Number: 245-845-8.
1.4 Formula: $C_{13}H_{20}O$.
1.5 Molecular Weight: 192.02.
1.6 IFRA: Rose ketone has an International Fragrance Association Standard (IFRA, 2007) – see Section 4.4.1. for details.

2. Physical properties

2.1 Log K_{ow} (calculated): 4.29.

3. Usage

cis-α-Damascone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 1–10 metric tonnes per annum.

The maximum skin level that results from the use of *cis*-α-damascone in formulae that go into fine fragrances has been reported to be 0.2% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.1% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.02 mg/kg for high end users of these products (see Table 1).

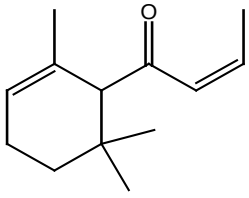

Fig. 1. *cis*-α-Damascone.

4. Toxicology data

4.1. Acute toxicity

No data available on this material.

4.2. Skin irritation

No data available on this material.

4.3. Mucous membrane (eye) iritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Dermal Sensitization Quantitative Risk Assessment (QRA)

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This new methodology represents a significant change over current risk assessment practices because it specifically addresses the elements of exposure-based risk assessment that are unique to the induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment.

Full details of this risk assessment approach can be found in the "QRA Expert Group, Dermal Sensitization Quantitative Risk Assessment (QRA) for Fragrance Ingredients, Technical Dossier, revised June 22, 2006", and IFRA/RIFM Quantitative Risk Assessment (QRA) for Fragrance Ingredients Booklet, May 11, 2006", at http://

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing *cis*-α-damascone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.1	0.0004
Face cream	0.80	2.00	1.000	0.003	0.1	0.0001
Eau de toilette	0.75	1.00	1.000	0.080	0.1	0.0010
Fragrance cream	5.00	0.29	1.000	0.040	0.1	0.0010
Antiperspirant	0.50	1.00	1.000	0.010	0.1	0.0001
Shampoo	8.00	1.00	0.010	0.005	0.1	0.0000
Bath products	17.00	0.29	0.001	0.020	0.1	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.1	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.1	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.1	0.0000
Total						0.0025

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
IFRA Standard based on the QRA

Limits in the finished product:	
For a description of the categories, refer to the QRA Information Booklet	
Category 1 – See Note (1)	0.003%
Category 2	0.004%
Category 3	0.02%
Category 4	0.05%
Category 5	0.02%
Category 6 – See Note (1)	0.07%
Category 7	0.008%
Category 8	0.1%
Category 9	0.5%
Category 10	0.8%
Category 11 – See Note (2)	

Note: The above limits apply to rose ketones used individually or in combination.
(1) IFRA would recommend that any material used to impart perfume or flavour in products intended for human ingestion should consist of ingredients that are in compliance with appropriate regulations for foods and food flavourings in the countries of planned distribution and, where these are lacking, with the recommendations laid down in the Code of Practice of IOFI (International Organisation of the Flavor Industry). Further information about IOFI can be found on its website (http://www.iofiorg.org).
(2) Category 11 includes all non-skin contact or incidental skin contact products. Due to negligible skin contact the concentration of a fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product.
For example, hypothetically if the usual concentration of a fragrance compound in the final product, for example a candle, is at 5%, then any individual fragrance ingredient (in this case a 'rose ketone') must not exceed 5% in the candle.

www.rifm/org/pub/publications.asp and http://www.ifraorg.org/News.asp.

An exposure-based Quantitative Risk Assessment (QRA) methodology has been used to determine acceptable exposure limits for rose ketone and a new IFRA Standard (IFRA, 2007) has been issued (See Tables 2 and 3).

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones

Table 3
Summary of the relevant sensitization data for the implementation of the QRA

CAS No	LLNA weighted mean EC3 values ($\mu g/cm^2$) [no. studies]	Human data			Potency Classification[b]	WoE NESIL ($\mu g/cm^2$)[c]
		NOEL – HRIPT (induction) ($\mu g/cm^2$)	Experimental NOEL – MAX (induction) ($\mu g/cm^2$)	LOEL[a] (induction) ($\mu g/cm^2$)		
57378-68-4	1579 [3]	NA	NA	1333	Moderate	100 [LLNA weighted mean for class = 1496 $\mu g/cm^2$]
43052-87-5	NA	133 (51)	0.2%	NA	Moderate	
24720-09-0	826 [1]	500 (DEP)	NA	NA	Moderate	
23696-85-7	308 [2]	100 (23)	NA	1000	Moderate	
23726-92-3	NA	67 (53)	NA	375	Moderate	
23726-91-2	600	1000 (pet/54)	NA	NA	Moderate	
23726-94-5	NA	NA	NA	NA	Moderate	
39872-57-6	NA	236 (DEP)	NA	2362	Moderate	
71048-82-3	NA	100 (24)	NA	1000	Moderate	
33673-71-1	NA	NA	NA	NA	Moderate	
70266-48-7	NA	1181 (DEP)	NA	NA	Moderate	

NOEL = No observed effect level; HRIPT = Human Repeat Insult Patch Test; MAX = Human Maximization Test; LOEL = Lowest Observed Effect Level; NA = Not available.
[a] Data derived from HRIPT or HMT.
[b] Gerberick et al., 2001.
[c] WoE NESIL limited to two significant figures.

When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, J. Lalko, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on *cis*-β-damascone

J. Lalko, A. Lapczynski *, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of *cis*-β-damascone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; *cis*-β-Damascone

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on *cis*-β-damascone. On-line databases that were surveyed

included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: 2-buten-1-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, (2*Z*)-; *cis*-β-damascone; Damasione; (*Z*)-1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (*Z*)-β-1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-buten-1-one.
1.2 CAS registry number: 23726-92-3.
1.3 EINECS number: 245-843-7.
1.4 Formula: $C_{13}H_{20}O$.
1.5 Molecular weight: 192.3.
1.6 JECFA: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 384) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent.
1.7 IFRA: *cis*-β-damascone has an International Fragrance Association Standard (IFRA, 2007) – see Section 4.4.1 for details.

2. Physical properties

2.1 Log K_{ow} (calculated): 4.42.
2.2 Molecular weight: 192.3.
2.3 Henry's law (calculated) 0.000114 atm m^3/mol 25C.
2.4 Log K_{ow} (calculated) 4.42.

3. Usage

cis-β-Damascone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 1–10 metric tonnes per annum.

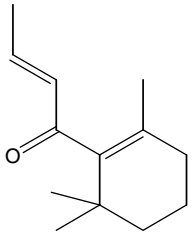

Fig. 1. *cis*-β-Damascone.

The maximum skin level that results from the use of *cis*-β-damascone in formulae that go into fine fragrances has been reported to be 0.02% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.07% (IFRA, 2002), which would result in a calculated conservative maximum daily exposure on the skin of 0.0018 mg/kg for high end users of these products (see Table 1).

4. Toxicology data

4.1. Acute toxicity

No data available on this material.

4.2. Skin irritation

4.2.1. Human studies (Table 2)

4.2.1.1. Irritation was evaluated as a part of a modified Shelanski human repeated insult patch test (HRIPT) conducted on 53 volunteers. A 0.2 ml aliquot of 0.05% *cis*-β-damascone in alcohol SDA39C was applied under semi-occlusion ($1\frac{1}{2} \times 1\frac{1}{2}$ in. Parke-Davis Readi-Bandages) to the back of each subject for 24 h. The sites were examined for reactions at patch removal and again at 24 h after patch removal just prior to the next patch application. A series of nine alternate-day applications were conducted over a three-week period on a Monday–Wednesday–Friday schedule. No irritation was observed (RIFM, 1980).

4.2.1.2. Primary irritation was evaluated as a part of an associated HRIPT study conducted in 18 subjects in panel I and 32 subjects in panel II. A 24-h semi-occlusive patch containing 0.5% of *cis*-β-damascone in 95% ethanol was applied to 18 panelists. Slight irritation was observed in 2/18 panelists and marked irritation was observed in 1/18 panelists. Thirty-two subjects in panel II received a 24-h occlusive patch application of 0.05% of the test material in 95% ethanol. Irritant reactions were observed in 2/32 subjects (RIFM, 1979a).

4.2.1.3. As part of an associated HRIPT, irritation was evaluated with 5% *cis*-β-damascone in petrolatum in 9 male and 41 female volunteers. A 0.2 g sample of *cis*-β-damascone was applied to the upper arm of each volunteer and then covered with a semi-occlusive patch for 24 h. After a 24 h rest period, volunteers were again patched at the same site. A total of nine applications were to be made over a three-week period; however, a number of reactions from slight to severe were observed in several subjects over the course of induction phase and it was decided to terminate the study after the eighth induction application was made. These reactions were considered sensitization reactions, not irritation reactions; the material was not classified as a primary irritant (RIFM, 1992a).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing *cis*-β-damascone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.07	0.0003
Face cream	0.80	2.00	1.000	0.003	0.07	0.0001
Eau de toilette	0.75	1.00	1.000	0.080	0.07	0.0007
Fragrance cream	5.00	0.29	1.000	0.040	0.07	0.0007
Antiperspirant	0.50	1.00	1.000	0.010	0.07	0.0001
Shampoo	8.00	1.00	0.010	0.005	0.07	0.0000
Bath products	17.00	0.29	0.001	0.020	0.07	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.07	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.07	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.07	0.0000
Total						0.0018

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of human irritation studies

Method	Dose (%)	Vehicle	Results	Reference
Induction phase (HRIPT)	0.5 (panel I) 0.05 (panel II)	95% ethanol	0.5% – irritation observed in 3/18 0.05% – irritation observed in 2/32	RIFM (1979a)
Induction phase (HRIPT)	0.05	Alcohol	0/53	RIFM (1980)

4.2.2. Animal studies

4.2.2.1. Irritation was assessed as part of an associated delayed contact hypersensitivity study in 20 Hartley guinea pigs (10/sex). A 0.4 ml aliquot of 1.5% *cis*-β-damascone in ethanol was applied for 6 h under occlusion (37 × 40 mm Parke-Davis Readi-Bandage covered with dental dam) to the clipped left shoulder of each animal. The treated sites were examined after each dosing and scored at 24 and 48 h. This procedure was performed once per week for three weeks for a total of three 6 h applications. Slight to moderate erythema was observed in 18/20 animals (RIFM, 1992a).

4.2.2.2. As a part of a Buehler sensitization study, irritation was evaluated in four Hartley guinea pigs. Two male and two female guinea pigs weighing 300–500 g received an occluded patch application of *cis*-β-damascone at 1.5% in 80% ethanol to the clipped skin on the dorsal left shoulder. Patches remained in place for 6 h. Reactions were read at 24 and 48 h after patch removal. No irritation was observed (RIFM, 1983).

4.2.2.3. A primary irritation test was conducted on six (3/sex) albino New Zealand rabbits. A 0.5 ml aliquot of 0.5% *cis*-β-damascone in alcohol SDA 39C was applied under occlusion (2 × 2 in. band-aid adhesive gauze patch) to abraded and intact skin on the back of each animal for 24 h. The sites were scored at 24 and 72 h. No irritation was observed (RIFM, 1979b).

4.3. Mucous membrane (eye) irritation

4.3.1

An eye irritation test was conducted using six albino New Zealand rabbits. A 0.1 ml aliquot of 0.5% *cis*-β-damascone in propylene glycol was instilled into right eye of each rabbit and the eyelids were gently held together for 1 s. The left eye of each rabbit served as control and remained untreated. Both eyes were examined at 24, 48 and 72 h and again on days 4 and 7. The primary irritation score was 1.33. Under the conditions of the test, *cis*-β-damascone was classified as practically non-irritating (RIFM, 1979c).

4.4. Skin sensitization

4.4.1. Dermal sensitization quantitative risk assessment (QRA)

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This new methodology represents a significant change over current risk assessment practices because it specifically addresses the elements of exposure-based risk assessment that are unique to the induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment.

Full details of this risk assessment approach can be found in the "QRA Expert Group, Dermal Sensitization Quantitative Risk Assessment (QRA) for Fragrance

Table 3
IFRA Standard based on the QRA

Limits in the finished product			
For a description of the categories, refer to the QRA Information Booklet			
Category 1 – see Note box (1)	0.003%	Category 7	0.008%
Category 2	0.004%	Category 8	0.1%
Category 3	0.02%	Category 9	0.5%
Category 4	0.05%	Category 10	0.8%
Category 5	0.02%	Category 11 – see Note box (2)	
Category 6 – see Note box (1)	0.07%		

Note box:
The above limits apply to rose ketones used individually or in combination.
(1) IFRA would recommend that any material used to impart perfume or flavor in products intended for human ingestion should consist of ingredients that are in compliance with appropriate regulations for foods and food flavorings in the countries of planned distribution and, where these are lacking, with the recommendations laid down in the Code of Practice of IOFI (International Organisation of the Flavor Industry). Further information about IOFI can be found on its website (www.iofiorg.org).
(2) Category 11 includes all non-skin contact or incidental skin contact products. Due to negligible skin contact the concentration of a fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product. For example, hypothetically if the usual concentration of a fragrance compound in the final product, for example a candle, is at 5%, then any individual fragrance ingredient (in this case a 'rose ketone') must not exceed 5% in the candle.

Ingredients, Technical Dossier, revised June 22, 2006", and "IFRA/RIFM Quantitative Risk Assessment (QRA) for Fragrance Ingredients Booklet, May 11, 2006", at http://www.rifm/org/pub/publications.asp and http://www.ifraorg.org/News.asp.

An exposure-based quantitative risk assessment (QRA) methodology has been used to determine acceptable exposure limits for *cis*-β-damascone and a new IFRA Standard (IFRA, 2007) has been issued (see Tables 3–5).

4.4.2. Human studies

4.4.2.1. A modified Shelanski HRIPT was conducted on 53 volunteers. A 0.2 ml aliquot of 0.05% *cis*-β-damascone in alcohol SDA39C was applied under semi-occlusion ($1\frac{1}{2} \times 1\frac{1}{2}$ in. Parke-Davis Readi-Bandages) to the back of each subject for 24 h. The sites were examined for reactions at patch removal and again at 24 h after patch removal just prior to the next patch application. A series of nine alternate-day applications were conducted over a three-week period on a Monday–Wednesday–Friday schedule. Eighteen days after application of the last induction patch, challenge applications were applied to virgin sites on the upper backs of all subjects. Challenge patches were removed after 24 h, and were graded approximately 15 min following patch removal. The challenge sites were also graded at 24, 48 and 72 h following patch removal. No sensitization was observed (RIFM, 1980).

4.4.2.2. A HRIPT study was conducted on 50 adult volunteers (9 males and 41 females). Both α- and β-damascone were tested on this same group of panelists. A 0.2 g sample of 5% *cis*-β-damascone in petrolatum was applied to the

Table 4
Summary of the relevant sensitization data for the implementation of the QRA

CAS no.	LLNA weighted mean EC3 values (μg/cm^2) [no. of studies]	Human data			Potency classification[b]	WoE NESIL (μg/cm^2)[c]
		NOEL – HRIPT (induction) (μg/cm^2)	Experimental NOEL – MAX (induction) (μg/cm^2)	LOEL[a] (induction) (μg/cm^2)		
57378-68-4	1579 [3]	NA	NA	1333	Moderate	100 (LLNA weighted mean for class = 1496 μg/cm^2)
43052-87-5	NA	133 (51)	0.2%	NA	Moderate	
24720-09-0	826 [1]	500 (DEP)	NA	NA	Moderate	
23696-85-7	308 [2]	100 (23)	NA	1000	Moderate	
23726-92-3	NA	67 (53)	NA	375	Moderate	
23726-91-2	600	1000 (pet/54)	NA	NA	Moderate	
23726-94-5	NA	NA	NA	NA	Moderate	
39872-57-6	NA	236 (DEP)	NA	2362	Moderate	
71048-82-3	NA	100 (24)	NA	1000	Moderate	
33673-71-1	NA	NA	NA	NA	Moderate	
70266-48-7	NA	1181 (DEP)	NA	NA	Moderate	

NOEL = no observed effect level; HRIPT = human repeat insult patch test; MAX = human maximization test; LOEL = lowest observed effect level; NA = not available.
[a] Data derived from HRIPT or HMT.
[b] Gerberick et al. (2001).
[c] WoE NESIL limited to two significant figures.

Table 5
Summary of human sensitization studies

Test method	Test concentration	Results	References
HRIPT	0.05%	No sensitization 0/53	RIFM (1980)
HRIPT	0.25%, 0.5%, 1.0%, and 5%	5% caused sensitization in 10/50	RIFM (1992a)
HRIPT	0.05%	No sensitization 0/28	RIFM (1979a)
HRIPT	0.05%	Sensitization observed in 6/17	RIFM (1979a)

upper arm of each subject for 24-h under semi-occlusion. After a 24-h rest period, subjects were again patched at the same site. A total of nine such applications were to be made over a three-week period, however, due to a number of reactions the study was ended after the eighth induction application. There was no challenge application. Reactions varied from slight to severe in several subjects over the course of this study. By the end of the eighth induction patch, there were reactions in 10 subjects. These reactions were judged to be sensitization reactions (RIFM, 1992a).

4.4.2.3. Four subjects who had participated in the above (4.4.1.3) HRIPT study with α- and β-damascone (two of these subjects had reacted to α- and β-damascone) were re-challenged approximately six months later with β-damascone. In addition, 2 subjects who had not participated in the original HRIPT were also tested. The subjects received patch applications of 0.25%, 0.5%, 1.0%, and 5% in petrolatum. The first patch application was followed $2\frac{1}{2}$ weeks later by a second patch application at the same concentration. The two subjects who had previously reacted to *cis*-β-damascone reacted very strongly to 0.25%, 0.5%, and 1.0% *cis*-β-damascone and did not receive a second patch application and were not tested with 5% *cis*-β-damascone. The two subjects who had not reacted to *cis*-β-damascone in the original HRIPT reacted to 5% *cis*-β-damascone but did not react to the three lower dose levels. Two subjects who were not previously patch tested did not react to *cis*-β-damascone at any dose level (RIFM, 1992a).

4.4.2.4. Three subjects who previously showed positive reactions to *cis*-β-damascone in HRIPT and three subjects who did not react *cis*-β-damascone were tested with a purified sample of *cis*-β-damascone at 1% in petrolatum. Sensitization reactions were observed in 2/3 subjects who had previously reacted to *cis*-β-damascone. No reactions were observed in the three subjects who had not previously reacted to *cis*-β-damascone (RIFM, 1992a).

4.4.2.5. A HRIPT study was conducted on 45 adult volunteers divided into two panels of 17 (panel I) and 28 (panel II). Volunteers in panel I received applications of 0.4 ml of 0.5% *cis*-β-damascone in ethanol under occlusion to the upper arm for 24 h. Each subject received a total of nine 24-h exposures over a three week period. The concentration for the last two induction applications was reduced to 0.05% and the challenge application was postponed by an additional four weeks because of a large number of strong residual reactions observed during induction. Approximately six weeks after the last induction patch, a semi-occlusive challenge application was made on a single naive site on subject's back using 0.05% *cis*-β-damascone in ethanol. Sensitization reactions were observed in 6/17 subjects. Subjects in panel II received induction applications of 0.05% of *cis*-β-damascone in ethanol. Beginning with the fourth application, the volume was reduced from 0.4 ml to 0.3 ml and the patch type was changed from occlusive to semi-occlusive. Following a two-week rest period, challenge applications were made using semi-occlusive applications of 0.05% *cis*-β-damascone in ethanol. No sensitization reactions were produced (0/28) (RIFM, 1979a).

4.4.2.6. Three subjects who had previously reacted to 1% δ-damascone in a HRIPT were cross-challenged with 0.1% *cis*-β-damascone. Twenty-four-hour occluded applications with 0.1% *cis*-β-damascone in alcohol SDA 39C were made to a naive site approximately three weeks after the completion of the HRIPT study. Reactions were graded after patch removal and again at 24, 48, and 72 h after patch removal. Cross-sensitization reactions were observed in 3/3 subjects (RIFM, 1982).

4.4.2.7. A multicenter trial was conducted in six dermatology centers from November 1998 to May 2000. A total of 1606 patients were patch tested with a series of fragrance materials from 0.2% mixture of α- and β-damascone (the concentration of each isomer was 0.1%) in petrolatum was applied to the back for 48 h using Finn Chambers® on Scanpor®, with the exception of one center that used van der Bend chambers. Readings of the test sites were conducted on days 2 and 4 at most centers, and readings on day 3 or 4 were used for overall evaluation of positive test results. Eight (0.5%) reactions were observed; in addition, 7 (0.4%) questionable reactions were also observed (Frosch et al., 2002).

4.4.2.8. A total of 202 patients with contact dermatitis were patch tested in Japan between September 1990 and April 1991. Patch tests were conducted using Finn Chambers® on Scanpor®. Reactions were assessed according to the guidelines of the International Contact Dermatitis Research Group. *cis*-β-Damascone at 2% in petrolatum did not produce any reactions (Kozuka et al., 1996).

4.4.3. Animal studies

4.4.3.1. Ten male and 10 female Hartley guinea pigs weighing 300–500 g were evaluated for sensitization using the Buehler method. The dorsal left shoulder of each animal was clipped free of hair and a 0.4 ml aliquot of 1.5% *cis*-β-damascone in 80% ethanol was applied under a

37 × 40 mm Parke-Davis Readi-Bandage and covered with dental dam. The patches were removed after 6 h and the sites were scored at 24 and 48 h. This procedure was performed once a week for three weeks. A positive control group consisting of five male and five female guinea pigs was similarly treated with 0.3% 1-chloro-2,4-dinitrobenzene (DNCB) in 80% ethanol. A naive control group of two female and two male guinea pigs remained untreated until challenge. Fourteen days after the last induction, all animals were challenged in the same manner on a naive site with 1.5% *cis*-β-damascone in ethanol. Twenty-four hours after the challenge, the animals were depilated and the test sites were graded after 2 h. The grading was repeated 24 h later (48-h score). Weak sensitization reaction was observed in 1/20 animals (RIFM, 1983).

4.4.3.2. A delayed contact hypersensitivity test was conducted in 20 Hartley guinea pigs (10/sex). During induction, a 0.4 ml aliquot of 1.5% *cis*-β-damascone in ethanol was applied for 6 h under occlusion (37 × 40 mm Parke-Davis Readi-Bandage covered with dental dam) to the clipped left shoulder of each animal. The procedure was repeated once per week for three weeks for a total of three 6 h induction applications. Fourteen days after the last induction application, the animals were challenged at a naive site on the left side with 1.5% *cis*-β-damascone in ethanol. A weak sensitization reaction was observed in one animal (RIFM, 1992b).

4.4.3.3. A Magnusson and Kligman (1969) guinea pig maximization test was conducted on 20 Hartley strain female guinea pigs. For induction each animal received an intradermal injection into the shoulder region with 10% *cis*-β-damascone diluted in liquid paraffin and Freund's complete adjuvant. Five days later 10% sodium lauryl sulfate in petrolatum was topically applied to the same area. After 24 h, 50% *cis*-β-damascone was applied topically for 48 h under occlusion. Two weeks after the induction period, challenge patches were applied to the animal's backs, using mini-plasters, for 24 h. Reactions were read at 24 and 48 h after removal of the mini-plasters. *cis*-β-Damascone produced sensitization at challenge concentrations of 2% (17/19), 5% (18/19), and 10% (18/19) (Kozuka et al., 1996).

4.5. Phototoxicity and photoallergy

4.5.1. Phototoxicity

4.5.1.1. Phototoxicity was evaluated as a part of an accompanying HRIPT study in which both α- and β-damascone were tested on the same group of panelists. Twenty healthy subjects (5 males and 15 females) were treated with an additional 0.2 g of 5% *cis*-β-damascone in petrolatum on the opposite arm. This site was irradiated with UV-A light using a Spectroline model B-100 Black light flood lamp (365 nm, 1680 μW/cm^2) for 15 min. The exposure distance was 15 in. from the lamp. After the exposure, the test site was covered with a semi-occlusive covering of gauze and loosely applied Dermical® tape. The patches were removed after 24 h and the skin sites were examined. The subjects were rested for 24 h after which the sites were again examined and *cis*-β-damascone was again applied as previously. No phototoxic effects were observed (RIFM, 1992a).

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

J. Lalko, A. Lapczynski, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on *trans*-β-damascone

A. Lapczynski *, J. Lalko, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of *trans*-β-damascone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; *trans*-β-Damascone

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on *trans*-β-damascone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: 2-Buten-1-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, (2E)-; *trans*-β-Damascone; (E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one.
1.2 CAS Registry No.: 23726-91-2.
1.3 EINECS No.: 245-842-1.
1.4 Formula: $C_{13}H_{20}O$.
1.5 Molecular weight: 192.02.
1.6 FEMA: Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient - GRAS 4. (3243).
1.7 JECFA: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 384) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent.
1.8 IFRA: Rose ketone has an International Fragrance Association Standard (IFRA, 2007) – see Section 4.4.1. for details.

2. Physical properties

2.1 Log K_{ow} (calculated): 4.42.
2.2 Flash point: >100 °C (CC).
2.3 Vapor pressure (calculated): 0.01 mm Hg 20 C.

3. Usage

trans-β-Damascone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 1–10 metric tonnes per annum.

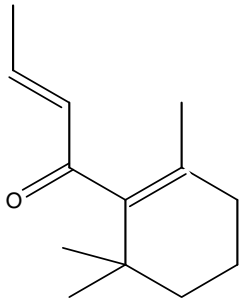

Fig. 1. *trans*-β-Damascone.

The maximum skin level that results from the use of *trans*-β-damascone in formulae that go into fine fragrances has been reported to be 0.02% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.07% (IFRA, 2002), which would result in a calculated conservative maximum daily exposure on the skin of 0.0018 mg/kg for high end users of these products (see Table 1).

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral toxicity of *trans*-β-damascone was evaluated in five male and five female Sprague–Dawley rats. All animals were administered 2 g/kg of the test material in 0.25% aqueous gum tragacanth by gavage at a dose volume of 10 ml/kg. The animals were observed frequently after dosing and once daily for 14 days for mortality or signs of toxicity. A gross necropsy was conducted on all animals. Two males and two females died within 4 h of dosing. All animals appeared normal throughout the study. Gross observations at necropsy were normal. The acute oral LD_{50} of *trans*-β-damascone was reported to be greater than 2 g/kg (RIFM, 1986).

4.1.1.2. The acute oral LD_{50} was evaluated in Wistar strain rats (5/sex/dose) with mean body weights of 113 g. *trans*-β-Damascone was administered by gavage at dose levels of 2.2, 2.5, 2.8, 3.1 and 3.4 g/kg in oil. One (1/10) death was observed at 2.2 g/kg, three (3/10) at 2.5 g/kg, seven (7/10) at 2.8 g/kg, five (5/10) at 3.1 g/kg and seven (7/10) at 3.4 g/kg. Clinical signs observed within the first 5 min included hypertonia, increased reflexes and motility, cardiac and respiratory frequencies and saliva secretion. Within 10–30 min, apathy, ataxia, ventral and lateral decubitus, hyptonia, ptosis of the eye, and spasms were observed; an increase in urinary or lachrymal secretion in some animals was also observed. Paralysis of the limbs and the head accompanied with a slow and forced respiration were observed from the 6th to 8th hour after dosing, especially in the two highest dose levels. Tremors were observed at 8 and 24 h. Reflexes were markedly reduced, pinching and auditory ones from 1 h after dosing, and ocular ones (corneal and to the light) after the 1st hour in the highest dose groups, and after 6 h in the other groups. All clinical signs had disappeared by 48 h in all survivors. The LD_{50} was calculated to be was calculated to be 2.9 g/kg (95% CI 2.6–3.2 g/kg) (RIFM, 1969; Posternak and Vodoz, 1975).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing *trans*-β-damascone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product	Ingredient/mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.07	0.0003
Face cream	0.80	2.00	1.000	0.003	0.07	0.0001
Eau de toilette	0.75	1.00	1.000	0.080	0.07	0.0007
Fragrance cream	5.00	0.29	1.000	0.040	0.07	0.0007
Antiperspirant	0.50	1.00	1.000	0.010	0.07	0.0001
Shampoo	8.00	1.00	0.010	0.005	0.07	0.0000
Bath products	17.00	0.29	0.001	0.020	0.07	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.07	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.07	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.07	0.0000
Total						0.0018

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	No. animals/ dose group	LD_{50}	References
Oral	Rat	5	>2 g/kg	RIFM (1986)
Oral	Rat	10	2.9 g/kg	RIFM (1969), Posternak and Vodoz (1975)
Dermal	Rabbit	3	>2 g/kg	RIFM (1979a)

Table 3
Summary of human irritation studies

Method	Dose (%)	Vehicle	Results	Reference
Induction phase (HRIPT)	0.5	Diethyl phthalate	0/104	RIFM (2000)
Induction phase (HRIPT)	1	Petrolatum	0/54	RIFM (1979b)
Maximization study	0.2	Petrolatum	0/23	RIFM (1985)

4.1.2. Dermal studies

4.1.2.1. The acute dermal toxicity of *trans*-β-damascone was evaluated in a group of six albino rabbits (3/sex) weighing 2–3 kg. *trans*-β-Damascone at 4 g/kg (50% solution in triethyl citrate) was applied to the clipped and abraded back of each animal for 24 h under occlusion. The animals were observed for signs of toxicity and mortality for 14 days. Gross necropsy was conducted on all animals. No effects were observed. The dermal LD_{50} was reported to be greater than 2 g/kg (RIFM, 1979a).

4.2. Skin irritation (Table 3)

4.2.1. Human studies

4.2.1.1. As a part of a HRIPT study, irritation was evaluated in 104 volunteers (16 male/88 female). A 0.2 ml aliquot of 0.5% *trans*-β-damascone in diethyl phthalate was applied to a 2 cm^2 Webril® pad which was attached to the back for 24-h under occlusion. The procedure was repeated three times a week for a total of nine applications. Sites were evaluated 24 h after removal of patches. No irritation was observed (RIFM, 2000).

4.2.1.2. As a part of associated human repeated Insult patch test (HRIPT) in 54 volunteers (17 male/37 female), irritation due to *trans*-β-damascone was evaluated during the induction phase. Approximately 0.2 g of 1% *trans*-β-damascone in white petrolatum was applied to a one inch square gauze pad which was secured to the upper back of each subject for 24-h under semi-occlusion. This procedure was repeated three times a week, Monday, Wednesday and Friday, for a total of nine applications. Each site was evaluated prior to re-application. No irritation was observed (RIFM, 1979b).

4.2.1.3. Irritation was evaluated during an associated maximization study. Twenty-three healthy male and female volunteers received an application of 0.2% *trans*-β-damascone in petrolatum under occlusion to the same site on the upper aspect of the arm for five alternate days 48-h periods. Patch sites were pretreated for 24-h with 5% aqueous sodium lauryl sulfate (SLS) under occlusion for the initial patch only. No irritation was observed (RIFM, 1985).

4.2.2. Animal studies

4.2.2.1. A concentration range finding study was conducted in 4 Hartley-Dunkin guinea pigs as part of a maximization test to determine the maximum non-irritant concentration of *trans*-β-damascone. The animals were pretreated with Freund's complete adjuvant and an 8×5 cm area of skin was clipped on both flanks. *trans*-β-Damascone at 0.125%, 0.25%, 0.5%, 0.625%, 1.0%, 1.25%, 2.5% and 5% in sterile distilled water was applied to a 2 cm^2 Whatman® No. 3 filter paper patch which was applied to the clipped flanks for 24 h under occlusion. The application sites were examined 24 and 48 h after patch removal. Irritation was not observed at any of the doses (RIFM, 1979c).

4.2.2.2. Primary irritation due to *trans*-β-damascone was evaluated in six albino rabbits. The animals were clipped free of hair over a wide area and the backs were abraded on one side. A 0.5 g aliquot of 50% of *trans*-β-damascone in triethyl citrate was applied to the abraded and intact skin sites for 24-h under occlusion. Reactions were read according to Draize at patch removal and again at 72 h according. The primary irritation score was 1.75. Under the conditions of the test, *trans*-β-damascone was not considered to be a primary skin irritant (RIFM, 1979d).

4.3. Mucous membrane (eye) irritation

4.3.1. Animal studies

4.3.1.1. A rabbit eye test was conducted in six healthy young adult albino rabbits. A 0.1 ml aliquot of 50% *trans*-β-damascone in triethyl citrate was instilled into the right eye of each rabbit without further treatment. The untreated left eye of each rabbit served as a control. The treated eyes were examined at 1, 2, 3, 5, and 7 days following instillation. Very slight conjunctival irritation, which cleared by day 3 was observed in 6/6 rabbits. The average irritation score was 3.3 on day 1, 0.3 on day 2 and 0 at day 3. *trans*-β-Damascone was not considered to be an eye irritant (RIFM, 1979e).

4.4. Skin sensitization (Table 4)

4.4.1. Human studies

4.4.1.1. A HRIPT study was conducted in male and female volunteers (16 male/88 female). A 0.2 ml aliquot of 0.5% of *trans*-β-damascone in diethyl phthalate (DEP) was applied to a 4 cm^2 Webril® pad which was then applied to the skin® for 24 h under occlusion. Nine induction applications were made on a Monday–Wednesday–Friday schedule over a three week period. After a rest period of 10–15 days, a 24-h occluded challenge patch with 0.5% *trans*-β-damascone in DEP was applied to a previously unexposed site. Reactions were read at 24 and 48 h after patch removal. No sensitization was observed (RIFM, 2000).

4.4.1.2. Sensitization was evaluated in another HRIPT which was conducted on 54 healthy volunteers (17 male/37 female). Approximately 0.2 g of 1% *trans*-β-damascone in white petrolatum was applied to a one inch square gauze pad which was applied to the upper back for 24-h under semi-occlusion. This procedure was repeated three times per week, Monday, Wednesday, and Friday, for a total of nine applications. Following a 14-day rest period, a semi-occlusive challenge patch with 1% *trans*-β-damascone in petrolatum was applied to the original test site and to a virgin site (volar forearm) for 24 h. Reactions were read at 24 and 48 h after application. No sensitization was observed (RIFM, 1979b).

Table 4
Summary of human sensitization studies

Test method	Test concentration (%)	Results	References
HRIPT	0.5	No sensitization 0/104	RIFM (2000)
HRIPT	1	No sensitization 0/54	RIFM (1979b)
Maximization study	0.2	No sensitization 0/23	RIFM (1985)

4.4.1.3. A maximization test was conducted in 23 male and female volunteers. *trans*-β-Damascone at 0.2% in petrolatum was applied under occlusion to the same site on the upper arm for five alternate day 48-h periods. Patch sites were pretreated for 24-h with 5% aqueous SLS under occlusion for the initial patch only. Following a 10–14 day rest period, challenge patches were applied under occlusion to fresh sites for 48 h. Challenge applications were preceded by 30-min applications of 7.5% aqueous SLS under occlusion on the left side of the back or the left arm whereas the test material and control were applied without SLS treatment on the right side. Reactions were read at 48 and 72 h after patch removal. No evidence of sensitization was observed (RIFM, 1985).

4.4.2. Animal studies

4.4.2.1. A Magnusson and Kligman maximization test was conducted on 10 Hartley–Dunkin guinea pigs weighing 300–500 g. Induction consisted of intradermal injections followed one week later by topical application. On either side of the clipped shoulder of the animal, three pairs of intradermal injections comprised of 0.1 ml of Freund's Complete Adjuvant, 0.1 ml of 5% of *trans*-β-damascone in dipropylene glycol, and 0.05 ml of *trans*-β-damascone emulsified with 0.05 ml of Adjuvant. One week after injections, a 4×2 cm patch of Whatman® No. 3 filter paper saturated with 5% of *trans*-β-damascone secured by overlapping impermeable plastic bandage (5 cm Elastoplast®) was applied for 48 h under occlusion. A control group of four guinea pigs was similarly treated with sterile distilled water in place of the test material. Two weeks after topical induction, the animals were challenged on the shaved flank by an occluded patch for 24 h using 2.5% and 5% of *trans*-β-damascone in distilled water. The test sites were examined at 24 and 48 h after removal of the patch. Sensitization reactions were observed in 1/10 animals with 2.5% and in 2/10 animals with 5%. The material was classified as a mild sensitizer (RIFM, 1979c).

4.4.2.2. A maximization test was conducted on 10 Hartley–Dunkin guinea pigs with initial weights of 300–500 g. Induction consisted of intradermal injections followed one week later by topical application. On either side of the clipped shoulder of the animal, three pairs of intradermal injections comprised of 0.1 ml of Freund's Complete Adjuvant, 0.1 ml of 1% of *trans*-β-damascone in dipropylene glycol, and 0.05 ml of *trans*-β-damascone emulsified

with 0.05 ml of Adjuvant were made. One week later, a 4 × 2 cm patch of Whatman® No. 3 filter paper saturated with 5% of *trans*-β-damascone was applied to the test site for 48 h under occlusion. A control group of four guinea pigs was similarly treated with sterile distilled water in place of *trans*-β-damascone. Two weeks after the topical induction, the animals were challenged on the shaved flank by a 24 h occluded patch using 0.5% and 1% of *trans*-β-damascone in distilled water. Reactions were read at 24 and 48 h after patch removal. No sensitization reactions were observed with 0.5%, but, sensitization was observed in 1/10 animals with 1%. Under the conditions of the study, *trans*-β-damascone was classified as a mild sensitizer (RIFM, 1979c).

4.4.3. Local lymph node assay

4.4.3.1. A Local Lymph Node Assay (LLNA) was conducted in female CBA/J Hsd mice (6/dose). A 25 μl aliquot of *trans*-β-damascone was applied daily to the dorsum of each ear for three consecutive days at concentrations of 0.1%, 0.25%, 0.5%, 1.0%, 2.5%, and 5% in acetone/olive oil (4:1). Isoeugenol at 0.5% and 5% was used as a positive control. Animals were allowed to rest for two days. On day 6, the mice were injected with 2 μC1 of ^{125}I-labelled iododeoxyuridine, 10^{-5} M FuDR in phosphate buffered saline. The mice were euthanized after 5 h and auricular lymph nodes were excised. The nodes were dissociated, washed and the radioactivity was measured using a gamma counter. All animals appeared healthy and there were no signs of irritation at the dosing site. The EC3 value was calculated to be 2.4% (600 μg/cm^2). Under the conditions of the test, *trans*-β-damascone was considered to be a sensitizer (RIFM, 2001).

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

4.7.1.

trans-β-Damascone was tested in a 90-day oral toxicity study in 32 male and female CF/Gif rats (16/sex). *trans*-β-Damascone was absorbed into cellulose and added to the diet such that the approximate daily dose was 2.26 mg/kg of bodyweight. A control group (16/sex) received the basic diet alone. Each diet was fed ad libitum. Weekly observations were made of growth, physical appearance and behavior. The efficiency of feed utilization was calculated. During the 7th week hematological studies were conducted on 16 animals (8/sex) and on all animals at the termination of the study. Each animal was sacrificed after 90 days and a gross necropsy was conducted. There were no mortalities and physical appearance and behavior were considered normal during the course of the study. Body weight gains were reduced by 4.8% and 0.4% for treated males and females, respectively. Feed consumption showed an increase for both males and females. A moderate decrease of feed efficiency was observed in both sexes equal to −9.04% in the males and −9.60% in the females. A significant increase in absolute liver and kidney weights was observed in females and a significant increase in relative liver and kidney weights for both males and females was observed; however, as these changes did not correlate with any histological modifications it was concluded that these changes were due to adaptation and not to pathology. Clinical hematology parameters did not reveal any significant changes. A slight modification in relative distribution of leukocytes was observed in females in week 7. In view of the fact that no toxicologically significant modifications were observed either in the hematological or histological examinations, it was concluded that the changes observed were not of any biological significance (Posternak and Vodoz, 1975; RIFM, 1969).

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual fragrance material review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, J. Lalko, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufactures of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on *delta*-damascone

J. Lalko, A. Lapczynski *, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc. 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of *delta*-damascone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; *Delta*-damascone

Contents

In 2006, a complete literature search was conducted on *delta*-damascone. On-line databases that were surveyed included Chemical Abstract Services and the National

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1. Synonyms: 2-Buten-1-one, 1-(2,6,6-trimethyl-3-cyclohexen-1-yl)- ; 1-(2,6,6-trimethyl-3-cyclohexen-1-yl)-2-buten-1-one; -1-(2,6,6-trimethyl-3-cyclohexen-1-yl)-2-buten-1-one; dihydro floriffone TD.
1.2. CAS Registry Number: 57378-68-4.
1.3. EINECS Number: 260-709-8.
1.4. Formula: $C_{13}H_{20}O$.
1.5. Molecular Weight: 192.3.
1.6. FEMA: Flavor and Extract Manufacturers' Association: Generally Recognized as Safe as a flavor ingredient - GRAS 12. (3622).
1.7. JECFA: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 386) concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent.

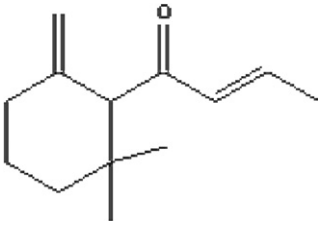

Fig. 1. *Delta*-damascone.

1.8. IFRA: *delta*-Damascone has an International Fragrance Association Standard (IFRA, 2007) – see section 4.4.1. for details.

2. Physical properties

2.1 Boiling point: 82 °C @ 2 mm Hg
2.2 Flash point: >200 °F; CC
2.3 Specific gravity: 0.930
2.4 Log K_{ow}: 4.16

3. Usage

delta-Damascone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 100–1000 less than 0.01 metric tonnes per annum.

The maximum skin level that results from the use of *delta*-damascone in formulae that go into fine fragrances has been reported to be 0.02% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.0940% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.0024 mg/kg for high end users of these products (see Table 1).

4. Toxicology data

4.1. Acute toxicity

4.1.1. Oral studies

4.1.1.1. Groups of BLU:Ha (1CR) albino mice (5/sex/dose) received a single oral (gavage) dose of 1.4, 1.6 1.9, 2.0, 2.1, 2.2 or 2.4 g/kg *delta*-damascone in corn oil. Mortality and/

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing *delta*-damascone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.094	0.0004
Face cream	0.80	2.00	1.000	0.003	0.094	0.0001
Eau de toilette	0.75	1.00	1.000	0.080	0.094	0.0009
Fragrance cream	5.00	0.29	1.000	0.040	0.094	0.0009
Antiperspirant	0.50	1.00	1.000	0.010	0.094	0.0001
Shampoo	8.00	1.00	0.010	0.005	0.094	0.0000
Bath products	17.00	0.29	0.001	0.020	0.094	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.094	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.094	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.094	0.0000
Total						0.0024

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

or systemic effects were observed over a 14-day period. A gross necropsy was conducted on all animals that died during the course of the study. No deaths were observed at the 1.4 g/kg dose group. Between 60% and 80% mortality was observed at all other dose levels with deaths occurring within three days of dose administration. Systemic effects observed in both sexes during the course of the study were lethargy, urinary incontinence, salivation, lacrimation, hyperactivity, tremors, and ataxia. At necropsy, animals of both sexes exhibited pale and mottled kidneys, as well as, pale to dark lungs, liver and spleen. Body weights of both sexes were observed to be maintained or increased in all surviving animals at the completion of the study. The LD_{50} in males was calculated to be 1.9 g/kg and in females was 1.9 g/kg. The combined LD_{50} of both sexes was calculated to be 1.8 g/kg (95% CI 1.4–2.4) (RIFM, 1978a; Moran et al., 1980).

4.1.2. Dermal studies

No data available on this material.

4.2. Skin irritation

Table 2.

4.2.1. Human studies

4.2.1.1. Irritation was evaluated during the induction phase of a human repeated insult patch test (HRIPT). A 0.4 ml aliquot of a 1% solution of *delta*-damascone in ethanol was applied to occlusive patches, which were then applied to the upper arm of each subject for 24 h. A total of nine applications were made over a three-week period. No irritation (0/15) was observed (RIFM, 1978b).

4.2.1.2. Irritation was evaluated during the induction phase of a HRIPT study. A 0.4 ml aliquot of a 0.1% solution of *delta*-damascone in ethanol was applied to occlusive patches, which were then applied to the upper arm of each subject for 24 h. A total of nine applications were made over a three-week period. No irritation (0/30) was observed (RIFM, 1978b).

4.2.2. Animal studies

No data available on this material.

4.3. Mucous membrane (eye) irritation

No data available on this material.

Table 2
Summary of human irritation studies

Method	Dose (%)	Vehicle	Results	Reference
Induction phase (HRIPT)	1	Ethanol	No reactions (0/15)	RIFM, 1978b
Induction phase (HRIPT)	0.1	Ethanol	No reactions (0/30)	RIFM, 1978b

4.4. Skin sensitization

4.4.1. Dermal sensitization quantitative risk assessment (QRA)

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This new methodology represents a significant change over current risk assessment practices because it specifically addresses the elements of exposure-based risk assessment that are unique to the induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment.

Full details of this risk assessment approach can be found in the "QRA Expert Group, Dermal Sensitization Quantitative Risk Assessment (QRA) for Fragrance Ingredients, Technical Dossier, revised June 22, 2006", and IFRA/RIFM Quantitative Risk Assessment (QRA) for Fragrance Ingredients Booklet, May 11, 2006", at http://www.rifm/org/pub/publications.asp and http://www.ifraorg.org/News.asp.

An exposure-based Quantitative Risk Assessment (QRA) methodology has been used to determine acceptable exposure limits for *delta*-damascone and a new IFRA Standard (IFRA, 2007) has been issued (see Tables 3 and 4).

4.4.2. Human studies (Table 5)

4.4.2.1. A repeated insult patch test (Shelanski and Shelanski, 1953) was carried out with 1% *delta*-damascone in alcohol SDA 39C on 54 male and female volunteers. A 0.2 ml

Table 3
IFRA Standard based on the QRA

Limits in the finished product:	
For a description of the categories, refer to the QRA Information Booklet	
Category 1 – See Note (1) 0.003%	Category 7 0.008%
Category 2 0.004%	Category 8 0.1%
Category 3 0.02%	Category 9 0.5%
Category 4 0.05%	Category 10 0.8%
Category 5 0.02%	Category 11 – See Note (2)
Category 6 – See Note (1) 0.07%	

Note: The above limits apply to rose ketones used individually or in combination.

(1) IFRA would recommend that any material used to impart perfume or flavour in products intended for human ingestion should consist of ingredients that are in compliance with appropriate regulations for foods and food flavourings in the countries of planned distribution and, where these are lacking, with the recommendations laid down in the Code of Practice of IOFI (International Organisation of the Flavor Industry). Further information about IOFI can be found on its website (http://www.iofiorg.org).

(2) Category 11 includes all non-skin contact or incidental skin contact products. Due to negligible skin contact the concentration of a fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product.

For example, hypothetically if the usual concentration of a fragrance compound in the final product, for example a candle, is at 5%, then any individual fragrance ingredient (in this case a 'rose ketone') must not exceed 5% in the candle

Table 4
Summary of the relevant sensitization data for the implementation of the QRA

CAS no.	LLNA weighted mean EC3 values (μg/cm^2) [no. studies]	Human data			Potency Classificatio[b]	WoE NESIL (μg/cm^2)[c]
		NOEL – HRIPT (induction) (μg/cm^2)	Experimental NOEL – MAX (induction) (μg/cm^2)	LOEL[a] (induction) (μg/cm^2)		
57378-68-4	1579 [3] class = 1496 μg/cm^2]	NA	NA	1333	Moderate	100 [LLNA weighted mean for
43052-87-5	NA	133 (51)	0.2%	NA	Moderate	
24720-09-0	826 [1]	500 (DEP)	NA	NA	Moderate	
23696-85-7	308 [2]	100 (23)	NA	1000	Moderate	
23726-92-3	NA	67 (53)	NA	375	Moderate	
23726-91-2	600	1000 (pet/54)	NA	NA	Moderate	
23726-94-5	NA	NA	NA	NA	Moderate	
39872-57-6	NA	236 (DEP)	NA	2362	Moderate	
71048-82-3	NA	100 (24)	NA	1000	Moderate	
33673-71-1	NA	NA	NA	NA	Moderate	
70266-48-7	NA	1181 (DEP)	NA	NA	Moderate	

NOEL = No observed effect level; HRIPT = Human Repeat Insult Patch Test; MAX = Human Maximization Test; LOEL = lowest observed effect level; NA = Not Available.
[a] Data derived from HRIPT or HMT.
[b] Gerberick et al., 2001.
[c] WoE NESIL limited to two significant figures.

Table 5
Summary of human sensitization studies

Test method	Test concentration (%)	Vehicle	Results	References
HRIPT	1	Alcohol SDA 39C	7/54 reactions	RIFM, 1982
HRIPT	1	Ethanol	2/15 reactions 2/15 questionable reactions	RIFM, 1978b
HRIPT	0.1	Ethanol	No reactions (0/24)	RIFM, 1978b

aliquot was applied to occlusive patches and allowed to dry prior to application to the backs of each volunteer. Induction consisted of nine, 24-hour patches applied over a four-week period. Following a fourteen-day rest period a challenge patch was applied to a fresh site on the backs for 24 h under occlusion. Reactions to challenge were read at patch removal and 24, 48 and 72 h thereafter. Seven reactions were observed during the induction phase. Based on the characteristics of these responses, which at a maximum included erythema with some degree of induration with or without papules and continuing past 14 days despite medication, they were considered to be sensitization reactions. Of these seven subjects, only one agreed to be challenged with a 24-hour occluded patch and when challenged reacted again. No reactions were observed in the remainder of the 47 subjects at challenge or during induction (RIFM, 1982).

4.4.2.2. Seven subjects who had reacted to 1% delta-damascone during the induction phase of an HRIPT [see section 4.4.2.1] were alternately challenged with four open applications of 0.2% *delta*-damascone in alcohol SDA 39C fourteen days after the last induction application. *delta*-Damascone was applied to the flexor surface of the left arm once daily over four consecutive days. Observations were made every 24 h following application. Reactions were observed in 3/7 subjects (RIFM, 1982).

4.4.2.3. To evaluate cross sensitization, 6 subjects who had reacted to 1% *delta*-damascone during the induction phase of an HRIPT [see section 4.4.2.1] were cross-challenged with *alpha*- or *beta*-damascone. Three subjects were cross challenged with 0.1% *alpha*-damascone in alcohol SDA 39C and 3 subjects were cross-challenged with 0.1% *beta*-damascone in alcohol SDA 39C. The test materials were applied to a naïve site for 24 h under occlusion. Reactions to challenge were read following patch removal and at 24, 48 and 72 h thereafter. Sensitization was observed in all three subjects tested with *alpha*-damascone and in all three subjects tested with *beta*-damascone (RIFM, 1982).

4.4.2.4. A HRIPT study was carried out with 1% *delta*-damascone in ethanol on 15 male and female volunteers. A 0.4 ml aliquot was applied to occlusive patches and allowed to dry prior to application to the back of each volunteer. Induction consisted of nine, 24-hour applications over a four-week period. Following a fourteen-day rest period, a challenge patch was applied to the original site and to a fresh site on the back for 24 h under occlusion. Reactions were read at patch removal and 24 and 48 h thereaf-

ter. Two (2/15) sensitization reactions were observed along with two (2/15) questionable reactions (RIFM, 1978b). No sensitization reactions were observed when 24 male and female volunteers were tested in the same manner as above with 0.1% *delta*-damascone in ethanol (RIFM, 1978b).

4.4.3. Local lymph node assay

4.4.3.1. A Local Lymph Node Assay (LLNA) was conducted on 48 mice, using 0.25, 0.5, 1.0, 2.5 and 5% *delta*-damascone in acetone and oil (4:1). The mice were treated daily for 3 consecutive days on the dorsum of both ears. The animals were observed daily for irritation and toxicity. Following the 3-day treatment period, the animals were allowed to rest for 2 days (day 4 and 5), and were then injected intravenously on day 6 with phosphate buffered saline containing 2 μCi of [125]I-labeled iododeoxyuridine and 10(-5) 2′-deoxy-5-fluorouridine (FUdR). Five hours later, the mice were sacrificed and the auricular nodes were excised. After preparation of the node cells, radioactivity was measured. The EC3 value was calculated to be 0.866% (217 μg/cm^2) (RIFM, 2002a). A second LLNA test was conducted using the same method and concentrations of *delta* damascone. The EC3 value was calculated to be 5.19% (1298 μg/cm^2) (RIFM, 2002b).

4.4.3.2. An LLNA was conducted in 25 female CBA/J female mice (5/dose). Each animal received a daily topical application of 25 μl of 7.5, 15 or 30% *delta*-damascone in EtOH:DEP (3:1) on the dorsal surface of each ear for 3 consecutive days. Control animals were treated with the vehicle alone. Three days after the third topical application all mice were injected intravenously through the tail vein with 250 μl sterile saline (PBS) containing 20 μCi 3H-methylthymidine (3H-thymidine). All mice were sacrificed 5 h after the intravenous injection. Draining auricular lymph nodes were excised and were pooled for each experimental group. Single cell suspensions were then prepared, washed with PBS, suspended in trichloroacetic acid (TCA) and left overnight at 2–8 °C. The samples were then resuspended in TCA and then transferred to a scintillation cocktail. 3H-TdR incorporation was then measured by β-scintillation counting and stimulation indices were determined for each experimental group. The EC3 value was calculated to be 9.6% (2400 μg/cm^2) (RIFM, 2004).

4.5. Phototoxicity and photoallergy

UV spectra reveals that *delta*-damascone does not absorb UV light at wavelengths in the range of 290–400 nm and therefore would have no potential to elicit photoirritation or photoallergy under the current conditions of use as a fragrance ingredient.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

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Review

Fragrance material review on *trans*,*trans*-δ-damascone

A. Lapczynski *, J. Lalko, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of *trans*,*trans*-δ-damascone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; *trans*,*trans*-δ-damascone

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on *trans*,*trans*-δ-damascone. On-line databases that were surveyed included chemical abstract services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms:2-Buten-1-one,1-(2,6,6-trimethyl-3-cyclohexen-1-yl)-, [1.α.(E),2.β.]-;[1.α.(E),2.β.]-1-(2,6,6-Trimethylcyclohex-3-en-1-yl)but-2-en-1-one.
1.2 CAS registry number: 71048-82-3.
1.3 EINECS number: 275-156-8.
1.4 Formula: $C_{13}H_{20}O$.
1.5 Molecular weight: 192.02.
1.6 IFRA: Rose ketone has an international fragrance association standard (IFRA, 2007) – see section 4.4.1. for details.

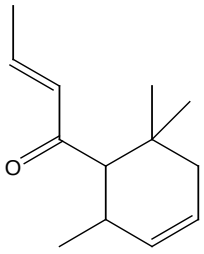

Fig. 1. *trans*,*trans*-δ-Damascone.

2. Physical properties

Log K_{ow} (calculated): 4.16

3. Usage

trans,*trans*-δ-Damascone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 0.1–1 metric tonnes per annum.

The maximum skin level that results from the use of *trans*,*trans*-δ-damascone in formulae that go into fine fragrances has been reported to be 0.02% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.094% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.0024 mg/kg for high end users of these products (see Table 1).

4. Toxicology data

4.1. Acute toxicity

4.1.1. Oral studies

4.1.1.1. A series of three dose range finding studies were conducted to determine the appropriate dose levels for an associated acute oral LD_{50} study. A total of six to eight albino mice (BLU: HA(ICR))(1/sex/dose), with initial weights between 14 and 36 g were used. *trans*,*trans*-δ-Damascone was administered at 0.1, 0.4, 1.6 g/kg (experiment 1); 0.96, 1.35, 1.9, 2.7 g/kg (experiment 2) and 2.2, 2.3, 2.4 and 2.5 g/kg (experiment 3) in corn oil at a constant volume of 20 ml/kg. Animals were observed for clinical

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing *trans*,*trans*-δ-damascone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.094	0.0004
Face cream	0.80	2.00	1.000	0.003	0.094	0.0001
Eau de toilette	0.75	1.00	1.000	0.080	0.094	0.0009
Fragrance cream	5.00	0.29	1.000	0.040	0.094	0.0009
Antiperspirant	0.50	1.00	1.000	0.010	0.094	0.0001
Shampoo	8.00	1.00	0.010	0.005	0.094	0.0000
Bath products	17.00	0.29	0.001	0.020	0.094	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.094	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.094	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.094	0.0000
Total						0.0024

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

signs and mortality over a 14-day period. No deaths were observed at 0.1, 0.4, 0.96 and 1.35 g/kg. One (1/2) animal died at 1.6, 2.2 and 2.3 g/kg and all (2/2) animals died at 1.9, 2.4, 2.5 and 2.7 g/kg. Clinical signs included decreased activity, salivation, anorexia, ataxia, tremors, urinary incontinence and decreased respiration (RIFM, 1979).

4.1.1.2. The acute oral LD_{50} was evaluated in male and female BU:HA (ICR) mice (5/sex/dose). A single oral dose of *trans*,*trans*-δ-damascone was administered as a corn oil solution at concentrations of 0.9, 1.3, 1.5, 1.6, 1.9, 2.0, 2.1, 2.2, 2.4 or 2.7 g/kg at a constant volume of 20 ml/kg. Animals were observed for 14 days following administration. Necropsy was conducted on all animals that died during the 14-day observation period. At the lowest dose, 2/10 deaths occurred; no deaths occurred at 1.3 g/kg; 3/10 deaths occurred at 1.5 g/kg; 6/10 deaths occurred at 2.2 g/kg; 8/10 deaths occurred at 1.6 and 2.1 g/kg; 9/10 deaths occurred at 1.9, 2.0, 2.4 and 2.7 g/kg. The LD_{50} in males was reported to be 1.5 g/kg (95% CI 1.5–1.6 g/kg); the LD_{50} in females was reported to be 1.6 g/kg (95% CI 1.4–1.8 g/kg); and the combined LD_{50} for both male and female mice was reported to be 1.6 g/kg (95% CI 1.0–2.8 g/kg). Clinical signs that were observed at all dose levels included decreased activity, ataxia and urinary incontinence; tremors were also observed at doses of 1.6 g/kg and above. Necropsy revealed dark lungs, pale livers, pale and mottled kidneys, and pale spleens (RIFM, 1979).

4.1.2. Dermal studies

No data available on this material.

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. Irritation was evaluated as a part of a human repeated insult patch test (HRIPT) conducted on 45 volunteers (panel I consisted of 15 volunteers and panel II consisted of 30 volunteers). *trans*,*trans*-δ-Damascone at 1% in alcohol SDA 39C was applied to a 4 cm^2 Webril (non-woven absorbent cotton fabric) swatch which was affixed to a 16 cm^2 adhesive square and then applied to the upper arm of each volunteer for 24 h under semi-occlusion. Application was every other day to the same test site until 9 applications were made. Reactions were scored according to Draize at patch removal. No irritation was observed (RIFM, 1978a).

4.2.2. Animal studies

4.2.2.1. A primary irritation test was conducted on three healthy Albino rabbits. A 0.5 ml aliquot of 1% *trans*, *trans*-δ-damascone in alcohol SDA 39C was applied to the intact and abraded skin of each animal for 24 h under occlusion. Reactions were scored according to Draize at patch removal and again 48 h after patch removal. No irritation was observed (RIFM, 1978b).

4.3. Mucous membrane (eye) irritation

4.3.1. Animal studies

4.3.1.1. An eye irritation test was conducted in three albino rabbits. A 0.1 ml aliquot of 1% *trans*,*trans*-δ-damascone in propylene glycol was instilled into the right eye of each animal without further treatment, while the left eye remained untreated and served as a control. Reactions were scored according to Draize every 24 h for 4 days and then again on day 7. Slight conjunctival irritation was observed in all 3 rabbits on day 1; all eyes were normal by the second day (RIFM, 1978c).

4.4. Skin sensitization

4.4.1. Dermal sensitization quantitative risk assessment (QRA)

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This new methodology represents a significant change over current risk assessment practices because it specifically addresses the elements of exposure-based risk assessment that are unique to the induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment.

Full details of this risk assessment approach can be found in the "QRA Expert Group, Dermal Sensitization Quantitative Risk Assessment (QRA) for Fragrance Ingredients, Technical Dossier, revised June 22, 2006", and IFRA/RIFM Quantitative Risk Assessment (QRA) for

Table 2
IFRA Standard based on the QRA

For a description of the categories, refer to the QRA information booklet	
Limits in the finished product	
Category 1 – see Notes (1) 0.003%	Category 7 – 0.008%
Category 2 – 0.004%	Category 8 – 0.1%
Category 3 – 0.02%	Category 9 – 0.5%
Category 4 – 0.05%	Category 10 – 0.8%
Category 5 – 0.02%	Category 11 – See Notes (2)
Category 6 – See Notes (1) 0.07%	

Notes: The above limits apply to rose ketones used individually or in combination.

(1) IFRA would recommend that any material used to impart perfume or flavour in products intended for human ingestion should consist of ingredients that are in compliance with appropriate regulations for foods and food flavourings in the countries of planned distribution and, where these are lacking, with the recommendations laid down in the Code of Practice of IOFI (International Organisation of the Flavor Industry). Further information about IOFI can be found on its website (www.iofiorg.org).

(2) Category 11 includes all non-skin contact or incidental skin contact products. Due to negligible skin contact the concentration of a fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product.

For example, hypothetically if the usual concentration of a fragrance compound in the final product, for example a candle, is at 5%, then any individual fragrance ingredient (in this case a 'rose ketone') must not exceed 5% in the candle.

Table 3
Summary of the relevant sensitization data for the implementation of the QRA

CAS no.	LLNA weighted mean EC3 values ($\mu g/cm^2$) [no. studies]	Human data			Potency classification[b]	WoE NESIL ($\mu g/cm^2$)[c]
		NOEL – HRIPT (induction) ($\mu g/cm^2$)	Experimental NOEL – MAX (induction) ($\mu g/cm^2$)	LOEL[a] (induction) ($\mu g/cm^2$)		
57378-68-4	1579 [3]	NA	NA	1333	Moderate	100 [LLNA weighted mean for class = 1496 $\mu g/cm^2$]
43052-87-5	NA	133 (51)	0.2%	NA	Moderate	
24720-09-0	826 [1]	500 (DEP)	NA	NA	Moderate	
23696-85-7	308 [2]	100 (23)	NA	1000	Moderate	
23726-92-3	NA	67 (53)	NA	375	Moderate	
23726-91-2	600	1000 (pet/54)	NA	NA	Moderate	
23726-94-5	NA	NA	NA	NA	Moderate	
39872-57-6	NA	236 (DEP)	NA	2362	Moderate	
71048-82-3	NA	100 (24)	NA	1000	Moderate	
33673-71-1	NA	NA	NA	NA	Moderate	
70266-48-7	NA	1181 (DEP)	NA	NA	Moderate	

NOEL = No observed effect level; HRIPT = Human repeat insult patch test; MAX = Human maximization test; LOEL = lowest observed effect level; NA = Not available.
[a] Data derived from HRIPT or HMT.
[b] Gerberick et al., (2001).
[c] WoE NESIL limited to two significant figures.

Fragrance Ingredients Booklet, May 11, 2006", at http://www.rifm/org/pub/publications.asp and http://www.ifraorg.org/News.asp.

An exposure-based Quantitative Risk Assessment (QRA) methodology has been used to determine acceptable exposure limits for rose ketone and a new IFRA Standard (IFRA, 2007) has been issued (See Tables 2 and 3).

4.4.2. Human studies

4.4.2.1. A HRIPT study was conducted on 39 volunteers (15 volunteers in panel I, and 24 volunteers in panel II). A 0.4 ml aliquot of 1% *trans*,*trans*-δ-damascone in alcohol SDA 39C was applied to a 4 cm^2 Webril swatch which was affixed to a 16 cm^2 adhesive square and then applied to the upper arm of each volunteer for 24 h under semi-occlusion. A series of nine induction applications were made over a period of three weeks. Challenge was conducted 2 weeks after the last induction application. Subjects from panel I were challenged with 1% *trans*,*trans*-δ-damascone in alcohol SDA 39C and subjects from panel II were challenged with 0.1% *trans*,*trans*-δ-damascone in alcohol SDA 39C. Reactions were scored according to Draize at 24 and 72 h after patch removal. No reactions were observed with 0.1% *trans*,*trans*-δ-damascone. Two (2/15) sensitization reactions plus two questionable reactions were observed with 1% *trans*,*trans*-δ-damascone (RIFM, 1978a).

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual fragrance material review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, J. Lalko, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on γ-damascone

J. Lalko, A. Lapczynski *, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of γ-damascone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Damascone

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on γ-damascone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification

1.1 Synonyms: 1-(2,2-dimethyl-6-methylenecyclohexyl)-but-2-en-1-one; 2-buten-1-one, 1-(2,2-dimethyl-6-methylenecyclohexyl.
1.2 CAS Registry Number: 35087-49-1.
1.3 EINECS Number: N/A.
1.4 Formula: $C_{13}H_{20}O$.
1.5 Molecular Weight: 192.30.
1.6 IFRA: γ-Damascone has an International Fragrance Association Standard (IFRA, 2007) – see Section 4.4.1. for details Fig. 1.

2. Physical properties

2.1 Boiling point: 254.4 °C.
2.2 Vapor pressure: 0.0245 mm Hg @ 25 °C.

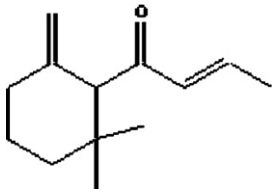

Fig. 1. γ-Damascone.

3. Usage

γ-Damascone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region less than 0.1 metric tonnes per annum.

The maximum skin level that results from the use of γ-damascone in formulae that go into fine fragrances has been reported to be 0.02% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has not been reported. Such as the maximum skin level concentration of 0.02% was used to calculate the conservative calculated maximum daily exposure on the skin to be 0.005 mg/kg for high end users of these products (see Table 1).

4. Toxicology data

4.1. Acute toxicity

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} of γ-damascone was evaluated in 20 (5/sex/dose) Sprague Dawley rats. Following overnight fasting, animals received a single oral (gavage) dose of γ-damascone suspended in cottonseed oil to give a dose volume of 10 ml/kg and dose levels of 2.0 or 5.0 g/kg body weight. All animals were observed for any signs of toxicity over a fourteen day period. No deaths were observed at 2.0 g/kg, but 5/10 animals from the 5.0 g/kg group died on day 2. Piloerection and perinasal staining were observed in most animals dosed at 2.0 or 5.0 g/kg. Animals from the 5.0 g/kg dose were also hypoactive within 2 h. The LD_{50} was reported to be greater than 2.0 g/kg (RIFM, 1987a).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing γ-damascone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.02	0.0001
Face cream	0.80	2.00	1.000	0.003	0.02	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.02	0.0002
Fragrance cream	5.00	0.29	1.000	0.040	0.02	0.0002
Antiperspirant	0.50	1.00	1.000	0.010	0.02	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.02	0.0000
Bath products	17.00	0.29	0.001	0.020	0.02	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.02	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.02	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.02	0.00.00
Total						0.0005

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} of γ-damascone was evaluated in 10 (5/sex) New Zealand white rabbits. γ-Damascone was applied for 24 h under occlusion to the clipped, dorsal skin of the trunk at a dose level of 2.0 g/kg. All animals were observed for a 14 day period for signs of toxicity and/or mortality. Gross necropsy was conducted on all animals. No deaths occurred. Loss of weight was observed in 2 animals. Necropsy examination revealed abnormalities in the lungs and colon in one rabbit. The LD_{50} was reported to be greater than 2.0 g/kg, based on no deaths at that dose (RIFM, 1987b).

4.2. Skin irritation

4.2.1. Human studies

No data available on this material.

4.2.2. Animal Studies

4.2.2.1. A primary irritation study was conducted in 4 New Zealand White rabbits. A 0.5 ml aliquot of 40%, 55% or 75% γ-damascone in ethanol or 100% γ-damascone was applied to 2.5 cm^2 piece of surgical lint B.P which was then applied to the back or flanks for 4 h under semi-occlusion. After the patches were removed, the treated sites were cleansed by gentle swabbing with cotton wool soaked in warm water. Reactions were read at 1, 24, 48, 72 and 168 h after patch removal. A material was considered to be an irritant if the mean values obtained for either erythema or edema equaled or exceeded 2. The mean erythema and edema scores for 40, 55 and 75% γ-damascone were all below 2; the mean erythema and edema scores for 100% γ-damascone were 2.0 and 1.0. Under the conditions of the test, 100% γ-damascone was considered to be an irritant (RIFM, 1986a,1986b).

4.2.2.2. Irritation was evaluated as a part of the acute dermal LD_{50} study described above. A single dermal application of 2.0 g/kg of neat γ-damascone produced well defined irritation and desquamation (RIFM, 1987b).

4.2.2.3. Irritation was evaluated prior to a Buehler guinea pig sensitization study. A 0.5 ml aliquot of γ-damascone was applied to a 20 mm^2 piece of surgical lint at concentrations of 10%, 20% or 50% in ethanol or 100%. The surgical lint was then placed on one of four sites on the backs of four Dunkin-Hartley guinea pigs and held in position by "Blenderm" surgical tape. Patches were removed after six hours and the treatment sites were examined 24 and 48 h later. No irritation was observed at 10%. Irritation reactions were observed at 20%, 50% and 100% (RIFM, 1986c).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Dermal Sensitization Quantitative Risk Assessment (QRA)

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This new methodology represents a significant change over current risk assessment practices because it specifically addresses the elements of exposure-based risk assessment that are unique to the induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment.

Full details of this risk assessment approach can be found in the "QRA Expert Group, Dermal Sensitization Quantitative Risk Assessment (QRA) for Fragrance Ingredients, Technical Dossier, revised June 22, 2006", and IFRA/RIFM Quantitative Risk Assessment (QRA) for Fragrance Ingredients Booklet, May 11, 2006", at http://www.rifm/org/pub/publications.asp and http://www.ifraorg.org/News.asp.

Table 2
IFRA Standard based on the QRA

Limits in the finished product:			
For a description of the categories, refer to the QRA Information Booklet			
Category 1 – See Note (1)	0.003%	Category 7	0.008%
Category 2	0.004%	Category 8	0.1%
Category 3	0.02%	Category 9	0.5%
Category 4	0.05%	Category 10	0.8%
Category 5	0.02%	Category 11 – See Note (2)	
Category 6 – See Note (1)	0.07%		

Note: The above limits apply to rose ketones used individually or in combination.

(1) IFRA would recommend that any material used to impart perfume or flavour in products intended for human ingestion should consist of ingredients that are in compliance with appropriate regulations for foods and food flavourings in the countries of planned distribution and, where these are lacking, with the recommendations laid down in the Code of Practice of IOFI (International Organisation of the Flavor Industry). Further information about IOFI can be found on its website (www.iofiorg.org).

(2) Category 11 includes all non-skin contact or incidental skin contact products. Due to negligible skin contact the concentration of a fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product.

For example, hypothetically if the usual concentration of a fragrance compound in the final product, for example a candle, is at 5%, then any individual fragrance ingredient (in this case a 'rose ketone') must not exceed 5% in the candle.

An exposure-based Quantitative Risk Assessment (QRA) methodology has been used to determine acceptable exposure limits for γ-damascone and a new IFRA Standard (IFRA, 2007) has been issued (see Tables 2 and 3).

4.4.2. Human studies

No data available on this material.

4.4.3. Animal studies

4.4.3.1. The sensitization potential of γ-damascone was evaluated in a Buehler sensitization test using two groups (10/group) Dunkin-Hartley guinea pigs. During the induction, a 0.5 ml aliquot of 20% γ-damascone in ethanol was applied to a piece of 20 mm^2 surgical lint which was then placed onto the clipped left flank of each animal. The sites were covered by "Blenderm" surgical tape and remained in place for six hours. The dosing procedure was repeated at weekly intervals on days 8 and 15. On day 28, the right flank of the animals in both test and control groups was clipped free of fur. The following day (Day 29) animals received a 6 h challenge application with 0.5 ml of 5% or 10% γ-damascone in ethanol. The test sites were examined at 24 and 48 h. Sensitization was observed in 1/10 guinea pigs with 5% γ-damascone and in 2/10 guinea pigs with 10% γ-damascone (RIFM, 1986c).

4.4.4. Local lymph node assay

4.4.4.1. A Local Lymph Node assay was conducted using CBA/J Hsd mice with 0.25%, 0.5%, 1.0%, 2.5% or 5% γ-damascone in acetone:oil (4:1). The mice were treated daily for 3 consecutive days on the dorsum of both ears. The animals were observed daily for irritation and toxicity. Following the 3-day treatment period, the animals were allowed to rest for 2 days (day 4 and 5), and were then injected intravenously on day 6 with phosphate buffered saline containing 2 μCi of [125]I-labeled iododeoxyuridine and 10(-5) 2'-deoxy-5-fluorouridine (FUdR). Approximately five hours later, the mice were sacrificed and the auricular nodes were excised. After preparation of the node cells, radioactivity was measured. The EC3 value was calculated to be 4.6% (1150 μg/cm^2). γ-Damascone was classified as a weak sensitizer (RIFM, 2001).

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

4.9.1. Bacterial study

4.9.1.1.. An Ames assay (Ames et al., 1975) was conducted using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 with or without S9 activation. γ-Damascone at doses up to 5000 μg/plate in dimethyl sulfoxide produced no mutagenic effects (RIFM, 1986d).

4.10. Carcinogenicity

No data available on this material.

Table 3
Summary of the relevant sensitization data for the implementation of the QRA

CAS no.	LLNA weighted mean EC3 values (μg/cm^2) [no. studies]	Human data			Potency classification[b]	WoE NESIL (μg/cm^2)[c]
		NOEL – HRIPT (induction) (μg/cm^2)	Experimental NOEL – MAX (induction) (μg/cm^2)	LOEL[a] (induction) (μg/cm^2)		
57378-68-4	1579 [3]	NA	NA	1333	Moderate	100 [LLNA weighted mean for class = 1496 μg/cm^2]
43052-87-5	NA	133 (51)	0.2%	NA	Moderate	
24720-09-0	826 [1]	500 (DEP)	NA	NA	Moderate	
23696-85-7	308 [2]	100 (23)	NA	1000	Moderate	
23726-92-3	NA	67 (53)	NA	375	Moderate	
23726-91-2	600	1000 (pet/54)	NA	NA	Moderate	
23726-94-5	NA	NA	NA	NA	Moderate	
39872-57-6	NA	236 (DEP)	NA	2362	Moderate	
71048-82-3	NA	100 (24)	NA	1000	Moderate	
33673-71-1	NA	NA	NA	NA	Moderate	
70266-48-7	NA	1181 (DEP)	NA	NA	Moderate	

NOEL = No observed effect level; HRIPT = Human Repeat Insult Patch Test; MAX = Human Maximization Test; LOEL = lowest observed effect level; NA = Not available.

[a] Data derived from HRIPT or HMT.

[b] Gerberick et al. (2001).

[c] WoE NESIL limited to two significant figures.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

J. Lalko, A. Lapczynski, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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RIFM (Research Institute for Fragrance Materials, Inc.), 1986d. Ames metabolic activation test to assess the potential mutagenic effect of γ-damascone. Unpublished report from Firmenich Incorporated, 13 March. Report number 39989 (RIFM, Woodcliff Lake, NJ, USA).

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RIFM (Research Institute for Fragrance Materials, Inc.), 1987b. Single dose dermal toxicity study of 2-buten-1-one, 1-(2,2-dimethyl-6-methylenecyclohexyl)- in the rabbit. Unpublished report from Firmenich Incorporated, 17 June. Report number 39985 (RIFM, Woodcliff Lake, NJ, USA).

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Review

Fragrance material review on dihydro-α-ionone

J. Lalko, A. Lapczynski *, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of dihydro-α-ionone when used as a fragrance ingredient is presented.

Keywords: Fragrance; Review; Dihydro-α-ionone

Contents

In 2006, a complete literature search was conducted on dihydro-α-ionone. On-line databases that were surveyed included Chemical Abstract Services and the National

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: 2-butanone, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-; dihydro-α-ionone; 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)butan-2-one.
1.2 CAS Registry number: 31499-72-6.
1.3 EINECS number: 250-657-4.
1.4 Formula: $C_{13}H_{22}O$.
1.5 Molecular weight: 194.32.
1.6 FEMA: Flavor and extract manufacturers' association states: Generally recognized as Safe as a flavor ingredient – GRAS 12 (3628) (FEMA, 1979).
1.7 JECFA: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 393) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent (JECFA, 1998).

2. Physical properties

2.1 Physical form: colorless to pale yellow oily liquid.
2.2 Log K_{ow} (calculated): 4.22.

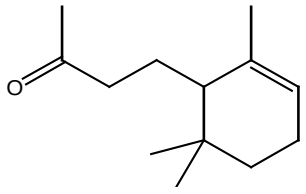

Fig. 1. Dihydro-α-ionone.

2.3 Flash point: >93.3 °C; CC.
2.4 Boiling point: 90 °C @ 0.1 mm Hg.
2.5 Refractive index @ 20 °C: 1.4791.
2.6 Solubility in alcohol: 1am/80.
2.7 Specific gravity 25 °C: 0.92.

3. Usage

Dihydro-α-ionone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of <0.1 metric tonnes per annum.

The maximum skin level that results from the use of dihydro-α-ionone in formulae that go into fine fragrances has not been reported. A default value of 0.02% is used, assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has not been reported. As such a default value of 0.02% is used to calculate maximum daily exposure on the skin of 0.0005 mg/kg for high end users of these products (see Table 1).

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} in rats was reported to be greater than 5.0 g/kg. Ten animals were administered a single oral dose of 5 g/kg of dihydro-α-ionone. Mortality and/or systemic effects were observed over a 14-day period. No deaths or systemic effects were observed (RIFM, 1976a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} of neat dihydro-α-ionone in rabbits was reported to be greater than 5.0 g/kg. Ten

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing dihydro-α-ionone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.02	0.0001
Face cream	0.80	2.00	1.000	0.003	0.02	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.02	0.0002
Fragrance cream	5.00	0.29	1.000	0.040	0.02	0.0002
Antiperspirant	0.50	1.00	1.000	0.010	0.02	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.02	0.0000
Bath products	17.00	0.29	0.001	0.020	0.02	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.02	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.02	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.02	0.0000
Total						0.0005

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	No. animals/ dose group	LD_{50}	References
Oral	Rat	10	>5 g/kg	RIFM, 1976a
Dermal	Rabbits	10	>5 g/kg	RIFM, 1976a

animals received a single dermal application of 5 g/kg of neat dihydro-α-ionone. Mortality and/or systemic effects were observed over a 14-day period. Two rabbits exhibited diarrhea on day one. One death occurred on day 13 (RIFM, 1976a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, 12% dihydro-α-ionone in petrolatum was tested in a 48-h occluded patch test on the back or volar forearms of 25 healthy, male and female volunteers. No irritation was observed (RIFM, 1976b).

4.2.2. Animal studies

4.2.2.1. As a part of an acute dermal LD_{50} study in rabbits, neat dihydro-α-ionone was evaluated for irritation after a single dermal application of 5.0 g/kg. Irritation was evaluated over a 14-day observation period. Slight erythema was observed in one animal and moderate erythema was observed in nine of the animals. Slight edema was observed in three animals and moderate edema was observed in seven animals (RIFM, 1976a).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (Kligman, 1966) was carried out with 12% dihydro-α-ionone in petrolatum on 12 male and 13 female volunteers. Induction applications were under occlusion to the same site on the volar forearms or backs of all subjects for five alternate-day 48-h periods. Patch test sites were pretreated for 24 h with 2.5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a 10-day rest period, a challenge patch (12% in petrolatum) was applied to a fresh site for 48 h under occlusion. The challenge sites were pretreated for one hour with 5–10% aqueous SLS. Reactions to challenge were read at patch removal and 24 h after patch removal. No reactions were observed (RIFM, 1976b).

4.4.2. Animal studies

4.4.2.1. A guinea pig open epicutaneous test (OET) was conducted on groups of 6–8 male and female guinea pigs weighting 300–450 g. Daily open applications were made for 3 weeks to a clipped 8-cm^2 area on the flank of each guinea pig. Reactions were read 24 h after each application. A total of 21 applications of 0.1 ml of 12% dihydro-α-ionone in an unspecified vehicle were made. At the challenge phase, both the test and control animals were treated on days 21 and 35 on the contralateral flank with the test material at the minimal irritating concentration and some lower primary non-irritating concentrations. No sensitization reactions were observed (Klecak, 1985).

4.5. Phototoxicity and photoallergy

UV spectra revealed that dihydro-α-ionone peaked within 235–255 nm range and showed minor absorption in the 260–300 nm region.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

J. Lalko, A. Lapczynski, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on dihydro-β-ionone

J. Lalko, A. Lapczynski *, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of dihydro-β-ionone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Dihydro-β-ionone

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on dihydro-β-ionone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual fragrance material review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: 2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-; dihydro-β-ionone; 4-(2,6,6-trimethyl-1-cyclohexenyl)butan-2-one.
1.2 CAS registry number: 17283–81–7.
1.3 EINECS number: 241–318–1.
1.4 Formula: $C_{13}H_{22}O$.
1.5 Molecular weight: 194.32.
1.6 FEMA: Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient – GRAS 12. (3626).
1.7 JECFA: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 394) concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent.

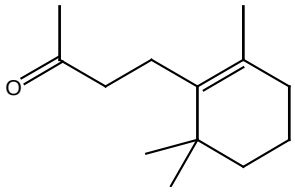

Fig. 1. Dihydro-β-ionone.

2. Physical properties

2.1 Log K_{ow} (calculated): 4.35.
2.2 Flash point: >200 °F;CC.
2.3 Vapor pressure (calculated): 0.007 mm Hg 20 °C.

3. Usage

Dihydro-β-ionone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 10–100 metric tonnes per annum.

The maximum skin level that results from the use of dihydro-β-ionone in formulae that go into fine fragrances has been reported to be 1.34% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5‰ use level in formulae for use in cosmetics in general has been reported to be 4.26% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.11 mg/kg for high end users of these products (see Table 1).

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral toxicity of dihydro-β-ionone was evaluated in male and female WIST (SPF) rats (3/sex/dose). Each animal received a single dose of 2.0 g/kg dihydro-β-ionone in polyethylene glycol PEG 300 via oral gavage. Animals were examined for clinical signs and mortality/viability once daily for a period of 14 days. On day 15, all animals were sacrificed and gross necropsies were conducted. No deaths occurred. On day 2, two females exhibited ruffled fur, hunched posture (but only

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing dihydro-β-ionone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product	Ingredient/mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	4.26	0.0161
Face cream	0.80	2.00	1.000	0.003	4.26	0.0034
Eau de toilette	0.75	1.00	1.000	0.080	4.26	0.0426
Fragrance cream	5.00	0.29	1.000	0.040	4.26	0.0412
Antiperspirant	0.50	1.00	1.000	0.010	4.26	0.0036
Shampoo	8.00	1.00	0.010	0.005	4.26	0.0003
Bath products	17.00	0.29	0.001	0.020	4.26	0.0001
Shower gel	5.00	1.07	0.010	0.012	4.26	0.0005
Toilet soap	0.80	6.00	0.010	0.015	4.26	0.0005
Hair spray	5.00	2.00	0.010	0.005	4.26	0.0004
Total						0.1085

[a] Upper 97.5‰ levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	No. animals/dose group	LD_{50}	References
oral	rats	6 (3/sex)	> 2.0 g/kg	RIFM (1999a)

one persisted through day three), and sedation. No changes were observed at necropsy. The acute oral LD_{50} was calculated to be greater than 2.0 g/kg based on no deaths at that dose (RIFM, 1999a).

4.1.2. Dermal studies

No data available on this material.

4.2. Skin irritation

4.2.1. Human studies

No data available on this material.

4.2.2. Animal studies

4.2.2.1. A primary skin irritation test was conducted on 3 (1 male and 2 female) young adult New Zealand white rabbits. Neat dihydro-β-ionone was applied to an area on the clipped left flank of each animal and then covered with a semi-occlusive dressing for approximately 4 h. Reactions were evaluated at 1, 24, 48 and 72 h after application. No irritation was observed. The primary irritation score was 0.00. Dihydro-β-ionone was classified as not irritating (RIFM, 1999b).

4.2.2.2. Irritation was evaluated in a pre-test conducted to determine the intradermal induction concentration to be used in a guinea pig maximization study. A single injection of 0.1 ml dihydro-β-ionone at 1%, 3% and 5% in PEG 400 was administered to the clipped flank of one male Himalayan spotted guinea pig. One concentration was tested at each site. Reactions were graded 24 h after the injection. One week prior to administration of the test material, 4 intradermal injections (0.1 ml/site) of a 1:1 (v/v) mixture of Freund's Complete Adjuvant (FCA)/physiological saline were administered to the neck of the same animal. Moderate and confluent erythema was observed at all concentrations (RIFM, 1999c).

4.2.2.3. In a pretest conducted to determine the epidermal induction and challenge concentrations for a guinea pig maximization study, two male Himalayan spotted guinea pigs were administered four intradermal injections (0.1 ml/site) of a 1:1 (v/v) mixture of Freund's Complete Adjuvant (FCA)/physiological saline in the neck. One week later, both flanks of each animal were clipped and shaved just prior to the administration of dihydro-β-ionone. For each dose level tested (25, 50, 75 in PEG 400% and 100%) a 3 cm^2 patch was saturated with approximately 0.2 ml aliquot of the test material. The patches were covered with aluminum foil and secured around the trunk of the animals with elastic plaster, then covered with impervious adhesive tape. The patches were removed after 24 h. Reactions were graded 24 and 48 h after patch removal. All concentrations produced discrete or patchy erythema. A second pretest was conducted on two additional animals in the same manner as the first pretest, with 1%, 5%, 10% and 15% dihydro-β-ionone in PEG 400. No irritation was produced with 1%, discrete or patchy erythema was produced by 5%, 10% and 15% (RIFM, 1999c).

4.2.2.4. As a part of an associated guinea pig maximization test on 10 male Himalayan spotted guinea pigs, irritation was evaluated during the induction phase. One week after intradermal induction injections were administered, a 2×4 cm patch saturated with 0.3 ml of neat dihydro-β-ionone was placed over the injection sites, covered with aluminum foil and secured around the trunk of the animals with elastic plaster, then covered with impervious adhesive tape. The patches were removed after 48 h of contact and reactions were graded 24 and 48 h after patch removal. At 24 h, 10/10 animals exhibited discrete or patchy erythema, which was still observed in 6/10 animals at 48 h (RIFM, 1999c).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

No data available on this material.

4.4.2. Animal studies

4.4.2.1. A guinea pig maximization test was conducted on 10 Himalayan spotted guinea pigs (Magnusson and Kligman, 1969). Three pairs of intradermal induction injections were administered on day 1 to both the control (2 injections of the FCA/saline mixture, 2 injections of PEG 400, and 2 injections of a 1:1 mixture of PEG 400 in the FCA/saline mixture) and treated animals (2 injections of a 1:1 mixture of FCA/physiological saline, 2 injections of 5% dihydro-β-ionone in PEG 400, and 2 injections of 5% dihydro-β-ionone in the FCA/saline mixture). On day 8, occluded 48-hours patches with neat dihydro-β-ionone were placed over the injection sites. On day 22, the challenge applications were made with two 24-hours occluded patches saturated with 1% dihydro-β-ionone (left flank) and PEG 400 (right flank). Reactions were graded 24 and 48 h after patch removal. The controls were treated with the material in the same manner as the test animals at challenge. No sensitization reactions were produced (RIFM, 1999c).

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

4.9.1. Bacterial studies

4.9.1.1. Dihydro-β-ionone was tested in a pre-incubation test using *Salmonella typhimurium* strain TA102 at doses up to 1000 μg/plate, in the presence of S9 mix. No dose-related increase in the number of revertant colonies was observed. Under the conditions of the test dihydro-β-ionone was not considered to be mutagenic (RIFM, 2000).

4.9.1.2. Dihydro-β-ionone was tested in a direct incorporation test using *S. typhimurium* strains TA1535 in the presence of S9 mix, and strain TA1537 in the absence and presence of S9 mix, at doses up to 1000 μg/plate. No dose-related increase in the number of revertant colonies was observed. Dihydro-β-ionone was not considered to be mutagenic (RIFM,2000).

4.9.1.3. The potential of dihydro-β-ionone to induce gene mutations according to the direct plate incorporation test was evaluated using *S. typhimurium* strains TA 98, TA100, TA102, TA1535 and TA1537 with and without S9 mix. No dose-related increase in the number of revertant colonies was observed. Dihydro-β-ionone at doses up to 1000 μg/plate was not considered to be mutagenic (RIFM, 2000).

4.10. Carcinogenicity

No data available on this material.

This individual fragrance material review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

J. Lalko, A. Lapczynski, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufactures of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufactures of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on dihydro-γ-ionone

J. Lalko, A. Lapczynski *, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of dihydro-γ-ionone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Dihydro-γ-ionone

Contents

In 2006, a complete literature search was conducted on dihydro-γ-ionone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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1. Identification (Fig. 1)

1.1 Synonyms: 2-Butanone, 4-(2,2-dimethyl-6- methylenecyclohexyl)-; dihydro-γ-ionone; 4-(2,2-dimethyl-6-methylenecyclohexyl)butan-2-one.
1.2 CAS Registry Number: 13720-12-2.
1.3 EINECS Number: 237-283-7.
1.4 Formula: $C_{13}H_{22}O$.
1.5 Molecular weight: 194.18.

2. Physical properties

2.1 Log K_{ow} (calculated): 4.3.

3. Usage

Dihydro-γ-ionone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of <0.1 metric tonnes per annum.

The maximum skin level that results form the use of dihydro-γ-ionone in formulae that go into fine fragrances has been reported to be 0.0001% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.0073% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.0002 mg/kg for high end users of these products (see Table 1).

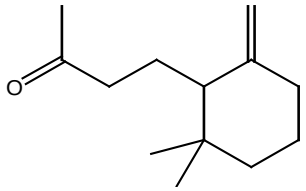

Fig. 1. Dihydro-γ-ionone.

4. Toxicology data

4.1. Acute toxicity

No data available on this material.

4.2. Skin irritation

No data available on this material.

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

No data available on this material.

4.4.2. Animal studies

4.4.2.1. A local lymph node assay was conducted in 25 female CBA/J female mice (5/dose). Each animal received a daily topical application of 25 μl of 7.5%, 15% or 30% dihydro-γ-ionone in EtOH:DEP (3:1) on the dorsal surface of each ear for three consecutive days. Control animals were treated with the vehicle alone. Three days after the third topical application all mice were injected intravenously through the tail vein with 250 μl sterile saline (PBS) containing 20 μCi 3*H*-methylthymidine (3*H*-thymidine). All mice were sacrificed 5 h after the intravenous injection. Draining auricular lymph nodes were excised and were pooled for each experimental group. Single cell suspensions were then prepared, washed with PBS, suspended in trichloroacetic acid (TCA) and left overnight at 2–8 °C. The samples were then resuspended in TCA and then transferred to a scintillation cocktail. 3*H*-TdR incorporation was then measured by β-scintillation counting and stimulation indices were determined for each experimental group. For each concentration of test material, a stimulation index (SI) relative to the concurrent vehicle-

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing dihydro-γ-ionone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.0073	0.0000
Face cream	0.80	2.00	1.000	0.003	0.0073	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.0073	0.0001
Fragrance cream	5.00	0.29	1.000	0.040	0.0073	0.0001
Antiperspirant	0.50	1.00	1.000	0.010	0.0073	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.0073	0.0000
Bath products	17.00	0.29	0.001	0.020	0.0073	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.0073	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.0073	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.0073	0.0000
Total						0.0002

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

treated control was calculated. The SI values were 1.39, 1.52 and 1.76 for 7.5%, 15% and 30%, respectively. Under the conditions of the study, dihydro-γ-ionone did not result in a stimulation index of 3 or greater and therefore was not considered to have the potential to produce skin sensitization (RIFM, 2004).

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

J. Lalko, A. Lapczynski, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on 4-(1,2-epoxy-2,6,6-trimethylcyclohexyl)-3-buten-2-one

J. Lalko, A. Lapczynski *, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of 4-(1,2-epoxy-2,6,6-trimethylcyclohexyl)-3-buten-2-one when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; 4-(1,2-Epoxy-2,6,6-trimethylcyclohexyl)-3-buten-2-one

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones when used as fragrance ingredients (Belsito et al., 2007) for an overall assessment of this material.

In 2005, a complete literature search was conducted on 4-(1,2-Epoxy-2,6,6-trimethyl cyclohexyl)-3-buten-2-one. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

1. Identification (Fig. 1)

1.1 Synonyms: 3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo [4.1.0] hept-1-yl)-; 5,6-epoxy-β-ionone; ionone epoxide, β; β-ionone-5,6-epoxide; 4-(2,2,6-trimethyl-7-oxabicyclo(4.1.0)hept-1-yl)-3-buten-2-one.
1.2 CAS Registry number: 23267-57-4.
1.3 EINECS number: 245-542-0.
1.4 Formula: $C_{13}H_{20}O_2$.
1.5 Molecular weight: 196.29.
1.6 FEMA: Flavor and Extract Manufacturers' Association: Generally recognized as safe as a flavor ingredient – GRAS 22 (4144) (FEMA, 2005).

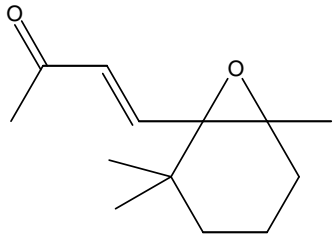

Fig. 1. 4-(1,2-Epoxy-2,6,6-trimethylcyclohexyl)-3-buten-2-one.

2. Physical properties

2.1 Log K_{ow} (calculated): 2.93.

3. Usage

4-(1,2-Epoxy-2,6,6-trimethylcyclohexyl)-3-buten-2-one is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of <0.1 metric tones per annum.

The maximum skin level that results from the use of 4-(1,2-epoxy-2,6,6-trimethylcyclohexyl)-3-buten-2-one in formulae that go into fine fragrances has been reported to be 0.003% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.0255% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.0006 mg/kg for high end users of these products (see Table 1).

4. Toxicology data

4.1. Acute toxicity

No data available on this material.

4.2. Skin irritation

No data available on this material.

4.3. Mucous membrane (eye) irritation

No data available on this material.

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing 4-(1,2-epoxy-2,6,6-trimethylcyclohexyl)-3-buten-2-one

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.0255	0.0001
Face cream	0.80	2.00	1.000	0.003	0.0255	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.0255	0.0003
Fragrance cream	5.00	0.29	1.000	0.040	0.0255	0.0002
Antiperspirant	0.50	1.00	1.000	0.010	0.0255	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.0255	0.0000
Bath products	17.00	0.29	0.001	0.020	0.0255	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.0255	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.0255	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.0255	0.0000
Total						0.0006

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

4.4. Skin sensitization

No data available on this material.

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental studies

No data available on this material.

4.9. Mutagenicity and genotoxicity

4.9.1. Mutagenicity

4.9.1.1. An Ames assay was conducted using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 with and without metabolic activation. No mutagenic effects were observed with doses up to 500 mg/plate (RIFM, 1988).

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

J. Lalko, A. Lapczynski, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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FEMA (Flavor Extract Manufacturers Association), 2005. GRAS flavouring substances 22. Food Technology 59, 24–62.

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Review

Fragrance material review on α-ionone

J. Lalko, A. Lapczynski *, V.T. Politano, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of α-ionone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; α-Ionone

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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doi:10.1016/j.fct.2007.09.046

In 2006, a complete literature search was conducted on α-ionone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: 3-buten-2-one,4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-; α-cyclocitrylideneacetone, α-irisone, 4-(2,2,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one.
1.2 CAS Registry Number: 127-41-3.
1.3 EINECS Number: 204-841-6.
1.4 Formula: $C_{13}H_{20}O$.
1.5 Molecular weight: 192.3.
1.6 Council of Europe (2000): α-ionone was included by the Council of Europe in the list of substances granted A – may be used in foodstuffs (COE No. 141).
1.7 FDA: α-ionone was approved by the FDA as GRAS (21 CFR 172.515).
1.8 FEMA (1965): Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient – GRAS 3. (2594).
1.9 JECFA: Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 388) Group ADI 0–0.1 mg/kg for α- and β-ionone singly or in combination (JECFA, 1998).

2. Physical properties

2.1 Flash point: >200 °F; CC.
2.2 Boiling point: 250 °C.
2.3 Log K_{ow} (calculated): 4.29.
2.4 Vapor pressure (calculated): <0.001 mm Hg 20 °C.
2.5 Specific gravity: 0.930.

3. Usage (Table 1)

α-Ionone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 100–1000 metric tonnes per annum.

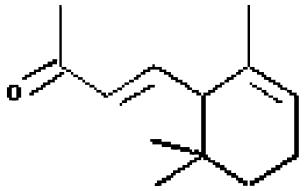

Fig. 1. α-Ionone.

The maximum skin level that results from the use of α-ionone in formulae that go into fine fragrances has been reported to be 1.00% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.50 percentile use level in formulae for use in cosmetics in general has been reported to be 2.01% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.05 mg/kg/day for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Male and female CF-1 mice (10/dose) weighing 17–25 g were orally administered α-ionone. The animals were observed for mortality over a 72-h period. The LD_{50} was calculated to be 6.66 ± 0.65 g/kg (RIFM, 1967).

4.1.1.2. Groups of mice (10/dose) were administered α-ionone by gavage. The animals were observed for mortality over a 10 day period. All deaths occurred within 24 h. The acute LD_{50} was calculated to be 7.0 g/kg (RIFM, 1980).

4.1.2. Intraperitoneal studies

4.1.2.1. As a part of micronucleus study, a pilot toxicity study was conducted on ICR mice. Two male mice received a single intraperitoneal injection (20 ml/kg) of 0.001, 0.01, 0.1 or 1.0 g/kg/body weight α-ionone, while five males and five females received 2.0 g/kg. Observations were made after administration and daily thereafter for 3 days. All animals died at 2.0 g/kg. Lethargy, piloerection and hunched position were observed in all males at 1.0 mg/kg. No effects were observed at 0.001, 0.01 and 0.1 g/kg (RIFM, 2006).

4.1.2.2. As a part of the same micronucleus study, a toxicity study was conducted in four groups of five male and five female ICR mice each. Each animal received a single intraperitoneal injection (2 ml/kg) of 1.2, 1.4, 1.6 or 1.8 g/kg α-ionone. Observations were made after dose administration and daily thereafter for 3 days. All animals died at 1.8 g/kg, 2/5 males and 3/5 females died at 1.6 g/kg, 2/5 males and all females died at 1.4 g/kg. Clinical signs included lethargy, piloerection, convulsions, hunched position and prostration. In addition, all mice at 1.4 g/kg had tremors. Based on these results, the high dose for the micronucleus test was set at 1.2 which was estimated to be the maximum tolerated dose (RIFM, 2006).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing α-ionone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product	Ingredient/mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	2.01	0.0076
Face cream	0.80	2.00	1.000	0.003	2.01	0.0016
Eau de toilette	0.75	1.00	1.000	0.080	2.01	0.0201
Fragrance cream	5.00	0.29	1.000	0.040	2.01	0.0194
Antiperspirant	0.50	1.00	1.000	0.010	2.01	0.0017
Shampoo	8.00	1.00	0.010	0.005	2.01	0.0001
Bath products	17.00	0.29	0.001	0.020	2.01	0.0000
Shower gel	5.00	1.07	0.010	0.012	2.01	0.0002
Toilet soap	0.80	6.00	0.010	0.015	2.01	0.0002
Hair spray	5.00	2.00	0.010	0.005	2.01	0.0002
Total						0.0512

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. A 48-h occluded patch test was conducted on the backs of 50 adult male volunteers. A 0.05 g aliquot of 32% α-ionone in acetone was placed on 15 mm patches which were then applied to the back of each subject for 48 h. The patches were removed and the sites were swabbed with dry gauze to remove residual test material. Reactions were evaluated at 30 min after patch removal and if necessary, at 72, 96 and 120 h after patch removal. α-ionone was found to be a moderate irritant (Motoyoshi et al., 1979).

4.2.2. Animal studies

4.2.2.1. Six Pitman-Moore improved strain miniature swine received a single dermal application of 50 mg of neat α-ionone on the clipped dorsal skin for 48 h under occlusion. After the 48-h exposure period, the patches were removed, and the reactions were then evaluated. No irritation was observed (Motoyoshi et al., 1979).

4.2.2.2. A 0.1 ml aliquot of neat α-ionone was applied to a 3 cm^2 area on the clipped dorsal skin of six male Hartley guinea pigs. Reactions were read 24 h after application. After the reading, the hair on the test areas was clipped again and the test material was applied 30 min later. A second set of readings and applications were made 48 h later. Following the 72-h reading, all the hair on the dorsal surface of each animal was clipped and 40 mg/kg of Evans blue dissolved in physiological saline was injected intravenously into each animal. α-Ionone was reported to be moderately irritating in guinea pigs (Motoyoshi et al., 1979).

Table 2
Summary of acute studies

Route	Species	No. animals/ dose group	LD_{50}	References
Oral	Mice	10	6.66 ± 0.65 g/kg	RIFM (1967)
Gavage	Mice	10	7.0 g/kg	RIFM (1980)

4.2.2.3. As a part of a modified Draize sensitization study, a preliminary irritation screen was conducted to determine the injection challenge concentration (ICC). Four inbred Hartley strain albino guinea pigs received intradermal injections to the shaved flanks with 0.1 ml aliquots of α-ionone at a range of concentrations. Reactions were read 24 h later. A concentration of 0.1% α-ionone (vehicle not reported) produced a slight but perceptible irritation and was selected as the ICC (Sharp, 1978).

4.2.2.4. As a part of a modified Draize sensitization test, a preliminary irritation screen was conducted to determine the application challenge concentration (ACC) using 4 inbred Hartley strain albino guinea pigs of the same sex. Different concentrations of α-ionone in aliquots of 0.1 ml (vehicle not reported) were applied to shaved flanks. Reactions were read 24 h later and the highest concentration causing no irritation was selected as the application challenge concentration. The ACC for α-ionone was found to be 30% (Sharp, 1978).

4.2.2.5. In a primary skin irritation test, 3 rabbits/dose received a single application of α-ionone on intact and abraded skin sites. Untreated skin on the same rabbit served as a control. Observations were made at 24 and 72 h. A dose of 5% α-ionone in DEP produced very slight erythema, at both intact and abraded skin sites, in 1/3 rabbits at 24 h, which cleared by 72 h. At 24 h, neat α-ionone produced very slight to well-defined erythema in all 3 rabbits at both intact and abraded skin sites (RIFM, 1967).

4.2.2.6. A closed primary skin irritation test was conducted in albino Angora rabbits (6/group) weighing 2.3–3.0 kg. A 0.1 g of neat α-ionone was applied to the clipped dorsal skin of each animal. The animals were wrapped with a plastic collar around the neck for 24 h. The collar was then removed and reactions were read. After reading, hair from the test area was clipped again and α-ionone was applied

30 min later. A second set of readings and applications was made 48 h later. After the 72 h reading, all hair on the dorsal surface of each animal was clipped. Each animal was injected intravenously with 40 mg/kg Evans blue dissolved in normal saline. One hour after the injection, the animals were killed by exanguination and the dorsal skin was removed. The dilating rate of blood vessels, swelling rate, (edema), blueing rate (as a result of increased capillary permeability) and reddening rate (erythema) on the test site were observed. Total scores of the living skin and isolated skin were referred to as the primary irritation index. α-Ionone produced severe irritation reaction (Motoyoshi et al., 1979).

4.3. Mucous membrane (eye) irritation (Table 3)

4.3.1. Animal studies

4.3.1.1. An eye irritation test was conducted in three rabbits. A 0.1 ml aliquot of 100% and 5% α-ionone in DEP was instilled into one eye of each of three rabbits with no further treatment. The untreated eye served as a control. Observations for irritation were made immediately after instillation and again at 1, 2, 4, 24, 48 and 72 h after instillation. Very slight conjunctival irritation was observed in all three rabbits at instillation, which cleared by 24 h (RIFM, 1967).

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. In a multicenter study, Frosch et al. (1995) reported the results of patch tests with 48 fragrance materials. α-Ionone at 1% and 5% in petrolatum was tested in 86 male and 119 female patients. α-Ionone was applied to the back for 48 h using Finn chambers® on Scanpor tape®. Reactions were assessed per ICDRG guidelines on days 2 and 3 or on days 2 and 4. No reactions were observed with 1% α-ionone; 5% α-ionone produced one irritant/questionable reaction.

4.4.2. Animal studies

4.4.2.1. The sensitization potential of α-ionone was evaluated in a guinea pig sensitization study using a modified Draize procedure. Ten male and female inbred Hartley strain albino guinea pigs/group with an average weight of 350 g were shaved on both flanks. A 0.1 ml aliquot of 0.25% α-ionone (vehicle not reported) was injected intradermally at 4 sites that overlap the 2 auxiliary and 2 inguinal lymph nodes. The guinea pigs were challenged 14 days later by an intradermal injection of 0.1% α-ionone (vehicle not reported) into one flank and a topical open application of 30% α-ionone (vehicle not reported) on the other flank. Reactions were scored 24 h after challenge treatments. A second challenge was carried out 7 days later. If no sensitization reactions were observed, the test was repeated. No reactions were observed (Sharp, 1978).

Table 3
Summary of eye irritation studies

Dose (%)	Vehicle	Results	References
100	N/A	No irritation	RIFM (1967)
5	DEP	No irritation	RIFM (1967)

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

4.6.1. Metabolism

4.6.1.1. Two rabbits were fed a single dose of 170 g of pure α-ionone. Urine was collected and ether soluble compounds were extracted. The major urinary metabolite was identified as 5-oxo-*cis*-tetrahydroionone (no other details reported) (Prelog et al., 1951).

4.7. Subchronic toxicity

4.7.1

α-Ionone was tested in a 90-days oral toxicity study using male and female Sprague-Dawley rats (15/sex/dose). α-Ionone was incorporated into the diet of each animal such that the daily dose was 10 or 100 mg/kg/body-weight/day. Renal functions and hematological studies were performed mid way through the treatment period and at the end of the study. In addition, the following data was collected: body weights, food intakes, water intakes and organ weights. Each animal was sacrificed after 90 days and a gross necropsy and histopathology was carried out. There were no mortalities and physical appearance and behavior were considered normal during the course of the study. Body weigh gains and feed intake were reduced slightly in both males and females in the high dose group. After six weeks, erythrocyte count and packed cell volumes showed a decrease in the 10 mg/kg male dose group. The males in the 100 mg/kg dose group showed an increase in neutrophil counts and a decrease in lymphocytes. These changes were not observed to persist to 13 weeks. Following water deprivation, the females in the high dose group produced a lower volume of concentrated urine when compared to controls. The males produced urine with a decrease in refractive index when compared to controls. The males of the high dose group exhibited a higher incidence of a minimal reaction for ketones in the urine. The females in the high dose group showed a decrease in serum glucose concentrations. The relative liver weights of both sexes were increased in the 10 mg/kg dose group. The NOAEL was considered to be 10 mg/kg body weight/day (RIFM, 1983).

4.7.2

Groups of 15 male and 15 female rats of the FDRL strain, weighing 75–85 g were fed diets containing α-ionone

in cotton seed oil at approximately 11 mg/kg/day (11.8 and 11.1 mg/kg/day in males and females, respectively) for 90 days. The dose selected was at least 100 times the maximum estimated human dietary intake level. Control group animals received the vehicle only. Hematological and blood chemistry determinations were made on 8 rats of each sex at six-weeks and in all rats at 12-weeks. All animals were sacrificed at 90 days and a gross necropsy was carried out. All major organs were collected including liver, kidneys, stomach, small and large intestines, spleen, pancreas, heart, lungs, bone marrow, muscle, brain, spinal cord, bladder, adrenals, thyroid, pituitary, gonads, salivary glands and lymph nodes, from half the animals in each group for histopathological examination. There were no adverse effects of α-ionone on body weight gain and food consumption. No effects were observed in hematology and blood chemistry parameters. Liver and kidney weights were not affected. There were no adverse effects on gross and microscopic appearance of major organs at necropsy (Oser et al., 1965; Bar and Griepentrog, 1967).

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

4.9.1. Mutagenicity

4.9.1.1. Oda et al. (1978) conducted a rec-assay in *Bacillus subtilis* strains H17 (rec+) and M45 (rec−) with α-ionone in DMSO. The 'no observed effect' concentration of α-ionone was 19 μg.

4.9.1.2. Mutagenic potency of 9 flavoring agents was evaluated in an Ames assay using *Salmonella typhimurium* strains TA98 and TA100 with and without aroclor 1254 induced rat liver S-9. α-ionone dissolved in dimethyl sulfoxide (DMSO) was added at concentrations of 0.01–50 μg/plate. No mutagenic effects due to α-ionone were observed (Kasamaki et al., 1982).

4.9.2. Genotoxicity

4.9.2.1. Genotoxicity of 9 flavor materials, including α-ionone, was evaluated using CH cell line B241 in culture stages between the 5th and 8th stages. One day after seeding, exponentially growing cells were exposed to each chemical in DMSO for 24 h. The cells were further incubated for another 24 h without the chemicals. α-ionone at 25 mM concentration caused significant increases in chromosome aberrations (Kasamaki et al., 1982).

4.9.2.2. A micronucleus test was conducted in seven groups (5/sex) of ICR mice. Mice in five groups received a single intraperitoneal injection of either the vehicle (corn oil) or α-ionone at dose of 0.3, 0.6 or 1.2 g/kg and were euthanized 24 h after treatment. Mice in other two groups were treated either with the vehicle or α-ionone at dose of 1.2 g/kg and were euthanized 48 h after treatment. Bone marrow cells (polychromatic erythrocytes) were collected 24 and 48 h after treatment and were examined microscopically for the presence of micronuclei. No statistically significant increase in the incidence of micronucleated polychromatic erythrocytes was observed (RIFM, 2006).

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

J. Lalko, A. Lapczynski, V.T. Politano, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on β-ionone

J. Lalko, A. Lapczynski *, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of β-ionone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; β-Ionone

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

In 2005, a complete literature search was conducted on β-ionone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

1. Identification (Fig. 1)

1.1 Synonyms: 3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-; β-cyclocitrylideneacetone; β-ionone; γ-irisone; 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one.
1.2 CAS Registry Number: 14901-07-6.
1.3 EINECS Number: 238-969-9.
1.4 Formula: $C_{13}H_{20}O$.
1.5 Molecular weight: 192.3.
1.6 COE: β-Ionone was included by the Council of Europe in the list of substances granted A – may be used in foodstuffs (COE No. 142).
1.7 FDA: β-Ionone was approved by the Food and Drug Administration as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and Extract Manufactures' Association states: Generally recognized as safe as a flavor ingredient – GRAS 3 (2595).
1.9 Joint FAO/WHO Expert Committee on Food Additives (JECFA): (JECFA No. 389) Group ADI 0–0.1 mg/kg for alpha and β-ionone singly or in combination (JECFA, 1998).

2. Physical properties

2.1 Log K_{ow} (calculated): 4.42.
2.2 Flash point: >200 °F; CC.
2.3 Vapor pressure (calculated): 0.006 mm Hg 20 °C.

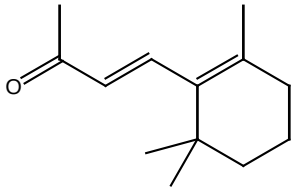

Fig. 1. β-Ionone.

3. Usage (Table 1)

β-Ionone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 100–1000 metric tones per annum.

The maximum skin level that results from the use of β-ionone in formulae that go into fine fragrances has been reported to be 2.34% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 4.34% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.11 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute LD_{50} of β-ionone in rats by the gavage route was calculated to be 7.12 g/kg within a 24-h period and 3.29 g/kg within a 10 day period. Animals received 5 daily administration of the test material. Observations were conducted up to 10 days (RIFM, 1980).

4.1.1.2. Male CFW mice (5/dose) weighing 17–22 g were dosed orally with β-ionone (vehicle not reported). The mice were observed for mortality for 72 h. The LD_{50} was calculated to be 5.33 ± 0.76 g/kg (RIFM, 1967a).

4.1.1.3. The acute LD_{50} of β-ionone in mice by the gavage route was calculated to be 2.0 g/kg (±3.2 g/kg). Animals received 5 daily administration of β-ionone. Observations were conducted up to 10 days (RIFM, 1980).

4.1.2. Intraperitoneal studies

4.1.2.1. The acute LD_{50} of β-ionone in mice was calculated to be 1.33 g/kg within a 24 h period, and 0.7 g/kg within a 10 day period. Ten animals per group were administered β-ionone by intraperitoneal injection daily for 5 days. Observations were conducted up to 10 days (RIFM, 1980).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing β-ionone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	4.34	0.0164
Face cream	0.80	2.00	1.000	0.003	4.34	0.0035
Eau de toilette	0.75	1.00	1.000	0.080	4.34	0.0434
Fragrance cream	5.00	0.29	1.000	0.040	4.34	0.0420
Antiperspirant	0.50	1.00	1.000	0.010	4.34	0.0036
Shampoo	8.00	1.00	0.010	0.005	4.34	0.0003
Bath products	17.00	0.29	0.001	0.020	4.34	0.0001
Shower gel	5.00	1.07	0.010	0.012	4.34	0.0005
Toilet soap	0.80	6.00	0.010	0.015	4.34	0.0005
Hair spray	5.00	2.00	0.010	0.005	4.34	0.0004
Total						0.1106

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute studies

Route	Species	No. animals/ dose group	LD_{50}	References
Oral	Rat	10	7.12 g/kg ± 1.0 g/kg 3.29 g/kg ± 0.5 g/kg	RIFM (1980a)
Oral	Mice	5	5.33 g/kg ± 0.76 g/kg	RIFM (1967a)
Oral	Mice	10	2.0 g/kg ± 0.32 g/kg	RIFM (1980)

4.1.3. Inhalation studies

4.1.3.1. Acute toxicity of β-ionone in the rat olfactory bulb was investigated following inhalation exposure for 5 weeks. Four Wistar rats, approximately 2 weeks old, weighing 28–39 g were placed in cylindrical Lucite cages. Fresh air was blown into the cages through charcoal filters and molecular sieves. Air flow was maintained at about 0.6 l/s through each cage. β-Ionone was introduced into the air stream at the concentration of 1.6×10^{-9} M. Control animals were exposed to filtered fresh air only. The rats were sacrificed after 1 and 5 weeks of exposure. Distribution of selective changes in the mitral cells of olfactory bulbs was examined and compared to controls. β-Ionone exposure produced moderate degeneration in the median and lateral surfaces of the mitral cell layer (Pinching and Doving, 1974).

4.2. Skin irritation

4.2.1. Human studies

No data available on this material.

4.2.2. Animal studies

4.2.2.1. Three rabbits received a single dermal application of neat β-ionone or 5% β-ionone in diethyl phthalate on abraded and intact skin. Untreated skin of the same rabbits served as a control. The skin sites were observed 24 and 48 or 72 h after application for signs of irritation. Application of neat β-ionone produced very slight to well-defined erythema on the abraded and intact skin at 24 h and well defined erythema at 72 h. There was very slight edema on the abraded and intact skin of all 3 rabbits at 24 h, which returned to normal in 2 rabbits by 72 h. Application of 5% β-ionone in diethyl phthalate resulted in very slight to well defined erythema on abraded and intact skin of 2 rabbits at 24 h, which cleared by 72 h. There was very slight edema on abraded and intact skin of 2 rabbits at 24 h, which cleared by 72 h (RIFM, 1967b).

4.2.2.2. As a part of phototoxicity study, acute dermal irritation was assessed in 5 female Hartley albino guinea pigs. Hair on the backs of animals was clipped. Four-hours after depilation, β-ionone at 5%, 10%, 30%, or 50% in acetone was applied to a circle of 1.5 cm diameter to the depilated area on both sides of the animal. A total of 8 applications were made. Immediately after application, one side was covered with aluminum foil. The test sites were observed for reactions at 24 and 48 h. No irritation was observed (RIFM, 1999).

4.3. Mucous membrane (eye) irritation

4.3.1. Animal studies (Table 3)

4.3.1.1. Eye irritation in rabbits was evaluated according to Draize et al. (1944). Neat β-ionone or 5% β-ionone in diethyl phthalate (0.1 ml) was instilled into the conjunctival sac of one eye of three rabbits. The untreated eyes served as controls. Eyes were observed immediately after application and at 1, 2, 4, 24, 48, and 72 h after application for any reactions. Fluorescein was used to check corneal damage. Very slight conjunctival irritation was observed in all three rabbits at 0, 1, 2, and 4 h with neat β-ionone. With 5% very

Table 3
Summary of eye irritation studies

Dose (%)	Vehicle	Results	References
100	N/A	No irritation	RIFM (1967b)
5	Diethyl phthalate	No irritation	RIFM (1967b)

slight conjunctival redness was present in all three rabbits only at instillation. There were no other effects observed (RIFM, 1967b).

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. In a multicenter study, extending over a three-year period, dermatological patients were tested for their sensitivity to fragrance materials. Sensitization due to β-ionone was tested in 205 consecutive patients. Patch tests were performed with 1% and 5% β-ionone in petrolatum using Finn Chambers® on Scanpor® tape applied for two days on the back of each patient. Reactions were recorded according to ICDRG on days 2 and 3 or on days 2 and 4. β-Ionone at 1% produced no irritation or sensitization. At 5% concentration, 2 patients showed questionable positive irritation reactions, but there was no sensitization (Frosch et al., 1995).

4.4.2. Animal studies

4.4.2.1. Skin sensitization potential of β-ionone was evaluated using Magnusson and Kligman maximization test in female Hartley albino Guinea pigs. Backs of nine animals (5 test and 4 controls) were clipped free of hair with an electric hair clipper and an electric shaver. Four-hours after depilation, the animals were induced intradermally with 10% β-ionone in 1:1 mixture of Freund's complete adjuvant (FCA) and physiological saline. Topical induction was carried out with 10% β-ionone in FCA. The animals were topically challenged with 5%, 10%, 20%, or 40% β-ionone in acetone (no additional details provided). Reactions were scored 48 h after challenge. No evidence of sensitization was observed. The test material was classified as a non-sensitizer (RIFM, 1999).

4.5. Phototoxicity and photoallergy

UV spectra revealed that β-ionone peaked within 285–295 nm range and showed minor absorption in the 300–340 nm region.

4.5.1. Human studies

No data available on this material.

4.5.2. Animal studies

4.5.2.1. Five female Hartley guinea pigs were clipped free of hair with an electric hair clipper and an electric shaver. Four hours after depilation, β-ionone at concentrations of 5%, 10%, 30% or 50% in acetone was applied on a circle of 1.5 cm in diameter in the depilated area on the right and left sides of the animal. Immediately after application, one side was covered with aluminum foil. The other side was irradiated with a bank of six ultraviolet lights (model FL-40 BLB lamps 40 watt tubes, emission 320–400 nm). The distance from the UV light source to the skin was 10 cm. Irradiation was continued for 70 min. The test sites were observed for reaction at 24 and 48 h after irradiation. There was no evidence of irritation with or without UV-A radiation at any of the doses. β-Ionone was considered to be a non-phototoxic to guinea pig skin (RIFM, 1999).

4.6. Absorption, distribution and metabolism

4.6.1. Distribution

4.6.1.1. As a part of a study to evaluate the effects of fragrance compounds on motility of mice, blood levels were measured after inhalation exposure. Female outbred Swiss mice were housed in groups of four under standardized conditions. Mice were exposed to individual compounds by inhalation for a period of 1 h. Air was passed into the cage through a glass tube containing the test material. Following exposure, blood samples were collected at 0, 30, 60, and 90 min of inhalation and analyzed using GC-MS. β-Ionone was detected only in trace levels (<0.1 ng/ml) (Buchbauer et al., 1993).

4.6.2. Metabolism

4.6.2.1. β-Ionone in aqueous suspension with gum Arabic was administered by stomach tube to a male albino rabbit weighing about 3.0 kg at a dose of 1 g/kg/day for seven days. The total administered dose was 23 g. Urine was collected everyday during the dosing period and for 4 days after the last dose. Urine samples were extracted and analyzed for β-ionone and its metabolites. The results showed that there were five free metabolites in urine. These included 3-oxo-β-ionone, 3-oxo-β-ionol, dihydro-3-oxo-β-ionol, 3-hydroxy-β-ionol and unchanged β-ionone. Additionally, two glucuronides of β-ionol were detected. Glucuronides were hydrolyzed with β-glucuronidase and were identified as the glucuronides of 3-oxo-β-ionol and dihydro-3-oxo-β-ionol (Ide and Toki, 1970).

4.6.2.2. Bielig and Hayasida (1940) fed β-ionone to three rabbits in daily increasing doses of 2–5 g with a total dose of about 30 g in one week. In another test, feeding continued for two weeks in daily doses of 4 g, which increased to 5 g towards the end. In this schedule, the dose was not administered on some days. Urine was collected from all animals and analyzed for the presence of metabolites. The metabolites identified included 3-oxo-β-ionone, β-ionol, dihydro-β-ionol, oxy-β-ionol, oxy-dihydro-β-ionol, and oxy-dihydro-β-ionone. Tetrahydro derivatives and multiple unsaturated products formed by dehydrogenation were not seen. Two separate feeding tests conducted in the Spring and in the Fall showed that conversion products of β-ionone which are hydrogenated to the -hydroxyl and -carbonyl groups were excreted in the Spring but not in the Fall.

4.6.2.3. Prelog and Meier (1950) studied metabolism of β-ionone in two canines. The animals were fed 100 g of pure β-ionone in the course of 18 days. During the study period 2840 ml of urine was collected. Various metabolites of β-

ionone were extracted from urine under acidic, basic, and neutral conditions by solvent extraction. Different fractions obtained were chromatographed and purified. Possible metabolites of β-ionone were identified and included 4-oxo-β-ionone and possibly 4-oxo-β-ionol. The position of oxygen introduced via biochemical oxidation was determined by conversion of both compounds into 4-oxo-tetrahydroionone. In addition to the two ketones, the urine from canines fed β-ionone also contained 4-oxy-β-ionols.

4.6.2.4. Longenecker et al. (1939) reported that feeding β-ionone to rats led to increased excretion of ascorbic acid in urine. Groups of rats (number/group not mentioned) were administered various compounds at different concentrations, each mixed with 1 ml of cotton seed oil and fed daily with 30–35 ml evaporated milk. β-Ionone was given at doses of 50 mg/kg/day and 100 mg/kg/day. Control animals received cottonseed oil and evaporated milk alone. Urine was collected for eight days and average excretion of ascorbic acid was determined. β-Ionone administration resulted in significantly elevated excretion of ascorbic acid in urine.

4.7. Subchronic toxicity

4.7.1. Oral studies

4.7.1.1. An oral subchronic test was conducted on 15 male and 15 female rats per dose level, with 60 controls rats (30 per sex). The animals were fed β-ionone for 90 days (13 weeks) in the diet such that the daily dose was 10 or 100 mg/kg/bodyweight. Observations for growth, physical appearance and behavior were made weekly. Hematological studies were conducted at 6 and 13 weeks, while urinalysis and renal function analysis were conducted at 5 and 12 weeks (males) or 13 weeks (females). Gross necropsy and histopathology examination was conducted when the animals were sacrificed after 90 days. No deaths occurred and physical appearance and behavior were normal during the treatment period. At 10 mg/kg significant increase in the refractive indices was observed at 0–2 h after water deprivation at week 12 in the males, with a non-significant decrease in the volume of concentrated urine. At week 5, a significant dose-related trend in the number of male rats with reaction for ketones in the urine was observed. At week 13, the males exhibited a significant decrease in serum glutamic pyruvic transaminase activity. The relative liver weights in females and males, expressed as mg/100 g bodyweight, were significantly higher than the controls. Histopathological changes were only found in the liver, but not at a significantly higher rate than the controls. In addition, the relative brain, liver, kidney and cecum weights in females were also significantly higher than the controls. Compared to the controls at 100 mg/kg, the bodyweights in females were significantly decreased. The mean food intake in both males and females was significantly decreased. At week 6, the packed cell volume and erythrocytes were significantly reduced in the males. At week 5 in the females, a significant increase in the refractive indices was observed at 0–2 h after water deprivation, with a non-significant decrease in the volume of concentrated urine. Based on the results the no-observe-effect level was 10 mg/kg/day (RIFM, 1983).

4.7.1.2. An oral 90-day subchronic study was conducted on 15 male and 15 female rats. β-Ionone at dose levels of 11.6 and 13.1 mg/kg/bodyweight/day in cotton-seed oil, for the males and females respectively, was administered ad libitum to the animals in the diet. Observations were made for growth and food consumption, and hematological and blood chemistry evaluations were made in 16 rats (8 per sex) at 6 weeks and in all rats at 12 weeks. Upon sacrifice at day 90, a gross necropsy was conducted. Liver and kidney weights were recorded and histological examinations of certain tissues were made in half of the animals from each dose group. A few animals and their corresponding controls exhibited a slight degree of reactive lymphatic hyperplasia, however, these were considered occasional aberrations and not dose related. No other effects were observed (Oser et al., 1965; Bar and Griepentrog, 1967).

4.8. Reproductive and developmental toxicity

4.8.1

The teratogenic potency of β-ionone was evaluated in timed pregnant LHK:LVG (SYR) hamsters weighing 99–183 g. A single oral dose of β-ionone at 48 (6 animals), 240 (9 animals), or 480 mg/kg (14 animals) in Tween 20: acetone (95:5) was administered on day 8 of pregnancy at a volume of 0.5 ml/kg body weight. Twenty animals served as controls and received vehicle alone. The animals were sacrificed on day 14 of pregnancy. The pregnant uteri were collected after laparotomy. Number of resorptions and dead fetuses were recorded. Living fetuses were examined for any malformations. Abnormal litters were considered those, which contained one or more malformed fetuses or three or more resorbed implantation sites. There were no clinical signs of toxicity due to β-ionone. There was no significant effect on maternal weight gain, incidence of abnormal litters, or mean litter fetal body weight (Willhite, 1986).

4.8.2

The reproductive and embryotoxic effect of β-ionone was evaluated in pregnant Wistar rats. Animals were administered a single dose of β-ionone dissolved in corn oil by gavage on pregnancy day 11, at dose levels of 250, 500, 750 and 1000 mg/kg. Animals were weighed on days 0, 11, and 21 of pregnancy, and sacrificed on day 21. At sacrifice, the uterus was weighed with its contents and the number of living and dead fetuses, implantation sites, and resorptions were recorded. In addition, the living fetuses were weighed and examined for externally visible anomalies with scoring from 0 (absence) to 4 (severe). With 250, 500 and 750 mg/kg, no effects were produced. Compared to the untreated controls, the uterus weight, the ratio

of resorptions per implantations and the percentage of resorptions per implantation per litter were substantially increased, and the ratio of live fetuses per implantations per litter was drastically decreased with 1000 mg/kg (Gomes-Carneiro et al., 2003).

4.9. Mutagenicity and genotoxicity

4.9.1. Mutagenicity

4.9.1.1. Mutagenic activity of tobacco smoke constituents was evaluated by Ames assay using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 with and without S-9 from aroclor induced rats. β-Ionone in ethanol was tested at 3 μmol/plate. There was no evidence of mutagenicity in any of the strains (Florin et al., 1980).

4.9.1.2. The *Salmonella* preincubation assay was conducted with and without S9 activation in *S. typhimurium* strains TA1535, TA98, TA100 and in either TA97 or TA1537. β-Ionone was tested at various doses ranging from 1–180 ug/plate in either DMSO, water, ethanol or acetone. There was no evidence of mutagenicity (Mortelmans et al., 1986).

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

J. Lalko, A. Lapczynski, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on *trans*-β-ionone

J. Lalko, A. Lapczynski *, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of *trans*-β-Ionone when used as a fragrance ingredient is presented.

Keywords: Fragrance; Review; *trans*-β-Ionone

Contents

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

In 2005, a complete literature search was conducted on *trans*-β-ionone. On-line databases that were surveyed included chemical abstract services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

1. Identification (Fig. 1)

1.1 Synonyms: 3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, (E)- ; (E)-β-Ionone;. β-Ionone; trans-β-Ionone; (E)-4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one.

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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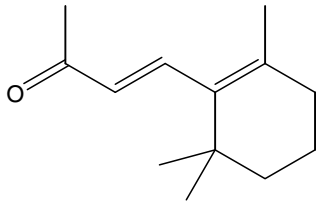

Fig. 1. (E)-β-Ionone.

1.2 CAS registry number: 79-77-6.
1.3 EINECS number: 201-224-3.
1.4 Formula: $C_{13}H_{20}O$.
1.5 Molecular weight: 192.02.

2. Physical properties

2.1 Log_{Kow} (calculated): 4.42.

3. Usage

Trans-β-Ionone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 10–100 metric tonnes per annum.

The maximum skin level that results from the use of *trans*-β-ionone in formulae that go into fine fragrances has been reported to be 1.46% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5%ile use level in formulae for use in cosmetics in general has been reported to be 3.11% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.08 mg/kg for high end users of these products (see Table 1).

4. Toxicology data

4.1. Acute toxicity

No data available on this material.

4.2. Skin irritation

No data available on this material.

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

No data available on this material.

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual fragrance material review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing *trans*-β-ionone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	3.11	0.0118
Face cream	0.80	2.00	1.000	0.003	3.11	0.0025
Eau de toilette	0.75	1.00	1.000	0.080	3.11	0.0311
Fragrance cream	5.00	0.29	1.000	0.040	3.11	0.0301
Antiperspirant	0.50	1.00	1.000	0.010	3.11	0.0026
Shampoo	8.00	1.00	0.010	0.005	3.11	0.0002
Bath products	17.00	0.29	0.001	0.020	3.11	0.0001
Shower gel	5.00	1.07	0.010	0.012	3.11	0.0003
Toilet soap	0.80	6.00	0.010	0.015	3.11	0.0004
Hair spray	5.00	2.00	0.010	0.005	3.11	0.0003
Total						**0.0792**

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

J. Lalko, A. Lapczynski, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on ionone

J. Lalko, A. Lapczynski *, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of ionone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Ionone

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* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on ionone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Cyclocitrylidenacetone α and β isomers; Ionone; α and β-Ionone; Ionone (mixed isomers).
1.2 CAS Registry Number: 8013–90–9.
1.3 EINECS Number: 232-396-8.
1.4 Formula: $C_{13}H_{20}O$.
1.5 Molecular weight: 192.3.

2. Physical properties

2.1 Flash point: >200 F; CC.
2.2 Boiling point: 123 °C at 11 mm Hg.
2.3 Log K_{ow} (calculated): 4.42.
2.4 Vapor pressure (calculated): 0.007 mm Hg at 20 °C.
2.5 Specific gravity: 0.93 g/mL.

3. Usage

Ionone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 100–1000 metric tonnes per annum.

The maximum skin level that results from the use of ionone in formulae that go into fine fragrances has been reported to be 1.57% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5% use level in formulae for use in cosmetics in general has been reported to be 3.0% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.08 mg/kg for high end users of these products (see Table 1).

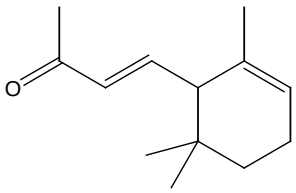

Fig. 1. Ionone.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Groups of 5 male and 5 female Osborne-Mendel rats were fasted for approximately 18 h. Undiluted ionone (60% alpha and 40% beta isomers) at different doses was administered by gavage. All animals were observed for toxic signs and mortality for two weeks. The LD_{50} was calculated to be 4.6 g/kg (95% CI 3.9–5.4 g/kg). Major toxic signs included depression and tremors. Deaths occurred between 4 h and 4 days (Jenner et al., 1964; Bar and Griepentrog, 1967).

4.1.1.2. An acute oral (gavage) study was conducted on 10 mice (5 male and 5 female). Animals were divided into three groups as follows: 1 male and 1 female in the 10.0 g/kg body weight group; 3 males and 3 females in the 5.0 g/kg body weight group; 1 male and 1 female in the 2.0 g/kg body weight group. Mortality and toxicity signs were observed for up to 7 days. No deaths were observed at 2 and 5 g/kg; one animal (1/2) died at 10 g/kg. Clinical signs included stress, laboured breathing, uncoordinated movement, hypothermia, lacrimation and bloated stomach. Necropsy of the animal that died revealed gross gaseous distension of the stomach and intestines and a pale mottled liver. Necropsy of the surviving animals revealed slight thickening of the cardiac region of the stomach and areas of bright red tissue in the lungs of animals at 5 and 10 g/kg. The acute oral LD_{50} was reported to be 10 g/kg (RIFM, 1980).

4.1.2. Intraperitoneal studies

4.1.2.1. Sporn et al. (1963) evaluated the acute toxicity of ionone in 620 white mice divided in three lots. Groups of mice weighing 15–18 g were given intraperitoneal injections of 1 ml of an oily solution of ionone at a range of concentrations. Mortality was recorded over a period of 7 days. The intraperitoneal LD_{50} was calculated to be 2.3 g/kg.

4.1.3. Subcutaneous studies

4.1.3.1. Groups of 10 male albino mice weighing 18–25 g were given subcutaneous injections of ionone (mixture of alpha- and beta-isomers) at a range of concentrations in sesame oil. General toxic signs included extreme excitement followed in rapid order by convulsions, respiratory depression and death. The LD_{50} was calculated to be 2.6 g/kg (95% CI 2.1–3.2 g/kg) (Wenzel and Ross, 1957).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. A 24-h closed patch test was conducted in adult male and female volunteers. Ionone was applied undiluted to an area of about 1 cm in diameter on the dermis of the

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing ionone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	3.0	0.0114
Face cream	0.80	2.00	1.000	0.003	3.0	0.0024
Eau de toilette	0.75	1.00	1.000	0.080	3.0	0.0300
Fragrance cream	5.00	0.29	1.000	0.040	3.0	0.0290
Antiperspirant	0.50	1.00	1.000	0.010	3.0	0.0025
Shampoo	8.00	1.00	0.010	0.005	3.0	0.0002
Bath products	17.00	0.29	0.001	0.020	3.0	0.0000
Shower gel	5.00	1.07	0.010	0.012	3.0	0.0003
Toilet soap	0.80	6.00	0.010	0.015	3.0	0.0004
Hair spray	5.00	2.00	0.010	0.005	3.0	0.0003
Total						*0.0764*

[a] Upper 97.5% levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	No. animals/ dose group	LD_{50}	References
Oral	Rat	10	4.6 g/kg	Jenner et al. (1964), Bar and Griepentrog (1967)
Oral	Mice	10	10 g/kg	RIFM (1980)
Subcutaneous	Mice	10	2.6 g/kg	Wenzel and Ross (1957)
Intraperitoneal	Mice	620	2.3 g/kg	Sporn et al. (1963)

inner portion of the lower arm. Immediately following application, the area was covered with an adhesive bandage. The tests were read at 24-h intervals for 5 days. No irritation was observed (Katz, 1946).

4.2.1.2. A 24–72 h patch test was conducted on 29 healthy male and female volunteers. Ionone at a concentration of 20% (in vaselinum aldum or unguentum hydrophilicum) was applied to the back of each volunteer. No reactions were observed. No reactions were also observed, when ionone at 2% (in unguentum simplex or unguentum hydrophilicum) was applied to the upper inside arm of 30 healthy volunteers or when 0.2% (in 99% ethanol or non-irritative cream base) was applied to the upper inside arm of 42 dermatoses patients (no additional details available) (Fujii et al., 1972).

4.2.2. Animal studies

4.2.2.1. A 4 h semi-occlusive patch test was conducted in 8 New Zealand rabbits. The dorsal region of each animal was clipped 3–4 days before the beginning of the study. A 0.5 ml aliquot of neat ionone was applied to a gauze pad which was attached to strips of adhesive tape and was then placed on the clipped dorsum of each animal. After removal of the patches the sites were wiped clean of excess test material. Reactions were graded immediately after patch removal and 24, 48 and 72 h after patch removal. Irritation was observed in all animals (RIFM, 1979).

4.2.2.2. As a part of an associated phototoxicity study irritation was evaluated in groups of 5 albino Wistar rats. A 0.1 ml aliquot of 10 or 30 ionone in ethanol or 100% ionone was applied to the clipped dorsal skin of each animal for 20 min. Excess test material was then wiped off with a cotton wool ball moistened with ethanol. Reactions were read 3, 6, 24, 48 and 72 h after treatment. Very slight erythema and edema was observed at all concentrations in all animals (RIFM, 1981).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. Ionone (75% alpha and 25% beta) was evaluated in Kligman maximization test conducted on 25 healthy adult volunteers. Ionone was applied under occlusion to the same site on the forearms of all subjects for 5 alternate-day 48 h periods. Patch sites were pre-treated for 24 h with 1 ml of 5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a 10 day rest period, a challenge patch of 8% test material was applied to a fresh site on the scapular back for 48 h under occlusion. Prior to challenge, 10% SLS was applied to the test site for 1 h before application of ionone. The challenge site was read at patch removal and again on each of two successive days. Ionone did not produce any sensitization reactions (Greif, 1967).

4.4.2. Animal studies

4.4.2.1. An open epicutaneous test (OET) was conducted to determine the sensitization potential of ionone. A 0.1 ml aliquot of ionone (concentration not reported) was applied to an 8 cm^2 area on the clipped flank skin of 6–8/group Himalayan white spotted guinea pigs weighing 400–500 g. The applications were repeated daily for 21 days on the same skin site. The application site was left uncovered and the reactions were read at 24 h after each application.

Challenge was conducted on days 21 and 35 by applying a 0.025 ml aliquot of ionone to skin areas measuring 2 cm^2 on the contralateral flank of all test animals as well as 6–8 untreated controls. Reactions were read at 24, 48 and 72 h. Ionone did not produce any sensitization reactions (Klecak et al., 1977).

4.4.2.2. A guinea pig open epicutaneous test (OET) was conducted on groups of 6–8 male and female guinea pigs weighting 300–450 g. An open application of a 0.1 ml aliquot of ionone (vehicle not specified) was applied daily to a clipped 8-cm^2 area on the flank of each guinea pig. Reactions were read 24 h after each application. A total of 21 open applications were made over a 21 day period. The 10 controls were either left untreated or treated with 0.1 ml aliquot of the vehicle for 21 days. At the challenge phase, both the test and control animals were treated on days 21 and 35 on the contralateral flank with 8% ionone. No sensitization was observed (Klecak, 1979, 1985).

4.4.2.3. A Draize test was conducted on groups of 6–8 male and female outbred Himalayan white-spotted guinea pigs. A dose of 0.05 ml of 0.1% solution of ionone in isotonic saline was injected intradermally on day 0 and further doses of 0.1 ml each were injected on 9 alternate days. The total dose injected was 0.95 mg. The treated animals and 6–8 untreated controls were challenged intradermally with 0.05 ml of 0.1% ionone on days 35 and 49. No sensitization was observed (Klecak et al., 1977).

4.4.2.4. A guinea pig maximization test was conducted on groups of 6–8 Himalayan white-spotted male and female guinea pigs. On day 0, the animals were injected intradermally with 0.1 ml of a 5% solution of ionone, 0.1 ml of a 5% emulsion of ionone in Freund's complete adjuvant (FCA) and 0.1 ml of FCA alone. Each injection was given twice. In addition, 250 mg of ionone dissolved in petrolatum at a concentration of 25% was applied on day 8 to a clipped area of the neck and was kept under occlusive bandage for 48 h. On day 21, ionone at a sub-irritant concentration in petrolatum was applied to the flank for 24 h under occlusion. Reactions were read at 24 and 48 h after removal of the patch. No sensitization was observed (Klecak et al., 1977).

4.4.2.5. Ishihara et al. (1986) conducted a guinea pig maximization test using the procedure of Magnusson and Kligman (1969). Induction and challenge were conducted with 10% ionone (vehicle not reported). No sensitization was reported (No further details provided).

4.4.2.6. To test the sensitization potential of ionone, a Freund's complete adjuvant test (FCAT) was conducted on groups of 6–8 male and female outbred Himalayan white spotted guinea pigs. A 0.05 aliquot ml of ionone was mixed with the same volume of FCA (50:50) and was then injected intradermally into the neck on days 0, 2, 4, 7, and 9. Control animals were similarly treated with FCA alone. Challenge was conducted on days 21 and 35 via a 24-h occlusive patch on the flank at a sub-irritant concentration in petrolatum. Reactions were read at 24 and 48 h after patch removal. No sensitization was observed (Klecak et al., 1977).

4.5. Phototoxicity and photoallergy

UV spectra revealed that ionone peaked within 290–295 nm range and showed minor absorption in the 300–320 nm region.

A phototoxicity test was conducted in three groups of 10 albino Wistar rats (5/sex/group). In group A, a 0.1 ml aliquot of neat ionone was applied to the clipped dorsum of each animal. The rats were left for 20 min and then any excess ionone was removed using a swab moistened with ethanol. Next, the animals were exposed to 12 J/cm^2 UV light from fluorescent black lamps (Philips TL40W/08, 300–400 nm) for 2.5 h. In group B, the animals were tested with ionone in exactly the same way as the animals from group A, but they were not exposed to UV light. In group C, the animals were first exposed to UV light and then treated with the test material under the same conditions as for group A. The test sites were examined immediately after irradiation and at 3, 6, 24, 48 and 75 h after treatment. No phototoxicity was observed (RIFM (1981)).

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

Groups of 10 male and 10 female Osborne-Mendel rats were given ionone (60% alpha, 40% beta) in the diet at 1000 ppm, 2500 ppm, or 10,000 ppm for 17 weeks (approximately equivalent to 50, 125, and 500 mg/kg body weight/day). As some of the test materials were quite volatile, they were examined over a 7-day period to determine the amount lost to evaporation; it was calculated that 1% of ionone was lost to evaporation. Body weight, food consumption, and general condition were recorded weekly. Hematological examination, which included white blood cell counts, red blood cell counts, hemoglobin, and hematocrit was conducted at the termination of the study. Tissues of all rats were examined macroscopically at the time of sacrifice. The viscera were removed and liver, kidneys, spleen, heart, and testes were weighed. These organs, the remaining abdominal and thoracic viscera, and one hind leg for bone, bone marrow, and muscle, were preserved for histopathological examination. Tissues from rats dying during the experiment were examined. Detailed microscopic examinations were generally done on 6 or 8 rats, evenly divided by sex, from the high-dose group and the control group. If changes attributable to ionone were found in the high dose group, additional animals at lower

dose levels would be examined. Histopathtology examination of the liver, revealed slight to moderate swelling of hepatocytes in high-dose animals, slight swelling in mid-dose animals, and very slight swelling of hepatocytes in the low-dose group. This hepatocellular swelling is presumably related to microsomal enzyme induction and not an "adverse" effect. No other effects were observed (Hagan et al., 1967; Bar and Griepentrog, 1967).

White rats (8/group) of the same age and weight were fed a synthetic diet containing 11% protein. The animals were administered 10 mg ionone (mixture of α- and β-ionone) dissolved in 0.1 ml oil on alternate days for 8 weeks. At the end of the study, the animals were sacrificed and liver homogenates were prepared. The activities of succinic dehydrogenase, aldolase, aspartic glutamic transaminase, alanine glutamic transaminase, DNA and nitrogen contents were measured. Enzyme activities were expressed in relation to the wet weight of the liver and in relation to liver nitrogen, DNA content and body weight. No effects were observed (Sporn and Dinu, 1964). Further studies were conducted on young white rats (8/group) of same weight and age. The animals were fed a synthetic diet containing 11% protein and wheat starch. Ionone was administered on alternate days at 10 mg dissolved in 0.1 ml oil for 8 weeks. After 3 weeks, when body weight had decreased by about 10 g, wheat starch was replaced by corn starch thus re-establishing normal conditions of growth. Animals were killed after 8 weeks and liver enzymes were measured as previously reported. At the end of the study, reduced body weights were observed in animals treated with ionone as compared to control animals, however, the differences were not statistically significant. No statistically significant differences were observed in absolute and relative liver weights in any of the treated animals. There were no differences in liver nitrogen when compared to controls. Liver DNA content was the same as that of the control group when the results were reported in relation to the wet liver weight or to hepatic nitrogen. When reported in relation to body weight, it was significantly higher in the treated group than in the control group. There was no effect on liver succinic dehydrogenase. Liver aldolase was significantly increased in the treated group when expressed in relation to liver wet weight, body weight, or liver DNA content but showed no effect when expressed in relation to liver nitrogen. The activity of glutamic-aspartate transaminase was decreased in the treated group when expressed in relation to liver wet weight and liver DNA content but not when expressed in relation to liver nitrogen or body weight. The activity of liver glutamic alanine transaminase was not affected by ionone administration (Sporn and Dinu, 1964).

The effect of ionone on the growth of rats was evaluated by Sporn et al. (1963). White rats (10/group) received a diet containing 19% protein as casein. In the first group, ionone (3 mg) was administered every two days as a 0.1 ml oil solution; the total dose administered during the entire 7 weeks study was 42 mg. In the second group, rats (8/group) received a diet containing 11% protein and 10 mg ionone every two days during a 8 week study; the total dose administered was 240 mg. In the third group, animals (8/group) received 11% protein but the carbohydrate intake included wheat starch, which was not well tolerated by rats causing lack of appetite, diarrhea and weigh loss. After 3 weeks, when the loss of weight was about 10 g, wheat starch was replaced by cornstarch. The growth rate returned to normal after carbohydrate replacement. These animals received 10 mg ionone every 2 days during an 8 week study period; total dose administered was 240 mg. When 3 or 10 mg ionone was administered every 2 days there were no effects on growth weight. There was no change in liver weight and no significant increase in liver nitrogen in any of the three experimental groups. Differential blood cell count measurement in rats of the first group receiving 19% protein and 3 mg ionone showed no adverse effect on any of the blood cell components at 4 and 8 weeks, as compared to control. Ascorbic acid content of the adrenal glands measured in this group of animals showed no significant changes as compared to control.

4.8. Reproductive and developmental toxicity

Sporn et al. (1963) studied the effects of ionone on reproduction of rats given 2 mg ionone in 0.1 ml oil solution on alternate days for 8 months (equivalent to a dose of approximately 8–10 mg/kg body weight/day). The female rats were studied through three reproduction cycles for number of pregnancies, weight, number of offspring, live pups, weight of pups at birth and after 7 and 21 days, and the viability of the pups after birth. The F1 generation (offspring) were allowed to reach maturity and treated with 15 mg/kg of ionone prior to being subject to reproductive toxicity testing. Their offspring, the F2 generation were evaluated for reproduction parameters. Ionone had no adverse effect on any of the parameters measured. Based on these data, no effects were observed for ionone at approximately 10 mg/kg body weight/day (Belsito et al., 2007).

4.9. Mutagenicity and genotoxicity

4.9.1. Mutagenicity

4.9.1.1. An Ames assay with *Salmonella typhimurium* strains TA100, TA1535, TA1538, TA98 and TA1537 was conducted with 0.001, 0.01, 0.1 and 1.0 μg/plate ionone in dimethyl sulfoxide (DMSO) with and without S-9 mix. No mutagenic effects were observed with 0.001 and 0.01 μg/plate. At 0.1 and 1.0 μg/plate, ionone was toxic to the bacteria (RIFM, 1980a).

4.9.1.2. The reverse mutation assay according to the plate incorporation test was conducted using *S. typhimurium* strains TA1535, TA1537, TA98, TA100 and TA102. The assay was performed in two independent experiments both with and without phenobarbital/beta-naphthoflavone induced rat liver microsomal activation (S9). Each

concentration, including the controls, was tested in triplicate. The ionone was tested at the following concentrations: 10, 33, 100, 333, 1000, 2500 and 5000 μg/plate in ethanol. Reduced background growth was observed in strain TA1537 at 1000 μg/plate and above with metabolic activation. Toxic effects, evident as a reduction in the number of revertants, were observed in strain TA102 with and without metabolic activation at 5000 μg/plate and in strains TA1537 (100–5000 μg/plate) and TA100 at 1000 μg/plate with metabolic activation. No substantial increase in revertant colony numbers of any of the five tester strains at any concentration level in the presence or absence of metabolic activation. Under the conditions of the study, ionone was considered to be non-mutagenic (RIFM, 2004).

4.9.1.3. Synthetic flavoring agents were tested for their genotoxic potential in the spore rec-assay using *Bacillus subtilis* strains M45 (rec−) and H17 (rec+). DNA damaging activity was measured by differences in growth inhibition zones. Ionone at the maximal dose of 20 μl/disk in dimethyl sufloxide (DMSO) was positive (no other doses were reported) (Yoo, 1986).

4.9.1.4. A mutation test using *Escherichia coli* WP2 uvrA (trp-) was conducted using 2.5–20 mg ionone/plate in DMSO. The mutation frequency of trp^+ revertants was measured. Ionone was not mutagenic in this test (Yoo, 1986).

4.9.2. Genotoxicity

4.9.2.1. The evaluation of genotoxic potential for some by-products of ozonation was reported by Ono et al. (1991). Genotoxicity was evaluated using a DNA repairing test (*umu*-test) using *S. typhimurium* strain TA1535/pSK1002 in the presence and absence of S9. Ionone (100 mg/l) produced positive effects at 2 h without S9.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

J. Lalko, A. Lapczynski, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on isodamascone

J. Lalko, A. Lapczynski *, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of isodamascone when used as a fragrance ingredient is presented.

Keywords: Fragrance; Review; Isodamascone

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on isodamascone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: 2-buten-1-one, 1-(2,4,4-trimethyl-2-cyclohexen-1-yl), Isodamascone (standard quality), 1-(2,4,4-trimethyl-2-cyclohexen-1-yl)-2-buten-1-one.
1.2 CAS Registry Number: 70266-48-7.
1.3 EINECS Number: no registration.
1.4 Formula: $C_{13}H_{20}O$.
1.5 Molecular weight: 192.02.
1.6 IFRA: Isodamascone has an International Fragrance Association Standard (IFRA, 2007) – see section 4.4.1. for details.

2. Physical properties

Log K_{ow} (calculated): 4.42.

3. Usage

Isodamascone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as non-cosmetic products such as household cleaners and detergents. Its use worldwide is not reported.

The maximum skin level that results from the use of isodamascone in formulae that go into fine fragrances has been reported to be 0.02% (IFRA, 2002), assuming use of the fragrance oil at levels up to 0.00% in the final product.

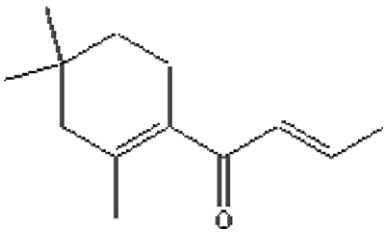

Fig. 1. Isodamascone.

The 97.5 percentile use level in formulae for use in cosmetics in general has not been reported. Such as the maximum skin level concentration of 0.02% was used to calculate the conservative calculated maximum daily exposure on the skin to be 0.005 mg/kg/day for a high end users of these products (Table 1).

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Male and female SPF-Wistar rats (5/sex/dose) with initial body weights between 145 and 210 g were dosed orally with 6.3, 7.94, 10 or 12.6 g/kg of isodamascone. Observations for mortality and/or systemic effects were made over a 14 day period. Gross necropsy was conducted on all animals. Clinical signs which were observed at all dose levels included decreased activity, irritability, abnormal gate and body posture, diarrhea, salivation and piloerection. Deaths occurred in all animals in the three highest dose groups by day 5; 8/10 deaths occurred at the lowest dose level, also by day 5. Necropsies of the animals that died during the study revealed redness of the gastro-intestinal mucous membrane. Gross observations at necropsy were normal for the two surviving animals. At 14 days, the LD_{50} was estimated to be 6.3 g/kg for all animals (RIFM, 1979a).

4.1.2. Dermal studies

No data available on this material.

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. Irritation was evaluated as a part of an associated human repeated insult patch test (HRIPT) in 65 (50 female and 15 male) healthy volunteers. During the induction phase, a 0.3 ml aliquot of 1% isodamascone in diethyl phthalate was applied under occlusion for 24 h using a 25 mm Hilltop Chamber® webril/adhesive patch. A series of nine, 24 h induction patches were completed on a Monday, Wednesday and Friday schedule over a period of three weeks. The test sites were observed after patch removal. Irritation was not observed with 1% isodamascone (RIFM, 1995).

4.2.2. Animal studies

4.2.2.1. As a part of a guinea pig maximization test (Magnusson and Kligman, 1969), a preliminary irritation screen was conducted using six male and female Pirbright white Bor:DHPW (SPF) guinea pigs. A closed patch of 25% or

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing isodamascone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product	Ingredient/mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.02	0.0001
Face cream	0.80	2.00	1.000	0.003	0.02	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.02	0.0002
Fragrance cream	5.00	0.29	1.000	0.040	0.02	0.0002
Antiperspirant	0.50	1.00	1.000	0.010	0.02	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.02	0.0000
Bath products	17.00	0.29	0.001	0.020	0.02	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.02	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.02	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.02	0.0000
Total						0.0005

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	No. of animals/dose group	LD_{50}	References
Oral	Rat	10 (5/sex)	6.3 g/kg	RIFM, 1979a

50% in peanut oil and 100% ionone was applied to two animals (1 sex/dose). Reactions were recorded 48 h after application. Irritation was not observed at 25% and 50%; at 100% slight to moderate erythema was observed (RIFM, 1991).

4.2.2.2. As a part of a guinea pig maximization test (Magnusson and Kligman, 1969), a preliminary irritation screen was conducted to determine the intradermal induction concentration. Pirbright white Bor:DHPW (SPF) guinea pigs (1/sex/dose) with initial body weights between 300 and 412 g received intradermal injections with 0.5%, 1%, 2.5% or 5% isodamascone in water and Freund's Complete Adjuvant. Reactions were read 48 h after injections. No irritation was observed at 0.5 and 1%; severe erythema with black discoloration at the injection sites was observed with 2.5% and 5%. Based on these results, a concentration of 1% was selected as the intradermal induction concentration (RIFM, 1991).

4.3. Mucous membrane (eye) irritation

4.3.1. Animal studies

4.3.1.1. An eye irritation test (Draize, 1959) was conducted in six New Zealand albino rabbits. A 0.1 ml aliquot of 1.5% isodamascone in petrolatum was instilled into the left eye of each rabbit with no further treatment. The untreated right eye of each rabbit served as the control. Observations were made at 1, 2 and 8 h and at 1, 2, 3, 4, 5, 6, 4 and 7 days after treatment. Slight conjunctival irritation with chemosis and discharge were observed in all rabbits. These effects cleared by 24 h in 4/6 rabbits and by day 2 in the remaining 2 rabbits. The primary irritation index was reported to be 1.0. Isodamascone was classified as non-irritating to the rabbit eye (RIFM, 1979).

4.4. Skin sensitization

4.4.1. Dermal sensitization quantitative risk assessment (QRA)

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This new methodology represents a significant change over current risk assessment practices because it specifically addresses the elements of exposure-based risk assessment that are unique to the induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment.

Full details of this risk assessment approach can be found in the "QRA Expert Group, Dermal Sensitization Quantitative Risk Assessment (QRA) for Fragrance Ingredients, Technical Dossier, revised June 22, 2006", and IFRA/RIFM Quantitative Risk Assessment (QRA) for Fragrance Ingredients Booklet, May 11, 2006," at http://www.rifm/org/pub/publications.asp and http://www.ifraorg.org/News.asp.

An exposure-based Quantitative Risk Assessment (QRA) methodology has been used to determine acceptable exposure limits for isodamascone and a new IFRA Standard (IFRA, 2007) has been issued (see Tables 3 and 4).

4.4.2. Human studies

4.4.2.1. A HRIPT study was conducted on 65 (50 female and 15 male) healthy volunteers using 1% isodamascone in DEP. Aliquots of 0.3 ml of isodamascone were applied to the left scapular area of each subject for 24 h under occlusion using 25 mm Hilltop Chambers® webril/adhesive patches. A series of 9, 24 h induction patches were completed on a Monday, Wednesday, Friday schedule over a period of three weeks. Following a two week rest period, a 24-h occluded challenge patch was applied to the right scapular area in the same way as in the induction phase.

Table 3
IFRA standard based on the QRA

Limits in the finished product:			
For a description of the categories, refer to the QRA information booklet			
Category 1 – see Note (1)	0.003%	Category 7	0.008%
Category 2	0.004%	Category 8	0.1%
Category 3	0.02%	Category 9	0.5%
Category 4	0.05%	Category 10	0.8%
Category 5	0.02%	Category 11 – see Note (2)	
Category 6 – see Note (1)	0.07%		

Note: The above limits apply to rose ketones used individually or in combination.
(1) IFRA would recommend that any material used to impart perfume or flavour in products intended for human ingestion should consist of ingredients that are in compliance with appropriate regulations for foods and food flavourings in the countries of planned distribution and, where these are lacking, with the recommendations laid down in the Code of Practice of IOFI (International Organisation of the Flavor Industry). Further information about IOFI can be found on its website (www.iofiorg.org).
(2) Category 11 includes all non-skin contact or incidental skin contact products. Due to negligible skin contact the concentration of a fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product. For example, hypothetically if the usual concentration of a fragrance compound in the final product, for example a candle, is at 5%, then any individual fragrance ingredient (in this case a 'rose ketone') must not exceed 5% in the candle.

The test sites were observed for reactions at 48, 72 and 96 h. No reactions were observed (RIFM, 1995).

4.4.3. Animal studies

4.4.3.1. Isodamascone was tested in a guinea pig maximization test (Magnusson and Kligman, 1969) in 20 male and female Pirbright white guinea pigs weighing 300–412 g. Induction consisted of two stages, intradermal injection followed one week later by a 48-h occluded patch application. A total of 6 intradermal injections were administered. They comprised of 2 injections of 0.1 ml of 50% Freund's Complete Adjuvant plus distilled water (1:1); 2 injections of 0.1 ml of a 1% solution of isodamascone in Freund's Complete Adjuvant in peanut oil; 2 injections of 0.1 ml of a 1% suspension of isodamascone in Freund's Complete Adjuvant and distilled water (1:1). The topical induction concentration was 100% isodamascone. Fourteen days after the topical induction application, guinea pigs were challenged on the shaved flank by a 24-h occluded application of 50% isodamascone in peanut oil. The treatment sites were examined for evidence of sensitization at 24 and 48 h after patch removal. No reactions were observed (RIFM, 1991).

4.5. Phototoxicity and photoallergy

4.5.1. Phototoxicity

4.5.1.1. Phototoxicity was evaluated during the induction phase of an associated photoallergy study in 20 Pirbright white guinea pigs weighing between 271 and 441 g. Isodamascone at 1.5% in petrolatum was applied to the shaved backs three times a week for three weeks. Following application, the test sites were irradiated with a weak erythema producing dose of UV (~320 nm). Reactions were scored according to Draize. One animal died during the induction phase but this was not considered to be treatment related. No phototoxic effects were observed in the remaining 19 animals (no further details provided (RIFM, 1979b).

4.5.2. Photoallergy

4.5.2.1. Photoallergy was evaluated in twenty Pirbright white guinea pigs weighing between 271 and 441 g. Isodamascone at a dose of 1.5% in petrolatum was applied to the shaved backs. Following application, the test sites were irradiated with a weak erythema producing dose of UV (~320 nm). Isodamascone was applied three times a week for three weeks. After a three week rest period, a single

Table 4
Summary of the relevant sensitization data for the implementation of the QRA

CAS no.	LLNA weighted mean EC3 values (μg/cm^2) [no. of studies]	Human data			Potency classification[b]	WoE NESIL (μg/cm^2)[c]
		NOEL – HRIPT (induction) (μg/cm^2)	Experimental NOEL – MAX (induction) (μg/cm^2)	LOEL[a] (induction) (μg/cm^2)		
57378-68-4	1579 [3]	NA	NA	1333	Moderate	100 [LLNA weighted mean for class = 1496 μg/cm^2]
43052-87-5	NA	133 (51)	0.2%	NA	Moderate	
24720-09-0	826 [1]	500 (DEP)	NA	NA	Moderate	
23696-85-7	308 [2]	100 (23)	NA	1000	Moderate	
23726-92-3	NA	67 (53)	NA	375	Moderate	
23726-91-2	600	1000 (pet/54)	NA	NA	Moderate	
23726-94-5	NA	NA	NA	NA	Moderate	
39872-57-6	NA	236 (DEP)	NA	2362	Moderate	
71048-82-3	NA	100 (24)	NA	1000	Moderate	
33673-71-1	NA	NA	NA	NA	Moderate	
70266-48-7	NA	1181 (DEP)	NA	NA	Moderate	

NOEL = no observed effect level; HRIPT = human repeat insult patch test; MAX = human maximization test; LOEL = lowest observed effect level; NA = not available.
[a] Data derived from HRIPT or HMT.
[b] Gerberick et al. (2001).
[c] WoE NESIL limited to two significant figures.

application of isodamascone was made to a site not previously exposed on the shaved back. The test sites were then irradiated with a UV dose lower than the erythema producing concentration (no further details reported). Reactions were scored according to Draize method at 2, 6 and 24 h after irradiation. The test sites were then depilated and scored again after 2, 6, 24 and 48 h. One animal died during the induction phase but this was not considered to be treatment related. Photoallergic effects were not observed in the remaining 19 animals (RIFM, 1979b).

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

J. Lalko, A. Lapczynski, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on isodamascone (isomer unspecified)

J. Lalko, A. Lapczynski *, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of isodamascone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Isodamascone

Contents

In 2006, a complete literature search was conducted on isodamascone. On-line databases that were surveyed included chemical abstract services and the national library of medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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1. Identification (Fig. 1)

1.1 Synonyms: 2-buten-1-one, 1-(2,4,4-trimethyl-2-cyclohexen-1-yl)-; 1-(2,4,4-trimethyl-2-cyclohexen-1-yl)-2-buten-1-one.
1.2 CAS registry number: 33673-71-1.
1.3 EINECS number: 251-63-0.
1.4 Formula: $C_{13}H_{20}O$.
1.5 Molecular weight: 192.3.
1.6 IFRA: isodamascone has an international fragrance association standard (IFRA, 2007) – see Section 4.4.1. for details.

2. Physical properties

2.1 Log K_{ow} (calculated): 4.29.
2.2 Vapor pressure (calculated): 0.0186 mm Hg 25 °C.

3. Usage

Isodamascone (isomer unspecified) is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of <0.01 metric tonnes per annum.

The maximum skin level that results from the use of isodamascone (isomer unspecified) in formulae that go into fine fragrances has been reported to be 0.0006% (IFRA, 2003), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has not been reported. As such the maximum skin level concentration of 0.02% was used to calculate the conservative calculated maximum daily exposure on the skin to be 0.005 mg/kg for high end users of these products (see Table 1).

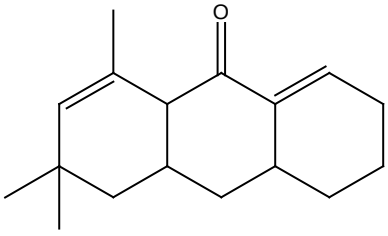

Fig. 1. Isodamascone (isomer unspecified).

4. Toxicology data

4.1. Acute toxicity

No data available on this material.

Table 2
IFRA standard based on the QRA

Limits in the finished product: For a description of the categories, refer to the QRA information booklet	
Category 1 – see Note (1) 0.003%	Category 7 0.008%
Category 2 0.004%	Category 8 0.1%
Category 3 0.02%	Category 9 0.5%
Category 4 0.05%	Category 10 0.8%
Category 5 0.02%	Category 11 – See Note (2)
Category 6 – see Note (1) 0.07%	

Note: The above limits apply to rose ketones used individually or in combination.
(1) IFRA would recommend that any material used to impart perfume or flavour in products intended for human ingestion should consist of ingredients that are in compliance with appropriate regulations for foods and food flavourings in the countries of planned distribution and, where these are lacking, with the recommendations laid down in the code of practice of IOFI (international organisation of the flavor industry). Further information about IOFI can be found on its website (http://www.iofiorg.org).
(2) Category 11 includes all non-skin contact or incidental skin contact products. Due to negligible skin contact the concentration of a fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product.
For example, hypothetically if the usual concentration of a fragrance compound in the final product, for example a candle, is at 5%, then any individual fragrance ingredient (in this case a 'rose ketone') must not exceed 5% in the candle.

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing isodamascone (isomer unspecified)

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.02	0.0001
Face cream	0.80	2.00	1.000	0.003	0.02	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.02.	0.0002
Fragrance cream	5.00	0.29	1.000	0.040	0.02	0.0002
Antiperspirant	0.50	1.00	1.000	0.010	0.02	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.02	0.0000
Bath products	17.00	0.29	0.001	0.020	0.02	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.02	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.02	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.02	0.0000
Total						0.0005

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.

Table 3
Summary of the relevant sensitization data for the implementation of the QRA

CAS no.	LLNA weighted mean EC3 values (μg/cm^2) [no. studies]	Human data			Potency classification[b]	WoE NESIL (μg/cm^2)[c]
		NOEL – HRIPT (induction) (μg/cm^2)	Experimental NOEL – MAX (induction) (μg/cm^2)	LOEL[a] (induction) (μg/cm^2)		
57378-68-4	1579 [3]	NA	NA	1333	Moderate	100 [LLNA weighted mean for class = 1496 μg/cm^2]
43052-87-5	NA	133 (51)	0.2%	NA	Moderate	
24720-09-0	826 [1]	500 (DEP)	NA	NA	Moderate	
23696-85-7	308 [2]	100 (23)	NA	1000	Moderate	
23726-92-3	NA	67 (53)	NA	375	Moderate	
23726-91-2	600	1000 (pet/54)	NA	NA	Moderate	
23726-94-5	NA	NA	NA	NA	Moderate	
39872-57-6	NA	236 (DEP)	NA	2362	Moderate	
71048-82-3	NA	100 (24)	NA	1000	Moderate	
33673-71-1	NA	NA	NA	NA	Moderate	
70266-48-7	NA	1181 (DEP)	NA	NA	Moderate	

NOEL = no observed effect level; HRIPT = human repeat insult patch test; MAX = human maximization test; LOEL = lowest observed effect level; NA = not available.
[a] Data derived from HRIPT or HMT.
[b] Gerberick et al. (2001).
[c] WoE NESIL limited to two significant figures.

4.2. Skin irritation

No data available on this material.

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Dermal sensitization quantitative risk assessment (QRA)

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This new methodology represents a significant change over current risk assessment practices because it specifically addresses the elements of exposure-based risk assessment that are unique to the induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment.

Full details of this risk assessment approach can be found in the "QRA expert group, dermal sensitization quantitative risk assessment (QRA) for fragrance ingredients, technical dossier, revised June 22, 2006", and IFRA/RIFM quantitative risk assessment (QRA) for fragrance ingredients booklet, May 11, 2006", at http://www.rifm/org/pub/publications.asp and http://www.ifraorg.org/News.asp.

An exposure-based quantitative risk assessment (QRA) methodology has been used to determine acceptable exposure limits for isodamascone and a new IFRA Standard (IFRA, 2007) has been issued (see Tables 1, 2 and 3).

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

J. Lalko, A. Lapczynski, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research

was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on α-isodamascone

A. Lapczynski *, J. Lalko, D. McGinty, S.P. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of α-isodamascone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; α-Isodamascone

Contents

In 2006, a complete literature search was conducted on α-isodamascone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: 2-Buten-1-one, 1-(2,4,4-trimethyl-2-cyclohexen-1-yl)-, (2E)-; 2-Buten-1-one, 1-(2,4,4-trimethyl-2-cyclohexen-1-yl)-, (E)-; (E)-1-(2,4,4-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one.
1.2 CAS Registry number: 39872-57-6.
1.3 EINECS number: 254-663-8.
1.4 Formula: $C_{13}H_{20}O$.
1.5 Molecular Weight: 192.02.
1.6 IFRA: Rose ketone has an International Fragrance Association Standard (IFRA, 2007) – see Section 4.4.1 for details.

2. Physical properties

2.1 Log K_{ow} (calculated): 4.29.
2.2 Molecular weight: 192.02.

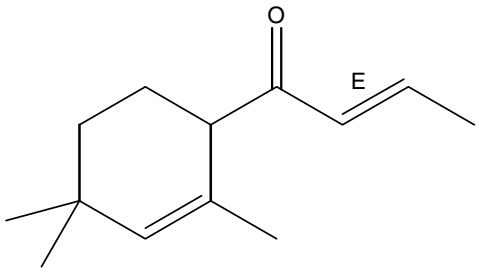

Fig. 1. α-Isodamascone.

3. Usage (Table 1)

α-Isodamascone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 0.1–1.0 metric tones per annum.

The maximum skin level that results from the use of α-isodamascone in formulae that go into fine fragrances has been reported to be 0.014% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.038% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.0010 mg/kg/day for a high end users of these products.

4. Toxicology data

4.1. Acute toxicity

No data available on this material.

4.2. Skin irritation (Table 2)

4.2.1. Human studies

4.2.1.1. Irritation was evaluated during the induction phase of a human repeated insult patch test (HRIPT). A 0.3 ml aliquot of 0.2% α-isodamascone in DEP was applied to 25 mm Hilltop Chambers® and allowed to volatilize up to 20 min. The patches were then applied under occlusion to the upper back of 103 male and female subjects. These patches were removed 24 h after application. A total of nine applications (3 times a week), to the same test site, were made over a three week period. No irritation was observed (RIFM, 1995).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing α-isodamascone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.038	0.0001
Face cream	0.80	2.00	1.000	0.003	0.038	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.038	0.0004
Fragrance cream	5.00	0.29	1.000	0.040	0.038	0.0004
Antiperspirant	0.50	1.00	1.000	0.010	0.038	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.038	0.0000
Bath products	17.00	0.29	0.001	0.020	0.038	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.038	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.038	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.038	0.0000
Total						0.0010

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60 kg adult.

Table 2
Summary of human irritation studies

Method	Dose (%)	Vehicle	Results	Reference
Induction phase (HRIPT)	0.2	DEP	No reactions (0/103)	RIFM (1995)
Induction phase (HRIPT)	2.0	DEP	Positive reaction (1/22)	RIFM (1994)

4.2.1.2. Irritation was evaluated in 22 female volunteers during the induction phase of a HIRPT study. A 0.3 ml aliquot of 2% α-isodamascone in diethyl phthalate was applied to a webril/adhesive patch (25 mm Hilltop Chambers®). A total of nine, 24 h occluded applications were made over a three-week period. Irritation was observed in one subject (RIFM, 1994).

4.2.2. Animal studies

No data available on this material.

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Dermal sensitization quantitative risk assessment (QRA)

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This new methodology represents a significant change over current risk assessment practices because it specifically addresses the elements of exposure-based risk assessment that are unique to the induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment.

Full details of this risk assessment approach can be found in the "QRA Expert Group, Dermal Sensitization Quantitative Risk Assessment (QRA) for Fragrance Ingredients, Technical Dossier, revised June 22, 2006", and IFRA/RIFM Quantitative Risk Assessment (QRA) for Fragrance Ingredients Booklet, May 11, 2006", at http://www.rifm/org/pub/publications.asp and http://www.ifraorg.org/news.asp.

An exposure-based Quantitative Risk Assessment (QRA) methodology has been used to determine acceptable exposure limits for α-rose ketone and a new IFRA Standard (IFRA, 2007) has been issued (see Tables 3 and 4).

4.4.2. Human studies (Table 5)

4.4.2.1. A HRIPT was conducted with α-isodamascone on 103 volunteers (33 males and 70 females). A 0.3 ml aliquot of 0.2% α-isodamascone in DEP was applied to a webril/adhesive patch (25 mm Hilltop Chambers®) and allowed to volatilize up to 20 minutes. The patches were then applied to the left scapular area under occlusion. These patches were removed 24 h after application. Patches were applied three times a week, on a Monday–Wednesday–Friday schedule. A total of nine applications were made over a three week period. After a rest period of approximately two weeks, an occluded challenge patch was applied to the right scapular area to a site not previously exposed and removed after 24 h. Reactions to challenge were read at patch removal and again at 24, 48 and 72 h after patch removal. No reactions were observed (RIFM, 1995).

Table 3
IFRA standard based on the QRA

Limits in the finished product:	
For a description of the categories, refer to the QRA Information Booklet	
Category 1 – See Note (1) 0.003%	Category 7 0.008%
Category 2 0.004%	Category 8 0.1%
Category 3 0.02%	Category 9 0.5%
Category 4 0.05%	Category 10 0.8%
Category 5 0.02%	Category 11 – See Note (2)
Category 6 – See Note (1) 0.07%	

Note:
The above limits apply to rose ketones used individually or in combination.
(1) IFRA would recommend that any material used to impart perfume or flavour in products intended for human ingestion should consist of ingredients that are in compliance with appropriate regulations for foods and food flavourings in the countries of planned distribution and, where these are lacking, with the recommendations laid down in the Code of Practice of IOFI (International Organisation of the Flavor Industry). Further information about IOFI can be found on its website (www.iofiorg.org).
(2) Category 11 includes all non-skin contact or incidental skin contact products. Due to negligible skin contact the concentration of a fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product.
For example, hypothetically if the usual concentration of a fragrance compound in the final product, for example a candle, is at 5%, then any individual fragrance ingredient (in this case a 'rose ketone') must not exceed 5% in the candle.

4.4.2.2. A HRIPT test was conducted in 22 female volunteers using 2.0% α-isodamascone in DEP. A 0.3 ml aliquot of α-isodamascone was applied to a webril/adhesive patch (25 mm Hilltop Chambers®). The test material was applied for 24 h under occlusion to the left upper back 3 times a week on a Monday–Wednesday–Friday schedule for 3 weeks (9 induction applications in total). Following a rest period of approximately 2 weeks, subjects were challenged at a naive site on the upper right back using a 24 h occluded patch. Reactions were read at patch removal and 24, 48, 72, and 96 h thereafter. Two sensitization reactions (2/22) were observed. The 2 subjects who had reacted during the study were subsequently re-challenged with 2.0% α-isodamascone and 2.0% isodamascone in DEP. Each subject reacted to both materials (RIFM, 1994).

4.4.3. Animal studies

No data available on the material.

Table 4
Summary of the relevant sensitization data for the implementation of the QRA

CAS no.	LLNA weighted mean EC3 values ($\mu g/cm^2$) [no. studies]	Human data			Potency classification[b]	WoE NESIL ($\mu g/cm^2$)[c]
		NOEL – HRIPT (induction) ($\mu g/cm^2$)	Experimental NOEL – MAX (induction) ($\mu g/cm^2$)	LOEL[a] induction) ($\mu g/cm^2$)		
57378-68-4	1579 [3]	NA	NA	1333	Moderate	100 [LLNA weighted mean for class = 1496 $\mu g/cm^2$]
43052-87-5	NA	133 (51)	0.2%	NA	Moderate	
24720-09-0	826 [1]	500 (DEP)	NA	NA	Moderate	
23696-85-7	308 [2]	100 (23)	NA	1000	Moderate	
23726-92-3	NA	67 (53)	NA	375	Moderate	
23726-91-2	600	1000 (pet/54)	NA	NA	Moderate	
23726-94-5	NA	NA	NA	NA	Moderate	
39872-57-6	NA	236 (DEP)	NA	2362	Moderate	
71048-82-3	NA	100 (24)	NA	1000	Moderate	
33673-71-1	NA	NA	NA	NA	Moderate	
70266-48-7	NA	1181 (DEP)	NA	NA	Moderate	

NOEL = No observed effect level; HRIPT = Human Repeat Insult Patch Test; MAX = Human Maximization Test; LOEL = lowest observed effect level; NA = Not available.
[a] Data derived from HRIPT or HMT.
[b] Gerberick et al. (2001).
[c] WoE NESIL limited to two significant figures.

Table 5
Summary of human sensitization studies

Test method	Test concentration	Vehicle	Results	References
HRIPT	0.2%	DEP	No reactions (0/103)	RIFM (1995)
HRIPT	2.0%	DEP	2/22 reactions	RIFM (1994)

4.5. Phototoxicity and photoallergy

UV spectra reveals that α-isodamascone does not absorb UV light at wavelengths in the range of 290–400 nm and therefore would have no potential to elicit photoirritation or photoallergy under the current conditions of use as fragrance ingredient.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, J. Lalko, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on α-irone

J. Lalko, A. Lapczynski *, D. McGinty, S.P. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of α-irone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; α-Irone

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

In 2005, a complete literature search was conducted on α-irone. On-line databases that were surveyed included chemical abstract services and the national library of medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

1. Identification (Fig. 1)

1.1 Synonyms: 3-buten-2-one, 4-(2,5,6,6-tetramethyl-2-cyclohexen-1-yl)-, cis- ; a-irone; cis-(2,6)-cis-(2(1),2(2))-a-irone ;6-methylionone; 6-methyl-a-ionone; 4-(2,5,6,6-tetramethyl-2-cyclohexen-1-yl)-3-buten-2-one.
1.2 CAS registry number: 79-69-6.
1.3 EINECS number: 201-219-6.
1.4 Formula: $C_{14}H_{22}O$.
1.5 Molecular weight: 206.33.
1.6 Council of Europe, 2000: α-irone was included by the Council of Europe in the list of substances granted A – may be used in foodstuffs (COE No. 145).
1.7 FDA (Food and Drug Administration): α-irone was approved by the Food and Drug Administration as a flavor (21 CFR 172.515).

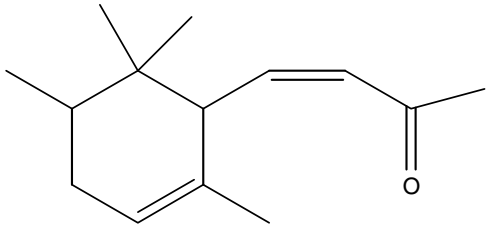

Fig. 1. α-Irone.

1.8 FEMA, 1965: flavor and extract manufacturers' association states: generally recognized as safe as a flavor ingredient – GRAS 3. (2597).

2. Physical properties

2.1 Flash point: >93.3 °C; CC.
2.2 Boiling point: 110–112 °C.
2.3 Log K_{ow} (calculated): 4.71.
2.4 Vapor pressure (calculated): <0.004 mm Hg 20 °C.
2.5 Specific gravity: 0.938.
2.6 Refractive index: 1.4970.

3. Usage (Table 1)

α-Irone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 1–10 metric tonnes per annum.

The maximum skin level that results from the use of α-irone in formulae that go into fine fragrances has been reported to be 0.29% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.22% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.01 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} in rats was reported to be greater than 5.0 g/kg. Ten Sherman–Wistar rats (5/sex) were administered a single oral dose of 5 g/kg of α-irone. Mortality and/or systemic effects were observed over a

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing α-irone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.22	0.0008
Face cream	0.80	2.00	1.000	0.003	0.22	0.0002
Eau de toilette	0.75	1.00	1.000	0.080	0.22	0.0022
Fragrance cream	5.00	0.29	1.000	0.040	0.22	0.0021
Antiperspirant	0.50	1.00	1.000	0.010	0.22	0.0002
Shampoo	8.00	1.00	0.010	0.005	0.22	0.0000
Bath products	17.00	0.29	0.001	0.020	0.22	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.22	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.22	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.22	0.0000
Total						0.0056

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute studies

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rat	10 (5/sex)	>5 g/kg	RIFM, 1972a
Oral	Mouse	10	7.4 ± 0.52 g/kg	RIFM, 1969
Dermal	Rabbits	3	>5 g/kg	RIFM, 1972a

14-day period. One female animal died on day 2, following prostration and coma soon after dosing. All other animals exhibited lethargy lasting 24–48 h after dosing (RIFM, 1972a).

4.1.1.2. The acute oral LD_{50} in mice was calculated to be 7.4 ± 0.52 g/kg. Groups of male and female CF-1 mice (10/dose) were administered, a single oral dose of α-irone. Mortality was observed over a 3-day period (no further details reported) (RIFM, 1969).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits was reported to be greater than 5.0 g/kg. Groups of three albino rabbits were administered a single dermal application of 5 g/kg of neat α-irone to either intact or abraded skin. Mortality and/or systemic effects were observed over a 15-day period. No deaths or systemic effects were observed during the course of the study (RIFM, 1972a).

4.1.3. Intraperitoneal studies

4.1.3.1. In a preliminary screen prior to a carcinogenesis assay, groups of male and female (5/group) A/He mice received six intraperitoneal injections of α-irone over a two-week period and were then observed for delayed toxicity over a 1–2 month period. A MTD (maximum tolerated dose) of 0.4 g/kg in tricaprylin was determined for α-irone (Stoner et al., 1973).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, 10% α-irone in petrolatum was tested in a 48-h closed patch test on the backs of five healthy, male volunteers. No irritation was observed (RIFM, 1972b).

4.2.2. Animal studies

4.2.2.1. As a part of an acute dermal LD_{50} study in rabbits, neat α-irone was evaluated for irritation after a single dermal application of 5.0 g/kg to either intact or abraded skin. Irritation was evaluated over a 14-day observation period. Mild erythema followed by drying and cracking of the skin was observed (RIFM, 1972a).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (Kligman, 1966) was carried out with 10% α-irone in petrolatum in 25 male volunteers. Application was under occlusion to the same site on the volar forearms of all subjects for five alternate-day 48-h periods. Patch test sites were pretreated for 24 h with 5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a 10-day rest period, a challenge patch was applied to a fresh site for 48 h under occlusion. The challenge sites were pretreated for 1 h with 10% aqueous SLS. Reactions to challenge were read at removal and 24 h after patch removal. No reactions were observed (RIFM, 1972b).

4.4.1.2. From November 1998 to May 2000, 1606 consecutive patients of contact dermatitis clinics at six dermatology departments were patch tested with a series of fragrance materials and 8% fragrance mix. α-irone at 10% in petrolatum was applied to the back of each volunteer for 48 h using Finn Chambers® on Scanpor®, with the exception of one center that used Van der Bend chambers®. Readings of the test sites were conducted at days 2 and 4. Reactions were observed in five patients (0.3%); doubtful reactions were observed in eight patients (0.5%) (Frosch et al., 2002).

4.4.2. Animal studies

4.4.2.1. An open epicutaneous test (OET) was conducted on groups of 6–8 guinea pigs, with 10% α-irone in an unspecified vehicle. For the induction phase, open applications were made to the shaved flanks for 21 consecutive days. On days 21 and 35, open challenge applications were conducted, and reactions were read at 24, 48 and 72 h. No reactions were observed (no further details reported) (Klecak, 1979, 1985).

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

4.7.1.

An oral 90-day subchronic study was conducted on 15 male and 15 female rats. α-Irone at dose levels of 5.2 and 5.9 mg/kg/bodyweight/day in cottonseed oil, for the males and females respectively, was administered ad libitum to the animals in the diet. Bodyweight and food consumption were regularly recorded. Haematological and blood chemical determinations were made on eight rats/sex at week 6 and on all rats at week 12. At necropsy, liver and kidney weights were recorded and histological examinations performed. No evidence of adverse toxic effects was observed in the males. Females exhibited an increased efficiency of food

utilization (13.7) as compared to controls (13.0 ± 0.26). In addition, slightly increased hematocrit (54%), hemoglobin (16.2 g/100 ml) and lymphocytes (81%) were observed when compared to controls (51.2% ± 0.88, 15.1 ± 0.36 g/100 ml and 73.7% ± 2.3, respectively) (Oser et al., 1965).

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available for this material.

4.10. Carcinogenicity

4.10.1.

α-Irone was examined for its ability to induce lung tumors in male and female A/He mice (15/sex/dose). The mice were 6–8 weeks old with an average initial weight of 18–20 g. Animals received intraperitoneal injections of α-irone in tricaprylin three times weekly for 8 weeks. Dose levels were set at the maximum tolerated dose (MTD) and a 1:5 dilution of the MTD. The MTD of 0.4 g/kg had been established in a preliminary toxicity screen. The total cumulative doses were 1.95 and 9.6 g/kg. Control groups received 0.1 ml tricaprylin alone or were untreated. The experiments were terminated 24 weeks after the first injection. Treated and control animals were sacrificed and a gross and microscopic examination of the lungs was carried out. Liver, kidney, spleen, thymus, intestine, and salivary and endocrine glands were also examined for abnormalities at necropsy. Four females and one male died in the high-dose group and one female and six males died in the low-dose group. Statistically significant increases in the incidence of lung tumors was observed between control animals and those treated with α-irone. The investigators concluded, based on average weight loss, mortality and a comparison of the mean tumor value to that of historical controls, that the vehicle as tested was unsuitable. Therefore, α-irone was re-tested in the same manner as above, with redistilled tricaprylin as vehicle. No significant differences in the incidence of lung tumors was observed between control animals and those treated with α-irone. No males or females died in the high-dose group. One male died in the low-dose group (Stoner et al., 1973).

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

J. Lalko, A. Lapczynski, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on methyl-α-ionone

A. Lapczynski *, J. Lalko, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of methyl-α-ionone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Methyl-α-ionone

Contents

In 2006, a complete literature search was conducted on methyl-α-ionone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: α-Cetone; α-cyclocitrylidenebutanone; α-cyclocitrylidenemethyl ethyl ketone; methyl-α-ionone; α-methylionone; 1-penten-3-one, 1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-, [R-(E)]-; (R-(E))-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)pent-1-en-3-one.
1.2 CAS Registry Number: 127-42-4.
1.3 EINECS Number: 204-842-1.
1.4 Formula: $C_{14}H_{22}O$.
1.5 Molecular weight: 206.33.
1.6 COE: Methyl-α-ionone was included by the Council of Europe in the list of substances granted A – may be used in foodstuffs (COE, 2000 No. 143).
1.7 FDA: Methyl-α-ionone was approved by the Food and Drug Administration as a flavor (21 CFR 172.515).
1.8 FEMA, 1965: Flavor and Extract Manufactures Association states. Generally recognized as safe as a flavor ingredient – GRAS 3 (2711).
1.9 JECFA: The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1998 No. 398) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent.
1.10 IFRA: Methyl ionone has an International Fragrance Association Standard (IFRA, 2007) – see Section 4.4.1. for details.

2. Physical properties

2.1 Physical form: Almost colorless or pale, straw-colored, oily liquid with floral, sweet-oily, violet odor.
2.2 Flash point: > 200 °F; CC.
2.3 Boiling point: 238 °C.
2.4 Log K_{ow} (calculated): 4.78.
2.5 Specific gravity: 0.93.

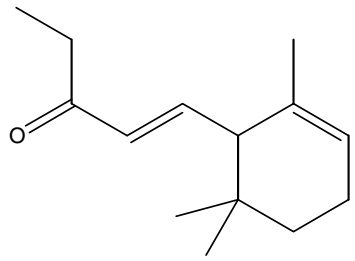

Fig. 1. Methyl-α-ionone.

3. Usage (Table 1)

Methyl-α-ionone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 10–100 metric tonnes per annum.

The maximum skin level that results from the use of methyl-α-ionone in formulae that go into fine fragrances has been reported to be 0.001% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.016% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0004 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity

No data available on this material.

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. Irritation was evaluated prior to a human repeated insult patch test. A 0.5-ml aliquot of 2% methyl-α-ionone in dimethyl phthalate was applied to patches, which were then applied to the inner surface of the left deltoid area of eight volunteers for 24 h under occlusion. No irritation was observed (RIFM, 1968).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Dermal sensitization quantitative risk assessment (QRA)

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This new methodology represents a significant change over current risk assessment practices because it specifically addresses the elements of exposure-based risk assessment that are unique to the induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment.

Full details of this risk assessment approach can be found in the "QRA Expert Group, dermal sensitization quantitative risk assessment (QRA) for "Fragrance Ingredients, Technical Dossier, revised June 22, 2006", and IFRA/RIFM quantitative risk assessment (QRA) for fragrance ingredients booklet, May 11, 2006", at http://

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing methyl-α-ionone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product	Ingredient/mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.016	0.0001
Face cream	0.80	2.00	1.000	0.003	0.016	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.016	0.0002
Fragrance cream	5.00	0.29	1.000	0.040	0.016	0.0002
Antiperspirant	0.50	1.00	1.000	0.010	0.016	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.016	0.0000
Bath products	17.00	0.29	0.001	0.020	0.016	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.016	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.016	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.016	0.0000
Total						0.0004

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

www.rifm/org/pub/publications.asp and http://www.ifraorg.org/News.asp.

Table 2
IFRA Standard based on the QRA

For a description of the categories, refer to the QRA information booklet	
Limits in the finished product	
Category 1 – See Note box (1) 2.0%	Category 7 – 5.4%
Category 2 – 2.6%	Category 8 – 2.0 %
Category 3 – 10.7%	Category 9 – 5.0 %
Category 4 – 32.1%	Category 10 – 2.5 %
Category 5 – 16.9%	Category 11 – See Note (2)
Category 6 – See Note (1) 51.4%	

Note:
(1) IFRA would recommend that any material used to impart perfume or flavor in products intended for human ingestion should consist of ingredients that are in compliance with appropriate regulations for foods and food flavorings in the countries of planned distribution and, where these are lacking, with the recommendations laid down in the Code of Practice of IOFI (International Organisation of the Flavor Industry). Further information about IOFI can be found on its website (www.iofiorg.org).
(2) Category 11 includes all non-skin contact or incidental skin contact products. Due to negligible skin contact the concentration of a fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product. For example, hypothetically if the usual concentration of a fragrance compound in the final product, for example a candle, is at 5%, then any individual fragrance ingredient (in this methyl ionone) must not exceed 5% in the candle.

An exposure-based quantitative risk assessment (QRA) methodology has been used to determine acceptable exposure limits for methyl ionone and a new IFRA Standard (IFRA, 2007) has been issued (see Tables 2 and 3).

4.4.2. Human studies

4.4.2.1. A human repeated insult patch test was conducted on 52 volunteers. A 0.5-ml aliquot of 2% methyl-α-ionone in dimethyl phthalate was applied to patches, which were then applied to the inner surface of right deltoid area for 48 h under occlusion. The patches were alternately applied to the right and left deltoid areas. A total of 10 applications were made over 3 weeks, however, the 8 subjects that were used for the preliminary irritation evaluation received a total of 11 applications. After a 2-week rest period, occluded challenge patches were applied to the inner surface of both the right and left deltoid areas for 48 h. Reactions were read at patch removal and also 24 h after patch removal. No reactions were observed (RIFM, 1968).

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

Table 3
Summary of the relevant sensitization data for the implementation of the QRA

LLNA weighted mean EC3 values (μg/cm^2) [no. studies]	Human data			Potency classification[b]	WoE NESIL (μg/cm^2)[c]
	NOEL – HRIPT (induction) (μg/cm^2)	Experimental NOEL – MAX (induction) (μg/cm^2)	LOEL[a] (induction) (μg/cm^2)		
5450 [1]	70866	NA	NA	Weak	71,000

[a] Data derived from HRIPT or HMT.
[b] Gerberick et al. (2001).
[c] WoE NESIL limited to two significant figures.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental studies

No data available on this material.

4.9. Mutagenicity and genotoxicity

4.9.1. Bacterial studies

4.9.1.1. In an Ames test (Ames et al., 1975) using *Salmonella typhimurium* strains TA1535, TA100, TA1537, TA1538 and TA98 with and without rat liver S9 metabolic activation, doses up to 3.6 mg/plate methyl-α-ionone in dimethyl sulfoxide were not mutagenic (Wild et al., 1983).

4.9.2. Insect studies

4.9.2.1. A Basc test using Berlin K (wild type) and Basc strains was performed on *Drosophila melanogaster*. Methyl-α-ionone was added to the diet at a dose level of 20 mM in 5% saccarose (with the possible addition of 2% ethanol and 2% Tween 80, details not provided). No significant increases in sex-linked recessive lethal (SRL) mutations were observed (Wild et al., 1983).

4.9.3. Mammalian studies

4.9.3.1. In a micronucleus test, groups of male and female NMRI mice (4/dose except at the highest which contained 12 animals) were given a single intraperitoneal injection of methyl-α-ionone at dose levels of 825, 1444 and 2063 mg/kg in olive oil. Control animals were dosed with olive oil alone. Animals were sacrificed 30 h later and bone marrow was extracted and smear preparations were made and stained. Polychromatic and normochromatic erythrocytes were then scored for the presence of micronuclei. Three animals died at the highest dose group. There was no evidence of a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in animals when compared to the concurrent vehicle control. The mean number of micronucleated polychromatic erythrocytes per 1000 polychromatic erythrocytes was 1.9 at 2063 mg/kg, 0.7 at 1444 mg/kg, 1.0 at 825 mg/kg and 1.7 for the control group. Methyl-α-ionone was considered to be non-genotoxic under the conditions of the test (Wild et al., 1983).

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, J. Lalko, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on alpha-*iso*-methylionone

A. Lapczynski *, J. Lalko, V.T. Politano, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of alpha-*iso*-methylionone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; alpha-*iso*-Methylionone

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on alpha-*iso*-methylionone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of the Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: 3-buten-2-one, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-; iraldeine γ; α-isomethylionone; isoraldeine 95, alpha-*iso*-methylionone; γ-methylionone; α-methyl ionone; methyl-γ-ionone, 3-methyl-4-(2,6,6-tri>2;methyl-2-cyclohexen-1-yl)-3-buten-2-one, raldeine γ.
1.2 CAS Registry number: 127-51-5.
1.3 EINECS number: 204-846-3.
1.4 Formula: $C_{14}H_{22}O$.
1.5 Molecular weight: 206.33.
1.6 COE: alpha-*iso*-methylionone was included by the Council of Europe in the list of substances granted A-may be used in foodstuffs (COE No. 169).
1.7 FDA: alpha-*iso*-methylionone was approved by the Food and Drug Administration as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and Extract Manufactures Association states; Generally Recognized as Safe as a flavor ingredient – GRAS 3 (2714).
1.9 JECFA: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 404) concluded that the substance does not present a safety concern at current levels of intake when used as a flavoring agent.
1.10 IFRA: alpha-*iso*-methylionone has an International Fragrance Association Standard (IFRA, 2007) – see Section 4.4.1. for details.

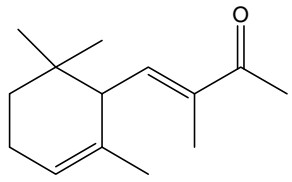

Fig. 1. alpha-*iso*-Methylionone.

2. Physical properties

2.1 Physical form: almost colorless or pale straw colored oil liquid.
2.2 Flash point: >93.3 °C; CC.
2.3 Boiling point: 238 °C.
2.4 Log K_{ow} (calculated): 4.84.
2.5 Specific gravity: 0.931.
2.6 Vapor pressure (calculated): 0.006 mm Hg 20 °C.

3. Usage (Table 1)

alpha-*iso*-Methylionone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 100–1000 metric tonnes per annum.

The maximum skin level that results from the use of alpha-*iso*-methylionone in formulae that go into fine fragrances has been reported to be 3.69% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 13% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.33 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Male and female CF-1 mice (10/dose) weighing 17–25 g were orally administered alpha-*iso*-methylionone. Mortality was observed over a 72 h period. The calculated LD_{50} was reported to be 8.7 ± 0.25 g/kg (RIFM, 1967).

4.1.1.2. In an acute range-finding toxicity test, 4–5 week old white mice received a single oral (gavage) administration of alpha-*iso*-methylionone at dose levels of 2, 5 and 10 g/kg. The animals were observed for signs of toxicity over a 7 day period. No deaths occurred at 2 and 5 g/kg. One animal died (1/2) at 10 g/kg. The acute LD_{50} was reported to be approximately 10 g/kg (RIFM, 1980).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing alpha-*iso*-methylionone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	13.0	0.0492
Face cream	0.80	2.00	1.000	0.003	13.0	0.0104
Eau de toilette	0.75	1.00	1.000	0.080	13.0	0.1300
Fragrance cream	5.00	0.29	1.000	0.040	13.0	0.1257
Antiperspirant	0.50	1.00	1.000	0.010	13.0	0.0108
Shampoo	8.00	1.00	0.010	0.005	13.0	0.0009
Bath products	17.00	0.29	0.001	0.020	13.0	0.0002
Shower gel	5.00	1.07	0.010	0.012	13.0	0.0014
Toilet soap	0.80	6.00	0.010	0.015	13.0	0.0016
Hair spray	5.00	2.00	0.010	0.005	13.0	0.0011
Total						0.3312

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute studies

Route	Species	No. animals/ dose group	LD_{50}	References
Oral	Mice	10	8.7 ± 0.25 g/kg	RIFM (1967)
Oral	Mice	10	~10 g/kg	RIFM (1980)
Oral	Rat	10	>5 g/kg	RIFM (1973)
Dermal	Rabbit	8	>5 g/kg	RIFM (1973)

4.1.1.3. Ten rats were administered a single oral dose of alpha-*iso*-methylionone at 5 g/kg body weight. Observations for mortality and systemic effects were made over a 14-day period. No deaths occurred. The acute oral LD_{50} was considered to be greater than 5 g/kg (RIFM, 1973).

4.1.2. Dermal studies

4.1.2.1. In an acute dermal LD_{50} study in 8 rabbits, 5 g/kg of neat alpha-*iso*-methylionone was applied to intact and abraded skin for 24 h under occlusion. Observations for mortality and systemic effects were made for 14 days after exposure. No deaths occurred. The acute dermal LD_{50} was reported to be greater than 5 g/kg (RIFM, 1973).

4.2. Skin irritation

4.2.1. Human studies (Table 3)

4.2.1.1. Irritation was evaluated during the induction phase of a human repeated insult patch test (HRIPT) in 106 volunteers (26 male and 80 females). A 0.3 ml aliquot of alpha-*iso*-methylionone at 60% in 3:1 DEP:EtOH, was applied under occlusion to the back of each volunteer using 25 mm Hilltop Chambers®. A series of nine, 24 h applications were made over a 3-week period based on Monday–Wednesday–Friday schedule. Reactions were read at patch removal. Irritation was observed in one subject (RIFM, 2004a). No irritation was observed when using the same method as above, 60% alpha-*iso*-methylionone in 3:1 EtOH:DEP was tested on 23 (6 male and 17 female) volunteers (RIFM, 2004b).

Table 3
Summary of human irritation studies

Test method	Test concentration	Results	References
Induction phase (HRIPT)	60% in 3:1 DEP:EtOH	1/106	RIFM (2004a)
Induction phase (HRIPT)	60% in 3:1 EtOH:DEP	0/23	RIFM (2004b,a)
HRIPT pre-test	2% in DEP	0/8	RIFM (1968)
Induction phase (HRIPT)	10% in alcohol	0/28	RIFM (1962)
MDPI	60% in 3:1 DEP:EtOH	0/12	RIFM (2002)
MDPI	60% in 3:1 EtOH:DEP	0/12	RIFM (2002)

4.2.1.2. As a part of a HRIPT, a pilot primary irritation test was conducted on 8 healthy volunteers. A 0.5 ml aliquot of 2% alpha-*iso*-methylionone in dimethyl phthalate was applied to absorbent patches, which were then placed on the inner surface of the left deltoid area for 48 h. The patches were secured in place with an impervious adhesive tape. Reactions were read at 24 and 48 h. No irritation was observed (RIFM, 1968).

4.2.1.3. Irritation was evaluated as a part of HRIPT study conducted on 28 volunteers. As a preliminary check, the sample patches (10% alpha-*iso*-methylionone in alcohol) were applied for 24 h to five volunteers. As no reactions were observed in these five subjects, the remaining 23 volunteers were patch tested. All applications were made as 24 h occluded patches with a 0.5 ml aliquot of 10% alpha-*iso*-methylionone in alcohol applied to the arm. The applications were made based on Monday–Wednesday–Friday schedule for a total of 11 applications for the pilot group (5 volunteers) and 10 applications for the remaining 23 volunteers. No irritation was observed (RIFM, 1962).

4.2.1.4. A modified dermal primary irritation (MDPI) test was conducted in 12 (2 male and 10 female) volunteers. alpha-*iso*-Methylionone was allowed to volatilize for at

least 15 min but no longer than 40 min prior to application. A 0.3 ml aliquot of alpha-*iso*-methylionone at 60% in either 3:1 DEP:EtOH or 3:1 EtOH:DEP, was applied under occlusion to the back of each volunteer using 25 mm Hill-top Chambers®. Each volunteer received two, 24 h applications (Monday–Wednesday). Reactions were scored at patch removal and 24 after patch removal. No irritation was observed (RIFM, 2002).

4.2.2. Animal Studies

4.2.2.1. In a primary skin irritation test, 5% in DEP or neat alpha-*iso*-methylionone was applied to abraded and intact rabbit skin (3/dose). Untreated skin on the same rabbit served as a control. Reactions were read at 24 and 72 h and were scored by the method of Draize (1955). Following application of neat alpha-*iso*-methylionone, well-defined erythema was observed on abraded and intact rabbit skin at 24 h. Very slight to well-defined erythema was still present at 72 h. There was very slight to well-defined edema in 2/3 rabbits at 24 h, which disappeared by 72 h. Application of 5% alpha-*iso*-methylionone showed no evidence of erythema or edema at 24 or 72 h (RIFM, 1967).

4.2.2.2. As a part of an acute toxicity study in 8 rabbits, neat alpha-*iso*-methylionone was applied to intact or abraded skin for 24 h under occlusion at a dose of 5 g/kg. The animals were observed for 14 days. Slight erythema was observed in 2/8 rabbits and moderate erythema was observed in 4/8 rabbits (RIFM, 1973).

4.2.2.3. A 4 h semi-occlusive patch test was conducted on three New Zealand white albino rabbits. A 0.5 ml aliquot of neat alpha-*iso*-methylionone was applied to a 2.5 cm square of surgical lint, which was then applied to the left flank, which had been clipped free of hair, and was held in place by an Elastoplast® elastic adhesive bandage. Patches were removed after 4 h of treatment and the skin sites were cleansed by gentle swabbing with cotton wool soaked in warm water. Reactions were assessed at 1, 24, 48, 72, and 168 h after patch removal. The test was conducted according to Annex V of EEC directive 79/831. Slight erythema and slight to moderate edema were observed in all 3 rabbits. The average erythema and edema scores were 2. Under the conditions of the test, alpha-*iso*-methylionone was considered to be an irritant (RIFM, 1984).

4.2.2.4. A 4 h semi-occlusive patch test was conducted on four female New Zealand white rabbits. A 0.5 ml aliquot of neat alpha-*iso*-methylionone was applied in the same manner as above and the test was conducted as described above (RIFM, 1984). No significant irritation was reported. Irritation observed at 24, 48, and 72 h had an average erythema score of 1.3 and an average edema score of 1.5. Under the conditions of this study alpha-*iso*-methylionone was considered to be non-irritating (RIFM, 1985).

4.2.2.5. A skin irritation test was conducted using 8 New Zealand white rabbits. A 0.5 ml aliquot of neat alpha-*iso*-methylionone was applied to clipped, intact skin on the dorsum of each rabbit for 4 h under a semi-occlusive patch. Reactions were assessed immediately after patch removal and again at 4, 48 and 72 h. Moderate irritation was observed in all animals (RIFM, 1979).

4.2.2.6. A preliminary irritation study was conducted as a part of an associated phototoxicity study. Groups of 5 albino Wistar rats received a 0.1 ml aliquot of 100, 30 or 10% alpha-*iso*-methylionone in ethanol applied for 20 min to the clipped dorsal skin of each animal. Reactions were evaluated at 3, 6, 24, 48 and 72 h after application. No irritation was observed at 10%. Irritation was observed in all animals at 30% and 100% (RIFM, 1981a).

4.2.2.7. As a part of a subchronic dermal toxicity study, irritation was evaluated daily for 90 days after application of 1% alpha-*iso*-methylionone in phenyl ethyl alcohol at a dose of 10 mg/kg. alpha-*iso*-Methylionone was applied to the clipped backs of 5 male and 5 female Sprague-Dawley rats. All rats were observed daily for skin reactions. There was no evidence of any irritation (RIFM, 1981).

4.2.2.8. Irritation was evaluated during an associated dermal subchronic study in 15 Sprague-Dawley rats per dose. An open application of 50, 170, 580 or 2000 mg/kg of alpha-*iso*-methylionone was made to the clipped backs, once daily for 90 days. All rats were observed daily for skin reactions. Erythema and edema with eschar formation were observed at all dose levels (RIFM, 1980b).

4.3. Mucous membrane (eye) irritation

4.3.1. Animal studies

4.3.1.1. A 0.1 ml aliquot of 12.5% alpha-*iso*-methylionone was instilled into the right eye of three normal, healthy albino rabbits. The untreated left eye of each animal served as its own control. Both eyes were examined every 24 h for 4 days and again on day 7. Scoring was done according to the Draize scale of ocular lesions. Instillation of alpha-*iso*-methylionone did not cause any corneal opacity or iris congestion. Intense conjunctival irritation with chemosis and discharge was observed in all three rabbits on days 1–4. All eyes were normal by day 7 (RIFM, 1963).

4.4. Skin sensitization

4.4.1. Dermal sensitization quantitative risk assessment (QRA)

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This new methodology represents a significant change over current risk assessment practices because it specifically addresses the elements of exposure-based risk assessment that are unique to the

induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment.

Full details of this risk assessment approach can be found in the "QRA Expert Group, Dermal Sensitization Quantitative Risk Assessment (QRA) for Fragrance Ingredients, Technical Dossier, revised June 22, 2006", and IFRA/RIFM Quantitative Risk Assessment (QRA) for Fragrance Ingredients Booklet, May 11, 2006", at http://www.rifm/org/pub/publications.asp and http://www.ifraorg.org/News.asp.

An exposure-based Quantitative Risk Assessment (QRA) methodology has been used to determine acceptable exposure limits for alpha-*iso*-methylionone and a new IFRA Standard (IFRA, 2007) has been issued (See Tables 4 and 5).

Table 4
IFRA standard based on the QRA

For a description of the categories, refer to the QRA information booklet	
Limits in the finished product:	
Category 1 – see note box (1)	2.0%
Category 2	2.6%
Category 3	10.7%
Category 4	32.1%
Category 5	16.9%
Category 6 – see note box (1)	51.4%
Category 7	5.4%
Category 8	2.0 %
Category 9	5.0 %
Category 10	2.5 %
Category 11 – see note box (2)	

Note box:
(1) IFRA would recommend that any material used to impart perfume or flavor in products intended for human ingestion should consist of ingredients that are in compliance with appropriate regulations for foods and food flavorings in the countries of planned distribution and, where these are lacking, with the recommendations laid down in the Code of Practice of IOFI (International Organisation of the Flavor Industry). Further information about IOFI can be found on its website (http://www.iofiorg.org).
(2) Category 11 includes all non-skin contact or incidental skin contact products. Due to negligible skin contact the concentration of a fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product. For example, hypothetically if the usual concentration of a fragrance compound in the final product, for example a candle, is at 5%, then any individual fragrance ingredient (in this case alpha-*iso*-methylionone) must not exceed 5% in the candle.

4.4.2. Human Studies (Table 6)

4.4.2.1. A HRIPT study was conducted on 106 volunteers (26 males and 80 females). During the induction phase a 0.3 ml aliquot of 60% alpha-*iso*-methylionone in 3:1 diethyl phthalate:ethanol was applied for 24 h to the back of each volunteer using a webril/adhesive patch (25 mm Hilltop Chambers®) under occlusion. A series of 9 induction patches were completed over a period of approximately 3 weeks. After a two week rest period a 24 h occluded challenge patch was applied to a virgin site. Reactions were evaluated at patch removal and then at 48, 72 and 96 h. No sensitization reactions were observed (RIFM, 2004a). No sensitization was observed when 60% alpha-*iso*-methylionone in 3:1 ethanol:diethyl phthalate was tested in the same manner as above on 23 volunteers (RIFM, 2004b).

4.4.2.2. A human repeated insult patch test was conducted on 52 healthy volunteers (35 females and 17 males). A 0.5 ml aliquot of 2% alpha-*iso*-methylionone (>95% alpha-isomer) in dimethyl phthalate was applied to individual absorbent patches. The patches were placed on alternating skin sites on the inner surface of the right and left deltoid area and secured in place with an impervious adhesive tape for 48 h (except on the weekends when the applications remained in place for 72 h until the following Monday). Reactions were read upon patch removal. A total of 10 such applications were made. Following a two-week rest period, a 48 h challenge patch was applied under occlusion, in duplicate, to the inner surface of both deltoid areas. Reactions to the challenge patch were read at 48 and 72 h after patch application. No sensitization reactions were observed (RIFM, 1968).

4.4.2.3. A HRIPT was conducted on 37 subjects (10 males and 27 females) using 0.5 ml of 12.5% alpha-*iso*-methylionone (vehicle not reported). alpha-*iso*-Methylionone was applied to a Webril swatch affixed to the center of a 1×3 in. strip of adhesive elastic bandage, which was then placed on the upper arm of each subject. Patches were applied to the same site for nine 24 h exposures on a Monday–Wednesday–Friday schedule for three successive weeks. After a two week rest period, a 24 h occluded challenge patch was applied to a virgin site. Reactions were read at 48 and 96 h. No sensitization was produced by 12.5% alpha-*iso*-methylionone (RIFM, 1964).

Table 5
Summary of the relevant sensitization data for the implementation of the QRA

LLNA weighted mean EC3 values (μg/cm^2) [no. studies]	Human data			Potency classification[b]	WoE NESIL (μg/cm^2)[c]
	NOEL – HRIPT (induction) (μg/cm^2)	Experimental NOEL – MAX (induction) (μg/cm^2)	LOEL[a] (induction) (μg/cm^2)		
5450 [1]	70,866	NA	NA	Weak	71,000

[a] Data derived from HRIPT or HMT.
[b] Gerberick et al. (2001).
[c] WoE NESIL limited to two significant figures.

Table 6
Summary of human sensitization studies

Test method	Test concentration	Results	References
HRIPT	60% in 3:1 DEP:EtOH	No reactions	RIFM (2004a)
HRIPT	60% in 3:1 EtOH:DEP	No reactions	RIFM (2004b)
HRIPT	2% in dimethyl phthalate	No reactions	RIFM (1968)
HRIPT	10% in alcohol	No reactions	RIFM (1962)
HRIPT	12.5% in unspecified vehicle	No reactions	RIFM (1964)

4.4.2.4. A HRIPT study was conducted on 28 volunteers. During the induction phase, a 0.5 ml aliquot of 10% alpha-*iso*-methylionone in alcohol was applied under occlusion to the forearm of each volunteer for 24 h. A total of 11 (for 5 pilot volunteers) and 10 (for remaining 23 volunteers) induction applications were conducted on a Monday–Wednesday–Thursday schedule over a three week period. After a two week rest period, challenge patches were applied. The test sites were observed at 48 and 72 h. No sensitization reactions were observed (RIFM, 1962).

4.4.2.5. In a multicenter study extending over a three year period, dermatitis patients were tested for their sensitivity to fragrance materials. alpha-*iso*-Methylionone was tested in 205 consecutive patients. Patch tests were performed with 1% and 5% alpha-*iso*-methyl ionone in white petrolatum using Finn Chambers® on Scanpor® applied for two days on the back. Reactions were recorded according to the ICDRG on days 2 and 3 or on days 2 and 4. alpha-*iso*-Methylionone at both 1% and 5% concentrations produced a questionable/irritant reaction in 1 patient (Frosch et al., 1995).

4.4.2.6. In a multicenter study, 119 cosmetic allergic patients (102 females and 17 males), ages 12–78, were patch tested to determine the causative allergens in cosmetic products. alpha-*iso*-Methylionone at 5% in petrolatum was applied using Van der Bend® patch test chambers and acrylate tape. Patches were removed after 48 h. Reactions were read 20 min after patch removal and again at 24 or 48 h. One patient reacted (deGroot et al., 1988).

4.4.2.7. Patch tests were conducted over the course a year at nine dermatology departments in Korea to determine the prevalence of allergic patch test responses in patients with suspected fragrance allergy. A total of 422 patients (83% women, 17% men) were patch tested with the Korean standard series and a fragrance series, with 18 specific fragrance ingredients added. Reactions to alpha-*iso*-methylionone were observed (no further details reported) (An et al. 2003; Eun et al. 2004).

4.4.3. Animal studies

4.4.3.1. A Local Lymph Node Assay (LLNA) was conducted in 25 CBA/J female mice (5/dose) according to the methods of Basketter et al. (2000) and OECD (2002). A daily topical application of 25 μl of 2.5%, 5%, 10%, 25%, or 50% alpha-*iso*-methylionone in EtOH:DEP (3:1) was made to the dorsal surface of each ear for 3 consecutive days. Control animals were treated with the vehicle alone. Three days after the third topical application all mice were injected intravenously through the tail vein with 250 μl phosphate buffered saline (PBS) containing 20 μCi 3H-methylthymidine (3H-thymidine). All mice were sacrificed 5 h after the intravenous injection. Draining auricular lymph nodes were excised and were pooled for each experimental group. Single cell suspensions were then prepared, washed with PBS, suspended in trichloroacetic acid (TCA) and left overnight at 4 °C. The samples were then resuspended in TCA and then transferred to a scintillation cocktail. 3H-TdR incorporation was then measured by β-scintillation counting and stimulation indices were determined for each experimental group. For each concentration of alpha-*iso*-methylionone, a stimulation index (SI) relative to the concurrent vehicle-treated control was calculated. The EC3 value was taken as a measure of relative potency. alpha-*iso*-Methylionone was considered to be a potential sensitizer under the conditions of the test with an EC3 value of 21.8% (5450 μg/cm^2) (RIFM, 2005a).

4.5. Phototoxicity and photoallergy

UV spectra revealed that alpha-*iso*-methylionone does not absorb UV light at wavelengths in the range of 290–400 nm and therefore would have no potential to elicit photoirritation or photoallergy under the current conditions of use as fragrance ingredient.

4.5.1. Phototoxicity

4.5.1.1. A phototoxicity test was conducted in three groups of 10 albino Wistar rats (5/sex/group). In group A, a 0.1 ml aliquot of 30% alpha-*iso*-methylionone in ethanol was applied to clipped dorsum of each animal. Twenty minutes later, any excess alpha-*iso*-methylionone was removed using a swab moistened with ethanol. Next, the animals were exposed to 12 J/cm^2 UV light from (Philips TL40W/08) fluorescent black lamps (300–400 nm) for 2.5 h at a distance of 33 cm. In group B, the animals were treated with alpha-*iso*-methylionone in exactly the same way as the animals from group A, but they were not exposed to UV light. In group C, the animals were first exposed to UV light and then treated with alpha-*iso*-methylionone under the same conditions as for group A. The test sites were examined immediately after irradiation and at 3, 6, 24, 48 and 75 h after treatment. No phototoxicity was observed (RIFM, 1981a).

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

4.7.1. Oral studies

4.7.1.1. alpha-*iso*-Methylionone was evaluated in a 90-day repeated dose oral (gavage) toxicity study in Sprague-Dawley Crl:CD® (SD) IGS BR strain rats (10/sex/dose). alpha-*iso*-Methylionone was administered by gavage for ninety consecutive days, at dose levels of 5, 30 or 500 mg/kg/day in corn oil. A control group of 10 males and 10 females was dosed with the vehicle alone. The animals were observed for clinical signs, body weight and food and water consumption. Haematology and blood chemistry were evaluated for all the animals at the end of the study. Ophthalmoscopic examination was also performed on control group and high dose animals. All animals were subjected to a gross necropsy and histopathological evaluation of selected tissues was performed. There were no unscheduled deaths and no clinical signs of toxicity were observed. No adverse effect on bodyweight, dietary intake or food efficiency and treatment-related haematology changes were detected. A statistically significant increase in liver and kidney weights, both absolute and relative, was observed in animals treated with 500 mg/kg/day. Males treated with 500 mg/kg/day also showed a significant increase in spleen weight. A significant increase in plasma creatinine, total protein and cholesterol was observed in animals from 500 mg/kg/day group. Also, males from this group showed significant increase in plasma albumin. No abnormalities were observed at necropsy. Histopathology revealed an enlargement of hepatocytes in the liver (generally regarded as adaptive in nature) in animals treated with 500 mg/kg/day, and a greater incidence of globular accumulations of eosinophilic material in the kidney's tubular epithelium of males treated with 30 and 500 mg/kg/day, and a higher incidence of follicular cell hypertrophy in thyroid and adipose infiltration of the bone marrow (indicative of morrow hyperplasia) in males treated with 500 mg/kg/day. The No Observed Effect Level (NOEL) was considered to be 30 mg/kg/day for females and 5 mg/kg/day for males. Because the kidney changes identified histopathologically were consistent with well documented changes that are peculiar to the male rat in response to treatment with some hydrocarbons, the No Observed Adverse Effect Level (NOAEL) for males may be considered to be 30 mg/kg/day (RIFM, 2006).

4.7.1.2. An oral 90-day subchronic study was conducted on male and female FDRL strain rats (15/sex), weighing 75–85 g. alpha-*iso*-Methylionone at a dose level of approximately 3.77 mg/kg body weight/day in cottonseed oil (3.55 and 4.10 mg/kg body weight/day for the males and females, respectively) was administered ad libitum to the animals in the diet. The dose selected was at least 100 times the maximum estimated human dietary intake level. Control animals received the vehicle alone. Observations were made for growth and food consumption, and hematological and blood chemistry evaluations were made in 16 rats (8/sex) at 6 weeks, and in all rats at 12 weeks. Upon sacrifice on day 90, a gross necropsy was conducted. Liver and kidney weights were recorded and all major organs were collected including liver, kidneys, stomach, small and large intestines, spleen, pancreas, heart, lungs, bone marrow, muscle, brain, spinal cord, bladder, adrenals, thyroid, pituitary, gonads, salivary glands, and lymph nodes, from half the animals in each group for histological examination. Hemoglobin and blood urea nitrogen were slightly decreased in the males but the hematocrit and erythrocyte counts were within the control ranges. There were no adverse effects of alpha-*iso*-methylionone on body weight gain and food consumption. Liver and kidney weights were not affected. There were no adverse effects on gross and microscopic appearance of major organs at necropsy (Oser et al., 1965; Bar and Griepentrog, 1967).

4.7.2. Dermal studies

4.7.2.1. alpha-*iso*-Methylionone was tested in a 90-day dermal toxicity study on rats. Ten Sprague-Dawley albino male and female rats (5/sex) received a dermal application of 1% alpha-*iso*-methylionone in phenethyl alcohol (PEA), at a dose of 10 mg/kg, once daily for 90 days. A control group of ten rats (5/sex) received applications of 1 mg/kg PEA. All animals were observed daily and skin reactions were recorded. Body weights were recorded weekly. At the study's termination, selected hematology, clinical chemistry and urinalysis parameters were evaluated. Necropsy was conducted on all animals. Gross observations were normal in all animals. The hematology, clinical chemistry and urinalysis parameters evaluated were comparable to the controls. Dermal reactions were normal for all animals (RIFM, 1981).

4.7.2.2. A dermal subchronic study was conducted on Sprague-Dawley rats (15/sex/dose). An open application of alpha-*iso*-methylionone at a dose of 50, 170, 580 or 2000 mg/kg was made to the clipped backs, once daily for 90 days. The controls (60 rats/sex) were not treated with the test material. Observations for signs of toxicity, including erythema and eschar formation, were performed daily. Body weight and food consumption data were measured weekly. Selective hematology, clinical chemistry and urinalysis assessment were conducted at weeks 7 and 13 of the study. A complete gross necropsy on all animals and a microscopic examination of tissues in the control and high dose animals were conducted at sacrifice. No treatment related deaths occurred. On the skin at the application site there was a dose-dependent increase in the severity of erythema, and eschar formation. Body weight gains were significantly reduced in females in the highest dose group and in males treated at 580 and 2000 mg/kg body weight/day. Total food consumption throughout the study was

significantly increased in females treated at the two highest dose levels and there was a significant decrease in food efficiency and food intake in both sexes in the two highest dose groups. The body weight changes may not represent a direct, test-material-related effect since many of these animals manifested severe skin lesions. There were hematological changes in the two highest dose groups and reduced serum glucose in the high dose animals, all largely attributable to the inflammation and infection at the site of application. A significant increase in serum BUN was reported in males in the top two dose groups. Urinalysis showed a significant increase in the incidence of albuminuria in males in the 3 highest dose groups. In the high dose males, abundant eosinophilic globules were observed in the kidney epithelium at necropsy. At necropsy there was a significant increase in the absolute and relative liver weights in both sexes at all dose levels. Increases, most of which attained statistical significance, in the absolute and relative weights of the kidneys were reported in all but the lowest dose groups of each sex. The absolute adrenal weights were significantly increased in the two highest dose groups of both sexes. The interpretation of the data is complicated by the severe skin damage at the application site, especially in the two highest dose groups. Depressed body weight gains and increased neutrophil count are probably attributable to infection and inflammation. Azotemia and proteinuria likely are a result of chronic severe tissue damage and infection. The liver weight increase probably resulted from induction of microsomal mixed-function oxidases. Increased adrenal weights probably reflect the response to stress caused by tissue damage and infection. Severe tissue destruction and infection in the skin may have combined to elicit increased kidney weight at higher doses and epithelial eosinophilic globules in the convoluted tubules of the outer cortex. To determine if these effects were specific to male rat nephropathy, a review of the histopathology of kidneys from rats in this study was conducted. This lesion occurred in a dose-responsive fashion in males only and was seen also in male control rats. It was accompanied by interstitial nephritis in control and treated rats. The findings suggest an endogenous disease process which was exacerbated by the application of the irritating test material and marked skin necrosis. On the basis of the review of the kidney histopathology data and considering the dermal inflammation and infection in these animals, the results of this study are concluded to show a systemic NOAEL of topical alpha-*iso*-methyl ionone of 50 mg/kg. Since erythema and eschar formation occurred in all treatment groups, a NOAEL for this effect could not be established (RIFM, 1980b; Belsito et al., 2007).

4.8. Developmental toxicity

4.8.1. Oral studies

4.8.1.1. As a part of associated developmental study, a dose range finding study was conducted to determine the appropriate doses for the definitive study. Forty (8/dose) presumed pregnant female Crl:CD(SD) IGS BR VAF/plus rats were administered alpha-*iso*-methylionone via gavage on days 7–17 of gestation at dose levels of 1.25, 2.5, 5 or 10 mg/kg/day in corn oil, at a final volume of 10 ml/kg. All animals were sacrificed on day of gestation (DG) 21 and were examined for the number and distribution of corpora lutea, implantation sites and uterine contents. A gross necropsy of the thoracic abdominal and pelvic viscera was performed. Fetuses were weighed and examined for gross external alterations and sex. A statistical evaluation was conducted. There were no deaths or clinical observations that were determined to be material related. All rats were pregnant. All caesarean-sectioning and litter observations were comparable between the five dosage groups. No fetal gross alterations occurred. Body weight gains were increased in all treated groups during the entire dosage period, the gestation period following the initiation of dosing and the entire gestation period, but this was not considered to be treatment related. Based on these results, doses of 3, 10 and 30 mg/kg/day were selected for the main study (RIFM, 2005b).

4.8.1.2. A developmental study was conducted on 100 (25/dose) presumed pregnant female Crl:CD(SD) IGS BR VAF/Plus rats. Animals were gavaged on days 7–17 of gestation with 0, 3, 10 or 30 mg/kg/day alpha-*iso*-methylionone in corn oil. Animals were observed twice daily for mortality and morbidity. Clinical observations of test material effects and observations for abortion and premature delivery were conducted before and approximately 1 h following dosing and once daily thereafter. Body weights were recorded prior to the start of the study and daily during dosage and post dosage periods. Feed consumption was recorded on days 0, 7, 10, 12, 15, 18 and 21. On day 21, all rats were sacrificed by CO_2 asphyxiation, caesarean sectioned and a gross necropsy was conducted on all animals. The uteri of apparently non-pregnant rats were examined while pressed between glass plates to confirm the absence of implantation sites. The number and distribution of corpora lutea were recorded. The uterus of each rat was removed and examined for pregnancy, number and distribution of implantations, fetal mortality and early and late resorptions. Each fetus was removed from the uterus with surviving fetuses being sacrificed by intraperitoneal injection of sodium pentobarbital. All fetuses were examined macroscopically for sex and the presence, shape and size of all organs. Fetal bodyweight were recorded. Approximately one half of each litter was examined for soft tissue alterations using a variation of the Wilson's staining technique. The remaining fetuses were eviscerated, cleared, stained and examined for skeletal alterations. All female rats survived to scheduled sacrifice. All clinical and necropsy observations were considered to be unrelated to the administration of alpha-*iso*-methylionone. Maternal body weights, bodyweight gains and absolute and relative feed consumption values were unaffected at dosages of alpha-*iso*-methylionone as high as 30 mg/

kg/day. Pregnancy occurred in 21 of 25 rats in each dosage group. Caesarean-sectioning and litter parameters were not affected by doses of alpha-*iso*-methylionone as high as 30 mg/kg/day. No fetal alterations occurred that were considered associated with alpha-*iso*-methylionone. It was concluded that the maternal and developmental no-observable-adverse-effect levels (NOAELs) for alpha-*iso*-methylionone were greater than 30 mg/kg/day (RIFM, 2005b; Politano et al., 2006).

4.9. Mutagenicity and genotoxicity

4.9.1. Mutagenicity

4.9.1.1.. In an Ames assay (Ames, 1975) with and without S9 activation, *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were treated with 9.3, 93, 930 and 9300 μg/plate alpha-*iso*-methylionone in dimethyl sulfoxide (DMSO). No effects were observed (RIFM, 1980a).

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of the Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, J. Lalko, V.T. Politano, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on methyl-β-ionone

A. Lapczynski *, J. Lalko, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of methyl-β-ionone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Methyl-β-ionone

Contents

In 2006, a complete literature search was conducted on methyl-β-ionone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: β-Cetone; β-cyclocitrylidenebutanone; β-iraldeine; methyl-β-ionone; β-methylionone; 1-penten-3-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-; 5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-4-penten-3-one; 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)pent-1-en-3-one.
1.2 CAS Registry Number: 127-43-5.
1.3 EINECS Number: 204-843-7.
1.4 Formula: $C_{14}H_{22}O$.
1.5 Molecular weight: 206.33.
1.6 COE: Methyl-β-ionone was included by the Council of Europe in the list of substances granted A – may be used in foodstuffs (COE No. 144).
1.7 FDA: Methyl-β-ionone was approved by the Food and Drug Administration as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and extract manufactures association states; generally recognized as safe as a flavor ingredient – GRAS 3 (2712).
1.9 JECFA: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 399) concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent.
1.10 IFRA: Methyl ionone has an International Fragrance Association Standard (IFRA, 2007) – see Section 4.4.1. for details.

2. Physical properties

2.1 Flash point: >200 °F; CC.
2.2 Boiling point: 238 °C.
2.3 Log K_{ow} (calculated): 4.91.
2.4 Vapor pressure (calculated): 0.004 mm Hg 20 °C.

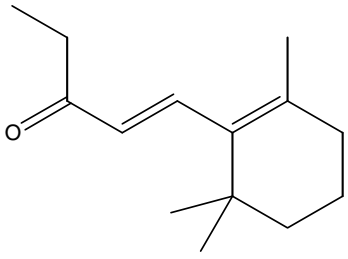

Fig. 1. Methyl-β-ionone.

3. Usage (Table 1)

Methyl-β-ionone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region 10–100 metric tonnes per annum.

The maximum skin level that results from the use of methyl-β-ionone in formulae that go into fine fragrances has been reported to be 0.02% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.1% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0025 mg/kg for high end users of these products (Table 1).

4. Toxicology data

4.1. Acute toxicity

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} in rats (5/sex) was reported to be greater than 2000 g/kg. Ten rats (5 male/5 female) received a single oral dose of 2.0 g/kg methyl-β-ionone. All animals were observed at 30 min, 1, 2 and 4 h after dosing, and then daily for 14 days. No deaths occurred. Fur staining was observed in all males 30 min after dosing and during the four days following dosing. This lasted until day 9 in 5 animals. No other effects were observed (RIFM, 1988a).

4.2. Skin irritation

4.2.1. Human studies

No data available on this material.

4.2.2. Animal studies

4.2.2.1. A primary irritation test was conducted in 3 female New Zealand albino rabbits. A 0.5 ml aliquot of methyl-β-ionone was applied to a 2.5 cm^2 patch which was then applied to a 6 cm^2 area on the back for 4 h under semi-occlusion. Reactions were read at 1, 24, 48 and 72 h and again on days 7 and 14. Well-defined erythema (3/3 rabbits) and very slight (1/3 rabbits) to slight (2/3 rabbits) edema were observed (RIFM, 1988b).

4.2.2.2. An irritation screen was conducted in 4 albino Dunkin Hartley guinea pigs prior to a guinea pig delayed dermal sensitization test. A 0.5 ml aliquot of methyl-β-ionone at 100% and 12.5%, 25% and 50% in ethanol was applied to a 2 cm^2 absorbent lint patch which was then applied to the skin for 6 h under occlusion. Reactions were evaluated 24 and 48 h after application. Slight erythema was observed in 4/4 guinea pigs with 100% methyl-β-ionone and slight erythema was observed in 2/4 guinea pigs

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing methyl-β-ionone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.1	0.0004
Face cream	0.80	2.00	1.000	0.003	0.1	0.0001
Eau de toilette	0.75	1.00	1.000	0.080	0.1	0.0010
Fragrance cream	5.00	0.29	1.000	0.040	0.1	0.0010
Antiperspirant	0.50	1.00	1.000	0.010	0.1	0.0001
Shampoo	8.00	1.00	0.010	0.005	0.1	0.0000
Bath products	17.00	0.29	0.001	0.020	0.1	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.1	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.1	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.1	0.0000
Total						0.0025

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

with 50%. No irritation was observed at 12.5% or 25% (RIFM, 1989). A second irritation screen was conducted using the same method as above but with methyl-β-ionone at 5%, 10%, 20% and 50% in light liquid paraffin. No irritation was observed (RIFM, 1989).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Dermal sensitization quantitative risk assessment (QRA)

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This new methodology represents a significant change over current risk assessment practices because it specifically addresses the elements of exposure-based risk assessment that are unique to the induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment.

Full details of this risk assessment approach can be found in the "QRA Expert Group, Dermal Sensitization Quantitative Risk Assessment (QRA) for Fragrance Ingredients, Technical Dossier, revised June 22, 2006", and IFRA/RIFM Quantitative Risk Assessment (QRA) for Fragrance Ingredients Booklet, May 11, 2006", at http://www.rifm/org/pub/publications.asp and http://www.ifraorg.org/News.asp.

An exposure-based quantitative risk assessment (QRA) methodology has been used to determine acceptable exposure limits for methyl ionone and a new IFRA Standard (IFRA, 2007) has been issued (see Tables 2 and 3).

4.4.2. Human studies

No data available on this material.

Table 2
IFRA Standard based on the QRA

For a description of the categories, refer to the QRA information booklet	
Limits in the finished product	
Category 1 – see Note (1)	2.0%
Category	2 2.6%
Category	3 10.7%
Category	4 32.1%
Category	5 16.9%
Category 6 – see Note (1)	51.4%
Category	7 5.4%
Category	8 2.0 %
Category	9 5.0 %
Category	10 2.5 %
Category 11 – see Note (2)	

Notes: (1) IFRA would recommend that any material used to impart perfume or flavour in products intended for human ingestion should consist of ingredients that are in compliance with appropriate regulations for foods and food flavourings in the countries of planned distribution and, where these are lacking, with the recommendations laid down in the Code of Practice of IOFI (International Organisation of the Flavor Industry). Further information about IOFI can be found on its website (www.iofiorg.org).
(2) Category 11 includes all non-skin contact or incidental skin contact products. Due to negligible skin contact the concentration of a fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product. For example, hypothetically if the usual concentration of a fragrance compound in the final product, for example a candle, is at 5%, then any individual fragrance ingredient (in this case methyl ionone) must not exceed 5% in the candle.

4.4.3. Animal studies

4.4.3.1. Sensitization was evaluated in a delayed dermal sensitization test (modified Buehler method) conducted on 10 healthy Dunkin Hartley guinea pigs. On day 1, a 0.5 ml aliquot of methyl-β-ionone at 50% in ethanol was applied to a 2 cm^2 absorbent lint patch which was then applied to the clipped left shoulder for 6 h under occlusion. This induction procedure was repeated again on days 8 and 15. One animal died during the induction phase but this was not considered to be treatment related. After a 14-day rest period, a 6-h occluded challenge application was made to the right and left clipped flank using the same

Table 3
Summary of the relevant sensitization data for the implementation of the QRA

LLNA weighted mean EC3 values (μg/cm^2) [no. studies]	Human data			Potency classification[b]	WoE NESIL (μg/cm^2)[c]
	NOEL–HRIPT (induction) (μg/cm^2)	Experimental NOEL – MAX (induction) (μg/cm^2)	LOEL[a] (induction) (μg/cm^2)		
5450 [1]	70866	NA	NA	Weak	71000

[a] Data derived from HRIPT or HMT.
[b] Gerberick et al. (2001).
[c] WoE NESIL limited to two significant figures.

patch technique as in the induction phase. All animals were challenged with 12.5% and 25% methyl-β-ionone in ethanol. One week after challenge, a 6-h occluded re-challenge application was made with 1% and 5% methyl-β-ionone in light liquid paraffin. Reactions were read 24 and 48 h after application. At primary challenge, 8/9 animals reacted to 25% methyl-β-ionone in ethanol and 4/9 animals reacted to 12.5% in ethanol. No reactions were observed when animals were re-challenged with 1% and 5% methyl-β-ionone in light liquid paraffin (RIFM, 1989).

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, J. Lalko, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Belsito, D., Bickers, D., Bruze, M., Calow, P., Greim, H., Hanifin, J.H., Rogers, A.E., Saurat, J.H., Sipes, I.G., Tagami, H., 2007. A toxicologic and dermatologic assessment of ionones when used as fragrance ingredients. Food and Chemical Toxicology 45 (1S1), S130–S167.

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Review

Fragrance material review on 6-methyl-β-ionone

J. Lalko, A. Lapczynski *, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of 6-methyl-β-ionone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; 6-Methyl-β-ionone

Contents

This individual fragrance material review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

In 2005, a complete literature search was conducted on 6-methyl-β-ionone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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1. Identification (Fig. 1)

1.1 Synonyms: 3-Buten-2-one, 4-(2,5,6,6-tetramethyl-1-cyclohexen-1-yl)-; b-ionone, 6-methyl-; b-irone; 6-methyl-b-ionone; 4-(2,5,6,6-tetramethyl-1-cyclohexen-1-yl)-3-buten-2-one.
1.2 CAS registry number: 79-70-9.
1.3 EINECS number: 201-220-1.
1.4 Formula: $C_{14}H_{22}O$.
1.5 Molecular weight: 206.29.

2. Physical properties

2.1 Log K_{ow} (calculated): 4.84.

3. Usage (Table 1)

6-Methyl-β-ionone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of <0.1 metric tonnes per annum.

The maximum skin level that results from the use of 6-methyl-β-ionone in formulae that go into fine fragrances has been reported to be 0.02% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5‰ use level in formulae for use in cosmetics in general has been reported to be 0.1% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.0025 mg/kg for high end users of these products.

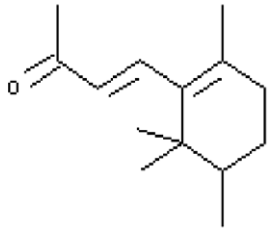

Fig. 1. 6-Methyl-β-ionone.

4. Toxicology data

4.1. Acute toxicity

No data available on this material.

4.2. Skin irritation

No data available on this material.

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

No data available on this material.

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing 6-methyl-β-ionone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.1	0.0004
Face cream	0.80	2.00	1.000	0.003	0.1	0.0001
Eau de toilette	0.75	1.00	1.000	0.080	0.1	0.0010
Fragrance cream	5.00	0.29	1.000	0.040	0.1	0.0010
Antiperspirant	0.50	1.00	1.000	0.010	0.1	0.0001
Shampoo	8.00	1.00	0.010	0.005	0.1	0.0000
Bath products	17.00	0.29	0.001	0.020	0.1	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.1	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.1	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.1	0.0000
Total						0.0025

[a] Upper 97.5‰ levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

4.10. Carcinogenicity

No data available on this material.

This individual fragrance material review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

J. Lalko, A. Lapczynski, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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IFRA (International Fragrance Association), 2001. Use Level Survey, July 2001.

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Review

Fragrance material review on *iso*-methyl-β-ionone

J. Lalko, A. Lapczynski *, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of *iso*-methyl-β-ionone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; *iso*-Methyl-β-ionone

Contents

In 2006, a complete literature search was conducted on *iso*-methyl-β-ionone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of the Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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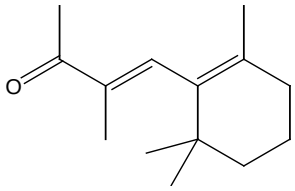

Fig. 1. *iso*-Methyl-β-ionone.

1. Identification (Fig. 1)

1.1 Synonyms: 3-buten-2-one, 3-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-; δ-iraldeine; isomethyl-β-ionone; *iso*-methyl-β-ionone; 3-methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-3-en-2-one.
1.2 CAS Registry Number: 79-89-0.
1.3 EINECS Number: 201-231-1.
1.4 Formula: $C_{14}H_{22}O$.
1.5 Molecular weight: 206.33.
1.6 Council of Europe: *iso*-methyl-β-ionone was included by the Council of Europe in the list of substances granted B – information required – 28 day oral study (COE No. 650).
1.7 FEMA: Flavor and Extract Manufactures Association states; Generally Recognized as Safe as a flavor ingredient – GRAS 22 (4151).

2. Physical properties

2.1 Log K_{ow} (calculated): 4.97.
2.2 Vapor pressure (calculated): 0.003 mm Hg 20 °C.

3. Usage (Table 1)

iso-Methyl-β-ionone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region 10–100 metric tonnes per annum.

The maximum skin level that results from the use of *iso*-methyl-β-ionone in formulae that go into fine fragrances has been reported to be 1.18% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 9.32% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.24 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity

No data available on this material.

4.2. Skin irritation

No data available on this material.

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

No data available on this material.

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing *iso*-methyl-β-ionone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	9.32	0.0353
Face cream	0.80	2.00	1.000	0.003	9.32	0.0075
Eau de toilette	0.75	1.00	1.000	0.080	9.32	0.0932
Fragrance cream	5.00	0.29	1.000	0.040	9.32	0.0901
Antiperspirant	0.50	1.00	1.000	0.010	9.32	0.0078
Shampoo	8.00	1.00	0.010	0.005	9.32	0.0006
Bath products	17.00	0.29	0.001	0.020	9.32	0.0002
Shower gel	5.00	1.07	0.010	0.012	9.32	0.0010
Toilet soap	0.80	6.00	0.010	0.015	9.32	0.0011
Hair spray	5.00	2.00	0.010	0.005	9.32	0.0008
Total						0.2375

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

J. Lalko, A. Lapczynski, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on methyl ionone (mixture of isomers)

J. Lalko, A. Lapczynski *, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of methyl ionone when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Methyl ionone (mixture of isomers)

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on methyl ionone. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used As Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: ionone, methyl-; isoraldeine; iralia; methyl ionone (mixture of isomers).
1.2 CAS Registry No.: 1335-46-2.
1.3 EINECS No.: 215-635-0.
1.4 Formula: $C_{14}H_{22}O$.
1.5 Molecular weight: 206.33.
1.6 IFRA: Methyl ionone (mixture of isomers) has an International Fragrance Association Standard (IFRA, 2007).

2. Physical properties

2.1 Physical form: almost colorless or pale, straw-colored, oily liquid with floral, sweet-oily, violet odor.
2.2 Flash point: >200 °F; CC.
2.3 Boiling point: 238 °C, 266 °C at 1013 mb.
2.4 Log K_{ow} (calculated): 4.84.
2.5 Specific gravity: 0.928; at 20 °C: 0.930 D20/4-0.929-0.932.
2.6 Vapor pressure (calculated): 0.005 mm Hg at 20 °C, 0.00613 mm Hg at 25 °C.
2.7 Molecular weight: 206.33.
2.8 Melting point (calculated): 59.38 °C.
2.9 Refractive index at 20 °C: 1.500 ND20-1.498-1.502.

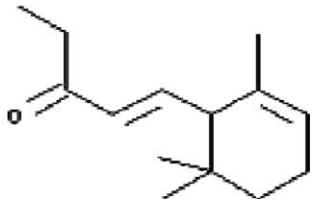

Fig. 1. Methyl ionone.

3. Usage (Table 1)

Methyl ionone is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region 100–1000 metric tonnes per annum.

The maximum skin level that results from the use of methyl ionone in formulae that go into fine fragrances has been reported to be 5.64% (IFRA, 2001), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 9.82% (IFRA, 2001), which would result in a conservative calculated maximum daily exposure on the skin of 0.25 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} was reported to be greater than 5 g/kg based on 0/10 deaths. Ten rats received a single oral dose of methyl ionone at 5 g/kg body weight. Mortality and toxic signs were observed over a period of 14 days (no additional information available) (RIFM, 1973).

4.1.1.2. An acute oral (gavage) study was conducted on 10 mice (5/sex). The animals were divided into three groups as follows: 1 male and 1 female in the 10.0 g/kg bodyweight group; 3 males and 3 females in the 5.0 g/kg body weight group; 1 male and 1 female in the 2.0 g/kg body weight group. Mortality and toxicity signs were observed for up to 7 days. No deaths were observed at 2.0 g/kg, one (1/6) animal died at 5 g/kg and all (2/2) animals died at 10 g/kg. Stress was observed in all animals. Animals dosed at 10 g/kg were cyanosed, somnolent, dehydrated and experienced heavy breathing. Necropsy of the animals that died revealed distended bladders with bright orange urine and irritation in the duodenum and ileum. Necropsy of the surviving animals, apart from gross thickening of the cardiac region of the stomach of male mice at 5 g/kg, were normal. The acute LD_{50} was reported to be between 5 and 10 g/kg (RIFM, 1980).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing methyl ionone

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	9.82	0.0372
Face cream	0.80	2.00	1.000	0.003	9.82	0.0079
Eau de toilette	0.75	1.00	1.000	0.080	9.82	0.0982
Fragrance cream	5.00	0.29	1.000	0.040	9.82	0.0949
Antiperspirant	0.50	1.00	1.000	0.010	9.82	0.0082
Shampoo	8.00	1.00	0.010	0.005	9.82	0.0007
Bath products	17.00	0.29	0.001	0.020	9.82	0.0002
Shower gel	5.00	1.07	0.010	0.012	9.82	0.0011
Toilet soap	0.80	6.00	0.010	0.015	9.82	0.0012
Hair spray	5.00	2.00	0.010	0.005	9.82	0.0008
Total						0.2502

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rats	10	> 5 g/kg	RIFM (1973)
Oral	Mice	10	Between 5 and 10 g/kg	RIFM (1980)
Dermal	Rabbits	8	> 5 g/kg	RIFM (1973)

4.1.2. Dermal studies

4.1.2.1. A single dermal application of neat methyl ionone (mixture of isomers) was applied at a dose of 5 g/kg to the skin of eight rabbits. Animals were observed for mortality and toxic signs for 14 days. No deaths were observed. The acute dermal LD_{50} of methyl ionone was reported to be greater than 5 g/kg (no additional information available) (RIFM, 1973).

4.1.3. Intraperitoneal studies

4.1.3.1. In a pilot toxicity study, the acute toxicity of methyl ionone (mixture of isomers) was evaluated in male and female ICR mice weighing 25–35 g. Male mice (2/dose) were dosed with a single intraperitoneal injection of 0.001, 0.001, 0.1 or 1.0 g/kg methyl ionone in corn oil. Additionally, male and female mice (5/dose) were administered 2.0 g/kg of methyl ionone (mixture of isomers) in corn oil. Control animals received vehicle alone. Mice were observed for clinical signs immediately after administration of methyl ionone (mixture of isomers) and daily for 3 days. Three deaths occurred (3/10) at the highest dose. Clinical signs included lethargy and piloerection in males at 1.0 g/kg and in females at 2.0 g/kg. Convulsions were observed in males and females at 2.0 g/kg and prostration and crusty eyes were observed in females at 2.0 g/kg (RIFM, 2000).

4.1.3.2. A toxicity study was conducted to determine the maximum tolerated dose (MTD) for a subsequent micronucleus assay. Male and female ICR mice weighing 25–35 g (5/dose) received a single intraperitoneal injection of methyl ionone in corn oil at doses of 1.5 and 1.75 g/kg body weight. Control mice received the vehicle alone. Mice were observed after dose administration and daily thereafter for 3 days for clinical signs. No deaths occurred. Clinical signs included lethargy and piloerection in both males and females in both dose groups. Based on these results and the results from the pilot toxicity study (see Section 4.1.3.1) in which 3/10 deaths occurred at 2.0 g/kg, the MTD was set at 1.85 g/kg (RIFM, 2000).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. A 24-h closed patch test was conducted in adult male and female volunteers. Neat methyl ionone was applied to an area of about 1 cm in diameter on the dermis of the inner portion of the lower arm. Immediately following application, the area was covered with an adhesive bandage for a period of 24 h. The sites were read at 24-h intervals for 5 days. Irritation was not observed (Katz, 1946).

4.2.1.2. A 48-h occluded patch test was conducted on the backs of 19 male and female subjects, with 5% methyl ionone in vaselinum aldum or unguentum hydrophilicum. Irritation was not produced (Fuji et al., 1972).

4.2.2. Animal studies

4.2.2.1. As a part of an acute toxicity study, eight rabbits received a single dermal application of neat methyl ionone (mixture of isomers) at 5 g/kg. Slight (2/8 rabbits) to moderate erythema (4/8 rabbits) was observed (RIFM, 1973).

4.2.2.2. A 4-h semi-occluded patch with 0.5 ml of neat methyl ionone was applied to the clipped skin of eight New Zealand white rabbits. After removal of the patches,

the sites were assessed at 4, 24, 48, and 72 h. Irritation was observed in all animals (8/8) (RIFM, 1979).

4.2.2.3. Irritation was evaluated prior to a phototoxicity study. Groups of five albino Wistar rats were topically treated on the clipped dorsal skin with a 0.1 ml aliquot of 10% or 30% methyl ionone (mixture of isomers) in ethanol, or 100% methyl ionone (mixture of isomers) without UV exposure. The sites were evaluated at 3, 6, 24, 48 and 72 h. Very slight to distinct erythema and edema were observed in all animals at all dose levels (RIFM, 1982).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Dermal sensitization quantitative risk assessment (QRA)

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This new methodology represents a significant change over current risk assessment practices because it specifically addresses the elements of exposure-based risk assessment that are unique to the induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment.

Full details of this risk assessment approach can be found in the "QRA Expert Group, dermal sensitization quantitative risk assessment (QRA) for Fragrance Ingredients, Technical Dossier, revised June 22, 2006", and IFRA/RIFM quantitative risk assessment (QRA) for Fragrance Ingredients Booklet, May 11, 2006", at http://www.rifm/org/pub/publications.asp and http://www.ifraorg.org/News.asp.

An exposure-based quantitative risk assessment (QRA) methodology has been used to determine acceptable exposure limits for methyl ionone (mixture of isomers) and a new IFRA Standard (IFRA, 2007) has been issued (see Tables 3 and 4).

4.4.2. Human studies

4.4.2.1. A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was conducted on 25 human volunteers. A total of five applications of a 48-h occluded patch with 10% methyl ionone (mixture of isomers) in an unspecified vehicle were made over 15 days. The test sites were pretreated for 24 h with 5% aqueous sodium lauryl sulfate (SLS). Following a 10-day rest period, the challenge patches were applied to fresh sites for 48 h under occlusion. Before the challenge patches were applied, the test sites were pretreated with 10% SLS for 1 h. The challenge sites were read at patch removal, and at 24 and 48 h after patch removal. No sensitization reactions were produced (0/25) (Greif, 1967).

Table 3
IFRA Standard based on the QRA

For a description of the categories, refer to the QRA Information Booklet	
Limits in the finished product	
Category 1 – see note (1)	2.0%
Category 2	2.6%
Category 3	10.7%
Category 4	32.1%
Category 5	16.9%
Category 6 – see note (1)	51.4%
Category 7	5.4%
Category 8	2.0%
Category 9	5.0%
Category 10	2.5%
Category 11 – see note (2)	

Note. (1) IFRA would recommend that any material used to impart perfume or flavour in products intended for human ingestion should consist of ingredients that are in compliance with appropriate regulations for foods and food flavourings in the countries of planned distribution and, where these are lacking, with the recommendations laid down in the Code of Practice of IOFI (International Organisation of the Flavor Industry). Further information about IOFI can be found on its website (www.iofiorg.org). (2) Category 11 includes all non-skin contact or incidental skin contact products. Due to negligible skin contact the concentration of a fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product. For example, hypothetically if the usual concentration of a fragrance compound in the final product, for example a candle, is at 5%, then any individual fragrance ingredient (in this case *alpha*-iso-methylionone) must not exceed 5% in the candle.

4.4.3. Animal studies

4.4.3.1. An open epicutaneous test was conducted to determine the sensitization potential of methyl ionone (mixture of isomers). A 0.1 ml aliquot of neat methyl ionone (mixture of isomers) was applied to an 8 cm^2 area on the clipped flank of 6–8 Himalayan white spotted guinea pigs weighing

Table 4
Summary of the relevant sensitization data for the implementation of the QRA

LLNA weighted mean EC3 values (μg/cm^2) [no. studies]	Human data			Potency classification[b]	WoE NESIL (μg/cm^2)[c]
	NOEL – HRIPT (induction) (μg/cm^2)	Experimental NOEL – MAX (induction) (μg/cm^2)	LOEL[a] (induction) (μg/cm^2)		
5450 [1]	70866	NA	NA	Weak	71000

[a] Data derived from HRIPT or HMT.
[b] Gerberick et al. (2001).
[c] WoE NESIL limited to two significant figures.

400–500 g, per group. The applications were repeated daily for 21 days on the same skin site. The application site was left uncovered and the reactions were read at 24 h after each application. Challenge was conducted on days 21 and 35 by applying a 0.025 ml aliquot of methyl ionone (mixture of isomers) to areas measuring 2 cm^2 on the contralateral flank of all animals as well as 6–8 untreated controls. Reactions were read at 24, 48 and/or 72 h. Methyl ionone (mixture of isomers) did not produce sensitization (Klecak et al., 1977).

4.4.3.2. A guinea pig open epicutaneous test (OET) was conducted on groups of 6–8 male and female guinea pigs weighting 300–450 g. Daily applications were made for 3 weeks to a clipped 8-cm^2 area on the flank of each guinea pig. The test sites were not covered and the reactions were read 24 h after each application. A 0.1 ml aliquot of methyl ionone (mixture of isomers) in an unspecified vehicle was applied daily for 21 days. Ten control animals were either left untreated or treated with a 0.1 ml aliquot of the vehicle for 21 days. At the challenge phase, both the test and control animals were treated on days 21 and 35 on the contralateral flank with methyl ionone (mixture of isomers) at 10%. No sensitization reactions were produced (Klecak, 1979, 1985).

4.4.3.3. Methyl ionone (mixture of isomers) was tested in another guinea pig sensitization study using a modified Draize procedure in groups of 6–8 male and female outbred Himalayan white-spotted guinea pigs. Induction consisted of 10 intradermal injections on alternate days with a dose of 0.05 ml of a 0.1% solution of methyl ionone (mixture of isomers) in isotonic saline starting on day 0 and further doses of 0.1 ml each were injected on nine alternate days. The animals were challenged on days 35 and 49 with an intradermal injection of 0.05 ml of a 0.1% solution of methyl ionone (mixture of isomers) in saline. Sensitization was not observed (Klecak et al., 1977).

4.4.3.4. A guinea pig maximization (Magnusson and Kligman, 1969) test was conducted on groups of 6–8 Himalayan white-spotted male and female guinea pigs. On day 0 the animals were injected intradermally with 0.1 ml of 5% methyl ionone (mixture of isomers), 0.1 ml of 5% methyl ionone (mixture of isomers) in Freund's complete adjuvant (FCA) and 0.1 ml of FCA alone. Each injection was given twice. In addition, 25% methyl ionone (mixture of isomers) in petrolatum was applied on day 8 to a clipped skin area of the neck for 48 h under occlusion. On day 21, an occlusive patch test with methyl ionone (mixture of isomers) at a sub-irritant concentration in petrolatum was applied to the flank for 24 h. Reactions were read at 24 and 48 h after removal of the patch. No sensitization was observed (Klecak et al., 1977).

4.4.3.5. Ishihara et al. (1986) conducted a guinea pig maximization test (Magnusson and Kligman, 1969). Induction and challenge were conducted with 10% methyl ionone (mixture of isomers) (vehicle not reported). Sensitization was not produced.

4.4.3.6. A Freund's complete adjuvant test (FCAT) was conducted on male and female guinea pigs. Five intradermal induction injections of a 0.1-ml aliquot of a 50:50 mixture of neat methyl ionone (mixture of isomers) and FCA, were made on days 0, 2, 4, 7 and 9. On days 21 and 35, the 24-h occluded challenge patch was applied to the flanks at a sub-irritant concentration of methyl ionone (mixture of isomers) in petrolatum. No sensitization reactions were produced (Klecak et al., 1977).

4.5. Phototoxicity and photoallergy

UV spectra revealed that methyl ionone (mixture of isomers) peaked at 230–235 nm range and showed minor absorption in the 245–320 nm region.

4.5.1. Phototoxcity

4.5.1.1. A phototoxicity study was conducted on two groups of four Colworth-Hartley guinea pigs. Animals were administered intradermal injections of 0.1 ml of methyl ionone (mixture of isomers) in 6% acetone/saline. A total of eight concentrations (0.1%, 0.25%, 0.5%, 1.0%, 2.5%, 5.0%, 10% and 25%) were tested on each animal. Concentrations of 1–25% were administered to one side of the clipped and shaved midline, while concentrations of 0.1–0.5% and the solvent alone were administered to the other side of the midline. After 20 min, the test sites of four animals were exposed to UV light (12 J/cm^2) for 3 h 7 min from Philips TL40W/08 fluorescent blacklamps (300–400 nm) at a distance of 35 cm (intensity, 1.07×10^3 W/cm^2). The second treated group of four animals acted as a control and were not irradiated. Reactions were evaluated at 3, 6, 24, and 48 h. Phototoxic reactions were observed at 25%; questionable reactions were observed at all other doses (RIFM, 1982a).

4.5.1.2. Phototoxicity was evaluated in groups of Colworth C57 BL mice (2/group). Methyl ionone (mixture of isomers) at 1.5, 5.0, 17, 60, 200 and 660 mg/kg in olive oil (concentrations selected such that a consistent volume of 10 ml/kg body weight was administered), was administered intraperitoneally into each mice. Thirty minutes after injection the animals were exposed to UV light (18 J/cm^2) for 4 h 16 min from Philips TL40W/08 fluorescent blacklamps (300–400 nm) at a distance of 33 cm (intensity, 1.17×10^3 W/cm^2). Reactions were evaluated at intervals up to 72 h after treatment. No phototoxicity was observed (RIFM, 1982b).

4.5.1.3. A phototoxicity test was conducted in three groups of 10 albino Wistar rats (5/sex/group). In the first group (Group A), 0.1 ml of 30% methyl ionone (mixture of isomers) in ethanol was applied to the clipped dorsum of each

animal. After 20 min, the animals were exposed to UV light (12 J/cm^2) for 2.5 h from Philips TL40W/08 fluorescent blacklight lamps (300–400 nm) at a distance of 33 cm (intensity, 1.34×10^3 W/cm^2). A second group (Group B), which was the control group, was treated with 0.1 ml of 30% methyl ionone (mixture of isomers) in ethanol, in exactly the same way as the animals from group A, but they were not exposed to UV light. In a third group (Group C), the animals were first exposed to UV light and then treated with the test material under the same conditions as for Group A. The test sites were examined immediately after irradiation and at 3, 6, 24, 48 and 75 h after treatment. Phototoxicity was observed (RIFM, 1981a). A second phototoxicity test was conducted with 30% methyl ionone (mixture of isomers) in another group of albino Wistar rats (5/sex/group) using the same protocol as above. Phototoxicity was also observed in this second test (RIFM, 1981b).

4.6. Absorption, distribution and metabolism

4.6.1. Percutaneous absorption

4.6.1.1. The in vitro skin absorption of methyl ionone (mixture of isomers) through excised pig skin was studied using glass penetration chambers. Methyl ionone (mixture of isomers) was a mixture of ^{14}C methyl ionone and non-radioactive methyl ionone dissolved in ethanol at a concentration of 10%. Sections of skin were stripped of their adipose and connective tissue and cut into 20 cm^2 diameter circles. A 5 cm^2 area on each circle was marked and radiolabeled (176.1 μCi/ml) methyl ionone (mixture of isomers) at a concentration of 10% in ethanol was applied to these areas at a dose of 600 μg/cm^2. The circles were mounted in diffusion cells with the lower part of the skin in constant contact with a physiological saline solution which was slightly agitated by a magnetic stirrer. After 6 h, the amount of methyl ionone (mixture of isomers) in the stratum corneum, stripped skin and chamber liquid was measured by liquid scintillation spectrometry. The amount of methyl ionone (mixture of isomers) that was absorbed was 9.6% of the applied dose (total amount absorbed was 41.4 μg/cm^2; 24.92 μg/cm^2 in the horny layer, 16.32 μg/cm^2 in the remaining skin tissue layers, and 0.16 μg/cm^2 in the chamber liquid). The amount of methyl ionone (mixture of isomers) recovered on the skin surface was approximately 60% of the applied dose (358.7 μg/cm^2). Approximately 30% of the applied dose was lost by evaporation (RIFM, 1984).

4.6.1.2. The same protocol as above was used to measure the in vitro skin penetration of 10% methyl ionone (mixture of isomers) in ethanol through "naked" rat skin. Radiolabeled (176.1 μCi/ml) methyl ionone (mixture of isomers) at a concentration of 10% in ethanol was applied to 5 cm^2 areas areas at a dose of 600 μg/cm^2. The amount of methyl ionone (mixture of isomers) in the stratum corneum, stripped skin and chamber liquid was measured at 1, 6 and 16 h by liquid scintillation spectrometry. The total amount of methyl ionone (mixed isomers) that was absorbed after 1 h was 22.4% of the applied dose (total amount absorbed was 134.28 μg/cm^2; 27.11 μg/cm^2 in the horny layer, 107.13 μg/cm^2 in the remaining skin tissue layers, and 0.04 μg/cm^2 in the chamber liquid). The penetration rate value was time-dependent and reached 48.2% of the applied dose (289.1 μg/cm^2) at 16 h. At this time, the amount of methyl ionone (mixture of isomers) recovered on the skin surface was approximately 11.7% of the applied dose (70.35 μg/cm^2). Approximately 30% of the applied dose was lost by evaporation (RIFM, 1984).

4.7. Subchronic toxicity

No data available.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

4.9.1. Bacterial studies

4.9.1.1. A bacterial reverse mutation assay was conducted using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and *Escherichia Coli* strain WP2uvrA in the presence or absence of Aroclor-1254 induced rat liver S9. The assay was performed in two phases using the plate incorporation method. In the preliminary toxicity assay using concentrations ranging from 6.7 μg/plate to 5000 μg/plate methyl ionone (mixture of isomers) in DMSO, toxicity was observed at $\geqslant$667 μg per plate and at 5000 μg per plate with strain TA100 in the absence and presence of S9 activation, respectively. Toxicity was observed at $\geqslant$1000 μg per plate and at $\geqslant$3333 μg per plate in the absence of S9 activation with strains TA1535 and TA1537, respectively. Based on the findings of the toxicity assay, the maximum dose plated in the mutagenicity assay was 5000 μg per plate. In the mutagenicity assay using concentrations of 25–5000 μg/plate in DMSO, no mutagenic effects were observed. Toxicity was observed at $\geqslant$1800 μg per plate with strain TA100 in the presence or absence of S9 activation. Toxicity was observed at $\geqslant$1800 μg per plate with the strain TA1537. Based on these results, methyl ionone (mixture of isomers) was reported to be negative in the bacterial reverse mutation assay (RIFM, 1999).

4.9.2. Mammalian studies

4.9.2.1. The clastogenic potential of methyl ionone (mixture of isomers) was tested in a chromosome aberration assay using Chinese hamster ovary (CHO) cells in the absence and presence of aroclor-induced S9 activation system. A preliminary toxicity test was performed to establish the dose range. Cells were exposed to concentrations

ranging from 0.206 to 2060 μg/ml in ethanol and treated in a medium for a 4-h period with and without S9 system and for 20-h period without S9 system. Complete cell growth inhibition (100%) was seen at dose levels ⩾206 μg/ml in all treatment groups. About 50 – 70% cell growth inhibition was observed at a dose of 61.8 μg/ml. Based on these findings, doses of 12.5–175 μg/ml were selected for the chromosome aberration assay. In this assay, cells were treated for 4 and 20 h without S9 and for 4 h with the S9-activation system. All cells were harvested at 20 h after treatment. Complete cell growth inhibition (100%) was observed at ⩾75 μg/ml after 4-h treatment with and without S9. Complete cell growth inhibition was seen after 20-h treatment without S9 at ⩾ 100 μg/ml. The chromosome aberration assay was therefore evaluated at 12.5, 25, and 50 μg/ml. At 50 μg/ml, cell growth inhibition was about 60–68% in all treatment groups. Methyl ionone (mixture of isomers) did not produce any significant structural or numerical chromosome aberrations after a 4-h treatment without S9, but in the presence of S9 there was an increase in structural chromosome aberrations at the highest dose of 50 μg/ml as compared to ethanol controls. However, since the increase in the percentage of structurally aberrant cells was within the range of historical control values, the chromosome aberrations observed in the presence of S9 after the 4-h treatment with methyl ionone (mixture of isomers) were considered not biologically significant. There was an increase in numerical chromosome aberrations at the dose of 25 μg/ml after 4-h treatment in the absence of S9. However, this increase was also within the historical control values. Structural chromosome aberrations were seen after 20 h exposure at doses of 12.5 and 25 μg/ml. There were no increases in numerical chromosome aberrations in this group. Based on these results, it was concluded that methyl ionone (mixture of isomers) was positive in the absence of S9 and negative in the presence of S9 for the induction of structural chromosome aberrations in CHO cells. Methyl ionone (mixture of isomers) was negative in both the absence and presence of S9 for the induction of numerical chromosome aberrations in CHO cells (RIFM, 2000).

4.9.2.2. A mouse micronucleus assay was conducted in male and female ICR mice (5/sex/dose). Methyl ionone (mixture of isomers) in corn oil was administered by intraperitoneal injection at a constant volume of 20 ml/kg body weight. The test animals were dosed with 462.5, 925, or 1850 mg/kg body weight methyl ionone (mixture of isomers). Bone marrow was collected 24 and 48 h after dose administration. Mortality was observed in only 1/15 male mice receiving 1850 mg/kg. This animal was replaced at the time of bone marrow collection with a replacement animal that also received 1850 mg/kg. Methyl ionone (mixture of isomers) did not induce a significant increase in micronucleated polychromatic erythrocytes in either male or female mice (RIFM, 2000).

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used As Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

J. Lalko, A. Lapczynski, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on 3-methyl-4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one

A. Lapczynski *, J. Lalko, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of 3-methyl-4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; 3-Methyl-4-(2,4,6-trimethyl-3-cyclohexen-l-yl)-3-buten-2-one

Contents

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

In 2005, a complete literature search was conducted on 3-methyl-4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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doi:10.1016/j.fct.2007.09.010

submit pertinent test data. All relevant references are included in this document.

1. Identification (Fig. 1)

1.1 Synonyms: 3-buten-2-one, 3-methyl-4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-; 3-methyl-4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one.
1.2 CAS Registry number: 67801-29-0.
1.3 EINECS number: 267-149-3.
1.4 Formula: $C_{14}H_{22}O$.
1.5 Molecular weight: 206.29.

2. Physical properties

2.1 Log K_{ow} (calculated): 4.81.
2.2 Molecular weight: 206.29.
2.3 Henry's law (calculated): 0.000283 atm m^3/mol 25C.

3. Usage (Table 1)

3-Methyl-4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of <0.1 metric tonnes per annum.

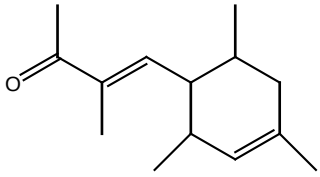

Fig. 1. 3-Methyl-4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one.

The maximum skin level that results from the use of 3-methyl-4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one in formulae that go into fine fragrances has been reported to be 0.02% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.521% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.0133 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity

No data available on this material.

4.2. Skin irritation

No data available on this material.

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

No data available on this material.

4.5. Phototoxicity and photoallergy

No data available on this material.

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing 3-methyl-4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.521	0.0020
Face cream	0.80	2.00	1.000	0.003	0.521	0.0004
Eau de toilette	0.75	1.00	1.000	0.080	0.521	0.0052
Fragrance cream	5.00	0.29	1.000	0.040	0.521	0.0050
Antiperspirant	0.50	1.00	1.000	0.010	0.521	0.0004
Shampoo	8.00	1.00	0.010	0.005	0.521	0.0000
Bath products	17.00	0.29	0.001	0.020	0.521	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.521	0.0001
Toilet soap	0.80	6.00	0.010	0.015	0.521	0.0001
Hair spray	5.00	2.00	0.010	0.005	0.521	0.0000
Total						0.0133

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, J. Lalko, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

References

Belsito, D., Bickers, D., Bruze, M., Calow, P., Greim, H., Hanifin, J.H., Rogers, A.E., Saurat, J.H., Sipes, I.G., Tagami, H., 2007. A toxicologic and dermatologic assessment of ionones when used as fragrance ingredients. Food and Chemical Toxicology 45 (1S1), S130–S167.

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Review

Fragrance material review on 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one

A. Lapczynski *, J. Lalko, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

In 2005, a complete literature search was conducted on 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

1. Identification (Fig. 1)

1.1 Synonyms: 3-Buten-2-one, 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-; 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one; iritone;
1.2 CAS Registry Number: 67801-38-1.
1.3 EINECS Number: 267-158-2.
1.4 Formula: $C_{13}H_{20}O$.
1.5 Molecular weight: 192.3.

2. Physical properties

2.1 Physical form: A pale straw-colored, slightly viscous liquid.
2.2 Log K_{ow} (calculated): 4.26.
2.3 Refractive index @ 20 °C: 1.494.

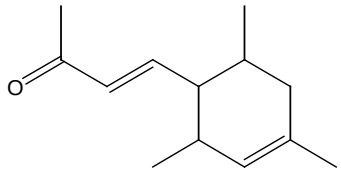

Fig. 1. 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one.

3. Usage

4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of <0.1 metric tones per annum.

The maximum skin level that results from the use of 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one in formulae that go into fine fragrances has been reported to be 0.007% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.045% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.0011 mg/kg for high end users of these products (see Table 1).

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1 The acute oral LD_{50} in rats was calculated to be 5.2 g/kg (95% C.I. 3.8–7.2 g/kg). Rats (10/dose) were administered a single oral dose of 3.51, 5.0, 7.12 or 10.14 g/kg of 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one. Observations for mortality and/or systemic effects were made over a 5-day period. At the completion of the study, a gross necropsy was conducted on all animals. At 3.51 g/kg, one (1/10) death occurred and the systemic effects observed in this animal included lethargy, flaccid muscle tone, ptosis, piloerection and ataxia and necropsy revealed red intestines and dark areas of the lung, kidney, liver and spleen. At 5.0 g/kg, 6/10 deaths occurred and the systemic effects observed in these animals included diarrhea, coma, lethargy, ptosis, piloerection and chromorhinorrhea. Five (5/10) deaths occurred at 7.12 g/kg and

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.0446	0.0002
Face cream	0.80	2.00	1.000	0.003	0.0446	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.0446	0.0004
Fragrance cream	5.00	0.29	1.000	0.040	0.0446	0.0004
Antiperspirant	0.50	1.00	1.000	0.010	0.0446	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.0446	0.0000
Bath products	17.00	0.29	0.001	0.020	0.0446	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.0446	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.0446	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.0446	0.0000
Total						0.0011

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity data

Route	Species	No. animals/ dose group	LD_{50}	References
Oral	Rat	10	5.2 g/kg	RIFM (1978)
Dermal	Rabbits	10	>5 g/kg	RIFM (1978)

9/10 deaths occurred at 10.14 g/kg. Systemic effects at both of these doses included diarrhea, lethargy, ataxia, ptosis, piloerection, convulsions, chromorhinorrhea, and slightly flaccid muscle. Necropsy findings in the three highest dose groups included exudate from the nose and mouth, red and yellow intestines, bloated stomach and dark/mottled areas of the lung, kidney, liver and spleen (RIFM, 1978).

4.1.2. Dermal studies

4.1.2.1 The acute dermal LD_{50} in rabbits was reported to be greater than 5.0 g/kg based on no deaths in 10 rabbits tested at that dose. The rabbits received a single dermal application of 5 g/kg of neat 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one. Observations for mortality and/or systemic effects were made over a 5-day period. Necropsy revealed brown anogential exudates from the mouth/nose, dark livers, white nodules in the liver, dark areas in the lungs and pale kidneys (RIFM, 1978).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1 As a part of a human maximization study, irritation was evaluated on the backs of 28 male volunteers. An occluded 48-hour patch with 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one at 20% in petrolatum did not produce any irritation (RIFM, 1978a).

4.2.2. Animal studies

4.2.2.1 As a part of an acute dermal LD_{50} study in rabbits, neat 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one was applied for 24 h under occlusion at a dose level of 5.0 g/kg. Irritation was evaluated over a 5-day observation period. Moderate (8/10 rabbits) to severe (2/10 rabbits) erythema and slight (5/10 rabbits) to moderate (5/10 rabbits) edema were observed (RIFM, 1978).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1 A human maximization test (Kligman, 1966) was carried out with 20% 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one in petrolatum on 28 healthy male volunteers. The induction application was under occlusion to the same site on the volar forearms of all subjects for five alternate-day 48-hour periods. Patch sites were pretreated for 24 h with 5.0% aqueous sodium lauryl sulfate (SLS) under occlusion for the initial patch only. Following a ten to fourteen day rest period, a challenge patch of 20% test material in petrolatum was applied to the back for 48 h under occlusion both with and without SLS. Reactions to challenge were read at removal and 24 h after patch removal. No reactions were observed (RIFM, 1978b).

4.4.2. Animal studies

No data available on this material.

4.5. Phototoxicity and photoallergy

UV spectra reveals that 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one does not absorb UV light at wavelengths in the range of 290–400 nm and therefore would have no potential to elicit photoirritation or photoallergy under the current conditions of use as fragrance ingredient.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, J. Lalko, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded

by the manufacturers of fragrances and consumer products containing fragrances.

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RIFM (Research Institute For Fragrance Materials, Inc.) 1978a. Report on human maximization studies with 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one. RIFM report number 1698, February, 27 (RIFM, Woodcliff Lake, NJ, USA).

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Review

Fragrance material review on 4-(3,5,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one

A. Lapczynski *, J. Lalko, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of 4-(3,5,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; 4-(3,5,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one

Contents

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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doi:10.1016/j.fct.2007.09.035

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing 4-(3,5,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.02	0.0001
Face cream	0.80	2.00	1.000	0.003	0.02	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.02	0.0002
Fragrance cream	5.00	0.29	1.000	0.040	0.02	0.0002
Antiperspirant	0.50	1.00	1.000	0.010	0.02	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.02	0.0000
Bath products	17.00	0.29	0.001	0.020	0.02	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.02	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.02	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.02	0.0000
Total						**0.0005**

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

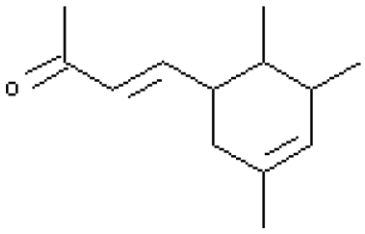

Fig. 1. 4-(3,5,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one.

When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

In 2005, a complete literature search was conducted on 4-(3,5,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

1. Identification (Fig. 1)

1.1 Synonyms: 3-Buten-2-one,4-(3,5,6-trimethyl-3-cyclohexen-1- yl)-.
1.2 CAS Registry Number: 67801-39-2.
1.3 EINECS Number: 267-159-8.
1.4 Formula: $C_{13}H_{20}O$.
1.5 Molecular Weight: 192.02.

2. Physical properties

2.1 Log K_{ow} (calculated): 4.26.

3. Usage

(4-3,5,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of less than 0.1 metric tonnes per annum.

The maximum skin level that results from the use of 4-(3,5,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one in formulae that go into fine fragrances has not been reported. A default value of 0.02% is used, assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has not been reported. As such the default value of 0.02% is used to calculate the maximum daily exposure on the skin of 0.0005 mg/kg for high end users of these products (see Table 1).

4. Toxicology data

4.1. Acute toxicity

No data available on this material.

4.2. Skin irritation

No data available on this material.

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

No data available on this material.

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Ionones When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, J. Lalko, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

Reference

Belsito, D., Bickers, D., Bruze, M., Calow, P., Greim, H., Hanifin, J.H., Rogers, A.E., Saurat, J.H., Sipes, I.G., Tagami, H., 2007. A toxicologic and dermatologic assessment of ionones when used as fragrance ingredients. Food and Chemical Toxicology 45 (1S1), S130–S167.

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Review

A toxicologic and dermatologic assessment of salicylates when used as fragrance ingredients ☆

The RIFM Expert Panel
D. Belsito [a], D. Bickers [b], M. Bruze [c], P. Calow [d], H. Greim [e], J.M. Hanifin [f], A.E. Rogers [g], J.H. Saurat [h], I.G. Sipes [i], H. Tagami [j]

[a] *University of Missouri (Kansas City), clo American Dermatology Associates, LLC, 6333 Long Avenue, Third Floor, Shawnee, KS 66216, USA*
[b] *Columbia University Medical Center, Department of Dermatology, 161 Fort Washington Avenue, New York, NY 10032, USA*
[c] *Malmo University Hospital, Department of Occupational & Environmental Dermatology, Sodra Forstadsgatan 101, Entrance 47, Malmo SE-20502, Sweden*
[d] *Institut for Miliovurdering, Environmental Assessment Institute, Linnésgade 18, 1st floor, Copenhagen 1361 K, Denmark*
[e] *Technical University of Munich, Institute for Toxicology & Environmental Hygiene, Hohenbachernstrasse 15-17, Freising-Weihenstephan D-85354, Germany*
[f] *Oregon Health Sciences University, Department of Dermatology L468, 3181 SW Sam Jackson Park Road, Portland, OR 97201-3098, USA*
[g] *Boston University School of Medicine, Department of Pathology and Laboratory Medicine, 715 Albany Street, L-804, Boston, MA 02118-2526, USA*
[h] *Hospital Cantonal Universitaire, Clinique et Policlinique de Dermatologie, 24, Rue Micheli-du-Crest, Geneve 14 1211, Switzerland*
[i] *Department of Pharmacology, University of Arizona, College of Medicine, 1501 North Campbell Avenue, P.O. Box 245050, Tucson, AZ 85724-5050, USA*
[j] *3-27-1 Kaigamori, Aoba-ku, Sendai 981-0942, Japan*

Abstract

An evaluation and review of a structurally related group of fragrance materials.

Keywords: Safety; Review; Salicylates; Fragrance

Contents

☆ All correspondence should be addressed to: A.M. Api, 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: amapi@rifm.org

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1. Chemical identity and exposure (Table 1)

This report summarizes scientific data relevant to the risk assessment of the use of salicylates as fragrance ingredients (Table 1). The 17 salicylates considered here include alkyl (*i.e.*, methyl-, ethyl-, butyl-, isobutyl-, pentyl-, isoamyl-, hexyl-, and ethyl hexylsalicylate), alkenyl (*i.e.*, *cis*-3-hexenyl-, *trans*-2-hexenyl-, 1,3-dimethyl-3-butenyl, and 3-methyl-2-butenyl salicylate), aromatic ring (*i.e.*, benzyl-, phenyl-, *p*-cresyl- and phenethyl salicylate) and other (*i.e.*, 4-methylsalicylate) derivatives of salicylic acid. Most of these substances are used as fragrance and flavor ingredients. This report presents and synthesizes animal and human data, including studies by various routes of exposure, and emphasizes the risk assessment for use of salicylates as fragrance ingredients. The scientific evaluation focuses on dermal exposure, which is considered to be the primary exposure route for fragrance materials. Where relevant, toxicity, metabolism and biological fate data from other routes of exposure have also been considered.

The current format for these RIFM publications includes a summary evaluation paper of the chemical group and individual Fragrance Material Reviews on the individual chemicals. The group summary is an evaluation of relevant data selected from the large bibliography of studies and reports on the individual chemicals. The selected data were deemed to be relevant based on the nature of the protocols, quality of the data, statistical significance, and appropriate exposure. These data are presented in tabular form in the group summary. The Fragrance Material Reviews on each individual salicylate contain a comprehensive summary of published and unpublished reports and comprehensive bibliographies.

Salicylates are ingredients of many fragrances. They may be found in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents.

Many of the salicylates assessed in this report have been evaluated and approved for use as flavor ingredients in foodstuffs. In the United States, 5 of the 17 salicylates (ethyl salicylate, isobutyl salicylate, isoamyl salicylate, benzyl salicylate, and phenethyl salicylate) have been approved for use as flavors by the Food and Drug Administration (FDA) in accordance with (21 CFR 172.515). In addition, methyl (2475), ethyl (2458), butyl (3650), isobutyl (2213), isoamyl (2084), benzyl (2151), phenyl (3960), phenethyl (2868) salicylate have been granted Generally Recognized

Table 1
Material identification, summary of volume of use, and dermal exposure

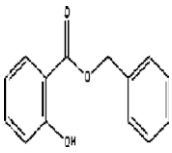
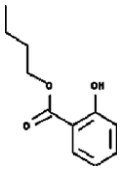
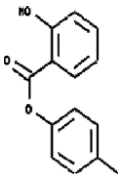
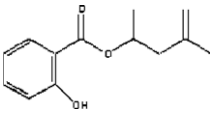
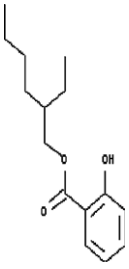
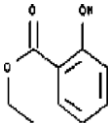
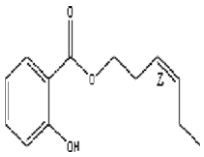
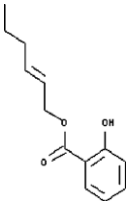
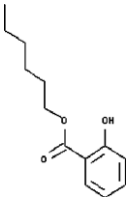
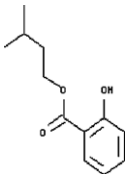
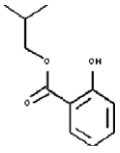
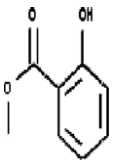
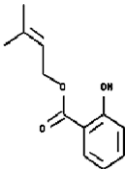
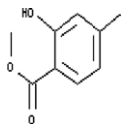
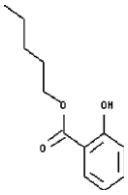
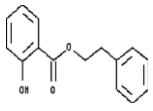
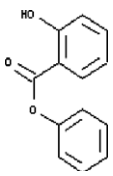
Material	Synonyms	Structure	Worldwide metric tons	Dermal systemic exposure in cosmetic products (mg/kg/day)	Maximum skin level (%)
Benzyl salicylate CAS# 118-58-1 Molecular weight: 228.25 Log K_{ow} (calculated): 4.31	Benzoic acid, 2-hydroxy-, phenylmethyl ester; Benzyl 2-hydroxybenzoate; Benzyl *o*-hydroxybenzoate; 2-Hydroxybenozic acid; Phenylmethyl 2-hydroxybenozate; Salicylic acid, benzyl ester		>1000	0.4023	6.71
Butyl salicylate CAS# 2052-14-4 Molecular weight: 194.23 Log K_{ow} (calculated): 4.08	Benzoic acid, 2-hydroxy-, butyl ester; *n*-Butyl *o*-hydroxybenzoate; *n*-Butyl salicylate		<0.01	0.0005[a]	0.02
p-Cresyl salicylate CAS# 607-88-5 Molecular weight: 228.25 Log K_{ow} (calculated): 4.37	Benzoic acid, 2-hydroxy-, 4-methylphenyl ester; *p*-Tolyl salicylate		< 0.01	0.0003	0.001
1,3-Dimethyl-3-butenyl salicylate CAS # 80118-10-1 Molecular weight: 220.26 Log K_{ow} (calculated): 4.91	Benzoic acid, 2-hydroxy-, 1,3-dimethyl-3-butenyl ester		0.1–1.0	0.0005[a]	0.02
Ethyl hexyl salicylate CAS# 118-60-5 Molecular weight: 250.34 Log K_{ow} (calculated): 5.97	Benzoic acid, 2-hydroxy-, 2-ethylhexyl ester; Dermoblock OS; Escalol 587; 2-Ethylhexyl 2-hydroxybenzoate; 2-Ethylhexyl salicylate; Eusolex OS; Heliosol 2; Neo Heliopan; Type OS; Neotan L; Salicylic acid 2-ethylhexyl ester; Trivent OS		0.1–1.0	0.0005[a]	0.02
Ethyl salicylate CAS# 118-61-6 Molecular weight: 166.18 Log K_{ow} (calculated): 3.09	Benzoic acid, 2-hydroxy-, ethyl ester; Ethyl 2-hydroxybenzoate; Ethyl *o*-hydroxybenzoate: Ethyl salicylate; salicylic ether		1–10	0.0002	0.14

Table 1 (*continued*)

Material	Synonyms	Structure	Worldwide metric tons	Dermal systemic exposure in cosmetic products (mg/kg/day)	Maximum skin level (%)
cis-3-Hexenyl salicylate CAS# 65405-77-8 Molecular weight: 220.27 Log K_{ow} (calculated): 4.84	Benzoic acid, 2-hydroxy-, 3-hexenyl ester; (*Z*)-3-Hexenyl 2-hydroxybenzoate; (Z)-3-Hexenyl salicylate, *cis*-3-Hexenyl salicylate		100–1000	0.10	2.02
trans-2-Hexenyl salicylate CAS# 68133-77-7 Molecular weight: 220.27 Log K_{ow} (calculated): 4.84	(*E*)-2-Hexenyl salicylate; salicylic acid, 2-hexenyl ester, (e)		<0.01	0.0955	0.17
Hexyl salicylate CAS# 6259-76-3 Molecular weight: 222.28 Log K_{ow} (calculated): 5.06	Benzoic acid, 2-hydroxy-, hexyl ester; Hexyl *o*-hydroxybenzoate		>1000	0.1108	2.86
Isoamyl salicylate CAS# 87-20-7 Molecular weight: 208.26 Log K_{ow} (calculated): 4.49	Benzoic acid, 2-hydroxy-, 3-methylbutyl ester; Isoamyl *o*-hydroxybenzoate; Isopentyl salicylate; 3-Methylbutyl *o*-hydroxybenzoate; 3-Methylbutyl salicylate		100–1000	0.1042	2.19
Isobutyl salicylate CAS# 87-19-4 Molecular weight: 194.23 Log K_{ow} (calculated): 4.0	Benzoic acid, 2-hydroxy-, 2-methylpropyl ester; Isobutyl *o*-hydroxybenzoate; 2-Methylpropyl *o*-hydroxybenzoate; 2-Methyl-1-propyl salicylate		10–100	0.0043	0.81
Methyl salicylate CAS# 119-36-8 Molecular weight: 152.15 Log K_{ow} (calculated): 2.5	Benzoic acid, 2-hydroxy-,methyl ester; 2-Carbomethoxyphenol; 2-hydroxybenzoic acid, methyl ester; Methyl 2-hydroxybenozate; Salicylic acid, methyl ester; Synthetic sweet birch oil; Synthetic teaberry oil; Synthetic wintergreen oil		10–100	0.0034	0.29
3-Methyl-2-butenyl salicylate CAS# 68555-58-8 Molecular weight: 206.24 Log K_{ow} (calculated): 4.41	Benzoic acid, 2-hydroxy-, 3-methyl-2-butenyl ester; Prenyl salicylate		1–10	0.0005[a]	0.02

(*continued on next page*)

Table 1 (*continued*)

Material	Synonyms	Structure	Worldwide metric tons	Dermal systemic exposure in cosmetic products (mg/kg/day)	Maximum skin level (%)
Methyl-4-methyl salicylate CAS# 4670-56-8 Molecular weight: 166–76 Log K_{ow} (calculated): 3.15	Benzoic acid, 2-hydroxy-4-methyl-, methyl ester; Methyl 2-hydroxy-4-methylbenzoate;		<0.1	0.0005[a]	0.02
Pentyl salicylate CAS# 2050-08-0 Molecular weight: 208.26 Log K_{ow} (calculated): 4.57	Amyl salicylate; Benzoic acid, 2-hydroxy-, pentyl ester; 2-Hydroxybenzoic acid, pentyl ester; Pentyl 2-hydroxybenzoate; Salicylic acid, pentyl ester		100–1000	0.1766	2.98
Phenethyl salicylate CAS# 87-22-9 Molecular weight: 242.28 Log K_{ow} (calculated): 4.8	Benzoic acid, 2-hydroxy-, 2-phenylethyl ester; Benzylcarbinyl 2-hydroxybenzoate; Benzylcarbinyl salicylate; 2-Phenylethyl 2-hydroxybenzoate, Phenylethyl salicylate; 2-Phenylethyl salicylate		1–10	0.0480	1.49
Phenyl salicylate CAS# 118-55-8 Molecular weight: 214.22 Log K_{ow} (calculated): 3.82	Benzoic acid, 2-hydroxy-, phenyl ester; 2-Hydroxybenzoic acid, phenyl ester; 2-Phenoxycarbonylphenol; Phenyl-2-hydroxybenzoate; Salol		<0.1	0.0005[a]	0.02

[a] A default value of 0.02% was used to calculate dermal systemic exposure.

as Safe (GRAS) status by the Flavor and Extract Manufacturers' Association.

The Council of Europe list of substances (Numbers 141, 145, 169, 144) that may be used in foodstuffs (*i.e.*, "A" status) includes only methyl salicylate (COE No. 433). Ethyl salicylate (COE No. 432), isobutyl salicylate (COE No. 434), pentyl salicylate (COE No. 613), isoamyl salicylate (COE No. 435), benzyl salicylate (COE No. 436), butyl salicylate (COE No. 614) and phenethyl salicylate (COE No. 437) were included by the Council of Europe in the list of substances granted "B status" (*i.e.*, those substances requiring information, in the case of the salicylates most often requiring hydrolysis data.

The International Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2001) has evaluated 7 of the 17 salicylates assessed in this report. The estimate of intake based on total annual production includes the assumption that only 10% of the population eats these agents. However, the analysis showed that >50% of the population would be expected to eat 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 body weight, which is below the current JECFA Acceptable Daily Intake (ADI) of 0–0.5 mg/kg body weight/day established for methyl salicylate (JECFA, 2001). The other six salicylates including ethyl-, butyl-, isobutyl-, isoamyl-, and phenethyl salicylate, were judged by the Committee not to present a safety concern at current estimated intake levels (JECFA, 2001).

Three salicylates, methyl salicylate, pentyl salicylate, and benzyl salicylate, are High Production Volume (HPV) materials and, as such, have been included in a Robust Summary and Test Plan for "Benzyl Derivatives", a document prepared by the Flavor and Fragrance High Production Volume Consortium.

Salicylates and their derivatives are present in many plant essential oils (Bauer and Garbe, 1985). Stofberg and Grundschober (1987) report that, in descending order,

isoamyl salicylate, methyl salicylate, ethyl salicylate, butyl salicylate and benzyl salicylate are naturally present in commonly eaten foodstuffs. Methyl salicylate occurs naturally in fruits, coffee, tea, and alcoholic beverages. It is also the chief component of wintergreen oil used in food and various over-the-counter health care products (*e.g.*, Ben-Gay and mouth rinses).

The annual worldwide use of the individual salicylates varies greatly and ranges from an estimated 0.01 metric tonnes (phenyl salicylate) to upwards of 2496 metric tonnes (benzyl salicylate) (Table 1). For most of the individual salicylates, annual worldwide production/use is in the range of 10–100 metric tonnes. For a number of the individual salicylates, notably, *trans*-2-hexenyl and methyl 4-methyl salicylate, no estimate of worldwide production/use was available.

1.1. Estimated consumer exposure

The availability of fragrance ingredients for potential consumer exposure is estimated in two ways (see Table 1). One estimates potential percutaneous absorption over the entire body due to the use of many different fragranced products. The other estimates potential dermal exposure due to the use of products, such as fine fragrances, that usually contain higher concentrations and are used on smaller localized skin sites. Potential skin exposure to the salicylates is estimated based on their concentrations in 10 types of cosmetic products (body lotion, face cream, eau de toilette, fragrance cream, anti-perspirant, shampoo, bath products, shower gel, toilet soap and hair spray). The concentration data in the 10 product types was multiplied by the amount of product applied, the number of applications/day for each product type, and a "retention factor" (ranging from 0.01 to 1.0) to account for the length of time a product may remain on the skin and/or the likelihood of it being removed by washing. The value produced represents the maximum skin concentration associated with each product type. As a conservative measure, the total maximum skin concentration was calculated to be the sum of the maximum skin concentrations for each of the 10 product categories.

The maximum skin exposure levels of the salicylates that form part of the formulae of fine fragrances varies widely and have been reported to range from 0.001% to 6.71%. For consideration of potential sensitization, the exposure is calculated as the percent concentration applied to the skin. Exposure to salicylates used in fine fragrance products is calculated based on the use of 20% of the fragrance mixture (the maximum used) in the fine fragrance consumer product (IFRA, 2004). The calculated exposures for the salicylates used in cosmetic products are listed in Table 1. Maximum daily exposures on the skin range from 0.0002 to 0.4023 mg/kg/day for the individual salicylates in high end users of cosmetic products containing these materials (see Table 1).

Maximum skin exposure data (the total of the 10 individual product categories) for each of the salicylates assessed were used to calculate potential systemic exposures. Systemic exposures (*i.e.*, the dose absorbed through the skin and available to the systemic circulation) were estimated based on dermal absorption rates. Where such data were lacking, as a conservative measure, dermal absorption was considered to be 100% (*i.e.*, the maximum skin exposure value was considered as the estimate of systemic exposure). Systemic exposure estimates were compared to indices of systemic toxicity such as NOAEL and LOAEL values from subchronic, chronic, and reproductive toxicity studies.

Exposure data were provided by the fragrance industry. Further explanation of how the data were obtained and of how exposures were determined have been previously reported by Cadby et al. (2002) and Ford et al. (2000).

2. Absorption, distribution and metabolism, and potential for enzyme induction

2.1. Absorption

2.1.1. Percutaneous absorption (Tables 2–5)

The percutaneous absorption of a number of the alkyl salicylates as well as of benzyl- and phenyl salicylate has been studied in humans, both *in vivo* (Brown and Scott, 1934a,b; Beutner et al., 1943; Cross et al., 1997; Cross et al., 1998; Yano et al., 1986; Treffel and Gabard, 1996) and *in vitro* (Watkinson et al., 1992; Treffel and Gabard, 1996; Cross et al., 1998), as well as in animals (Siddiqi and Ritschel, 1972; Yano et al., 1991; Jimbo, 1983; RIFM, 1983a; Boehnlein et al., 1994; Higo et al., 1995; Riviere et al., 2000, 2001; Duncan et al., 2002), The most extensive dermal absorption data exist for methyl salicylate.

2.1.1.1. Human studies (Tables 2 and 3)

2.1.1.1.1. In vivo human studies. Beutner et al. (1943), using crude methods, reported that application of a substance containing 20% methyl salicylate and 80% anhydrous lanolin resulted in average salicylic acid excretion of approximately 2%.

More recent dermal studies indicate that there is considerable penetration of methyl salicylate or pentyl salicylate into the dermis and subcutaneous tissue (Yano et al., 1986; Cross et al., 1997, 1998). Through the use of microdialysis probes placed in the skin adjacent to the site where a 20% methyl salicylate preparation was applied under occlusion every 2–3 h, for 24 h, 30.7% of the methyl salicylate was found to have penetrated into the dermis and/or subcutaneous tissue (Cross et al., 1997). Similarly, Yano et al. (1986) reported 92.9% absorption of methyl salicylate in the subcutaneous tissue following the application of 0.5 mg methyl salicylate, under occlusion for 4 h, to the forearms of 28 male volunteers. In the same experiment absorption of 58.6% and 17.1% was reported for ethyl

Table 2
Summary of human *in vivo* percutaneous absorption data

Material	Method	Results	References
Butyl salicylate	4 h occluded application to the forearm	17.1%	Yano et al. (1986)
Pentyl salicylate	1 h open application to the hand	43 mg (average excretion)	Brown and Scott (1934b)
Ethyl salicylate	4 h occluded application to the forearm	58.6%	Yano et al. (1986)
Ethyl hexyl salicylate	30 min open application to the back	1–50%	Treffel and Gabard (1996)
Methyl salicylate	20 min open application	~22% (calculated uptake of salicylic acid)	Pratzel et al. (1990)
Methyl salicylate	8 h occluded application using 2, 4 or 8 patches	8.6–29.5 ng/ml	Martin et al. (2004)
Methyl salicylate	6 h open application to the chest and back	1–2.6%	Danon et al. (1986)
Methyl salicylate	1 h continuous massage with 2 cm^3 every 5 min at 38 °C	138 mg (average excretion)	Brown and Scott (1934b)
Methyl salicylate	1 h occluded application to the forearm	278–292 mg (average excretion of sodium salicylate)	Brown and Scott (1934a)
Methyl salicylate	1 h open application to trunk	Traces (sodium salicylate excretion)	Brown and Scott (1934a)
Methyl salicylate	1 h open application by adding 2 cm^3 every minute	284 mg (average excretion)	Brown and Scott (1934b)
Methyl salicylate	1 h open application	300 mg (at 0.16% suspension)–429 mg (at 5% suspension) (average excretion)	Brown and Scott (1934b)
Methyl salicylate	24 h open application to the chest, abdominal and thigh	~2% (average salicylic acid excretion)	Beutner et al. (1943)
Methyl salicylate	4 h occluded application to the forearm	92.9%	Yano et al. (1986)
Methyl salicylate	Open application to the thigh every 12 h for 4 days	15.5–22%	Morra et al. (1996)
Methyl salicylate	6 h open application to forearm	30.7%	Cross et al. (1998)
Methyl salicylate	10 h occluded application to forearm	12–20%	Roberts et al. (1982)

Table 3
Summary of human *in vitro* percutaneous absorption data

Material	Method	Results	References
Benzyl salicylate	72 h exposure; abdominal skin	0.031%	Jimbo (1983)
Ethyl hexyl salicylate	2 min, 0.5, 2 and 6 h exposures; abdominal skin	40–113%	Treffel and Gabard (1996)
Isoamyl salicylate	72 h exposure; abdominal skin	0.008%	Jimbo (1983)
Methyl salicylate	24 h exposure; full thickness breast skin	11.2 μg/cm^2/h	Cross et al. (1998)
Methyl salicylate	24 h exposure; epidermal membrane	32.8 μg/cm^2/h	Cross et al. (1998)
Octyl salicylate[a]	48 h exposure; full thickness abdominal skin	0.23–0.65%	Walters et al. (1997)
Octyl salicylate[a]	24 h exposure; abdominal skin	7.1 μg	Jiang et al. (1997)

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

salicylate and butyl salicylate, respectively (Yano et al., 1986).

Martin et al. (2004) reported on a dermal absorption test in which 24 human volunteers were exposed to 74.88 mg methyl salicylates for 8 h under 2, 4 or 8 patches. Ten blood samples were obtained from each subject at different time points over 24 h. The average maximum plasma concentrations in $C_{max} \pm SD$ for methyl salicylates were 29.5 ± 10.5 ng/ml (8 patches, 599 mg methyl salicylate), 16.8 ± 6.8 ng/ml (4 patches, 300 mg), and 8.6 ± 3.8 ng/mL (2 patches, 150 mg). The material was not detected beyond 8 h. The researchers were unable to determine the absolute dermal bioavailability of methyl salicylate.

Roberts et al. (1982) reported dermal application to humans of 5 g of various formulations containing methyl salicylate at concentrations of 12.5–50% under occlusive patches for 10 h. Only about 12–20% of the methyl salicylate applied was absorbed into systemic circulation. In a human crossover study, Morra et al. (1996) topically applied 5 g of ointment containing 12.5% methyl salicylate to six men and six women twice daily for 4 days. Salicylic acid and associated metabolites (salicyluric acid and glucuronides) were recovered in the urine and accounted for 15.5% (day 1) to 22% (days 2–4) of the topically applied dose, indicating significant systemic absorption.

2.1.1.1.2. In vitro human studies. *In vitro* percutaneous studies in human skin preparations demonstrate that salicylates penetrate dermal tissues. Cross et al. (1998) reported a permeability of methyl salicylate (flux calculated from the cumulative amount versus time) of 11.2 ± 0.7 μg/cm^2/h for full thickness skin and 32.8 ± 2.0 μg/cm^2/h for epidermal membrane following application of 20% commercial formulation (containing 20% methyl salicylate, 7% glycol salicylate and 10% triethanolamine salicylate (TEASA) to the stratum corneum. This represents total 24-h absorption of approximately 0.2%. Lesser amounts were retained within the skin sample. Treffel and Gabard (1996) measured skin penetration of ethyl hexyl salicylate

(3%) in either an emulsion gel or petrolatum jelly, using static diffusion Franz cells. Four applications times were investigated (2 min, 0.5 h, 2 h and 6 h). The total recovery in dermis, epidermis and wash was 68–113% with emulsion gel and 40–54% with petrolatum jelly. Ethyl hexyl salicylate was not detected in receptor fluids.

In earlier *in vitro* dermal penetration experiments, Jimbo (1983) reported that 0.008% of a 0.2 ml aliquot of isoamyl salicylate traversed human skin over a 72-h period. The corresponding value for benzyl salicylate was 0.031%.

Based on physico-chemical properties and assuming an applied dose of 40 μg/cm^2 and a body surface area of 1.4 m^2, Watkinson et al. (1992) calculated a whole body exposure of 13,000 μg for methyl salicylate over a 12-h period. This is equivalent to a dermal bioavailability of about 2.3%. Much lower total body exposures were calculated for butyl salicylate (380 μg over a 12-h period equivalent to a dermal absorption rate of 0.068%), pentyl salicylate (96 μg over a 12-h period equivalent to a dermal absorption rate of 0.017%), hexyl salicylate (27 μg over a 12-h period equivalent to a dermal absorption rate of 0.005%), and ethyl hexyl salicylate (3.3 μg over a 12-h period equivalent to a dermal absorption rate of 0.0006%).

2.1.1.2. Animal studies (Tables 4 and 5)

2.1.1.2.1. In vivo animal studies. Permeation rate constants for methyl salicylate have been determined in Yorkshire–Landrace cross barrow pigs (Duncan et al., 2002). In this study, methyl salicylate was applied neat to the ear, epigastrum, perineum, and inguinal crease and blood concentrations were measured 6 h after exposure. The initial flux rates were calculated to be 0.063 μg/cm^2/min, 0.025 μg/cm^2/min, 0.044 μg/cm^2/min, and 0.012 μg/cm^2/min at the respective sites. Flux rates for methyl salicylate applied to the tail of a rat were reported by Siddiqi and Ritschel (1972), which ranged from 0.001 μg/cm^2/min at pH 3 to 0.003 μg/cm^2/min at pH 6. Flux rates for ethyl salicylate in the same model were 0.003 μg/cm^2/min at pH 2 and pH 3 with no absorption at pH 6 or pH 8; flux rates reported for phenyl salicylate were: 0.005 μg/cm^2/min at pH 2, 0.004 μg/cm^2/min at pH 3 and 0.003 μg/cm^2/min at pH 6 (Siddiqi and Ritschel, 1972).

2.1.1.2.2. In vitro animal studies. The percutaneous absorption of salicylates has been studied in several *in vitro* animal systems where neat, or within a liquid vehicle, salicylate was applied to the skin surface. The amount of salicylate and/or associated metabolites in a receptor fluid beneath the skin preparation at later time(s) was determined. Percutaneous absorption of methyl salicylate as a percent of the applied dose was reported by Boehnlein et al. (1994) to be 55% for viable male hairless guinea pig skin over a 24-h period. Riviere et al. (2000, 2001) reported that only 2.4% of an applied dose of methyl salicylate passed through a perfused porcine skin preparation after 8 h. Using intact rat skin preparations, RIFM (1983a) reported that application of a 1%, 3%, or 10% solution of benzyl salicylate in ethanol for 24 h resulted in test substance migrations of 62.7%, 58.8%, and 40.3%, respectively, into the receptor fluid. In skin preparations from guinea pigs, 16 h after application of a 1%, 3%, or 10% solution of benzyl salicylate in ethanol, 3.5%, 1.7%, and 0.9%, respectively, migrated through the skin into the receptor fluid.

Overall, the percutaneous absorption data demonstrate that salicylates are dermally absorbed and that significant amount of salicylate can be retained within epidermis, dermis, and subcutaneous tissue. The human *in vivo* data, derived mostly from experiments conducted with methyl salicylate, support dermal bioavailability in the range of 2–43% (Beutner et al., 1943; Brown and Scott, 1934a; Cross et al., 1998; Roberts et al., 1982; Morra et al., 1996). Limited data on *in vitro* or calculated absorption of other salicylates indicate that certain longer chain alkyl derivatives may be absorbed to a lesser extent than methyl salicylates (Brown and Scott, 1934a,b; Siddiqi and Ritschel, 1972; Yano et al., 1986; Watkinson et al., 1992). As a result, the use of the dermal bioavailability of methyl salicylate to characterize the dermal absorption of the other salicylates represents a conservative measure.

2.1.2. Oral absorption

Limited data are available from which to characterize the oral bioavailability of the 17 salicylates assessed in this report.

Davison et al. (1961) reported that oral dosing of six human volunteers with 420 mg of methyl salicylate (~6 mg/kg body weight) resulted in both methyl salicylate and free salicylate in the plasma at 15 and 90 min post-exposure. At 90 min, plasma concentrations of methyl salicylate and free salicylate were 2.8 and 10.5 mg/l,

Table 4
Summary of non-human mammalian *in vivo* percutaneous absorption data

Material	Method	Results	References
Ethyl salicylate	45 min open application to the rats tail	0–1.97 μg/mm^2/h	Siddiqi and Ritschel (1972)
Methyl salicylate	6 h open application to the ear, epigastrum, perineum and inguinal crease of pig	0.012–0.063 μg/cm^2/min	Duncan et al. (2002)
Methyl salicylate	45 min open application to the rats tail	0.76–1.77 μg/mm^2/h	Siddiqi and Ritschel (1972)
Methyl salicylate	1 and 6 h occluded application to the dorsal skin of mice	0.64 μmol/g at 1 h 0.29 μmol/g at 6 h	Yano et al. (1991)
Phenyl salicylate	45 min open application to the rats tail	2.18–2.90 μg/mm^2/h	Siddiqi and Ritschel (1972)

Table 5
Summary of non-human mammalian *in vitro* percutaneous absorption Data

Material	Method	Results	References
Benzyl salicylate	24 h exposure; excised naked rat skin	40.3–62.7%	RIFM (1983a)
Benzyl salicylate	16 h exposure; excised pig skin	0.9–3.5%	RIFM (1983a)
Butyl salicylate	10 h exposure; hairless mouse skin	0.014 μmol/cm^2/h	Higo et al. (1995)
Ethyl salicylate	10 h exposure; hairless mouse skin	0.72 μmol/cm^2/h	Higo et al. (1995)
Methyl salicylate	10 h exposure; hairless mouse skin	2.8 μmol/cm^2/h	Higo et al. (1995)
Methyl salicylate	24 h exposure; viable male hairless guinea pig skin	55%	Boehnlein et al. (1994)
Methyl salicylate	8 h exposure; perfused porcine pig skin	2.39%	Riviere et al. (2000, 2001)

respectively. In the same publication, Davison et al. (1961) administered methyl salicylate in 2% methylcellulose at a dose of 500 mg/kg body weight (as salicylic acid equivalents) by oral gavage to rats. This resulted in plasma free salicylate concentrations of 217 mg/l and 278 mg/l at 20 and 60 min post-exposure, respectively. No parent methyl salicylate was detected. While these data demonstrate systemic exposure from the oral route, they provide no quantitative estimate of oral bioavailability. It has been well documented that salicylic acid, the chief hydrolysis product of the alkyl, alkenyl, and benzyl–phenyl-substituted salicylates, is rapidly and extensively absorbed from the gastrointestinal tract of both humans (Alpen et al., 1951; Shen et al., 1991; Janssen et al., 1996) and laboratory animals (Alam et al., 1981; McMahon et al., 1990; Short et al., 1991).

Oral absorption studies conducted on closely related hydroxy- and alkoxy-substituted benzyl derivatives indicate rapid and nearly complete absorption following ingestion (Sammons and Williams, 1941; Bray et al., 1947, 1948, 1952; Clarke et al., 1958; Dirscherl and Wirtzfeld, 1964; Jones et al., 1956; Strand and Scheline, 1975). For example, in a study in which groups of 4–8 rabbits were administered gavage doses of 4-hydroxybenzoic acid every 3–7 days at 100, 250, 500, 1000, or 1500 mg/kg body weight, the total urinary recovery of the test material and associated metabolites ranged from 84% to 104% (Bray et al., 1947). In a subsequent study, urinary metabolites as a percent of the dose following single oral administration of 250 mg/kg of 2-hydroxybenzoic acid to two groups of four rabbits were 85% ether soluble acid, 4% ester glucuronide and 5% ether glucuronide. After administration of 500 mg/kg of 2-hydroxybenzoic acid, urinary metabolites were 85% ether soluble acid, 3% ester glucuronide and 14% ether glucuronide (Bray et al., 1948).

Administration of butyl *p*-hydroxybenzoate, a benzyl ester similar to the salicylates, at an oral dose of 1000 mg/kg body weight or 50 mg/kg body weight intravenously, resulted in urinary recoveries of 48% (oral) and 40% (intravenously) of the total administered test material almost entirely as the *p*-hydroxybenzoic acid (Jones et al., 1956). These data indicate nearly complete bioavailability (*i.e.*, oral and i.v. dosing recoveries were similar). Jones et al. (1956) further reported similar results with methyl- and ethyl-*p*-hydroxybenzoate, but with greater percent recoveries from both the oral and intravenous routes of exposure. The authors concluded that butyl *p*-hydroxybenzoate and other alkyl esters are readily absorbed *via* the oral route.

The oral administration to humans of 2,4-dihydroxybenzoic acid in 1000 mg doses, every 3 h for 2–16 days, as a treatment for rheumatic fever, yielded urinary excretion of metabolites accounting for 42.7–75.8% of the dose (Clarke et al., 1958).

As a result, for the assessment of potential effects of oral exposures to the salicylates from their use as fragrance ingredients, an oral bioavailability of 100% is assumed.

2.1.3. Inhalation absorption

The potential for absorption of methyl salicylate *via* inhalation (Buchbauer et al., 1993) was determined in female Swiss mice exposed to 20–50 mg of methyl salicylate over 1 h; only traces of salicylate were detected in the plasma at the end of the inhalation period.

2.2. Distribution and pharmacokinetics

Data describing the distribution and pharmacokinetics of salicylates are limited to plasma levels following dermal application in humans (Roberts et al., 1982; Morra et al., 1996) and oral dosing in humans and rats (Wolowich et al., 2003; Davison et al., 1961).

Morra et al. (1996) reported that dermal application of 5 g of 12.5% methyl salicylate ointment to the anterior aspect of the thigh of 6 men and 6 women twice daily for 4 days resulted in salicylic acid serum concentrations of 0.31–0.91 mg/l within 1 h of dosing. Maximum salicylic acid serum concentrations of 2 and 6 mg/l were reached following the seventh application on Day 4. Earlier, Roberts et al. (1982), reported steady-state serum salicylate concentrations in the range of 2.5 mg/l after dermal application of products containing 12.5% methyl salicylate and 7.6 mg/l for formulations containing 50% methyl salicylate.

With regard to oral exposure, Wolowich et al. (2003) determined plasma concentrations of salicylate in four humans following ingestion of Bengay® cream containing 900 or 2700 mg methyl salicylate, or of 1000 mg of wintergreen oil, which contained 98% methyl salicylates. Following consumption of the low-dose of Bengay® cream, serum salicylate t_{max} values ranged from 1.5 to 4 h; C_{max} values were 36–51 mg/l. Corresponding t_{max} and C_{max} values for

the high-dose Bengay® cream were reported as 4–12 h and 120–201 mg/l, respectively. Consumption of the wintergreen oil resulted in a serum salicylates t_{max} of 2.4 h and a C_{max} of 70 mg/l.

As a part of a reproductive study, methyl salicylate in doses of 172–1400 mg/100 g body weight (vehicle not reported) was applied directly to the shaved skin of LVG – strain pregnant female hamsters for 2 h followed by a thorough water wash. A peak blood salicylate level of 50 mg/100 ml was measured 5–6 h after treatment (Overman and White, 1978, 1983). When methyl salicylate was administered by oral intubation at 175 mg/100 g body weight, the plasma salicylate level reached a peak of 125 mg/100 ml at approximately 2 h after treatment, and then returned to normal over a period of 12–24 h (Overman and White, 1978, 1983).

In a rat study, Davison et al. (1961) reported mean values for total salicylate (methyl salicylate and salicylic acid) plasma concentrations of 217 mg/l and 278 mg/l, 20 and 60 min after gavage administration of methyl salicylate in 2% methylcellulose at 500 mg/kg body weight (as salicylic acid equivalents). Concentrations of total salicylate of 8 mg/l and 42 mg/l, were detected in the brain 20 and 60 min post-exposure, respectively.

Pharmacokinetic data are available on orally administered structurally related hydroxyl-, alkyl- and alkoxy-benzyl compounds, including 2- and 4-hydroxybenzoic acid, (Bray et al., 1947, 1948), butyl-*p*-hydroxybenzoate (Jones et al., 1956), vanillin (Dirscherl and Wirtzfeld, 1964; Strand and Scheline, 1975), and salicylaldehyde (Bray et al., 1952). Studies in rats, rabbits, dogs, and humans, showed rapid distribution to the plasma with rapid and near complete excretion *via* the kidneys. Urinary species included minor amounts (or none) of parent compound, with the majority present as glucuronide, glycine, or sulfate conjugates of benzoic acid derivatives or in free acid forms (Bray et al., 1947, 1948, 1952; Jones et al., 1956; Davison et al., 1961; Strand and Scheline, 1975). Given the rapid and near complete excretion of salicylates and related compounds in the urine, one can conclude that absorbed salicylates and their metabolites are widely distributed via blood, with little retention in tissues.

2.3. Metabolism (Fig. 1)

The 17 compounds assessed in this report include the core salicylate moiety that upon hydrolysis yield salicylic acid and the alcohol of the corresponding alkyl, alkenyl, benzyl, phenyl, phenethyl, *etc*. side chain. This is consistent with information on other alkyl- and alkoxy- benzyl derivatives whereby aromatic esters are hydrolyzed in *vivo* by carboxylesterases, or esterases, especially the A-esterases (Heymann, 1980; Anders, 1989). Potential differences in the metabolism of the individual salicylates would be related to the manner in which the hydrolyzed side chain undergoes further oxidation/reduction and/or conjugation reactions as described below.

For the one exceptional compound, methyl 4-methylsalicylate, the only difference in the core salicylate moiety is the methylation of the benzene ring at the *para* position. This difference would not be expected to change significantly the metabolic profile following hydrolysis of the parent compound to methanol and 4-methylsalicylic acid.

In vivo metabolic data are available for methyl salicylate (Hanzlik and Wetzel, 1920; Robinson and Williams, 1956; Davison et al., 1961; Infurna et al., 1990). One human metabolism study is available on phenyl salicylate (Fishbeck et al., 1975).

Carboxylesterases show extensive tissue distribution (Heymann, 1980) with respect to hydrolysis of methyl salicylate. *In vitro* studies demonstrate greatest activity in the liver, but also extensive activity in the intestines, kidney, pancreas and spleen (Davison et al., 1961). Both the liver and intestines can contribute to the pre-systemic hydrolysis of salicylates.

Davison et al. (1961) reported that oral consumption of 0.42 ml of methyl salicylate by 6 human volunteers resulted in the rapid appearance of salicylic acid in the plasma. At both 15 and 90 min, salicyclic acid was two- and fourfold higher in plasma than methyl salicylate. This is indicative of extensive hydrolysis during oral absorption. Davison et al. (1961) similarly demonstrated that hydrolysis of methyl salicylate following administration to male mongrel dogs at 300 mg/kg body weight was 95% complete within 1 h. Gavage dosing of rats with 300 mg methyl salicylate/kg body weight resulted in the appearance of hydrolyzed free salicylate in both the plasma and brain tissue within 20 min (Davison et al., 1961). Salicylic acid was also found in the plasma of pregnant rats exposed dermally with 2000 mg methyl salicylate/kg body weight/day on gestational days 6 through 15 (Infurna et al., 1990).

In a study with a single human volunteer, Fishbeck et al. (1975) reported that ingestion of 1 ounce (~28 g or ~400 mg/kg body weight) of phenyl salicylate in capsule form every h for 8 h resulted in a rapid increase in free urinary phenol concentration, which peaked at 260 mg/L during the 8-h period following the final dose. At 48 h after ingestion, free urinary phenol had decreased to 5.5 mg/L.

In mouse skin preparations, *in vitro* metabolism studies have shown variable results with respect to the degree of hydrolysis, from <5% of methyl salicylate that migrated through the skin to 25–30% of ethyl salicylate and 100% of butyl salicylate (Higo et al., 1995). In an *in vitro* guinea pig skin preparation, 38% of the absorbed methyl salicylate was metabolized to salicylic acid in nonviable skin. In viable skin, 57% of methyl salicylate metabolized to 21% salicyluric acid and 36% salicylic acid (Boehnlein et al., 1994).

Based on numerous metabolic studies in both humans and animals, salicylic acid undergoes metabolism primarily in the liver. At low, non-toxic doses, approximately 80% of salicylic acid is further metabolized in the liver *via* conjugation with glycine and subsequent formation of salicyluric acid. Salicylic acid also undergoes glucuronide conjugation

to form acyl and phenolic glucuronides (Levy and Tsuchiya, 1972; Goldsmith, 1979; Vree et al., 1994a,b). Metabolism of salicylic acid is characterized by first order kinetics at low doses and zero order kinetics at doses that saturate glycine conjugation capacity (Done, 1960; Levy and Tsuchiya, 1972). A small amount of salicylic acid is oxidized to gentisic acid, a product that in turn may be subject to glucuronide conjugation.

The activity of salicylic acid metabolic pathways (*i.e.*, extensive glycine and/or glucuronide conjugation followed by partial degradation of the conjugates) is evidenced by the finding of glucuronide, glycine, or sulfate conjugates as the major urinary metabolites of several alkyl- and alkoxy-benzyl derivatives. These compounds are close structural analogues of the salicylates, in rats, rabbits, dogs, and humans (Bray et al., 1947, 1948, 1952; Jones et al., 1956; Davison et al., 1961; Strand and Scheline, 1975).

For each of the salicylates, following hydrolysis to salicylic acid, the resulting side chains, hydroxylated alkyl, alkenyl, and phenyl moieties, could be expected to be further metabolized. In the case of the alcohols formed following hydrolysis (*e.g.*, methanol, ethanol, butanol, pentanol, hexanol, *etc.*) further metabolism would result in the formation of the corresponding aldehydes and acids, with eventual degradation to CO_2 by the fatty acid pathway and the tricarboxylic acid cycle. The secondary alcohols formed by hydrolysis of isobutyl and isoamyl salicylate, would primarily be conjugated with glucuronic acid and excreted. They could also interconvert to the corresponding ketones (JECFA, 1998).

Salicylates bearing alkenyl side chains, namely the *cis*-3-hexenyl, *trans*-2-hexenyl, 1,3-dimethyl-3-butenyl, and 3-methyl-2-butenyl side chains, may undergo epoxidation and subsequent hydroxylation at points of unsaturation. However, since both the alkyl and alkenyl side chains would be hydroxylated at one terminus following hydrolysis of the corresponding salicylate, a significant proportion of these hydrolysis products would be excreted in the urine precluding further metabolism and epoxidation (JECFA, 1998).

In the case of hydrolysis of the salicylates containing aromatic side chains, phenyl salicylate and benzyl salicylate, phenol and benzyl alcohol, respectively, would be formed. In the case of the phenethyl side chain, hydrolysis yields 2-phenylethanol. Phenol is subject to conjugation

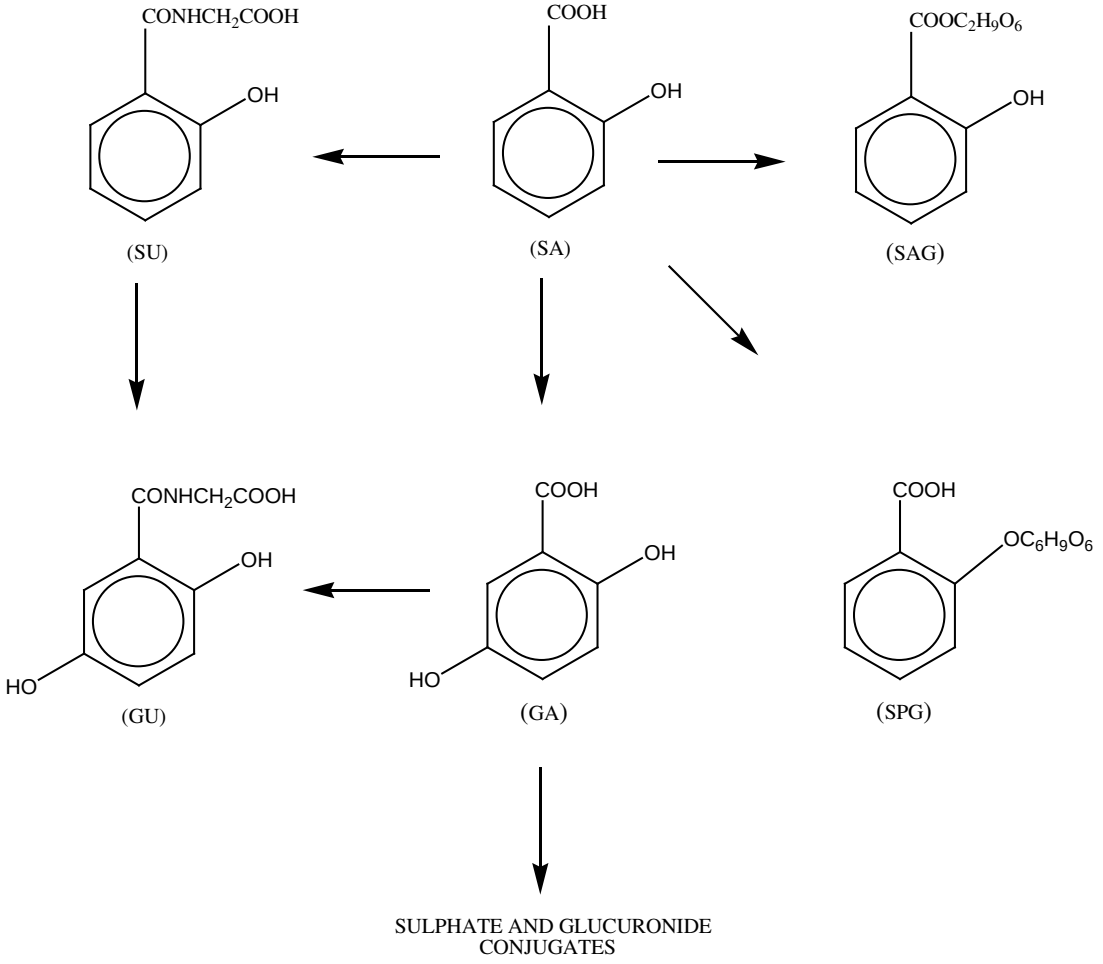


Fig. 1. Biotransformation of salicylic acid.

with glucuronic acid to form phenyl glucuronide and sulfation to form phenyl sulfate. These products have been shown to be the major metabolites of phenol in many species (Inder, 1999). Benzyl alcohol is rapidly oxidized to benzoic acid, conjugated with glycine, and excreted in the urine as the hippuric acid derivative (Williams, 1959). 2-Phenylethanol is oxidized to 2-phenylacetic acid, conjugated with glutamine (primarily in humans), taurine, or glycine and rapidly excreted in the urine (Williams, 1959; James et al., 1972, 1973).

In summary, all 17 salicylates assessed in this report are expected to undergo extensive hydrolysis, primarily in the liver, to salicylic acid. In the case of methyl 4-methylsalicylate, hydrolysis would yield 4-methylsalicylic acid. Substitution of the benzene ring, as with benzoic acid (JECFA, 1996, 2001), however, would not materially affect the metabolism of 4-methly salicylic acid in comparison to salicylic acid. As a result, salicylic acid represents a common metabolite for this group of salicylates. In the liver, salicylic acid is conjugated with either glycine or glucuronide and excreted in the urine as salicyluric acid and acyl and phenolic glucuronides. The hydrolyzed side chains are metabolized by common and well-characterized metabolic pathways leading to the formation of innocuous end products. Primary alcohols are metabolized to corresponding aldehydes and acids, and ultimately to CO_2, while secondary alcohols are conjugated with glucuronide and excreted. Unsaturated alcohols may undergo further oxidation at the point of unsaturation while the aromatic side chains (benzyl, phenyl, and phenethyl) are either directly conjugated (phenol), or oxidized to the corresponding acid prior to conjugation and excretion in the urine. The expected metabolism of the salicylates does not present any obvious toxicological concerns.

3. Toxicological studies

3.1. Acute toxicity (Tables 6a–6c)

The acute dermal toxicity of the salicylates is very low. Rabbit dermal LD_{50} values have been reported to be >5000 mg/kg body weight for 15 of the 16 salicylates tested (Table 6a), findings likely related to the limited degree of dermal absorption, the retention of salicylate in the skin, and the relatively moderate toxicity of salicylic acid itself upon systemic exposure (*i.e.*, oral LD_{50} value of 891 mg/kg body weight in rats) (Sax, 1979). The acute dermal LD_{50} for 1,3-dimethyl-3-butenyl salicylate has been reported as >2000 mg/kg body weight which was the highest dose tested.

Overall, the acute oral toxicity of the salicylates is moderate, with toxicity generally decreasing with increasing size of the ester R-group (Table 6b). For example, the oral LD_{50} for methyl salicylate ranges from 890 to 2820 mg/kg body weight in rats (Giroux et al., 1954; Jenner et al., 1964; Bar and Griepentrog, 1967; RIFM, 1982a). For the longer carbon chain salicylates, acute oral LD_{50}'s range from 1320 to >5000 mg/kg body weight (RIFM, 1982a,b) (RIFM, 1974a) (RIFM, 1975a) (RIFM, 1968a, 1976a). The acute oral toxicity of the unsaturated salicylates (*cis*-3-hexenyl-, *trans*-2-hexenyl, 1,3-dimethyl-3-butenyl, and 3-methyl-2-butenyl) is likewise low to moderate with rat oral LD_{50}'s in the 3200 to >5000 mg/kg body weight range (RIFM, 1975a, 1978a) as are the acute oral toxicities of the aromatic salicylates (1300 to >5000 mg/kg body weight) (RIFM, 1970a, 1973a, 1975b). Differences in acute oral toxicity are likely related to the relative proportion of the molecular weight released as salicylic acid follows hydrolysis. Parenteral injection increases the toxicity (Table 6c).

Table 6a
Acute dermal toxicity studies

Material	Species	No. animals/dose/group	LD_{50} (mg/kg)[b]	References
Benzyl salicylate	Rabbits	3	14,150	RIFM (1970b)
Butyl salicylate	Rabbits	4	>5000	RIFM (1975b)
p-Cresyl salicylate	Rabbits	10	>5000	RIFM (1980a)
1,3-Dimethyl-3-butenyl salicylate	Rabbits	6	>2000	RIFM (1981a)
Ethyl hexyl salicylate	Rabbits	4	>5000	RIFM (1974a)
Ethyl salicylate	Rabbits	10	>5000	RIFM (1976a)
cis-3-Hexenyl salicylate	Rabbits	10	>5000	RIFM (1975a)
trans-2-Hexenyl salicylate	Rabbits	10	>5000	RIFM (1978a)
Hexyl salicylate	Rabbits	10	>5000	RIFM (1975a)
Homomenthyl salicylate[a]	Rabbits	10	>5000	RIFM (1978a)
Isobutyl salicylate	Rabbits	8	>5000	RIFM (1973a)
3-Methyl-2-butenyl salicylate	Rabbits	10	>5000	RIFM (1978a)
Methyl salicylate	Rabbits	10	>5000	RIFM (1973a)
Octyl salicylate[a]	Rabbits	10	>5000	RIFM (1976a)
Pentyl salicylate	Rabbits	10	>5000	RIFM (1982b)
Phenyl salicylate	Rabbits	4	>5000	RIFM (1975b)
Phenethyl salicylate	Rabbits	9	>5000	RIFM (1973a)

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.
[b] Units have been converted to make easier comparisons; original units are in the Fragrance Material Reviews.

Table 6b
Acute oral toxicity studies

Material	Species	No. animals/dose/group	LD_{50} (mg/kg)[a]	References
Benzyl salicylate	Rats	6	2230 (C.I. 1930–2580)	RIFM (1970a)
Butyl salicylate	Rats	10	1700 (95% C.I. 1260–2290)	RIFM (1975b)
p-Cresyl salicylate	Rats	10	1300 (95% C.I. 990–1790)	RIFM (1980a)
1,3-Dimethyl-3-butenyl salicylate	Rats	10	>5000	RIFM (1981b)
Ethyl hexyl salicylate	Rats	10	>5000	RIFM (1974a)
Ethyl salicylate	Rats	10	1320 (C.I. 1010–1630)	RIFM (1976a)
cis-3-Hexenyl salicylate	Rats	10	~5000	RIFM (1975a)
trans-2-Hexenyl salicylate	Rats	10	4430 (C.I. 3860–5100)	RIFM (1978a)
Hexyl salicylate	Rats	10	>5000	RIFM (1975a)
Homomenthyl salicylate[b]	Rats	10	>5000	RIFM (1978a)
Isoamyl salicylate	Rats	10	>5000	RIFM (1982a)
Isobutyl salicylate	Rats	10	1560 (95% C.I. 1320–1800)	RIFM (1973a)
Methyl salicylate	Rats	10	2820 (95% C.I 2480–3210)	RIFM (1982a)
Methyl salicylate	Rats	N/A[c]	1250	Giroux et al. (1954)
Methyl salicylate	Rats	N/A[c]	887 (95% C.I. 720–1100)	Jenner et al. (1964), Bar and Griepentrog (1967)
Methyl salicylate	Mice	10	1390 (95% C.I. 1250–1540)	Ohsumi et al. (1984)
Methyl salicylate	Mice	N/A[c]	1110	Davison et al. (1961)
Methyl salicylate	Mice	16	1440[d]	NTP (1984)
Methyl salicylate	Guinea pigs	N/A[c]	1060 (95% C.I. 870–1300)	Jenner et al. (1964)
3-Methyl-2-butenyl salicylate	Rats	10	3200 (C.I. 2600–3900)	RIFM (1978a)
Octyl salicylate[b]	Rats	10	4800 ± 300	RIFM (1968a)
Octyl salicylate[b]	Rats	10	>5000	RIFM (1976a)
Pentyl salicylate	Rats	10	4100 (95% C.I. 3300–5000)	RIFM (1982b)
Pentyl salicylate	Rats	10	2000	RIFM (1990)
Phenethyl salicylate	Rats	10	>5000	RIFM (1973a)
Phenyl salicylate	Rats	10	3000	RIFM (1975b)

[a] Units have been converted to make easier comparisons; original units are in the Fragrance Material Reviews.
[b] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.
[c] Data not reported in reference.
[d] mg/kg/day.

Table 6c
Miscellaneous acute toxicity studies

Material	Dose route	Species	No. animals/dose group	LD_{50} (mg/kg)[a]	References
Ethyl hexyl salicylate	i.p. injection (in propylene glycol)	Mice	10	200–300	Doull et al. (1962)
Methyl salicylate	i.p. injection (in alcohol)	Rats	3	750–1000	Giroux et al. (1954)
Methyl salicylate	i.p. injection (in alcohol)	Guinea pigs	3	750–1000	Giroux et al. (1954)

[a] Units have been converted to make easier comparisons; original units are in the Fragrance Material Reviews.

3.2. Subchronic toxicity (Table 7)

The results of subchronic dermal and oral studies with salicylates are summarized in Table 7 and are described below.

3.2.1. Dermal studies

Of the 17 salicylates assessed, only methyl salicylate (Giroux et al., 1954; Webb and Hansen, 1952; Webb and Hansen, 1963) has been tested in repeat dose dermal toxicity studies (1 rabbit and 1 dog study).

In the rabbit study, groups of three animals of mixed sex were administered methyl salicylate of 99% purity to sites on the back clipped free of hair. Dermal exposures of 590, 1180, 2360, and 4720 mg/kg body weight/day were administered 5 days/week and allowed to remain on the application site for 6.5 h. The experiment was terminated after 28 days, the time at which all the high-dose animals had died, following weight loss and depressed activity. In one of the high-dose animals, evidence of nephrotoxicity was reported. At 2360 mg/kg, sloughing of epidermal scales was observed in 2/3 rabbits. No effects were noted in rabbits exposed to 590 and 1180 mg/kg body weight/day (Webb and Hansen, 1962, 1963).

Giroux et al. (1954) applied methyl salicylate dermally to three beagle dogs twice daily (5000 mg/kg/day body weight) for 16 days. The animals showed decreased urine output, albuminuria, increased BUN, and decreased "alkaline reserve". After a 10-day recovery period, the only treatment-related effect that remained was trace albuminuria.

It is apparent that at extreme exposure levels, on the order of near 5 g/kg body weight/day or more, repeated

Table 7
Dermal and oral subchronic toxicity studies

Material	Method	Dose[a] (mg/kg/day)	Species (no./dose group)	Results	References
Isoamyl salicylate	Oral (diet) 13 week toxicity study	4.7–4.8 (50 ppm in the diet) 46–47 (500 ppm in the diet), 420–480 (5000 ppm in the diet)	Wistar rats (15/sex/dose)	4.7–4.8 mg/kg/day: no adverse toxic effects 46–47 mg/kg/day: significantly increased relative kidney weight in females (considered the NOAEL) 420–489 mg/kg/day: 1 death in females. Treated rats of both sexes were visibly smaller and showed significantly reduced body weights in comparison to controls (by 15% in males and by 9% in females). Significant decreases in feed consumption and increase in water intake in females were noted. Clinical sings of respiratory infection were present in about 50% of the animals from Week 3 onward. No effects on hematological or urinary parameters after 13 weeks treatment. Increased relative kidney weights in both sexes and increased relative spleen and liver weights in females No histopathological effects at any dose level	Drake et al. (1975)
Isoamyl salicylate	Oral (diet) 2 week toxicity study	46–47 (500 ppm in the diet) 420–480 (5000 ppm in the diet)	Wistar rats (5/sex/dose)	46–47 mg/kg/day: significantly decreased RBC in females 420–480 mg/kg/day: increased relative liver weights in males and females No histopathological effects at any dose level	Drake et al. (1975)
Isoamyl salicylate	Oral (diet) 6 week toxicity study	46–47 (500 ppm in the diet) 420–480 (5000 ppm in the diet)	Wistar rats (5/sex/dose)	46–47 mg/kg/day: increased relative spleen weight in males 420–480 mg/kg/day: increased relative spleen, cecal and testes weights in males and increased relative liver weights in females No histopathological effects at any dose level	Drake et al. (1975)
Isoamyl salicylate	Oral (diet) 98 day study	420–480 (5000 ppm in the diet)	Wistar rats (5/sex/dose)	No effects	Drake et al. (1975)
Methyl salicylate	Dermal 96-day toxicity study	590 1180 2360 4720	Rabbits (3 of mixed sex/dose)	590 and 1180 mg/kg/day: no effects 2360 mg/kg: sloughing of epidermal scales in 2/3 rabbits 4720 mg/kg: all rabbits showed weight loss, depressed activity, and died by Study Day 28. Nephrotoxicity observed in one animal	Webb and Hansen (1962, 1963)
Methyl salicylate	Dermal 16-day toxicity study	5000 mg/kg/day	Beagle dogs (3)	Decreased urine output, albumin in the urine, increased BUN, and decreased alkaline reserve. After a 10 day recovery period, the only feature that remained was a trace of albumin in the urine.	Giroux et al. (1954)
Methyl salicylate	Oral (diet) 12-week toxicity study	100 (0.2% in the diet) 180 (0.36% in the diet) 320 (0.63% in the diet) 560 (1.13% in the diet) 1000 (2.0% in the diet)	Sprague–Dawley rats (5/sex/dose)	100 and 180 mg/kg/day: no effects 320 mg/kg/day: decreased body weight gain in males 560 and 1000 mg/kg/day: decreased body weight gain and increased bone density of the metaphyses of the femur, humerus, tibia, and radius	Abbott and Harrisson (1978)
Methyl salicylate	Oral (diet) 11-week toxicity study	300 (0.6% in the diet) 450 (0.9% in the diet) 600 (1.2% in the diet) 1000 (2.0% in the diet)	Sprague–Dawley rats (10/sex/dose)	300 and 450 mg/kg/day: no effects on incidence or progression of bone lesions 600 mg/kg/day: bone changes apparent on X-ray at Week 5 with an increased incidence of cancellous bone by Week 8 1000 mg/kg/day: bone changes apparent on X-ray, and an increased incidence of cancellous bone, apparent by Week 2	Abbott and Harrisson (1978)
Methyl salicylate	Oral (diet) 11-week toxicity study	1000 (2.0% in the diet)	Sprague–Dawley rats (15 males)	20% mortality compared to 0% in controls and increased bone density of the metaphyses of various long bones	Abbott and Harrisson (1978)
Methyl salicylate	Oral (diet) 12-week toxicity study	300 (0.6% in the diet) 1000 (2.0% in the diet)	Sprague–Dawley rats (5 males/dose) No controls	300 mg/kg/day: no effects 1000 mg/kg/day: 100% mortality after 6 weeks of treatment and all rats had bone lesions following whole body X-ray examination	Abbott and Harrisson (1978)

(continued on next page)

Table 7 (*continued*)

Material	Method	Dose[a] (mg/kg/day)	Species (no./dose group)	Results	References
Methyl salicylate	Oral (diet) 6-week toxicity study	300 (0.6% in the diet) 300 (0.6% in the diet in feed portions equivalent to the 1000 mg/kg/day group) 1000 (2.0% in the diet) *Ad libitum* and pair fed controls	Sprague–Dawley rats (10 males/dose) No controls	300 mg/kg/day (fed *ad libitum*): reduced growth rate compared to controls 300 mg/kg/day (pair-fed to the same feed consumption of the 1000 mg/kg/day group): no increased mortality compared to pair-fed controls. Body weight was similar to that of the 1000 mg/kg bw/day group 1000 mg/kg/day: mortality occurred in 90% of animals, survivors showed decreased body weight	Abbott and Harrisson (1978)
Methyl salicylate	Oral (diet) 17-week toxicity study	50 (0.1% in the diet) 500 (1.0% in the diet)	Osborne–Mendel rats (10/sex/dose)	50 mg/kg/day: no effects 500 mg/kg bw/day: reduced body weight gains	Webb and Hansen (1963)
Methyl salicylate	Oral (diet) 71-day toxicity study	1000 (2.0% in the diet)	Osborne–Mendel rats (3/sex/dose)	All males were dead by Day 19, with all females expired by Day 71. Rough hair coat and stunting of growth was noted. Increased bone density in the metaphyses of all bones was observed. Treatment induced labored breathing and hemorrhages in the glandular stomach. Lung damage was noted in 4 animals	Webb and Hansen (1963)
Methyl salicylate	Oral (diet) 10-week toxicity study	~550 (1.12% in the diet) 1000 (2.0% in the diet)	Rats (numbers not reported)	550 mg/kg/day: increased incidence of cancellous bone 1000 mg/kg/day: increased mortality, decreased feed consumption and body weight, and increased incidence of cancellous bone	Harrisson et al. (1963)
Methyl salicylate	Oral (capsule) 6.5–7.5-month toxicity study	150 300 500 800 (6 days/week)	Beagle dogs (3/sex/dose)	150 and 300 mg/kg/day: increased relative kidney and liver weights, but no histopathological correlates In 300 mg/kg/day dogs allowed a 6-week recovery period, no increase in relative kidney or liver weights was found 500 mg/kg/day: 4/6 dogs died. Decreased body weight reported in one survivor. Relative kidney and liver weights were increased 800 mg/kg/day: all dogs died by the second week of study. Relative liver and kidney weights were increased Histological examination revealed general increase in liver cell size and alteration in cytoplasmic granulality. No other histopathological correlates were found	Abbott and Harrisson (1978)
Methyl salicylate	Oral (capsule) 6–8 month toxicity study	50 100 167 (6 days/week)	Beagle dogs (4/sex/dose at 50 and 100 mg/kg/day 6/sex/dose at 170 mg/kg/day)	50, 100, and 167 mg/kg/day: no effects on liver or kidney weights, body weights, or on routine hematological and clinical chemistry evaluation. During the second month of the study treated dogs showed signs of seborrhea oleosum, a condition that remitted following addition of lard to the diet of all dogs. One dog at each of the 3 dose levels exhibited hyperemic foci of the pyloric mucosa	Abbott and Harrisson (1978)
Methyl salicylate	Oral (capsule) 59 day toxicity study	50 100 250 500 800 1200 (for 6 days/week)	Beagle dogs (1/sex/dose)	50, 150, and 250 mg/kg/day: no effects 500 mg/kg/day: all dogs died within the first month of the study. Two of these dogs had diarrhea and weakness during their last 3 days 800 and 1200 mg/kg/day: all dogs died within the first month of study. Several dogs vomited with 3–4 h of dosing. There was evidence of marked fatty metamorphosis of the liver	Webb and Hansen (1962, 1963)
Phenyl salicylate	Oral (capsule) 51-day toxicity study	125 250 500	Beagle dogs	125 mg/kg/day: no effects 250 and 500 mg/kg/day: decreased appetite, body weight gains and activity levels. Dark urine and feces were observed. Transient increases in non-segmented neutrophil leukocytes and of serum GPT and GOT	Fishbeck et al. (1975) and Kociba et al. (1976)

[a] Units have been converted to make easier comparisons; original units are in the Fragrance Material Reviews.

dermal methyl salicylate is nephrotoxic and potentially lethal. The studies reported few toxicological endpoints and are not suitable for use in risk assessment.

3.2.2. Oral studies

Subchronic oral toxicity studies have been conducted on methyl salicylate (Webb and Hansen, 1963; Harrisson et al., 1963; RIFM, 1978), isoamyl salicylate (Drake et al., 1975), and phenyl salicylate (Fishbeck et al., 1975; Kociba et al., 1976). The results of these studies are summarized in Table 7.

The effects of methyl salicylate were assessed by Webb and Hansen (1963) in groups of 10 male and 10 female Osborne–Mendel rats fed methyl salicylate at 0%, 0.1% or 1.0% in the diet for 17 weeks. The dietary concentrations equated to oral doses of 0, 50 and 500 mg/kg body weight/day, respectively (Table 14 of Appendix I in FDA's Priority-based assessment of Food Additives (PAFA)). Bodyweight gain and selected organ weight and pathology were assessed. The high dose (500 mg/kg body weight/day) was associated with reduced bodyweight gain but had no effect on organ weights or histopathology. No effects were reported in the lower-dose groups. An NOAEL of 0.1% in the diet, equivalent to 50 mg/kg body weight/day, was identified.

Additional studies of effects of dietary methyl salicylate on bone density were conducted by Abbott and Harrisson (1978).

Abbott and Harrisson (1978) conducted a series of six experiments to assess the body weight and bone formation effects of methyl salicylate fed to Sprague–Dawley rats of both sexes at various dietary concentrations and exposure periods.

Rats were fed diets containing 0.2%, 0.36%, 0.63%, 1.13%, or 2.0% methyl salicylate for 11 weeks. These dietary concentrations equated to nominal doses of 100, 180, 320, 560, and 1000 mg/kg body weight/day, respectively. The intended dietary concentrations were fed for 7 weeks of the study after gradual escalation of the dose. Rats of both sexes fed 0.2% and 0.36% methyl salicylate and females in the 0.63% group showed normal body weight gain. Males fed 0.63% and males and females fed 1.13% and 2.0% exhibited decreased weight gains. X-ray examinations showed increased bone density at the metaphyses of the femur, humerus, tibia and radius in the animals of both sexes at the two highest levels of methyl salicylate. The nature of the bone density increase was not well defined. Based on these limited criteria, the NOAEL was 0.36% dietary methyl salicylate, or 180 mg/kg body weight/day. In some but not all subsequent similar studies, higher doses were better tolerated.

Abbott and Harrisson (1978) reported that supplementation of the diet with 0.3% calcium blocked the development of increased bone density, reduced mortality and supported normal weight gain with rats fed 1.2% methyl salicylate (600 mg/kg body weight/day) for 12 weeks.

Abbott and Harrisson (1978) also conducted two subchronic oral toxicity studies of methyl salicylate in beagle dogs, one being 6.5–7.5 months in duration with a 2 month recovery period and the other covering a six month span with a 5-month recovery period. In the first study, groups of three male and three female dogs were administered methyl salicylate in capsule form to provide doses of 150, 300, 500, 800 mg/kg/day 6 days/week. Two males and four females served as controls. All high dose dogs died in week 2 and 4 of 6 dogs administered 500 mg/kg died during the study. All showed increased relative liver and kidney weights. None of the dogs administered 150 or 300 mg/kg/day exhibited weight loss during the test period, but they showed increased relative kidney and liver weights that were not associated with any histopathological changes. There were no effects on clinical chemistry or urinalysis parameters. In 300 mg/kg/day dogs allowed a 6-week recovery period, no increases in relative kidney or liver weights were reported. A NOAEL of 300 mg/kg/day can be derived from these data.

In the second dog study, methyl salicylate was administered *via* capsule at 50, 100, or 170 mg/kg/day, 6 days/week, to groups of four male and four female beagles for 6 months. Two high-dose and control dogs of each sex were allowed a 2-month recovery period. For all doses, there were no treatment-related effects on body weights, liver or kidney weights, or on the results of hematological and clinical chemistry evaluations. During the second month of the study, all treated dogs showed signs of seborrhea oleosum and pyoderm; the severity of this condition varied directly with the dose of test compound; the addition of lard to the diets of all animals caused a remission of this skin condition. The NOAEL was 170 mg/kg/day, the highest dose tested.

The oral toxicity of isoamyl salicylate was assessed in groups of 15 male and 15 female Wistar rats fed diets containing concentrations of 50, 500, or 5000 ppm for 13 weeks (Drake et al., 1975). Actual doses were 0, 4.7–4.8 mg/kg body weight/day, 46–47 mg/kg body weight/day, and 420–489 mg/kg body weight/day. One high-dose female died during the study. At the highest dose level, body weight gain and feed intake were significantly depressed; increased relative kidney weights in both sexes and increased relative spleen and liver weights in females were reported. Approximately 50% of the high-dose animals displayed signs of respiratory infection. Increased relative kidney weights were also reported in mid-dose (47 mg/kg body weight/day) females. There were no histopathological or hematological abnormalities in any animals. The results of the Drake et al. (1975) study support a NOAEL value of 47 mg/kg body weight/day since the only finding at this dose was of increased relative kidney weights in females that had no histopathological correlates.

3.2.3. Summary of subchronic toxicity studies

The dermal studies conducted on methyl salicylate are limited in design and in reporting detail and are not useful

for the purposes of risk assessment. They showed that extreme doses of methyl salicylate (*i.e.*, ~5 g/kg body weight/day) may be associated with nephrotoxicity (Webb and Hansen, 1963).

The most appropriate methyl salicylate oral data are those from the 17-week study in Osborn–Mendel rats reported by Webb and Hansen, 1963, and the 6–12 week experiments in Sprague–Dawley rats reported by Abbott and Harrisson (1978).

In the 17-week study (Webb and Hansen, 1963), a NOAEL of 0.1% in the diet, equivalent to ~50 mg/kg body weight/day, was identified. The results of Abbott and Harrisson (1978), suggest a NOAEL value of 180 mg/kg body weight/day. These results must be used with caution since the studies, while well conducted and reported, are limited in endpoints evaluated. In dogs administered methyl salicylate for 6 months a NOAEL of 170 mg/kg body weight/day was reported Abbott and Harrisson (1978).

Study of isoamyl salicylate in a well-conducted and reported 13-week toxicity assay in Wistar rats resulted in a NOAEL of 47 mg/kg body weight/day (Drake et al., 1975).

A systemic NOAEL of 50 mg/kg body weight/day can be used for quantitative human health risk assessment of the use of the salicylates as fragrance compounds. Given the data on methyl- and isoamyl-salicylates there do not appear to be large differences in the toxicity of the individual salicylates.

3.3. Chronic toxicity (Table 8)

Chronic toxicity studies have been conducted on methyl salicylate, two in rats (Packman et al., 1961; Webb and Hansen, 1962, 1963) and one in dogs (Webb and Hansen, 1962, 1963). Although the studies are relatively old, the protocol and results of the rat and dog studies conducted by Webb and Hansen (1962, 1963) were reported in adequate detail and included hematological studies, gross pathology, and limited histopathological examinations of key organs and tissues.

Webb and Hansen (1962, 1963) administered methyl salicylate in the diet to groups of 24–25 male and 25–26 female Osborne–Mendel rats at dietary concentrations of 0, 0.1%, 0.5%, 1.0%, or 2.0% in the diet providing doses of approximately 0, 50, 250, 500, and 1000 mg/kg body weight/day for two years. While these two references do not provide complete details and no statistics were reported, a summary of the results are given below.

All rats in the 1000 mg/kg group died by the 49th week. Body weights of both sexes were significantly decreased in both the 500 and 1000 mg/kg body weight/day groups. An increased amount of cancellous bone was present in the metaphyses in rats treated at either 500 or 1000 mg/kg body weight/day, with a more marked effect at the highest dose level. The relative testes weights of males were significantly increased as were the relative weights of the heart and kidneys of females in the 500 mg/kg body weight/day group. Gross pituitary gland lesions were found in 10 rats

Table 8
Chronic studies

Material	Method	Dose[a] (mg/kg/day)	Species	Results	References
Methyl salicylate	2 year oral (diet) study	50 (0.1% in the diet), 250 (0.5% in the diet), 500 (1.0% in the diet), 1000 (2.0% in the diet)	Osborne–Mendel rats	50 mg/kg/day: no effects 250 mg/kg/day: gross pituitary lesions reported in 10 animals 1 male and 2 females were diagnosed with malignant pituitary tumors 500 mg/kg/day: significant reduction in body weight gains and rough hair coat were reported. Increased testes weight in males and increased heart and kidney weights in females. Slight increase in cancellous bone in the metaphysic 1000 mg/kg/day: 50% mortality after 8 weeks, with 100% mortality after 49 weeks. Decreased body weight gain, rough hair coats, and evidence of pneumonia were reported. Moderate to marked increase in cancellous bone in the metaphysic was observed	Webb and Hansen (1962, 1963)
Methyl salicylate	2 year oral (diet) study	35 (700 ppm in the diet) 100 (2100 ppm in the diet)	Albino rats	No effects	Packman et al. (1961)
Methyl salicylate	2 year oral (capsule) study	50 150 350	Beagle dogs	50 mg/kg/day: no effects 150 and 350 mg/kg/day: growth retardation and body weight loss. Increased relative liver weights and grossly enlarged livers were observed at necropsy. Microscopy revealed hepatocellular hypertrophy. 350 mg/kg/day: 1 female died (not treatment related)	Webb and Hansen (1962, 1963)

[a] Units have been converted to make easier comparisons; original units are in the Fragrance Material Reviews.

(both sexes combined) treated at 250 mg/kg body weight/day (0.5% in the diet) compared to 4 in the controls. Microscopic examinations revealed malignant pituitary gland tumors in one treated male and two treated females. The incidences of benign pituitary tumors and all other tumors, mainly mammary gland neoplasms, did not differ between treated and control groups. The lack of complete details precluded further independent analyses of tumor incidence. The authors concluded that the NOAEL in rats was 50 mg/kg body weight/day (*i.e.*, 0.1% in the diet) (Webb and Hansen, 1963).

Webb and Hansen (1963) studied groups of two male and two female purebred beagles fed methyl salicylate in capsule form at doses of 0, 50, 150, or 350 mg/kg body weight/day, 6 days/week for 2 years.

One high-dose animal died of hepatitis apparently unrelated to methyl salicylate. Hematological analyses at 1, 3, 6, 12 and 24 months and complete necropsy examination were normal, except that dogs treated at 150 and 350 mg/kg body weight/day had enlarged livers with hepatocellular swelling. No other pathology was reported in any of the animals. Reduced body weight was reported in the 350 and 150 mg/kg body weight/day groups. Webb and Hansen (1963) considered the NOAEL to be 50 mg/kg body weight/day.

The chronic oral toxicity data for methyl salicylate are consistent with the oral subchronic toxicity data from the same laboratory in that the NOAEL value is 50 mg/kg body weight/day (Webb and Hansen, 1963) in both rats and dogs.

3.3.1. Repeated-dose toxicity of salicylate metabolites

The alcohols and acids that are formed as metabolites of salicylates are without significant toxicity. The hydrolyzed side chains are metabolized by common and well-characterized metabolic pathways. These primary alcohols (butanol, pentanol, hexanol, octanol, and propanol) and their corresponding aldehydes and acids have also been evaluated by JECFA who found them to have no safety concerns based on their current levels as food flavors. In a 90-day study in rats, butanol has been shown to have a subchronic NOAEL of 125 mg/kg (IRIS, 1998) while butanoic acid, formed from butanol, has been shown to have NOAELs of 250 and 500 mg/kg in chronic studies in dogs and rats, respectively. Hexanoic acid was reported to have a NOAEL of 500 mg/kg in a chronic study in rats. Hexanal was reported to have a NOAEL of 110 mg/kg in a subchronic study in rats (Komsta et al., 1988).

Secondary alcohols such as isobutanol or isoamyl alcohol that are formed by hydrolysis of isobutyl or isoamyl salicylate were evaluated in a 90 day study conducted in rats. Isobutanol have been reported to have a NOAEL of 1450 mg/kg while NOAELs of 340 and 1250 mg/kg/day have been reported for isoamyl alcohol, male and female rats, respectively (Schilling et al., 1997). They also have been evaluated by JECFA who reported no safety concerns for them or their corresponding aldehydes and acids.

In the case of hydrolysis of the salicylates containing aromatic side chains, such as phenyl salicylate, benzyl salicylate and ethyl hexyl salicylate, phenol, benzyl alcohol and ethylhexanol, respectively, would be formed. In 2-year studies in mice and rats, benzyl alcohol showed no evidence of carcinogenic activity at doses up to 400 mg/kg in rats and 200 mg/kg in mice and benzoic acid which is rapidly oxidized from benzyl alcohol has been shown to have a chronic NOAEL of 1% (approximately equivalent to 500 mg/kg/day) in the diet of rats (Kieckebusch and Lang, 1960). In the case of the phenethyl side chain, hydrolysis yields 2-phenylethanol. Phenethyl alcohol has been reported to have a NOAEL of 500 mg/kg/day in a 13-week dermal study in rats (Owston et al., 1981). In a 3-month study in rats the NOAEL for ethylhexanol was reported to be 125 mg/kg/day (BASF, 1991). The NOAELs for ethylhexanoic acid, a metabolite of ethylhexanol, were reported to be approximately 66 and 192 mg/kg/day for rats and mice, respectively, in a 13-week study (Juberg et al., 1998). Both phenethyl and benzyl alcohol were also evaluated by JECFA who reported no safety concerns for them.

3.4. Mutagenicity and genotoxicity

Of the 17 salicylates considered, 3, including methyl-, benzyl-, and phenyl salicylate have been tested for genotoxicity in various *in vitro* test systems. Only ethyl hexyl salicylate has been subject to *in vivo* genotoxicity testing.

In several of the genetic toxicity studies, protocols and results were insufficiently described, rendering the data reported uninterpretable. Studies that did not report the concentration/dose of the test material were not ascribed significant weight, but are reported in the summary tables. Detailed conditions and results of the available genetic toxicity studies are presented in Tables 9 and 10 and are described below.

3.4.1. Bacterial studies (Table 9)

In Ames assays using *Salmonella typhimurium*, methyl salicylate (Ishidate et al., 1984; Mortelmans et al., 1986), ethyl hexyl salicylate (RIFM, 1990), phenyl salicylate (Szybalski, 1958; Zeiger et al., 1987) and benzyl salicylate (Zeiger et al., 1987) have all been reported to be without mutagenic activity, both in the absence or in the presence of S9 mix.

Kuboyama and Fujii (1992) reported weak positive results for methyl salicylate tested with S9 mix prepared from golden hamsters pretreated with PCBs in corn oil but not when tested at the same doses in the presence and in the absence of S9 prepared from either rats or mice. However, the positive results are hardly interpretable due to the lack of cytotoxicity data. Methyl salicylate was also non-mutagenic in two separate Rec assays (Oda et al., 1978; Kuboyama and Fujii, 1992).

Table 9
Mutagenicity and genotoxicity: bacterial studies

Material	Test system	Species	Concentrations	Results	References
Benzyl salicylate	Ames pre-incubation assay with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	3.3–333 μg/plate	Negative	Zeiger et al. (1987)
Homomenthyl salicylate[a]	Ames pre-incubation assay with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	10–10,000 μg/plate	Negative	Zeiger et al. (1987)
Methyl salicylate	Rec-assay	*Bacillus subtilis* in strains H 17 (rec+) and M 45 (rec−)	23 μg/disk	Negative	Oda et al. (1978)
Methyl salicylate	Rec-assay	*Bacillus subtilis* in strains H 17 (rec+) and M 45 (rec−)	5000 μg/disk	Negative	Kuboyama and Fujii (1992)
Methyl salicylate	Ames pre-incubation assay with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	1–333 μg/plate	Negative	Mortelmans et al. (1986)
Methyl salicylate	Ames assay with and without S9 activation	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, and TA1537	0–10,000 μg/plate	Negative	Ishidate et al. (1984)
Methyl salicylate	Ames assay with and without rat, mouse, guinea pig and hamster S9	*S. typhimurium* TA98, TA100	100 μg/disk	Positive Hamster	Kuboyama and Fujii (1992)
Octyl salicylate[a]	Ames assay with and without S9 activation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and TA1538	0.001–5 μl/plate	Negative	RIFM (1977a)
Octyl salicylate[a]	*Saccharomyces cerevisiae* mutation assay (overlay method) with and without S9 activation	*Saccharomyces cerevisiae* D4	0.001–5 μl/plate	Negative	RIFM (1977a)
Phenyl salicylate	Ames pre-incubation assay with and without S9 activation	*S. typhimurium* TA97, TA98, TA100, TA1535 and TA1537	3.33–333 μg/plate	Negative	Zeiger et al. (1987)
Phenyl salicylate	Ames pre-incubation assay with and without S9 activation	*S. typhimurium* TA97, TA98, TA100, TA1535 and TA1537	1–100 μg/plate	Equivocal results	Zeiger et al. (1987)
Phenyl salicylate	Reverse mutation assay in *E. coli*	*Escherichia coli* streptomycin dependent mutants	1–100 μg/plate	Negative	Szybalski (1958)

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

Table 10
Mutagenicity and genotoxicity: mammalian studies

Material	Test system	Species	Dose or concentration	Results	References
Ethyl hexyl salicylate	*In vivo* mouse micronucleus assay	NMRI mice	2000 mg/kg bw by i.p. injection	Negative	Haarmann and Reimer (1991)
Methyl salicylate	*In vitro* chromosome aberration assay	Chinese hamster fibroblast cells without exogenous metabolic activation	0–250 μg/ml	Negative	Ishidate et al. (1984)

3.4.2. Mammalian studies (Table 10)

Methyl salicylate was negative in *in vitro* tests for clastogenic potential in Chinese hamster fibroblast cells (Ishidate et al., 1984). One *in vivo* genotoxicity study on ethyl hexyl salicylate has been reported by RIFM (1990). In a micronucleus assay (OECD Guideline 474), in which NMRI mice were dosed orally with 2000 mg/kg body weight; there were no increases in the incidence of micronucleated polychromatic erythrocytes at the 24-, 48-, or 72-h sampling intervals.

3.4.3. Summary of the genotoxicity data

In Ames assays (Ishidate et al., 1984; Mortelmans et al., 1986; RIFM, 1990; RIFM, 1977a; Bonin et al., 1982; Zeiger et al., 1987; Szybalski, 1958; Oda et al., 1978; and Kuboyama and Fujii, 1992) using rat or mouse S9 there have been negative results for the six salicylates tested. A weak response was observed with methyl salicylate in the presence of S9 isolated from PCB-treated hamsters. Based on the results it is unlikely that the salicylates are mutagenic.

The fully reported *in vitro* chromosome aberration/SCE assays of methyl salicylate showed no evidence of clastogenicity. An *in vivo* mouse micronucleus assay demonstrated ethyl hexyl salicylate to be non-genotoxic (RIFM, 1990). The *in vitro* genotoxicity data are concluded to show no evidence of genotoxic activity.

The core hydrolysis product, salicylic acid, has not shown evidence of a genotoxic effect in an *in vivo* chromosome aberration and SCE assay in mice (Giri et al., 1996). Other structurally related alkyl- and alkoxy-benzyl derivatives are generally without genotoxic effect (Adams et al., 2005). Other metabolites of the salicylates are simple alcohols and acids. Therefore, the salicylates as a group are concluded to be without mutagenic/genotoxic potential.

3.5. Carcinogenicity

No recent 2-year rodent bioassays are available that investigate the carcinogenic potential of any of the 18 salicylates. Two older 2-year studies in rats, one reported in some detail (Webb and Hansen, 1963), and the other only in abstract form (Packman et al., 1961), are available on methyl salicylate and are discussed above under ''chronic toxicity''. While limited in design and reporting, the studies of Webb and Hansen (1963) and Packman et al. (1961) provide no evidence to indicate that the salicylates are carcinogenic. The studies are summarized in Table 8 along with the chronic toxicity studies. Also, methyl salicylate has been tested for carcinogenic potential in the A/He strain of mouse, a strain susceptible to carcinogen-induced lung tumorigenesis (Stoner et al., 1973). In addition to studies relevant to the assessment of carcinogenic activity, methyl salicylate has been studied for anti-carcinogenic potential in several older assays (Strong, 1932a,b; Boyland and Huntsman-Mawson, 1938).

3.5.1. Non-standard carcinogenicity studies

Methyl salicylate has been tested for carcinogenic activity by the intraperitoneal route in mice (Stoner et al., 1973). Methyl salicylate was injected three times weekly in tricaprylin for 8 weeks to groups of 15 A/He mice of each sex at doses of 100 or 500 mg/kg body weight, providing total doses of 2400 or 12,000 mg/kg body weight or approximately 43 or 214 mg/kg body weight/day. In the low-dose group, 2/15 (13%) males and 1/15 (6%) females developed lung tumors while in the high-dose group, 1/15 males (6%) and 5/15 (33%) females developed lung tumors. In comparison, 22/80 (28%) male and 16/80 (20%) female control mice developed lung tumors. There was no evidence of carcinogenic potential of methyl salicylate.

3.5.2. Anti-carcinogenic effects

The anti-tumor activity of wintergreen oil (99% methyl salicylate) was evaluated in 32 mice of the A strain, a strain that commonly develops spontaneous tumors of the mammary gland (Strong, 1932a). The oil was added to the diet at: 1, 2, or 3 drops of oil to 1 g of diet daily for an unspecified period after tumors had developed. There was no detectable effect on animal survival or tumor growth rate. In a related study, the effect of wintergreen oil in the diet on the occurrence of spontaneous mammary gland carcinomas was studied in 45 female D strain mice. Average time to tumor formation was 18 months in treated mice and 12.1 months in controls (Strong, 1932b).

3.5.3. Summary of the carcinogenicity data

In summary, the 2-year rat studies conducted by Webb and Hansen (1963) and Packman et al. (1961) and the study in A/He mice (Stoner et al., 1973) provide no evidence to indicate that methyl salicylate is carcinogenic. Given the genetic toxicity data and the well-characterized metabolism of the salicylates and closely related compounds, it can be concluded that the salicylates are unlikely to possess carcinogenic activity.

3.6. Reproductive and developmental toxicity (Table 11)

A number of reproductive (Collins et al., 1971; NTP, 1984a,b; Morrissey et al., 1989) and developmental toxicity (Warkany and Takacs, 1959; Bertone and Monie, 1965; Pyun, 1970; Woo and Hoar, 1972; Overman and White, 1978, 1983; Overman, 1979; Kavlock et al., 1982; Daston et al., 1988; Infurna et al., 1990) studies have been conducted on the salicylates. These studies have focused almost exclusively on methyl salicylate, due to the known reproductive toxicity of salicylic acid (Kimmel et al., 1971; Tanaka et al., 1973a,b; Waltman et al., 1973), the major metabolite of this group of chemicals.

Reproductive and developmental toxicity of salicylic acid associated with cosmetics exposure in humans was evaluated by the CIR (Cosmetic Ingredient Review) Expert Panel, who concluded that systemic exposure from facial cosmetic products containing 2% salicylic acid is expected to be in range of approximately 20% of that following ingestion of a single baby aspirin, which is a dose widely recognized as carrying no maternal or fetal risk (CIR, 2003).

There have been several animal reproductive studies conducted with salicylic acid. Cekanova et al. (1974) evaluated the teratogenic effects of salicylic acid in NMRI mice *via* oral administration of 500 and 1000 mg/kg of salicylic acid during gestation days 9 or 17. These exposures resulted in fetal resorption and rib and vertebral malformations. The severity of the effects depended on the time of administration (at 1000 mg/kg, higher resorption was observed in animals that received salicyclic acid on GD 17). Salicylic acid is also known as the causative agent of aspirin-induced teratogenesis in rats (Kimmel et al., 1971). When administered to groups of pregnant Sprague–Dawley rats on GD 12–21 at 20, 80 and 200 mg/kg, no effects were observed at 20 and 80 mg/kg but teratogenic effects were observed at 200 mg/kg (Davis et al., 1994).

Several of the developmental toxicity studies of methyl salicylate used intraperitoneal (Woo and Hoar, 1972; Kavlock et al., 1982; Daston et al., 1988) or subcutaneous injection (Warkany and Takacs, 1959; Bertone and Monie, 1965; Pyun, 1970). Since these routes of exposure are of limited relevance to potential exposure to salicylates *via* fragrances, the results are not discussed in detail, but are summarized below. Further details regarding the results of the particular studies are available in the Fragrance Material Review for methyl salicylate.

In a 3-generation study, rats were fed methyl salicylate at doses of 500, 1500, 3000 or 5000 ppm in the diet (25, 75, 150 or 250 mg/kg body weight) 100 days before the first mating and then throughout the experiment. Litter parameters were decreased in the F_2 generation, and weanling weights were decreased in all generations in animals fed

Table 11
Reproductive and developmental toxicity

Material	Method	Concentration(s)/ doses	Species	Results	References
Methyl salicylate	Oral (diet study) 3 generation study	25, 75, 150 and 250 mg/kg (500, 1500, 3000 and 5000 ppm)	Rats	500–1500 ppm – NOAEL 3000–5000 ppm – decrease in litter size, number of live born progeny; decrease in average number of survivors to day 4 and average number to weaning	Collins et al. (1971)
Methyl salicylate	Oral (diet study) 2 generation study	125 and 250 mg/kg (0.25% and 0.5%)	Rats	2500 ppm – decrease in litter size; 5000 ppm – decrease mating performance and reproductive and viability indices; increased deaths between birth and postnatal day 5	Abbott and Harrisson (1978)
Methyl salicylate	Oral (diet study)	125 and 250 mg/kg (2500 and 5000 ppm)	Mice	125 and 250 mg/kg – NOAEL	Abbott and Harrisson (1978)
Methyl salicylate	Oral (gavage study) Continuous breeding test	25, 50 and 100 mg/kg/ day in corn oil	Mice	25, 50 and 100 mg/kg/day – NOAEL	NTP (1984a), Chapin and Sloane (1997)
Methyl salicylate	Oral (gavage study) Continuous breeding test	100, 250 and 500 mg/ kg/day in corn oil	Mice	100 mg/kg/day – NOAEL 250 mg/kg/day – reduced pup weights 500 mg/kg/day – decrease in the number of live pups per litter, the percentage of live born pups and pup weights	NTP (1984b) and Morrissey et al. (1989), Chapin and Sloane (1997)
Methyl salicylate	Oral (single gavage administration on the 7th GD	1750 mg/kg	Hamster	1750 mg/kg – neural tube malformation	Overman and White (1978, 1983)
Methyl salicylate	Single 2 h dermal application	3500 and 5250 mg/kg	Hamster	3500 and 5250 mg/kg – neural tube malformation; lethal	Overman and White (1978, 1983)
Methyl salicylate	Dermal application on GD days 6–15	1000 and 2000 mg/kg per day	Rat	1000 mg/kg – incidence of total resorption (100%) 2000 mg/kg – maternal toxicity (25%)	Infurna et al. (1990)
Methyl salicylate	Single subcutaneous injection on GD day 9,10 or 11	118 and 590 mg/day (0.1–0.5 cm^3)	Rat	0.1–0.5 cm^3 – maternal toxicity; total resorption; external malformations and skeletal anomalies	Warkany and Takacs (1959)
Methyl salicylate	Single subcutaneous injection on day GD 10 or 11	118 mg/day (0.1 ml/ day)	Rat	Resorption; malformations; exencephaly and retarded fetal growth; hydronephrosis; etopic kidney	Bertone and Monie (1965)
Methyl salicylate	Intraperitoneal injections on GD day 10 and 11	59 and 118 mg/day (0.05 and 0.1 ml/day)	Rat	59–118 – maternal toxicity; resorption; malformation; hydronephrosis	Woo and Hoar (1972)
Methyl salicylate	Intraperitoneal injections on GD day 9 and 10	200 and 400 mg/kg/ day	Rat	200 and 400 mg/kg – maternal toxicity; malformations; decreased fetal weight; reduction of fetal body weight index;	Kavlock et al. (1982)
Methyl salicylate	Intraperitoneal injections on GD day 11–14	200, 250, 300, 350, 375, 400 and 450 mg/ kg/day	Rat	200–450 mg/kg/day – decreased maternal body weight gain, etopic kidneys; maternal lethality; reduced fetal weight; dilated renal pelvis	Daston et al. (1988)
Phenyl salicylate	Oral administration on GD day 7–9 or 7–12	100, 200, 300 and 400 mg/kg/day	Rat	Malformations observed	Nagaham et al. (1966)

150 or 250 mg/kg body weight; fertility index was decreased in F_2 and F_3 in animals fed 250 mg/kg. There were no abnormalities in the offspring. The NOAEL was 75 mg/kg (Collins et al., 1971).

In an earlier 2-generation reproductive toxicity study (Abbott and Harrisson, 1978) rats were fed methyl salicylate at dietary concentrations of 0.25 or 5% (125 or 250 g/kg body weight) from 60 days before the first mating

and throughout the entire study period. At the dose of 125 mg/kg body weight the only reported effect of methyl salicylate treatment was a decrease in litter size. In the 250 mg/kg body weight dose group, decreases in mating performance, reproductive indices and viability indices were noted but these findings were not statistically significant, and deaths between birth and postnatal day 5 were increased. There were no effects at either dose on the incidence of gross abnormalities or on growth, appearance and behavior of the pups surviving to weaning. Given the report of decreased litter size in the low-dose group, a NOAEL level could not be determined.

In a similar study with mice, (Abbott and Harrisson, 1978) there were no significant effects of treatment on reproductive performance or on the growth/survival of the young. The NOAEL was 250 mg/kg body weight/day, the highest dose tested in the study.

Two additional reproductive toxicity studies of methyl salicylate in mice were conducted as part of the National Toxicology Program (NTP) Fertility Assessment by Continuous Breeding study (NTP, 1984a,b; Morrissey et al., 1989; Chapin and Sloane, 1997) and utilized gavage dosing.

In the first study mice were administered methyl salicylate by gavage (in corn oil) at 25, 50 or 100 mg/kg/day during the 7-day pre-mating and a 98-day cohabitation period (NTP, 1984a). Treatment was not associated with any adverse effects on fertility, number of pups/litter, percentage of live pups, or on pup weight. Necropsy of the F_1 animals, reared and dosed with methyl salicylate, revealed no adverse effects on terminal body and organ weights or on sperm motility, density and morphology (NTP, 1984a; Chapin and Sloane, 1997). A NOAEL for reproductive effects of 100 mg/kg body weight/day was identified, the highest dose tested in the study. In the second study that utilized doses of 0, 100, 250, and 500 mg methyl salicylate/kg body weight/day (NTP, 1984b; Morrissey et al., 1989; Chapin and Sloane, 1997), decreases in the number of live pups per litter, the percentage of pups born alive, and pup weights were reported in the high-dose group. Pup weights were reduced by approximately 3% in animals treated at 250 mg/kg body weight/day (Chapin and Sloane, 1997). The NOAEL was 100 mg/kg body weight/day, consistent with the results of the first study.

Two developmental toxicity studies have been conducted in hamsters using methyl salicylate by the dermal or oral routes of exposure (Overman and White, 1983; Infurna et al., 1990).

Overman and White (1978, 1983) administered methyl salicylate topically (no vehicle reported) at approximate doses of 3500 and 5250 mg/kg body weight to LVG-strain pregnant hamsters at 7th day of gestation. At the same time, another group of pregnant hamsters were treated by oral intubation with methyl salicylate at 1750 mg/kg body weight. Embryos from both treatment groups were recovered at GD9. Some were allowed to continue their development but few survived to the 12th day (many embryos died between GD9 and GD12). The incidence of neural tube closure defects was 72% in the embryos after oral administration of 1750 mg/kg and 6% and 53% after topical application of 3500 and 5250 mg/kg body weight, respectively. The study showed that methyl salicylate can be teratogenic in hamsters when applied topically, although a very high dose is necessary to achieve the same blood level and teratogenic effects seen after oral treatment.

In a dermal study reported in an abstract only, undiluted methyl salicylate was applied to the skin of pregnant rats on gestation days 6–15, initially at a dose of 2000 mg/kg body weight/day. Due to maternal toxicity (25% mortality) and severe dermal irritation, the dose was reduced to 1000 mg/kg body weight/day on gestation days 10–15. There were 100% total resorptions. Topical methyl salicylate at doses in excess of 1000 mg/kg body weight/day was clearly maternally toxic and embryotoxic in the rat (Infurna et al., 1990).

3.6.1. Summary of the reproductive and developmental toxicity

In summary, the reproductive and developmental toxicity data on methyl salicylate demonstrate that, under conditions of sufficient exposure, there is a pattern of embryotoxicity and teratogenesis that is similar to those caused by salicylic acid in comparable doses. The abnormalities include neural tube defects and malformations of the skeleton and viscera. In hamsters, 3500 mg/kg body weight/day by dermal exposure was embryotoxic and teratogenic, producing neural tube defects. However in well-designed and reported studies of methyl salicylate exposure in diet or by gavage NOAELs for reproductive toxicity are of 75–100 mg/kg body weight/day (Abbott and Harrisson, 1978; Collins et al., 1971; NTP, 1984a,b; Chapin and Sloane, 1997), and are consistent with NOAELs available from subchronic and chronic toxicity studies. These NOELs are also consistent with studies on the reproductive toxicity of salicylic acid, which reported a NOEL of 80 mg/kg. The Cosmetic Ingredient Review Board Expert Panel (CIR, 2003) concluded that the total calculated exposure to salicylates and salicylic acid in cosmetic products does not pose a risk for reproductive or developmental effects in humans since serum levels would not approach those associated with adverse effects. Moreover, as documented in a developmental toxicity study in hamsters (Overman and White, 1979; Overman and White, 1983), dermal exposure results in low serum salicylate concentrations. On a dose/bodyweight basis, dermal exposure results in markedly lower systemic exposure as compared to parenteral exposure.

Further, the reproductive and developmental toxicity of alcohol products that are formed upon hydrolysis of salicylates was evaluated by the Maximum workplace concentration (Maximale Arbeitsplatzkonzentration, a.k.a. MAK) commission (for details see "The MAK-Collection for Occupational Health and Safety") and concluded that 2-ethyl hexanol, methanol, ethanol, butyl alcohol, octanol and isobutyl alcohol show no reproductive/developmental

Table 12
Skin irritation studies in humans

Material	Method	Concentration	Subjects	Results	References
Benzyl salicylate	Maximization pre-test (48-h occluded patch)	30% in petrolatum	5 volunteers	No irritation (0/5)	RIFM (1975c)
Benzyl salicylate	Maximization pre-test (48-h occluded patch)	30% in petrolatum	22 volunteers	Questionable irritation observed in 2/22	RIFM (1975d)
Benzyl salicylate	HRIPT pre-test (48-h occluded patch)	5% in dimethyl phthalate	8 volunteers	No irritation (0/8)	RIFM (1968b)
Benzyl salicylate	Induction phase HRIPT (24-h occluded patch, nine applications)	10% in alcohol	35 volunteers	No irritation (0/35)	RIFM (1975h)
Benzyl salicylate	Induction phrase HRIPT (24-h occluded patch, nine applications)	15% in 3:1 DEP:ethanol	101 volunteers	No irritation (0/101)	RIFM (2004c)
Benzyl salicylate	48-h occluded patch	20% in vaselium aldum	5 volunteers	No irritation (0/5)	Fujii et al. (1972)
Benzyl salicylate	24–72 h occluded patch	2% in unguentum simplex	30 volunteers	No irritation (0/30)	Fujii et al. (1972)
Benzyl salicylate	24-h occluded patch	5% in vaseline	25 volunteers	No irritation (0/25)	RIFM (1997a)
Benzyl salicylate	4-h occluded patch	100% (0.2 ml aliquot)	30 volunteers	No irritation (0/30)	Basketter et al. (2004)
Butyl salicylate	Maximization pre-test (48-h closed patch)	2% in petrolatum	5 volunteers	No irritation (0/5)	RIFM (1975c)
p-Cresyl salicylate	Maximization pre-test (48-h occluded patch)	4% in petrolatum	25 volunteers	No irritation (0/25)	RIFM (1980b)
1,3-Dimethyl-3-butenyl salicylate	Induction phase (HRIPT) (24-h occluded patch, nine applications)	10% in petrolatum	50 volunteers	No irritation (0/50)	RIFM (1981c)
Ethyl hexyl salicylate	Maximization pre-test (48-h occluded patch)	4% in petrolatum	23 volunteers	No irritation (0/23)	RIFM (1974b)
Ethyl salicylate	Maximization pre-test (48-h occluded patch)	12% in petrolatum	25 volunteers	No irritation (0/25)	RIFM (1976c)
cis-3-Hexenyl salicylate	Maximization pre-test (48-h occluded patch)	3% in petrolatum	5 male volunteers	No irritation (0/5)	RIFM (1975c)
trans-2-Hexenyl salicylate	Maximization pre-test (48-h occluded patch)	20% in petrolatum	33 male volunteers	No irritation (0/33)	RIFM (1978b)
Hexyl salicylate	Maximization pre-test (48-h occluded patch)	3% (vehicle not specified)	22 volunteers	No irritation (0/22)	RIFM (1975d)
Hexyl salicylate	Induction phrase HRIPT (24-h occluded patch, nine applications)	30% in 3:1 DEP:ethanol	103 volunteers	Slight irritation observed in 3/103	RIFM (2004a)
Hexyl salicylate	A 24-h occluded patch	0.3%, 3%, and 30% in 3:1 DEP:ethanol	56 volunteers	No irritation (0/56)	RIFM (2004b)
Hexyl salicylate	4-h occluded patch	100%	30 volunteers	No irritation (0/30)	Basketter et al. (2004)
Homomenthyl salicylate[a]	Maximization pre-test (48-h occluded patch)	8% in petrolatum	25 volunteers	No irritation (0/25)	RIFM (1977b)
Isoamyl salicylate	48-h occluded patch	20% in vaselium aldum or unguentum hydrophilicum	29 volunteers	No irritation (0/29)	Fujii et al. (1972)
Isoamyl salicylate	24–72 H occluded patch	2% in unguentum simplex or unguentum hydrophilicum	30 volunteers	No irritation (0/30)	Fujii et al. (1972)
Isoamyl salicylate	48-h occluded patch	32% in acetone	50 volunteers	No irritation (0/50)	Motoyoshi et al. (1979)
Isobutyl salicylate	Maximization pre-test (48-h occluded patch)	10% in petrolatum	5 male volunteers	No irritation (0/5)	RIFM (1973c)
3-Methyl-2-butenyl salicylate	Maximization pre-test (48-h occluded patch)	20% in petrolatum	25 volunteers	No irritation (0/25)	RIFM (1978c)
Methyl salicylate (wintergreen oil; 80–99% methyl salicylate)	Maximization pre-test (48-h occluded patch)	12% in petrolatum	25 volunteers	No irritation (0/25)	RIFM (1976b)
Methyl salicylate	Maximization pre-test (48-h occluded patch)	8% (vehicle not specified)	27 volunteers	No irritation (0/27)	RIFM (1973b)
Methyl salicylate	24-h occluded patch test	25 ml of 30% or 60% solutions	9 volunteers	Irritation observed	Green and Shaffer (1992)
Octyl salicylate[a]	Maximization pre-test (48-h occluded patch)	5% in petrolatum	25 volunteers	No irritation (0/25)	RIFM (1976c)

Table 12 (*continued*)

Material	Method	Concentration	Subjects	Results	References
Octyl salicylate[a]	Induction phrase HRIPT (24 h occluded patch, nine applications)	100%	25 volunteers	No irritation (0/25)	RIFM (1976d)
Octyl salicylate[a]	24-h occluded patch	5% in mineral oil	10 volunteers	No irritation (0/10)	RIFM (1971)
Pentyl salicylate	Maximization pre-test (48-h occluded patch)	10% in petrolatum	27 volunteers	No irritation (0/27)	RIFM (1982c)
Phenyl salicylate	Maximization pre-test (48-h occluded patch)	6% in petrolatum	5 volunteers	No irritation (0/5)	RIFM (1975c)
Phenethyl salicylate	Maximization pre-test (48-h occluded patch)	8% in petrolatum	5 volunteers	No irritation (0/5)	RIFM (1973c)

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

potential when used at levels ranging from 200–8000 ml/m^3 for inhalation studies and 130–300 mg/kg for dietary studies. Dermal studies conducted with phenyl ethyl alcohol resulted in a NOAEL value of 0.43 ml/kg.

3.7. Skin irritation

3.7.1. Human studies (Table 12)

The salicylates have been well-studied for their potential to produce skin irritation in humans. Approximately 958 male and female volunteers were tested in standard 24 or 48-h closed patch tests. No evidence of skin irritation was reported for methyl salicylate, ethyl salicylate, butyl salicylate, isobutyl salicylate, isoamyl salicylate, ethyl hexyl salicylate, *cis*-3-hexenyl salicylate, *trans*-2-hexenyl salicylate, 1,3-dimethyl-3-butenyl salicylate, 3-methyl-2-butenyl salicylate, pentyl salicylate, phenyl salicylate, phenethyl salicylate and *p*-cresyl salicylate.

Transient and minimal irritation reactions were observed in a 24-h closed patch test with hexyl salicylate at 30% in diethyl phthalate (DEP): ethanol. Questionable reactions were also observed with benzyl salicylate when tested at 30% in petrolatum in a 48-h close patch test.

The human studies provide strong evidence to indicate that the salicylates are non-irritating to skin at concentrations relevant to fragrances. Any potential for irritation is limited to high concentrations (*i.e.*, 30%) well in excess of known use levels in fragrance products. For details of the individual studies, see Table 12.

3.7.2. Animal studies (Table 13)

In addition to a large complement of human studies, many of the salicylates have been tested in animal models of skin irritation using either rabbits or guinea pigs. The salicylates have been extensively studied in guinea pig models of skin irritation. These include pre-tests conducted prior to or part of skin sensitization assays including open epicutaneous tests (OET), Draize assays, or as a part of phototoxicity and/or photoallergy studies. Methyl salicylate and pentyl salicylate produced no irritation reactions with concentrations up to 1%, while phenethyl salicylate and benzyl salicylate produced no irritation reactions with concentrations up to 0.03%. No irritation reactions were also observed with isoamyl salicylate, hexyl salicylate and *cis*-3-hexenyl salicylate at concentrations higher than 50%.

Sixteen of seventeen salicylates (methyl 4-methyl salicylate, pentyl salicylate and phenethyl salicylate were not included) were evaluated in irritation assays conducted in rabbits. Butyl salicylate, benzyl salicylate, 1,3-dimethyl-3-butenyl salicylate and phenyl salicylate showed no irritation reactions with concentrations up to 100%, hexyl salicylate showed no irritation with concentrations up to 25% and methyl salicylate with concentrations lower than 1%. The rest of the salicylates had shown irritation reactions at a concentration of 100%.

In miniature swine irritation studies conducted as a part of a phototoxicity assay, neat concentrations of methyl salicylate (wintergreen oil) produced irritation reactions while neat hexyl salicylate was not reported to induce any signs of irritation. For these salicylates, similar findings were reported in mice.

Further details of these and other studies of dermal irritation are provided in Table 13 and in the monographs for each individual fragrance compound.

3.7.3. Summary of the skin irritation data

The potential for irritation by most of the salicylates assessed in this report has been well characterized in both humans and in experimental animals.

The human studies performed with 16 of the 17 salicylates show little if any evidence of irritation. Only one irritation reaction was observed with 10% pentyl salicylate. No other irritations were reported for any substance in any test system at concentrations below 30%. Overall, the human skin irritation studies, including studies conducted as part of skin sensitization assays, demonstrate the salicylates to be essentially non-irritating.

The animal data are mixed and are concluded to indicate that the salicylates are likely to be skin irritants when topically applied at neat concentrations. At lower dermal concentrations the salicylates appear to have only limited capacity to irritate skin in animal models. For the most

Table 13
Skin irritation studies in animals

Material	Method	Concentration	Species	Results	References
Benzyl salicylate	Pre-test for an OET (24-h open application)	0.03–100% as a single application (vehicle not specified)	Himalayan white-spotted guinea pigs (6–8/sex/group)	0.03%: no irritation 0.1%: considered as the minimal irritating concentration 0.3–100%: irritation observed	Klecak et al. (1977)
Benzyl salicylate	Induction phase of an OET	0.03–100% applied daily for 21 days (vehicle not specified)	Himalayan white-spotted guinea pigs (6–8/sex/group)	0.03%: no irritation 0.1%: considered as the minimal irritating concentration 0.3–100%: irritation observed	Klecak et al. (1977)
Benzyl salicylate	Pre-test for Draize assay (open application)	2% (vehicle not specified)	4 Hartley albino guinea pigs	No irritation	Sharp (1978)
Benzyl salicylate	Primary skin irritation study (4-h semi-occlusive patch)	100%	4 Female New Zealand White Rabbits	No irritation	RIFM (1984) and RIFM (1985)
Benzyl salicylate	Pre-test for sensitization assay (24-h closed patch test)	10% in SDA 39C alcohol	3 Albino rabbits	No irritation	RIFM (1975e)
Benzyl salicylate	Irritation evaluated during an associated LD_{50} study	100%	3 Rabbits	No irritation	RIFM (1970b)
Benzyl salicylate	Irritation studied as part of a phototoxicity study (no occlusion, 24- and 48-h assay)	5%, 10%, and 30% in acetone	5 female Hartley guinea pigs	5%: no irritation 10%: irritation (slight erythema only) in 1/5 animals 30%: irritation (slight erythema only) in 5/5 animals	RIFM (1997b)
Benzyl salicylate	Irritation studied as part of a phototoxicity/ photoallergy study (1.5-h occlusive patch)	5%, and 10% in alcohol	two male and two female Dunkin–Hartley guinea pigs	5% and 10%: no irritation	RIFM (1983b)
Butyl salicylate	Irritation evaluated during an associated LD_{50} study	100%	4 Rabbits	No irritation	RIFM (1975b)
p-Cresyl salicylate	Irritation evaluated during an associated LD_{50} study	100%	10 Rabbits	Irritation observed	RIFM (1980a)
1,3-Dimethyl-3-butenyl salicylate	Primary irritation study (24-h occluded patch)	100%	6 Rabbits	No irritation	RIFM (1981d)
Ethyl hexyl salicylate	Irritation evaluated during an associated LD_{50} study	100%	4 Rabbits	Irritation observed	RIFM (1974a)
Ethyl salicylate	Irritation evaluated during an associated LD_{50} study	100%	10 Rabbits	Irritation observed	RIFM (1976a)
cis-3-Hexenyl salicylate	Irritation evaluated during an associated LD_{50} study	100%	10 Rabbits	Irritation observed	RIFM (1975a)
cis-3-Hexenyl salicylate	Irritation studied as part of a phototoxicity test (24- and 48-open application)	5%, 10%, 30%, and 50% in acetone	4 Female Hartley guinea pigs	No irritation	RIFM (1999)
trans-2-Hexenyl salicylate	Irritation evaluated during an associated LD_{50} study	100%	10 Rabbits	Irritation observed	RIFM (1978a)
Hexyl salicylate	Primary skin irritation study (4-h semi-occlusive patch)	100%	3 New Zealand White Rabbits	Irritation observed	RIFM (1984) and RIFM (1985)
Hexyl salicylate	Primary skin irritation study (4-h occlusive patch)	10%, 15%, 50%, and 100% in DEP	4 Female New Zealand White Rabbits	10%, 15%, 25%, and 50%: no irritation 100%: irritation observed	RIFM (1986a)

Table 13 (*continued*)

Material	Method	Concentration	Species	Results	References
Hexyl salicylate	Irritation evaluated during an associated LD_{50} study	100%	10 Rabbits	Irritation observed	RIFM (1975a)
Hexyl salicylate	Primary skin irritation study (4-h occlusive patch)	10%, 15%, 25%, 50%, and 100% in DEP	4 Female New Zealand White Rabbits	10%, 15%, and 25%: no irritation 50% and 100%: irritation observed	RIFM (1986b)
Hexyl salicylate	Pre-test for Draize assay (dermal application)	5% (vehicle not specified)	4 Hartley albino guinea pigs	No irritation	Sharp (1978)
Hexyl salicylate	Irritation studied as part of a phototoxicity test	100%	6 Mice (hairless)	No irritation	RIFM (1975f)
Hexyl salicylate	Irritation studied as part of a phototoxicity test	100%	Miniature swine	No irritation	RIFM (1975f)
Hexyl salicylate	Irritation studies as part of a photoallergy test (2-h exposure with Hilltop chambers)	1%, 5%, 10%, 50%, 100% in 3:1 DEP:ethanol	Male albino hairless guinea pigs (5/group)	No irritation	RIFM (2003)
Hexyl salicylate	Preliminary irritation study	10%, 25% and 50% in acetone	4 Albino guinea pigs	10%; no irritation 25 and 50%: irritation observed	RIFM (1981e)
Homomenthyl salicylate[a]	Irritation evaluated during an associated LD_{50} study	100%	10 Rabbits	Irritation observed	RIFM (1978a)
Isoamyl salicylate	Primary irritation (24-h occluded patch)	100%	6 Albino Angora rabbits	Irritation observed	Motoyoshi et al. (1979)
Isoamyl salicylate	Primary irritation (48-h occluded patch)	100%	6 Pitman–Moore miniature swine	No irritation	Motoyoshi et al. (1979)
Isoamyl salicylate	Primary irritation (24-h open application)	100%	6 Male Hartley guinea pigs	Irritation observed	Motoyoshi et al. (1979)
Isoamyl salicylate	Primary irritation test	15% and 100%	Rabbits	No irritation	RIFM (1970c)
Isobutyl salicylate	Primary irritation test	15% and 100%	Rabbits	No irritation	RIFM (1970c)
Isobutyl salicylate	Irritation evaluated during an associated LD_{50} study	100%	8 Rabbits	Irritation observed	RIFM (1973a)
3-Methyl-2-butenyl salicylate	Irritation evaluated during an associated LD_{50} study	100%	10 Rabbits	Irritation observed	RIFM (1978a)
3-Methyl-2-butenyl salicylate	Primary irritation (24-h occluded patch)	1.25% in 98% SDA 39C alcohol	3 Rabbits	No irritation	RIFM (1968c)
Methyl salicylate	Irritation evaluated during an associated LD_{50} study	100%	10 Rabbits	Irritation observed	RIFM (1973a)
Methyl salicylate	Primary irritation study (24- and 72-h occluded patch)	1%, 3%, and 6% in water	Rabbits (3/group)	1%: no irritation 3% and 6%: irritation observed	Yankell (1972)
Methyl salicylate	Primary irritation study (24- and 72-h occluded patch)	1%, 3%, and 6% in PEG 400	Rabbits (3/group)	1%, 3% and 6%: irritation observed	Yankell (1972)
Methyl salicylate	Primary irritation study (24- and 72-h occluded patch)	1%, 3%, and 6% in 70% ethanol	Rabbits (3/group)	1%, 3% and 6%: irritation observed	Yankell (1972)
Methyl salicylate	Primary irritation study (24- and 72-h occluded patch)	1%, 3%, and 6% in 70% ethanol plus emollients	Rabbits (3/group)	1%, 3% and 6%: irritation observed	Yankell (1972)
Methyl salicylate	Pre-test for an open epicutaneous test (OET) (24-h primary irritation)	0.03–100% as a single application (vehicle not specified)	Himalayan white-spotted guinea pigs (6–8/sex/group)	0.03%, 0.1%, 1%: no irritation 3%: minimal irritating concentration 10–100%: irritation observed	Klecak et al. (1977)

(*continued on next page*)

Table 13 (*continued*)

Material	Method	Concentration	Species	Results	References
Methyl salicylate	Pre-test for an OET (24 h primary irritation)	0.03–100% applied daily for 21 days (vehicle not specified)	Himalayan white-spotted guinea pigs (6–8/sex/group)	0.03%, 0.1%, 1%: no irritation 3%: considered as the minimal irritating concentration 10–100%: irritation observed	Klecak et al. (1977)
Methyl salicylate (wintergreen oil, 80–99% methyl salicylate)	Irritation evaluated as part of a photoirritation study	100%	6 Mice (hairless)	Irritation observed	RIFM (1976e)
Methyl salicylate (wintergreen oil, 80–99% methyl salicylate)	Irritation evaluated as part of a photoirritation study	100%	Miniature swine	Irritation observed	RIFM (1976e)
Methyl salicylate	Irritation studied as part of a mouse ear swelling test	1%, 2.5%, 10%, and 20% in 4:1 acetone to olive oil	Mice	1%, 2.5%, 10%: no irritation 20%: established as the minimal irritating concentration producing significant increase in ear swelling	Howell et al. (2000)
Methyl salicylate	Irritation studied as part of a mouse ear swelling test	2.5, 5.0, 7.5 and 10% in ethanol	Mice	Irritation observed	Patrick et al. (1985, 1987) and Patrick and Maibach (1986)
Octyl salicylate[a]	Irritation evaluated during an associated LD_{50} study	100%	10 Rabbits	Irritation observed	RIFM (1976a)
Pentyl salicylate	Pre-test for an OET (24-h primary irritation)	0.03–100% as a single application (vehicle not specified)	Himalayan white-spotted guinea pigs (6–8/sex/group)	0.03%, 0.1%, 1%, and 3%: no irritation 10%: considered as the minimal irritating concentration 30–100%: irritation observed	Klecak et al. (1977)
Pentyl salicylate	Induction phase of an OET	0.03–100% applied daily for 21 days (vehicle not specified)	Himalayan white-spotted guinea pigs (6–8/sex/group)	0.03%, 0.1%, 1%: no irritation 3%: considered as the minimal irritating concentration 10–100%: irritation observed	Klecak et al. (1977)
Pentyl salicylate	Pre-test for Draize assay (dermal application)	10% (vehicle not specified)	4 Hartley albino guinea pigs	No irritation	Sharp (1978)
Pentyl salicylate	Preliminary irritation evaluated prior to MAX study	10, 25 and 50%	4 Dunkin–Hartley guinea pigs	No irritation 10%: selected as challenge application 40%: selected as topical induction application	RIFM (1981f)
Pentyl salicylate	Irritation evaluated during an associated LD_{50} study	100%	10 Rabbits	Irritation observed	RIFM (1982b)
Phenethyl salicylate	Pre-test of an OET (24-h primary irritation)	0.03–100% as a single application (vehicle not specified)	Female Himalayan white-spotted guinea pigs (6–8/sex/group)	0.03%: no irritation 0.1%: considered as the minimal irritating concentration 0.3–100%: irritation observed	Klecak et al. (1977)
Phenethyl salicylate	Induction phase of an OET	0.03–100% applied daily for 21 days (vehicle not specified)	Himalayan white-spotted guinea pigs (6–8/sex/group)	0.03%: no irritation 0.1%: considered as the minimal irritating concentration 0.3–100%: irritation observed	Klecak et al. (1977)

Table 13 (*continued*)

Material	Method	Concentration	Species	Results	References
Phenethyl salicylate	Irritation evaluated prior to guinea pig MAX test	10, 25 and 50% in acetone	Albino Dunkin–Hartley guinea pigs	Irritation observed	RIFM (1981g)
Phenyl salicylate	Irritation evaluated during an associated LD_{50} study	100%	4 Rabbits	No irritation	RIFM (1975b)

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

Table 14
Mucous membrane (eye) irritation studies (in rabbits)

Material	Concentration(s)	Results	References
Benzyl salicylate	10% in SDA 39C alcohol	Irritation observed	RIFM (1975g)
1,3-Dimethyl-3-butenyl salicylate	100%	No irritation	RIFM (1981h)
Isoamyl salicylate	15% (vehicle not reported) and 100%	No irritation	RIFM (1970c)
3-Methyl-2-butenyl salicylate	5% in 75% ethanol	No irritation	RIFM (1970d)
Methyl salicylate	100%	Irritation observed	Carpenter and Smyth (1946)
Methyl salicylate	1.25% in SDA 39 C alcohol	Irritation observed	RIFM (1963)
Isobutyl salicylate	15% (vehicle not reported) and 100%	No irritation	RIFM (1970c)
Octyl salicylate[a]	100%	No irritation	RIFM (1978d)

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

part any minimal evidence of skin irritation was associated with concentrations ranging from 0.3% to 3.0%.

3.8. Mucous membrane (eye) irritation (Table 14)

In comparison to skin irritation, the potential for the salicylates to induce eye irritation has been studied only in a limited manner, and on fewer representatives of this class of compounds.

Strong irritation reactions with tissue necrosis and marked conjunctival irritations were observed with methyl salicylate at 100% and 1.25% (in alcohol SDA 39C), respectively. Irritation reactions were also observed with 10% benzyl salicylate in alcohol SDA 39C.

No irritation was observed when neat 1,3-dimethyl-3-butenyl salicylate was tested. Isobutyl salicylate, isoamyl salicylate, octyl salicylate and 3-methyl-2-butenyl salicylate have shown no evidence of eye irritation at concentrations of 2–5%.

Additional information about the eye irritation potential of the salicylates are provided in Table 14.

3.9. Skin sensitization

3.9.1. Human studies (Table 15)

All the salicylates under review, except for methyl 4-methylsalicylate, have been evaluated for the potential to induce sensitization in humans in either a maximization test or in a repeated insult patch test (HRIPT).

Sensitization reactions were observed in 2 maximization studies conducted with 20% benzyl salicylate in petrolatum. A number of other studies (both maximization and HRIPT) with benzyl salicylate have reported no such reactions, even at concentrations up to 30% in petrolatum. One non-specific reaction (clinically appeared to be due to irritation) was observed with 10% pentyl salicylate in petrolatum. Two other maximization studies showed no sensitization reactions at that concentration.

The rest of evaluated salicylates showed no sensitization potential when tested at concentrations of 1.25–100%.

3.9.2. Cross sensitization

Cross sensitization reactions in humans who were induced with 30% hexyl salicylate and challenged with 15% benzyl salicylate (both substances dissolved in 3:1 DEP:ethanol) have reportedly been observed.

Individual studies are summarized in Table 15.

3.9.3. Animal studies (Table 16)

Mixed results were obtained when salicylates were evaluated for skin sensitization. Twelve of the 17 salicylates assessed in this report have been subjected to testing, either in standard guinea pig models [open epicutaneous test (OET), Draize tests, closed epicutaneous tests (CET), optimization assays, cumulative contact enhancement tests (CCET), Freund's complete adjuvant tests (FCAT), *etc.*] or in the mouse local lymph node assay (LLNA).

The salicylates with aromatic side chains, including benzyl salicylate (most notably), phenyl salicylate and phenethyl salicylate have been reported in a number of studies to induce skin sensitization at concentrations as low as 0.1%. However, despite the number of studies that have reported these salicylates to be effective skin sensitizers, several others, including those using standard guinea pig models have failed to observe such sensitization potential with concentrations up to 25%.

With regard to the alkyl-side chain salicylates, sensitization reactions were observed with methyl salicylate

Table 15
Skin sensitization studies in humans

Material	Method	Concentration(s)	Subjects	Results	References
Benzyl salicylate	MAX	20% in petrolatum	25 volunteers	Sensitization observed in 2/25	RIFM (1980c)
Benzyl salicylate	MAX	20% in petrolatum	25 volunteers	Sensitization observed in 1/25	RIFM (1979)
Benzyl salicylate	MAX	30% in petrolatum	25 volunteers	No sensitization reactions	RIFM (1970e)
Benzyl salicylate	MAX	30% in petrolatum	25 volunteers	No sensitization reactions	RIFM (1975c)
Benzyl salicylate	MAX	30% in petrolatum	22 male volunteers	No sensitization reactions	RIFM (1975d)
Benzyl salicylate	HRIPT	15% in 3:1 DEP:ethanol	101 volunteers	No sensitization reactions	RIFM (2004c)
Benzyl salicylate	HRIPT	10% in alcohol SD 39	35 volunteers	No sensitization reactions	RIFM (1975h)
Benzyl salicylate	HRIPT	5% in dimethyl phthalate	52 volunteers	No sensitization reactions	RIFM (1968b)
Butyl salicylate	MAX	2% in petrolatum	25 male and female volunteers	No sensitization reactions	RIFM (1975c)
p-Cresyl salicylate	MAX	4% in petrolatum	25 volunteers	No sensitization reactions	RIFM (1980b)
1,3-Dimethyl-3-butenyl salicylate	HRIPT	10% in petrolatum	50 volunteers	No sensitization reactions	RIFM (1981c)
Ethyl hexyl salicylate	MAX	4% in petrolatum	23 male volunteers	No sensitization reactions	RIFM (1974b)
Ethyl salicylate	MAX	12% in petrolatum	25 male and female volunteers	No sensitization reactions	RIFM (1976c)
cis-3-Hexenyl salicylate	MAX	3% in petrolatum	25 volunteers	No sensitization reactions	RIFM (1975c)
trans-2-Hexenyl salicylate	MAX	20% in petrolatum	33 male volunteers	No sensitization reactions	RIFM (1978b)
Hexyl salicylate	MAX	3% in petrolatum	22 volunteers	No sensitization reactions	RIFM (1975d)
Hexyl salicylate	HRIPT	30% in 3:1 DEP:ethanol	103 volunteers	No sensitization reactions	RIFM (2004a)
Homomenthyl salicylate[a]	MAX	8% in petrolatum	25 volunteers	No sensitization reactions	RIFM (1977b)
Isobutyl salicylate	MAX	10% in petrolatum	25 volunteers	No sensitization reactions	RIFM (1973c)
3-Methyl-2-butenyl salicylate	MAX	20% in petrolatum	25 volunteers	No sensitization reactions	RIFM (1978c)
Methyl salicylate (wintergreen oil ; 80–99% methyl salicylate)	MAX	12% in petrolatum	25 volunteers	No sensitization reactions	RIFM (1976b)
Methyl salicylate	MAX	8% in petrolatum	27 volunteers	No sensitization reactions	RIFM (1973b)
Methyl salicylate	HRIPT	1.25% (vehicle not specified)	39 volunteers	No sensitization reactions	RIFM (1964)
Octyl salicylate[a]	MAX	5% in petrolatum	25 volunteers	No sensitization reactions	RIFM (1976c)
Octyl salicylate[a]	HRIPT	100%	25 volunteers	No sensitization reactions	RIFM (1976d)
Pentyl salicylate	MAX	10% in petrolatum	27 volunteers	Non specific reaction observed in 1/27	RIFM (1982c)
Pentyl salicylate	MAX	10% in petrolatum	20 volunteers	No sensitization reactions	RIFM (1970e)
Pentyl salicylate	MAX	10% (vehicle not specified)	26 volunteers	No sensitization reactions	RIFM (1979)
Phenethyl salicylate	MAX	8% in petrolatum	25 volunteers	No sensitization reactions	RIFM (1973c)
Phenyl salicylate	MAX	6% in petrolatum	25 volunteers	No sensitization reactions	RIFM (1975c)

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

and pentyl salicylate at concentrations higher or equal to 30%. Hexyl salicylate was sensitizing in modified Draize test at 5%. Ethyl salicylate and isobutyl salicylate were not sensitizers at concentrations up to 10%, and *cis*-3-hexenyl salicylate was not a sensitizer at concentrations up to 20%.

Sensitization of benzyl salicylate, methyl salicylate and phenethyl salicylate was also evaluated in mice using the LLNA. Methyl salicylate was considered not a sensitizer at concentrations up to 30% (EC3 value not calculable). Benzyl salicylate and phenethyl saliyclate were considered sensitizers when tested at concentrations 2.5, 5.0, 10, 25 or 50% with EC3 values of 1.5–2.9% and at 1.0, 2.5, 5.0, 10 and 25% with EC3 values of 2.1%, respectively.

Additional information on the individual studies is provided in Table 16.

3.9.4. Summary of the skin sensitization data

The potential for most of those salicylates in this report to cause skin sensitization has been well characterized in both humans and in experimental animals.

In humans, only benzyl salicylate produced sensitization in 2/25 volunteers in one study and in 1/25 in another. All of the other maximization studies in humans failed to show any evidence of skin sensitization reactions. The HRIPTs all reported no evidence of skin sensitization potential.

Overall, the animal data indicate that salicylates bearing aromatic side chains have some potential for skin sensitization. Most of the studies showing positive results are those considered the most sensitive assays, for example, the guinea pig maximization test, FCAT, and the LLNA. In particular, sensitization was often noted for the salicylates bearing aromatic side chains in studies involving intradermal injection at either the induction and/or the challenge

Table 16
Skin sensitization studies in animals

Material	Method	Concentration(s)	Species	Results	References
Benzyl salicylate	OET	Induction and challenge: 30% (vehicle not specified)	Guinea pigs (minimum six animals)	No reactions	Klecak (1985)
Benzyl salicylate	OET	Induction and challenge: 10% (vehicle not specified)	Guinea pigs (6–8 males and females)	No reactions	Klecak (1979)
Benzyl salicylate	OET	Induction and challenge: 0.03–100% (vehicle not specified)	Himalayan white-spotted guinea pigs (6–8 males and females)	0.03%: minimum eliciting concentration 30%: minimum sensitizing concentration	Klecak et al. (1977)
Benzyl salicylate	Cumulative contact enhancement test (CCET)	Induction: 30% in ethanol topically Challenge: 1%, 3%, or 10% topically	Hartley albino guinea pigs (10 females/group)	Sensitization observed	Kashima et al. (1993)
Benzyl salicylate	CCET	Induction: 3%, 10%, 30% and 100% topically Challenge: concentration not specified topically under occlusive patch under occlusive patch; also intradermal injection with FCA	Pirbright and Hartley guinea pigs (6–10 of each strain/group)	10%: no reactions 30%: sensitization in 3/6 Pirbright guinea pigs 100%: sensitization in 1/10 Hartley guinea pigs	Tsuchiya et al. (1982)
Benzyl salicylate	CCET	Induction: 100% topically under occlusive patch; also intradermal injection with FCA Challenge: 50% topically under occlusive patch	Tortoise shell guinea pigs (10, sex not specified)	Sensitization observed	Imokawa and Kawai (1987)
Benzyl salicylate	CET	Induction: 30% (vehicle not specified) Challenge: 1% (vehicle not specified)	Guinea pigs (20, sex not specified)	Sensitization observed in 3/20	Ishihara et al. (1986)
Benzyl salicylate	Modified Draize test	Induction and challenge: 0.1% by intradermal injection in isotonic saline	Himalayan white-spotted guinea pigs (6–8 males and females)	No reactions	Klecak et al. (1977)
Benzyl salicylate	Modified Draize test	Intradermal induction: 1.25% (vehicle not specified) Intradermal challenge: 0.5% Topical Challenge: 2% (vehicle not specified)	Hartley albino guinea pigs (4 or 6 of each sex, 10 total)	No reactions	Sharp (1978)
Benzyl salicylate	Guinea pig maximization test	Intradermal induction: 10% in FCA Topical induction: 10% in acetone Topical Challenge: 5%, 10%, or 20% in acetone	Albino Dunkin–Hartley guinea pigs (8 females)	Sensitization observed	RIFM (1997c)
Benzyl salicylate	Guinea pig maximization test	Intradermal induction: 10% in FCA Topical induction: 50% (vehicle not reported) Topical Challenge: 5%, 10%, or 20% (vehicle not reported)	Hartley guinea pigs (20 females/group)	Sensitization observed in 2/20 at 20% Questionable reactions observed in 3/20 at 5%, 5/20 at 10%, and 4/20 at 20%	Kozuka et al. (1996)
Benzyl salicylate	Guinea pig maximization test	Intradermal induction: 10% in liquid paraffin Topical induction: 30% in ethanol Topical Challenge: 0.003%, 0.01%, or 0.03% in ethanol	Hartley guinea pigs (10 females/group)	Sensitization observed	Kashima et al. (1993)
Benzyl salicylate	Guinea pig maximization test	Intradermal induction: 5% in FCA Topical induction: 25% in petrolatum Topical Challenge: sub-irritant concentration (<0.1%) in petrolatum	Male and female Himalayan guinea pigs (numbers not specified)	No reactions	Klecak et al. (1977)

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Table 16 (*continued*)

Material	Method	Concentration(s)	Species	Results	References
Benzyl salicylate	Guinea pig maximization test	Intradermal induction: 1% (vehicle not specified) Topical induction: 100% Topical Challenge: 100%	Hartley guinea pigs (10/group)	No reactions	Tsuchiya et al. (1982)
Benzyl salicylate	Guinea pig maximization test	Induction and challenge: 10% (no further details provided)	Guinea pigs (sex and number not specified)	Sensitization observed	Ishihara et al. (1986)
Benzyl salicylate	Sensitization evaluated as part of a photoallergy study	Induction: 10% in ethanol Challenge: 10% in ethanol	Dunkin–Hartley guinea pigs (25/group)	No reactions	RIFM (1983b)
Benzyl salicylate	FCAT	Induction: 50% in FCA by intradermal injection Topical challenge: <0.1% (vehicle not specified)	Himalayan white-spotted guinea pigs (6–8 males and females)	No reactions	Klecak et al. (1977)
Benzyl salicylate	Modified FCAT	Induction: 10% in FCA by intradermal injection Challenge: 10% in acetone	Pirbright guinea pigs (10)	Sensitization observed	Hausen and Wollenweber (1988)
Benzyl salicylate	Optimization test	Intradermal induction: 1% in saline Intradermal challenge: 0.1% in saline Topical challenge: 10% in petrolatum	Pirbright guinea pigs (10/sex)	Sensitization observed in 1/20 after intradermal challenge and in 7/20 after topical challenge	Maurer et al. (1980)
Benzyl salicylate	Delayed contact hypersensitivity assay using the AP2 test method	Induction: 30% in ethanol Challenge: 1%, 3%, or 10% in ethanol	10 Female Hartley guinea pigs	Sensitization observed at all dose levels	Kashima et al. (1993)
Benzyl salicylate	LLNA	10% in 4:1 acetone:olive oil	4 Female CBA/JN mice/group	EC3%: 1.5	Yoshida et al. (2000)
Benzyl salicylate	LLNA	2.5%, 5.0%, 10%, 25%, and 50% in 3:1 DEP:ethanol	4 Female CBA/Ca mice/group	EC3%: 2.9	RIFM (2005)
Ethyl salicylate	CET	Induction: 30% (vehicle not specified) Challenge: 1% (vehicle not specified)	10 Guinea pigs (sex not specified)	No reactions	Ishihara et al. (1986)
cis-3-Hexenyl salicylate	OET	Induction and challenge: 3% (vehicle not specified)	6–8 Guinea pigs	No reactions	Klecak (1985)
cis-3-Hexenyl salicylate	Guinea pig maximization tested	Intradermal induction: 10% in FCA Topical induction: 10% in FCA Topical challenge: 5%, 10%, 20%, and 40% in 50:50 PEG:acetone	5 Female Hartley guinea pigs	5%, 10%, 20%: no reactions 40%: sensitization observed in 2/5	RIFM (1999)
Hexyl salicylate	Modified Draize test	Intradermal induction: 0.1% (vehicle not specified) Intradermal challenge: 0.1% (vehicle not specified) Topical challenge: 5% (vehicle not specified)	Hartley albino guinea pigs (4 or 6 of each sex, 10 total)	Sensitization observed	Sharp (1978)
Hexyl salicylate	Sensitization evaluated as part of a photoallergy study	Induction: 100% in 3:1 DEP:ethanol Challenge: 100% or 50% in 3:1 DEP:ethanol	Male albino hairless guinea pigs (5/group)	No reactions	RIFM (2003)
Hexyl salicylate	Guinea pig maximization test	Intradermal induction: 1% in 0.01% Dobs/saline Topical induction: 40% in acetone Topical Challenge: 10% in acetone	Dunkin–Hartley guinea pigs	No reactions	RIFM (1981e)

Table 16 (*continued*)

Material	Method	Concentration(s)	Species	Results	References
Isoamyl salicylate	CET	Induction: 30% (vehicle not specified) Challenge: 1% (vehicle not specified)	Guinea pigs (5, sex not specified)	No reactions	Ishihara et al. (1986)
Isoamyl salicylate	Modified Draize test	Induction and challenge: intradermal injection of 0.1% in 5% ethanol and water	Male white guinea pigs	No reactions	RIFM (1970c)
Isobutyl salicylate	OET	Induction and challenge: 10% (vehicle not specified)	Guinea pigs (minimum of six animals)	No reactions	Klecak (1979, 1985)
Isobutyl salicylate	Modified Draize test	Induction and challenge: 0.1% by intradermal injection in 5% ethanol and water	Male white guinea pigs	No reactions	RIFM (1970c)
Methyl salicylate	Open epicutaneous test (OET)	Induction and challenge: 0.03–100% (vehicle not specified)	Himalayan white-spotted guinea pigs (6–8 males and females)	1%: minimum eliciting concentration 30%: minimum sensitizing concentration	Klecak et al. (1977)
Methyl salicylate	OET	Induction and challenge: 8% (vehicle not specified)	Guinea pigs (minimum of six animals)	No reactions	Klecak (1979, 1985)
Methyl salicylate	Closed epicutaneous test (CET)	Induction: 30% (vehicle not specified) Challenge: 1% (vehicle not specified)	Guinea pigs (5, sex not specified)	No reactions	Ishihara et al. (1986)
Methyl salicylate	Modified Draize test	Induction and challenge: 0.1% in isotonic saline *via* intradermal injection	Himalayan white-spotted guinea pigs (6–8 males and females)	No reactions	Klecak et al. (1977)
Methyl salicylate	Guinea pig maximization test	Intradermal induction: 2.5% in Dobs Topical induction: 100% Topical Challenge: 10% in acetone/PEG 400	Dunkin–Hartley albino guinea pigs (9–10, sex not specified)	No reactions	Kimber et al. (1991) and Basketter and Scholes (1992)
Methyl salicylate	Guinea pig maximization test	Intradermal induction: 5% in FCA Topical induction: 25% in petrolatum Topical challenge: sub-irritant concentration (<3%) in petrolatum	Male and female Himalayan guinea pigs (numbers not specified)	No reactions	Klecak et al. (1977)
Methyl salicylate	Freund's complete adjuvant test (FCAT)	Induction: 50% in FCA by intradermal injection Challenge: <3% (vehicle not specified) topically under occlusive patch	Himalayan white-spotted guinea pigs (6–8 males and females)	No reactions	Klecak et al. (1977)
Methyl salicylate	Optimization test	Intradermal induction: 0.1% in saline Intradermal challenge: 0.1% in saline Topical challenge: 10% in petrolatum	Pirbright guinea pigs (10/sex)	Sensitization observed in 2/20 after intradermal challenge and in 0/20 after topical challenge	Maurer et al. (1980)
Methyl salicylate	LLNA	1.0%, in 4:1 acetone/olive oil 2.5%, in 4:1 acetone/olive oil 5.0% in 4:1 acetone/olive oil 10.0% in 4:1 acetone/olive oil 20.0% in 4:1 acetone/olive oil	5 CBA/Ca female mice per group	EC3: >20%	Kimber et al. (1991, 1995, 1998)
Methyl salicylate	LLNA	5.0% in 4:1 acetone/olive oil	4 CBA/JN female mice per group	5.0%: SI = 0.7	Yoshida et al. (2000)
Methyl salicylate	LLNA	1.0%, in dimethylformamide 5.0% in dimethylformamide 25.0% in dimethylformamide	4 CBA/Ca female mice per group	1.0%: SI = 1.0 5.0%: SI = 1.2 25.0%: SI = 3.0	Montelius et al. (1994)
Methyl salicylate	LLNA	5.0% in 4:1 acetone/olive oil 10.0% in 4:1 acetone/olive oil 25.0% in 4:1 acetone/olive oil	4 CBA/Ca female mice per group	5.0%: SI = 1.3 10.0%: SI = 1.0 25.0%: SI = 0.8	Basketter and Scholes (1992)

(*continued on next page*)

Table 16 (*continued*)

Material	Method	Concentration(s)	Species	Results	References
Methyl salicylate	LLNA	1.0%, in 4:1 acetone/olive oil 20.0% in 4:1 acetone/olive oil	5 CBA/JHsd female mice per group	1.0%: SI = <3.0 20.0%: SI = <3.0	Ladics et al. (1995)
Methyl salicylate	Guinea pig lymph node cell proliferation assay (GPLNA)	10% in DMSO	Female Hartley albino guinea pigs (numbers not specified)	SI = 0.78	Yoshida et al. (2000)
Pentyl salicylate	OET	Induction and challenge: 0.03–100% (vehicle not specified)	Himalayan white-spotted guinea pigs (6–8 males and females)	3%: minimum eliciting concentration 30%: minimum sensitizing concentration	Klecak et al. (1977)
Pentyl salicylate	OET	Induction and challenge: 10% (vehicle not specified)	Guinea pigs (minimum of six animals)	No reactions	Klecak (1979)
Pentyl salicylate	Modified Draize test	Intradermal induction and challenge: 0.1% in isotonic saline	Himalayan white-spotted guinea pigs (6–8 males and females)	No reactions	Klecak et al. (1977)
Pentyl salicylate	Modified Draize test	Intradermal induction: 0.05% (vehicle not specified) Intradermal challenge: 0.05% (vehicle not specified) Topical challenge: 10% (vehicle not specified)	Hartley albino guinea pigs (4 or 6 of each sex, 10 total)	No reactions	Sharp (1978)
Pentyl salicylate	Guinea pig maximization test	Intradermal induction: 5% in FCA Topical induction: 25% in petrolatum Topical challenge: sub-irritant concentration (<10%) in petrolatum	Male and female Himalayan guinea pigs (numbers not specified)	No reactions	Klecak et al. (1977)
Pentyl salicylate	FCAT	Intradermal induction: 50% in FCA Topical challenge: <10% (vehicle not specified)	Himalayan white-spotted guinea pigs (6–8 males and females)	No reactions	Klecak et al. (1977)
Pentyl salicylate	Optimization test	Intradermal induction: 0.1% in saline Intradermal challenge: 0.1% in saline Topical challenge: 10% in petrolatum	Pirbright guinea pigs (10/sex)	No reactions	Maurer et al. (1980)
Pentyl salicylate	Guinea pig Maximization test	Intradermal induction: 1% in 0.01% Dobs/saline Topical induction: 40% in acetone Topical Challenge: 10% in acetone	Albino Dunkin–Hartley guinea pig	No reactions	RIFM (1981f)
Phenethyl salicylate	OET	Induction and challenge: 8% (vehicle not specified)	Guinea pigs (minimum of six animals)	Sensitization observed	Klecak (1985)
Phenethyl salicylate	OET	Induction and challenge: 8% (vehicle not specified)	Guinea pigs (6–8, sex not specified)	No reactions	Klecak (1979)
Phenethyl salicylate	OET	Induction and challenge: 0.03–100% (vehicle not specified)	Himalayan white-spotted guinea pigs (6–8 males and females)	0.03%: minimum eliciting concentration 30%: minimum sensitizing concentration	Klecak et al. (1977)
Phenethyl salicylate	CET	Induction: 30% (vehicle not specified) Challenge: 1% (vehicle not specified)	Guinea pigs (8, sex not specified)	No reactions	Ishihara et al. (1986)
Phenethyl salicylate	Guinea pig maximization test	Intradermal induction: 5% in FCA Topical induction: 25% in petrolatum Topical challenge: sub-irritant concentration (<0.1%) in petrolatum	Male and female Himalayan guinea pigs (numbers not specified)	Sensitization observed	Klecak et al. (1977)

Table 16 (*continued*)

Material	Method	Concentration(s)	Species	Results	References
Phenethyl salicylate	Guinea pig maximization test	Intradermal induction: 0.5% in acetone/PEG400/Tween80/saline Topical induction: 50% in acetone Topical challenge: 10% in acetone	Albino/Dunkin–Hartley guinea pigs	Sensitization observed	RIFM (1981g)
Phenethyl salicylate	FCAT	Intradermal induction: 50% in FCA Topical challenge: <0.1% (vehicle not specified)	Himalayan white-spotted guinea pigs (6–8 males and females)	Sensitization observed	Klecak et al. (1977)
Phenethyl salicylate	LLNA	1.0% in 1:3 ethanol/DEP 2.5% in 1:3 ethanol/DEP 5.0% in 1:3 ethanol/DEP 10% in 1:3 ethanol/DEP 25% in 1:3 ethanol/DEP	4 CBA/Ca female mice per group	EC3 – 2.1%	RIFM (2006)
Phenethyl salicylate	Draize test	Induction and challenge: 0.1% in saline	Male and female Himalayan guinea pigs	No reactions	Klecak et al. (1977)
Phenyl salicylate	Buehler sensitization assay	Induction: 25% (vehicle not specified) Challenge: 25% (vehicle not specified)	Guinea pigs (number not stated, but either 10 or 20)	No reactions	Basketter and Gerberick (1996)
Phenyl salicylate	CET	Induction: 30% (vehicle not specified) Challenge: 1% (vehicle not specified)	Guinea pigs (5, sex not specified)	No reactions	Ishihara et al. (1986)
Phenyl salicylate	FCAT	Intradermal induction: 50% in FCA Topical challenge: 0.3% and 1.0% in 9:1 ethanol:olive oil	Hartley guinea pigs (9 females/group)	0.3%: sensitization observed in 8/9 1.0%: sensitization observed in 9/9	Marchand et al. (1982)

dose. Sensitization by topical applications generally required concentrations of 1–30% for the induction or challenge dose, or both.

3.10. Phototoxicity and photoallergenicity (Tables 17–19)

3.10.1. Human studies

Three salicylates, hexyl salicylate, 1,3-dimethyl-3-butenyl salicylate, and benzyl salicylate have been assessed for phototoxicity/photoallergenicity potential in humans (see Tables 17a, 18a). No phototoxic reactions were observed with hexyl salicylate, 1,3-dimethyl-3-butenyl salicylate or benzyl salicylate at concentrations ranging from 0.3% to 30%. Benzyl salicylate and 1,3-dimethyl-3-butenyl salicylate were also evaluated for photoallergy using the photopatch technique. 1,3-Dimethyl-3-butenyl salicylate was tested at 10% and did not produce any photoallergic reactions. Benzyl salicylate produced reactions at concentrations of 2% and higher.

3.10.2. Animal studies

Four of the 17 salicylates have been studied for phototoxic and/or photoallergenic potential in animals. These include methyl salicylate, hexyl salicylate, *cis*-3-hexenyl salicylate, and benzyl salicylate (see Tables 17b, 18b).

Hexyl salicylate, methyl salicylate and *cis*-3-hexenyl salicylate did not produce any phototoxic reactions at concentrations of 50% and 100% in both guinea pigs and mice. With neat benzyl salicylate no reactions were observed in hairless mice, but application of 3% in acetone produced phototoxic reactions in guinea pigs. However, these reactions were seen only at the 24-h reading. By 72 h the skin sites had returned to normal. Two additional studies on benzyl salicylate showed no reactions in guinea pigs at 10% or 30%.

Photoallergy was evaluated with 10% benzyl salicylate and neat hexyl salicylate. No reactions were observed.

UV spectra have been obtained for 10 salicylates (benzyl salicylate, butyl salicylate, *p*-cresyl salicylate, ethyl hexyl salicylate, *cis*-3-hexenyl salicylate, hexyl salicylate, isoamyl

Table 17a
Phototoxicity studies in humans

Material	Concentration	Subjects	Results	References
1,3-Dimethyl-3-butenyl salicylate	10% in petrolatum	20 volunteers	No reactions	RIFM (1981c)
Hexyl salicylate	0.3–30% in 3:1 DEP:EtOH	56 volunteers	No reactions	RIFM (2004b)
Benzyl salicylate	3% and 10% in 1:1 EtOH:acetone	6 volunteers	No reactions	RIFM (1983c)
Octyl salicylate[a]	5% in ethanol	10 volunteers	No reactions	RIFM (1975i)

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

Table 17b
Phototoxicity studies in animals

Material	Concentration	Species	Results	References
Hexyl salicylate	100%	Miniature swine	No reactions	Forbes et al. (1977)
Hexyl salicylate	100%	Miniature swine	No reactions	RIFM (1975f)
Hexyl salicylate	50% in 3:1 DEP:EtOH or 100%	Guinea pigs	No reactions	RIFM (2003)
Hexyl salicylate	100%	Mice	No reactions	Forbes (1977)
Hexyl salicylate	100%	Mice	No reactions	RIFM (1975f)
Benzyl salicylate	1% or 3% in acetone	Guinea pigs	1% no reactions 3% positive reactions	RIFM (1982d)
Benzyl salicylate	25% or 100% in methanol	Mice	No reactions	RIFM (1983d)
Benzyl salicylate	5–30% in acetone	Guinea pigs	No reactions	RIFM (1997b)
Benzyl salicylate	10% in EtOH	Guinea pigs	No reactions	RIFM (1983b)
cis-3-Hexenyl salicylate	5–50% (vehicle not specified)	Guinea pigs	No reactions	RIFM (1999)

Table 18a
Photoallergy studies in humans

Material	Concentration	Subjects	Results	Reference
1,3-Dimethyl-3-butenyl salicylate	10% in petrolatum	20 volunteers	No reactions	1981c

Table 18b
Photoallergy studies in animals

Material	Concentration	Species	Results	References
Hexyl salicylate	50% in 3:1 DEP:EtOH or 100%	Guinea pigs	No reactions	RIFM (2003)
Benzyl salicylate	10% in EtOH	Guinea pigs	No reactions	RIFM (1983b)

salicylate, isobutyl salicylate, phenethyl salicylate and phenyl salicylate). They all absorbed UVB light peaking around 200–340 nm and returning to baseline at 330–340 nm (see Table 19). Based on the UV spectra and review of phototoxic/photoallergy data, salicylates would not be expected to elicit phototoxicity or photoallergy under the current conditions of use as a fragrance ingredient.

3.11. Environmental toxicity

In addition to a human health assessment, environmental assessment of fragrance materials is performed according to a standard framework (Salvito et al., 2002). This screens chemicals in the RIFM/FEMA Database for their potential to present a hazard to the aquatic environment by considering their removal in wastewater treatment, minimal dilution in the mixing zone, and the application of a large uncertainty factors to ecotoxicological endpoints determined using quantitative structure–activity relationships. This screening, based on conservative assumptions, identifies priority materials that may require further study to quantitatively assess potential environmental risks. None of the materials in the salicylate group were identified as a priority material for risk assessment refinement.

Table 19
Summary of UV spectra data

Material	UV spectra range of absorption (nm)
Benzyl salicylate	200–340
Butyl salicylate	220–340
p-Cresyl salicylate	200–340
Ethyl hexyl salicylate	220–340
cis-3-Hexenyl salicylate	220–340
Hexyl salicylate	200–330
Homomenthyl salicylate[a]	220–340
Isoamyl salicylate	200–340
Isobutyl salicylate	200–340
Phenyl salicylate	200–340
Phenethyl salicylate	200–340

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

However, there are environmental data in the RIFM/FEMA Database for materials within the salicylate group. These include biodegradation, acute *Daphnia* and fish studies, and algal population growth inhibition data. Data are available for eight materials. Overall, these materials appear to be readily biodegradable; the acute toxicities range from 0.7 to >10 mg/L.

In addition, three papers describe the fate of some of the salicylate compounds in the environment. In a study by DiFrancesco et al. (2004), hexyl salicylate was spiked into wastewater treatment plant sludge amended to soil in a series of experiments to determine its dissipation in the soil compartment and potential to leach from the upper 10 cm of soil. Hexyl salicylate was undetected after 3 months in the soil compartment and not detected in the leachate.

As several of these materials have both biogenic and other commercial sources, their identification in the environment is not necessarily indicative of sources from fragrance

compounds. For example, methyl salicylate has been reported in the environment (Kolpin et al., 2004 and Alvarez et al., 2005). The use of methyl salicylate, for example, as a flavor was noted in Salvito et al. (2002) as a possible explanation for the higher than expected influent concentration measured in some wastewater treatment plants when compared to its predicted influent concentration based on its volume of use as a fragrance ingredient. Their infrequent identification and relatively low concentrations in the environment is not indicative of their use in fragrance compounds. Furthermore, Simonich et al. (2000) reported that removal of benzyl salicylate, hexyl salicylate and methyl salicylate in a variety of wastewater treatment plants in Europe and the United States exceeded 90% in secondary treatment plants. This was confirmed in work reported in Simonich et al., 2002).

The salicylates, as used in fragrance compounds, present a negligible environmental risk and would not be considered persistent, bioaccumulative or toxic chemicals as indicated by applying the RIFM framework (Salvito et al., 2002) and reviewing the available environmental data.

4. Summary

The salicylates are dermally absorbed to varying extents and, a significant amount can be retained briefly within the epidermis, dermis, and subcutaneous tissue. The human data, derived primarily from experiments conducted with methyl salicylate, support dermal bioavailability in the range of 12–30.7%. Limited data on other salicylates indicates that the longer chain alkyl derivatives are absorbed to a lesser extent.

Few data were available from which to characterize the oral bioavailability of the salicylates assessed in this report. Oral absorption studies conducted on closely related hydroxyl- and alkoxy-substituted benzyl derivatives indicate rapid and nearly complete absorption following ingestion. In addition, it has been well documented that salicylic acid, the chief hydrolysis product of the alkyl, alkenyl, and benzyl-/phenyl-substituted salicylates, is rapidly and extensively absorbed from the gastrointestinal tract of both humans and laboratory animals. For the assessment of potential oral exposures to the salicylates assessed here, bioavailability is assumed to be 100%.

The salicylates reviewed are expected to undergo extensive hydrolysis, primarily in the liver, to salicylic acid or, in the case of methyl 4-methylsalicylate, 4-methylsalicylic acid. Substitution of the benzene ring would not materially affect the metabolism of 4-methylsalicylic acid in comparison to salicylic acid. In the liver, salicylic acid is conjugated with either glycine or glucuronide and excreted in the urine as salicyluric acid and acyl and phenolic glucuronides. The hydrolyzed side chains are metabolized by common and well-characterized metabolic pathways leading to the formation of innocuous end products: primary alcohols metabolized to corresponding aldehydes and acids, and ultimately to CO_2, and secondary alcohols conjugated with glucuronide and excreted. Unsaturated alcohols may undergo further oxidation at the point of unsaturation while the aromatic side chains (benzyl, phenyl, and phenethyl) are either directly conjugated (phenol) or oxidized to the corresponding acid prior to conjugation and excretion in the urine. The expected metabolism of the salicylates does not present any toxicological concerns.

The acute dermal toxicity of the salicylates is very low, with LD_{50} values in rabbits reported to be greater than 5000 mg/kg body weight. The acute oral toxicity of the salicylates is moderate, with toxicity generally decreasing with increasing size of the ester R-group (LD_{50} range). The aromatic salicylates are of low to moderate acute oral toxicity (1300 to >5000 mg/kg body weight). Differences in acute oral toxicity are likely related to the relative proportion of the molecular weight released as salicylic acid following hydrolysis.

Dermal subchronic toxicity studies have been conducted on methyl salicylate in rabbits and dogs and indicated that extreme doses (*i.e.*, ~5 g/kg body weight/day) may be associated with nephrotoxicity. The lowest "no effect" dose reported was 1180 mg/kg body weight/day in rabbits. The most appropriate oral toxicity studies are on methyl salicylate and isoamyl salicylate in rats. The NOAEL for each compound was approximately 50 mg/kg body weight/day. This NOAEL value could be used for quantitative human health risk assessment of the use of the salicylates as fragrance compounds.

There appear to be no major differences in the toxicity of the individual salicylates, given the data on methyl-, isoamyl-, and phenyl salicylate. Additional subchronic toxicity data on the other salicylates would establish these observations more definitively.

The chronic toxicity data, (2 years exposure) for methyl salicylate are consistent with the oral subchronic toxicity data in that the lowest NOAEL value identified was 50 mg/kg body weight/day in both rats and dogs. In rats, growth retardation occurred at doses in excess of 50 mg methyl salicylate/kg body weight/day, and increased bone density at doses in excess of 300–450 mg/kg body weight/day. In dogs, growth retardation and non-specific signs of hepatotoxicity were reported to occur at doses of 150 and 350 mg/kg body weight/day. These are observations to be investigated if such high exposures are ever considered.

Methyl salicylate has been extensively tested in genotoxicity studies, and there are relevant data on a few other salicylates. Ames and other bacterial mutation data demonstrated that those salicylates that have been tested are without mutagenic activity. Given that structurally related alkyl- and alkoxy-benzyl derivatives are generally without genotoxic effects (Adams et al., 2005) and noting that metabolites of the salicylates are simple alcohols and acids, the salicylates as a group are considered to be non-genotoxic.

The 2-year studies of oral methyl salicylate in rats showed no evidence of carcinogenicity. Similarly, no evidence of carcinogenicity was reported following i.p. injection of methyl salicylate in mice. Given these results, the genetic toxicity

data, and the metabolism of the salicylates, it appears that the salicylates are unlikely to be carcinogenic.

Reproductive and developmental toxicity data on methyl salicylate in rats demonstrate that, under conditions of high maternotoxic exposure, there is a pattern of embryotoxicity and teratogenesis similar to that characterized for salicylic acid. It shows neural tube defects and malformations of the skeleton and visceral organs. The no-effect-levels for reproductive toxicity (*e.g.*, fertility, neonatal growth and survival, *etc.*) are 75–100 mg/kg lower than levels reported to cause teratogenic effects and are consistent with the NOAEL determined from subchronic and chronic toxicity studies. The Cosmetic Ingredient Review Board Expert Panel (CIR, 2003) has concluded that the total use of salicylates and salicylic acid in cosmetic products would not pose a risk for reproductive or developmental effects in humans since potential serum levels of salicylic acid would not approach those associated with adverse effects. Moreover, as documented in a developmental toxicity study in hamsters (Overman and White, 1979; Overman and White, 1983), dermal exposure to methyl salicylate results in much lower serum salicylate concentrations compared to oral or parenteral exposure.

At concentrations likely to be encountered by humans through the use of the salicylates as fragrance ingredients, these chemicals are considered non-irritating to human skin. Application of neat material or injection of salicylates into the skin may be associated with mild to moderate skin irritation.

Methyl salicylate is a strong eye irritant at concentrations in excess of 1.0%. The other salicylates appear to have much weaker potential for eye irritation, with concentrations in the range of 10–100% producing at most mild conjunctival irritation. Given these data, and the maximum use concentrations, it is concluded that under the conditions of use (*i.e.*, presence as fragrance ingredients at low concentrations in cosmetic products), the salicylates assessed in this report, perhaps with the exception of methyl salicylate, would be expected to be non-irritating to mucous membranes (eyes).

Except for the aromatic side-chain-bearing salicylates, this group of chemicals is considered to have at most limited skin sensitization potential. However, benzyl salicylate has been reported to cause skin sensitization in several human studies and in a number of animal studies. Other salicylates with aromatic side chains have also shown sensitization in standard guinea pig tests. The International Fragrance Association (IFRA) has established a Standard (2007) on the use of benzyl salicylate as a fragrance ingredient (please see the individual Fragrance Material Review on benzyl salicylate for more information on this IFRA Standard). Alkyl- and aliphatic ring side-chain salicylates appear to have no sensitization potential.

Based on the available data, it can be concluded that the salicylates included in this summary are not phototoxic or photoallergenic.

5. Conclusion

The Panel has noted that:

- The salicylates are well absorbed by the oral route, and oral bioavailability is assumed to be 100%. Absorption by the dermal route in humans is more limited with bioavailability in the range of 11.8–30.7%.
- The salicylates are expected to undergo extensive hydrolysis, primarily in the liver, to salicylic acid. In the liver, salicylic acid is conjugated with either glycine or glucuronide and is excreted in the urine as salicyluric acid and acyl and phenolic glucuronides. The hydrolyzed side chains are metabolized by common and well-characterized metabolic pathways leading to the formation of innocuous end products. The expected metabolism of the salicylates does not present toxicological concerns.
- The acute dermal toxicity of the salicylates is very low, with LD_{50} values in rabbits reported to be greater than 5000 mg/kg body weight. The acute oral toxicity of the salicylates is moderate, with toxicity generally decreasing with increasing size of the ester R-group and with LD_{50}'s between 1000 and >5000 g/kg.
- In dermal subchronic toxicity studies, extreme doses of methyl salicylate (~5 g/kg body weight/day) possibly were nephrotoxic but the data were minimal. The subchronic oral NOAEL is concluded to be 50 mg/kg body weight/day. At higher doses, in excess of 300–450 mg/kg body weight/day, methyl salicylate is associated with increased density of the metaphyses of the long bones in rats. The oral NOAEL of 50 mg/kg body weight/day can be used in the risk assessment of the use of the salicylates as fragrance ingredients.
- Oral chronic toxicity data for methyl salicylate are consistent with the oral subchronic toxicity data in that the lowest NOAEL value identified was 50 mg/kg body weight/day in both rats and dogs.
- Genetic toxicity data, for methyl salicylate, a few other salicylates and for structurally related alkyl- and alkoxy-benzyl derivatives are negative for genotoxicity. Since the metabolites of the salicylates are simple alcohols and acids, the salicylates as a group are considered to be non-genotoxic.
- Limited long-term oral studies in rats and an i.p. injection study in mice using methyl salicylate provided no evidence of carcinogenicity. Given the metabolism of salicylate and the evidence that they are non-genotoxic, it can be concluded that the salicylates are without carcinogenic potential.
- The reproductive and developmental toxicity data on methyl salicylate demonstrate that high, maternally toxic doses result in a pattern of embryotoxicity and teratogenesis similar to that characterized for salicylic acid. The no-adverse-effect levels for reproductive toxicity (*e.g.*, fertility, neonatal growth and survival, *etc.*) are lower than doses reported to be teratogenic and are consistent with the NOAELs available from subchronic and

chronic toxicity studies. The Cosmetic Ingredient Review Board has concluded that use of salicylates and salicylic acid in cosmetic products would not pose a risk for reproductive or developmental effects in humans.

- At concentrations likely to be encountered by humans through the use of the salicylates as fragrance ingredients, these chemicals are considered to be non-irritating to the skin.
- The salicylates in general have no or very limited skin sensitization potential. However, benzyl salicylate has been reported to cause skin sensitization in several human studies and in a number of animal studies and Nakayama (1998) has classified benzyl salicylate as a common cosmetic sensitizer and primary sensitizer. IFRA (2007) has established a Standard on the use of benzyl salicylate as a fragrance ingredient. Other salicylates with aromatic side chains have also shown sensitization in standard guinea pig tests.
- The salicylates are non-phototoxic and have no photoirritant or photoallergenic activity.
- The use of the salicylates in fragrances produces low levels of exposure relative to doses that elicit adverse systemic effects in laboratory animals exposed by the dermal or oral route. The estimates for maximum systemic exposure to salicylates of humans using cosmetic products range from 0.0002 to 0.4023 mg/kg/day based on the assumption of 100% bioavailability. Considering that bioavailability of the salicylates is actually likely in the range of 11.8–30.7%, systemic exposures are likely lower, in the range of 0.00002–0.124 mg/kg body weight/day.
- Based on the above considerations, and using the NOAEL values of 50 mg/kg body weight/day identified in the subchronic (Webb and Hansen, 1963; Abbott and Harrisson, 1978; Drake et al., 1975) and the chronic toxicity studies (Packman et al., 1961; Webb and Hansen, 1962, 1963), a margin of safety for systemic exposure of humans to the individual salicylates in cosmetic products may be calculated to range from 125 to 2,500,000 (depending upon the assumption of either 12–30% or 100% bioavailability following dermal application) times the maximum daily exposure.

Conflict of interest statement

D. Belsito, D. Bickers, M. Bruze, P. Calow, H. Greim, J.M. Hanifin, A. E. Rogers and J.H. Saurat are members of the Expert Panel of the Research Institute for Fragrance Materials, an independent group of experts who evaluate the safety of fragrance materials that is supported by the manufacturers of fragrances and consumer products containing fragrances. I.G. Sipes and H. Tagami are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on benzyl salicylate

A. Lapczynski *, D. McGinty, L. Jones, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of benzyl salicylate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Benzyl salicylate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on benzyl salicylate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Amyl(iso) salicylate; Benzoic acid, 2-hydroxy-, phenylmethyl ester; Benzyl 2-hydroxybenzoate; Benzyl *o*-hydroxybenzoate; Benzyl salicylate; 2-Hydroxybenozic acid; Phenylmethyl 2-hydroxybenozate; Salicylic acid, benzyl ester.
1.2 CAS Registry number: 118-58-1.
1.3 EINECS number: 204-262-9.
1.4 Formula: $C_{14}H_{12}O_3$.
1.5 Molecular weight: 228.25.
1.6 COE: Benzyl salicylate was included by the Council of Europe in the list of substances B-information required-hydrolysis study (COE No. 436).
1.7 FDA: Benzyl salicylate was approved by the Food and Drug Administration as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and Extract Manufactures' Association states: Generally Recognized as Safe as ingredient – Gras 3 (2151).
1.9 JECFA: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 904) concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent.

2. Physical properties

2.1 *Physical form*: A clear colorless to pale yellow liquid with a faint sweet odor, may solidify below room temperature.
2.2 *Boiling point*: 335 °C.
2.3 *Flash point*: >212 F; CC.
2.4 *Log* K_{ow} (*calculated*): 4.31.
2.5 *Refractive index at* 20 °C (*I.B*): 1.579–1.582.

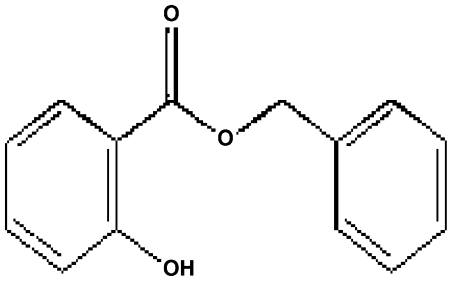

Fig. 1. Benzyl salicylate.

2.6 *Specific gravity*: 1.18 g/ml.
2.7 *Vapor pressure*: <0.001 mm Hg 20 °C.

3. Usage

Benzyl salicylate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region greater than 1000 metric tonnes per annum.

The maximum skin level that results from the use of benzyl salicylate in formulae that go into fine fragrances has been reported to be 6.71% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 15.79% (IFRA, 2002), which would result in a maximum daily exposure on the skin of 0.40 mg/kg for high end users (see Table 1).

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Three groups of rats (6/dose) weighing approximately 100–200 g were dosed orally (gavage) at levels of 1.25, 2.5, or 5.0 g/kg. The calculated LD_{50} was 2.23 g/kg (1.93–2.58 g/kg). Observations for mortality and systemic effects were made over a 7-day period. At 1.25 g/kg, no deaths (0/6) were observed; 4/6 deaths were observed at 2.5 g/kg and all (6/6) animals died at 5.0 g/kg. The principal toxic effect observed before death was depression (RIFM, 1970a).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing benzyl salicylate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	15.79	0.0598
Face cream	0.80	2.00	1.000	0.003	15.79	0.0126
Eau de toilette	0.75	1.00	1.000	0.080	15.79	0.1579
Fragrance cream	5.00	0.29	1.000	0.040	15.79	0.1526
Antiperspirant	0.50	1.00	1.000	0.010	15.79	0.0132
Shampoo	8.00	1.00	0.010	0.005	15.79	0.0011
Bath products	17.00	0.29	0.001	0.020	15.79	0.0003
Shower gel	5.00	1.07	0.010	0.012	15.79	0.0017
Toilet soap	0.80	6.00	0.010	0.015	15.79	0.0019
Hair spray	5.00	2.00	0.010	0.005	15.79	0.0013
Total						**0.4023**

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rat	6	2.23 g/kg	RIFM (1970a)
Dermal	Rabbit	3	14.15 g/kg	RIFM (1970b)

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} of benzyl salicylate was calculated to be 14.15 g/kg (95% CI 4.56–43.86 g/kg) when evaluated in groups of three albino rabbits. Neat benzyl salicylate was applied to the clipped area at dosages of 5, 10, or 20 g/kg and held in close contact with the skin under saran wrap and bandages for an exposure period of 24 h. The animals were observed daily for a period of seven days for any signs of systemic toxicity. On the fifth experimental day, blood was drawn from the marginal ear vein for hematology and clinical chemistry evaluation. No effects were observed at 5.0 g/kg. Animals that received the 10 and 20 g/kg dosage were depressed and showed slow respiration. On the fifth day, one of three rabbits (1/3) at the 10 g/kg level and two (2/3) at the 20 g/kg level died. Among animals dying, intoxication persisted, and gradually deepened to severe depression, loss of the righting reflex, coma and death. Survivors appeared normal by the fifth day. No significant gross pathology was noted in animals that died during the study. Hematological and clinical chemistry values from the survivors were within normal limits when compared to control animals, except for a low hemoglobin value that was noted for the only survivor animal treated at the 20 g/kg level (RIFM, 1970b).

4.2. Skin irritation

4.2.1. Human studies (Table 3)

4.2.1.1. Irritation was evaluated during the induction phase of an associated repeated insult patch test (RIPT) that was conducted on 29 males and 72 females. A 0.3 ml of 15% benzyl salicylate in 3:1 DEP:EtOH was applied to a webril/adhesive patch (25 mm Hilltop® Chamber System) and then applied to the left side of the back of each subject. Patches remained in place for 24 h. Nine induction patches were completed based on Monday–Wednesday and Friday schedule over a period of approximately three weeks. Reactions were scored at patch removal. No irritation was observed (RIFM, 2004a).

4.2.1.2. Irritation was evaluated during the induction phase of an associated RIPT conducted on 17 male and 18 female volunteers. A 0.5 ml aliquot of 10% benzyl salicylate in alcohol SDA 39 C was applied to a 1×1 inch Webril® swatch patch affixed to the center of a 1×2 inch elastic bandage. Test patches were applied to the upper arms of the subjects and remained in place for 24 h. A series of nine applications were made during the induction phase. Reactions were scored 48 h after application. No irritation was observed (RIFM, 1975 c).

4.2.1.3. As a part of an associated RIPT study, eight male and female volunteers were used to determine the primary irritation of benzyl salicylate. A 0.5 cc of 5% benzyl salicylate in dimethyl phthalate was applied to individual absorbent patches. The patches were secured with impervious adhesive tape and applied to the inner surface of the left deltoid area for 48 h. Reactions were read at 24 and 48 h. No irritation was observed (RIFM, 1968).

4.2.1.4. In a pre-test for a human maximization study, no irritation was observed following 48-h closed patch with 30% benzyl salicylate in petrolatum applied to normal sites on the volar forearms of five volunteers (RIFM, 1975a).

4.2.1.5. Two irritant reactions were observed in a pre-test for a human maximization study conducted with 30% benzyl salicylate in petrolatum. A 48-h patch with benzyl salicylate was applied under occlusion to the backs of 22 male volunteers (RIFM, 1975b).

Table 3
Summary of human irritation studies

Method	Dose (%)	Vehicle	Results		References
			Reactions	Incidence (%)	
Induction (HRIPT)	15	3:1 DEP:EtOH	0/101	0	RIFM (2004a)
Induction (HRIPT)	10	Alcohol SDA39C	0/35	0	RIFM (1975c)
Induction (HRIPT)	5	Dimethyl phthalate	0/8	0	RIFM (1968)
Maximization (pre-test)	30	Petrolatum	0/5	0	RIFM (1975a)
Maximization (pre-test)	30	Petrolatum	2/22	9.09	RIFM (1975b)
48 h closed patch test	20	Vaselinum Aldum or Unguentum Hydrophilicum	0/5	0	Fujii et al. (1972)
48 h closed patch test	2	Unguentum Simplex or Unguentum Hydrophilic	0/30	0	Fujii et al. (1972)
48 h closed patch test	0.2	Ethanol or non-irritative cream base	5/313	1.6	Fujii et al. (1972)
24 h closed patch test	5	Vaseline	0/25	0	RIFM (1997a)
4 h closed patch test	100	N/A	0/30	0	Basketter et al. (2004)

4.2.1.6. Irritation was evaluated in a 48-h closed patch test conducted in five male and female volunteers. Benzyl salicylate at 20% in vaselinum aldum or unguentum hydrophilicum applied to the back of each subject produced no irritation (Fujii et al., 1972). Irritation was not observed when a 24–72 h closed patch with 2% benzyl salicylate in unguentum simplex or unguentum hydrophilic was applied to the upper inside of arm of 30 male and female volunteers (Fujii et al., 1972). Five (5/313) positive reactions were observed when 0.2% benzyl salicylate in 99% ethanol or a non-irritative cream base was applied under occlusion for 24–48 h to the upper inside of arm of 313 volunteers (Fujii et al., 1972).

4.2.1.7. A 24-h closed patch test was conducted on 12 male and 13 female volunteers. Benzyl salicylate at 5% in petrolatum was applied to Torii test plasters which were then applied to the upper arms of each subject. Reactions were scored 1 and 24 h after removal of the plaster. No irritation was observed (RIFM, 1997a).

4.2.1.8. The potential of benzyl salicylate to produce irritation was evaluated in a 4-h patch test which was conducted on 30 volunteers. A 0.2 ml aliquot of neat benzyl salicylate was applied to a 25 mm Hilltop® Chambers which were then applied to the skin of the upper outer arm for up to 4 h. Benzyl salicylate was applied progressively for 15 and 30 min through 1, 2, 3 and 4 h, each, to a new skin site. Reactions were score at 24, 48 and 72 h after patch removal. No irritation was observed (Basketter et al., 2004).

4.2.2. Animal studies (Table 4)

4.2.2.1. A 24-h closed patch test was conducted using three Albino rabbits. A 0.5 ml aliquot of 10% benzyl salicylate in alcohol SDA 39 C was applied to intact and abraded skin on each animal using Webril® patches and Blenderm® surgical tape. Reactions were evaluated according to Draize at 24 and 72 h. No irritation was observed (RIFM, 1975d).

4.2.2.2. A 4-h semi-occlusive patch test was conducted on four female New Zealand white albino rabbits. A 0.5 ml aliquot of neat benzyl salicylate was applied to a 2.5 cm square surgical lint that was then placed on an area of clipped, intact dorsal skin. The lint patches were held in place by encircling the trunk of the animal with a length of "Elastoplast®" elastic adhesive bandage 10 cm wide. After 4 h, the adhesive tapes were removed and the treated sites were cleansed by gentle swabbing with cotton wool soaked in warm water. One hour after removal of the patches and excess test material, the treated sites were assessed for reactions. Similar examinations were made at 24, 48, 72 and 168 h after patch removal. No irritation was observed (RIFM, 1984, 1985).

Table 4
Summary of animal irritation studies

Method	Dose	Species	Results	References
24-h closed patch test	10% in alcohol SDA39C	Rabbit	No irritation	RIFM (1975d)
4-h semi-occlusive patch test	100%	Rabbit	No irritation	RIFM (1984) RIFM (1985)
Irritation evaluated as a part of LD_{50} study	100%	Rabbit	No irritation	RIFM (1970b)
Irritation evaluated during induction phase of OET	0.1%	Guinea pig	Minimal irritating concentration	Klecak et al. (1977)
Irritation evaluated during pre-test for OET	0.1%	Guinea pig	Minimal irritation concentration	Klecak et al. (1977)
Irritation evaluated during phototoxocity test	5% and 10% in ethanol	Guinea pig	No irritation	RIFM (1983a)
Irritation evaluated during phototoxicity test	5%, 10% and 30% in acetone	Guinea pig	No irritation observed at 5% irritation observed at 10% and 30%	RIFM (1997b)
Irritation evaluated as a part of Draize test	2%	Guinea pig	Irritation observed (ACC)	Sharp (1978)

4.2.2.3. As a part of acute dermal LD_{50} study described above, irritation was not observed with neat benzyl salicylate at 5, 10 or 20 g/kg when applied to a clipped area and held in contact with the skin for 24 h under occlusion (RIFM, 1970b).

4.2.2.4. Benzyl salicylate was evaluated for irritation, at several dose levels during the induction phase of an open epicutaneous test (OET). A 0.1 ml aliquot of benzyl salicylate was applied to an area measuring 8 cm^2 on the clipped flank of 6–8 male and female outbred Himalayan white–spotted guinea pigs. The application site was left uncovered and reactions were read after 24 h. The minimal irritating concentration after 21 applications was 0.1% (vehicle not specified) (Klecak et al., 1977).

4.2.2.5. Prior to an OET test, benzyl salicylate at a range of concentrations was evaluated for irritation in 6–8 male and female outbred Himalayan white-spotted guinea pigs. A 0.025 ml aliquot was applied with a pipette to an area measuring 2 cm^2 on the clipped flank. The application site was left uncovered and reactions were read after 24 h. The concentration of 0.1% was the lowest concentration to produce mild erythema in at least 25% of the animals and this dose was selected as the minimal irritating concentration after one application (Klecak et al., 1977).

4.2.2.6. A preliminary test to determine the irritation potential for a definitive phototoxicity and photoallergy study was conducted using four (2/sex) adult albino Dunkin-Hartley guinea pigs. A 0.5 ml aliquot of 5% and 10% benzyl salicylate in absolute ethanol was applied to the depilated skin of each animal under an occlusive patch for 90 min. Evaluation of the test site was made at 1, 6, 24 and 48 h. No irritation was observed (RIFM, 1983a).

4.2.2.7. Irritation was evaluated at non-irradiated sites in an associated phototoxicity study that was conducted using five female albino Hartley-Dunkin guinea pigs. Aliquots of 0.02 ml of benzyl salicylate at 5%, 10% or 30% in acetone were applied to a clipped site on each animal. Reactions were scored according to Draize at 24 and 48 h after application. No irritation was observed at 5%. Irritation was observed at 10% and 30% (RIFM, 1997b).

4.2.2.8. As a part of a modified Draize sensitization study (Draize, 1959), a preliminary irritation screen was conducted to determine the injection challenge concentration (ICC) using four inbred Hartley strain albino guinea pigs of the same sex with an average weight of 450 g. Animals were given intradermal injections on shaved flanks with 0.1 ml aliquots of benzyl salicylate in an unspecified vehicle at a range of concentrations. Reactions were read 24 h after injection. The concentration giving slight but perceptible irritation with no edema was 0.5% and it was selected as the ICC (Sharp, 1978).

4.2.2.9. As a part of a modified Draize sensitization study (Draize, 1959), a preliminary irritation screen was conducted to determine the application challenge concentration (ACC) using four inbred Hartley strain albino guinea pigs of the same sex with an average weight of 450 g. Animals received open applications on shaved flanks with 0.1 ml aliquots of benzyl salicylate in an unspecified vehicle at a range of concentrations. Reactions were examined for erythema at 24 h after application. The highest concentration causing no irritation was 2% and it was selected as the ACC (Sharp, 1978).

4.3. Mucous membrane (eye) irritation

4.3.1

A Draize rabbit eye test (Draize, 1959) was conducted using three albino rabbits. A 0.1 ml aliquot of 10% benzyl salicylate in alcohol SDA 39 C was instilled into the right eye of each animal with no further treatment. The untreated left eye of each animal served as a control. The eyes were examined every 24 h for 4 days then again on days 7 and 10. Irritant effects were observed. Mild conjunctival irritation was observed in all 3 rabbits and; corneal opacity was observed in one rabbit. All eyes were clear on the seventh day (RIFM, 1975e).

4.4. Skin sensitization

4.4.1. Dermal sensitization quantitative risk assessment (QRA)

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This new methodology represents a significant change over current risk assessment practices because it specifically addresses the elements of exposure-based risk assessment that are unique to the induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment.

Full details of this risk assessment approach can be found in the ''QRA Expert Group, Dermal Sensitization Quantitative Risk Assessment (QRA) for Fragrance Ingredients, Technical Dossier, revised June 22, 2006'', and IFRA/RIFM Quantitative Risk Assessment (QRA) for Fragrance Ingredients Booklet, May 11, 2006'', at http://www.rifm/org/pub/publications.asp and http://www.ifraorg.org/News.asp.

An exposure-based Quantitative Risk Assessment (QRA) methodology has been used to determine acceptable exposure limits for benzyl salicylate and a new IFRA Standard (IFRA, 2007) has been issued (see Tables 5 and 6).

4.4.2. Human studies

4.4.2.1. Induction studies (Table 7)

4.4.2.1.1. A Human Repeated Insult Patch test (HRIPT) was conducted on 101 volunteers (29 males and 72 females). During the induction phase, 0.3 ml of benzyl salicylate was applied to a webril/adhesive patch (25 mm

Table 5
IFRA Standard based on the QRA

Limits in the finished product: For a description of the categories, refer to the QRA Information Booklet			
Category 1 – see Note (1)	0.5%	Category 7	1.3%
Category 2	0.7%	Category 8	2.0%
Category 3	2.7%	Category 9	5.0%
Category 4	8.0%	Category 10	2.5%
Category 5	4.2%	Category 11 – see Note (2)	
Category 6 – see Note (1)	12.8%		

Note:
(1) IFRA would recommend that any material used to impart perfume or flavour in products intended for human ingestion should consist of ingredients that are in compliance with appropriate regulations for foods and food flavourings in the countries of planned distribution and, where these are lacking, with the recommendations laid down in the Code of Practice of IOFI (International Organisation of the Flavor Industry). Further information about IOFI can be found on its website (http://www.iofiorg.org).
(2) Category 11 includes all non-skin contact or incidental skin contact products. Due to negligible skin contact the concentration of a fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product.
For example, hypothetically if the usual concentration of a fragrance compound in the final product, for example a candle, is at 5%, then any individual fragrance ingredient (in this case benzyl salicylate) must not exceed 5% in the candle.

Table 6
Summary of the relevant sensitization data for the implementation of the QRA

LLNA weighted mean EC3 values ($\mu g/cm^2$) (no. studies)	Human data			Potency Classification[2]	WoE NESIL ($\mu g/cm^2$)[3]
	NOEL – HRIPT (induction) ($\mu g/cm^2$)	Experimental NOEL – MAX (induction) ($\mu g/cm^2$)	LOEL[1] (induction) ($\mu g/cm^2$)		
725 [1]	17,717	20,690	NA	Weak	17,700

NOEL = no observed effect level; HRIPT = human repeat insult patch test; MAX = human maximization test; LOEL = lowest observed effect level; NA = not available.
[1] Data derived from HRIPT or HMT.
[2] Gerberick et al., 2001.
[3] WoE NESIL limited to two significant figure.

Hilltop® Chamber System), and then applied to the back of each volunteer. Patches remained in place for approximately 24 h. Nine induction patches were completed over a 3-week period. After a 2-week rest period, challenge patches were applied to a virgin site on the back and kept in place for 24 h. The test sites were scored at 48, 72 and 96 h. Under the conditions of the study, 15% benzyl salicylate in 3:1 DEP:EtOH did not induce dermal sensitization (RIFM, 2004a).

4.4.2.1.2. To evaluate the potential for cross-reactivity an HRIPT was conducted on 103 volunteers (29 male and 74 females). Using the same method as described above, subjects were induced with 30% hexyl salicylate in 3:1 DEP:EtOH, and cross-challenged with 15% benzyl salicylate in 3:1 DEP:EtOH. No cross-reactions were observed (RIFM, 2004b).

4.4.2.1.3. Thirty five subjects (17 males and 18 females) completed a HRIPT with 10% benzyl salicylate in alcohol SDA 39C. An aliquot of 0.5 ml of benzyl salicylate was applied to a 1×1 inch Webril swatch affixed to the center of a 1×2 inch elastic bandage. These patches were then applied to the upper arms for 24 h under semi-occlusion. Nine induction applications were made over a 3-week period. After a 2-week rest period, a 24-h challenge application was made to the same site and to a virgin site in the same manner as the induction applications. Reactions were scored 48 and 96 h after application. No sensitization reactions were observed (RIFM, 1975c).

4.4.2.1.4. A HRIPT was conducted on 52 volunteers using a modified Draize method (Draize, 1959). An aliquot of 5 ml of 5% benzyl salicylate in dimethyl phthalate was applied to a patch which was then applied to the inner surface of the right deltoid area of each subject and secured by means of overlying strips of impervious adhesive tape, which were then further occluded with additional overlying strips of similar tape. The patches remained in place for 48 h, and then they were removed, observed and recorded. A series of ten induction patches were applied. The challenge patches were applied after a 2-week rest period in the same manner as the induction patches except they were applied in duplicate, one set to the inner surface of each deltoid area. Patches remained in place for 48 h. Reactions were read at patch removal and again at 72 and 144 h. No sensitization reactions were observed (RIFM, 1968).

4.4.2.1.5. A human maximization test was carried out with 20% benzyl salicylate in petrolatum on 25 male and female volunteers. Application was under occlusion to the same site on the volar forearms of all subjects for five alternate day, 48-h periods. After a 14-day rest period, a challenge patch was applied. Reactions were read at 48

Table 7
Human studies for skin sensitization

Test method	Test concentration	Results		References
		Reactions	Incidence (%)	
HRIPT	15% in DEP:EtOH	0/101	0	RIFM (2004a)
HRIPT	10% in alcohol SD39	0/35	0	RIFM (1975c)
HRIPT	5% in dimethyl phthalate	0/52	0	RIFM (1968)
MAX	20% in petrolatum	2/25	8	RIFM (1980)
MAX	30% in petrolatum	0/25	0	RIFM (1975a)
MAX	30% in petrolatum	0/25	0	RIFM (1970c)
MAX	20% in petrolatum	1/25	4	RIFM (1979)
MAX	30% in petrolatum	0/22	0	RIFM (1975b)

and 96 h. Sensitization was observed in two (2/25) volunteers (RIFM, 1980).

4.4.2.1.6. A human maximization test was carried out on 25 healthy male and female volunteers with 30% benzyl salicylate in petrolatum. Benzyl salicylate was applied under occlusion to the same site on the volar forearms of each subject for five alternate day, 48-h periods. Patch sites were pre-treated for 24 h with 5% aqueous sodium lauryl sulfate (SLS) under occlusion. After a rest period, a challenge patch was applied. Challenge sites were read at patch removal and 24 h later. No sensitization reactions were observed (RIFM, 1975a).

4.4.2.1.7. A maximization test was conducted with 30% benzyl salicylate in petrolatum. Benzyl salicylate was applied to the same site on the volar forearm of 25 male volunteers under occlusion for five alternate day, 48-h periods. Each application was preceded by 24-h occlusive applications of 5% aqueous SLS. Following a 10-day rest period, challenge patches of benzyl salicylate were applied to fresh sites on the backs of each subject under occlusion for 48 h. The challenge sites were pre-treated for 1 h with 10% aqueous SLS. Challenge sites were read at 48 and 72 h. No sensitization reactions were observed (RIFM, 1970c).

4.4.2.1.8. A maximization test was conducted on 25 healthy Japanese-American volunteers using 20% benzyl salicylate in petrolatum which was applied under occlusion to the volar forearms of all subjects for five alternate day, 48-h periods. The patch site was pre-treated for 24 h with 5% aqueous SLS under occlusion. Following a 10–14 day rest period, a challenge patch of benzyl salicylate was applied to fresh sites for 48 h under occlusion. The challenge sites were pretreated for 30 min with 3% aqueous SLS under occlusion on the left side of the back whereas benzyl salicylate was applied without SLS on the right side. Additional SLS controls were placed on the left and petrolatum on the right. One (1/25) sensitization reaction was observed (RIFM, 1979).

4.4.2.1.9. A maximization test was carried out on 22 healthy male volunteers. Benzyl salicylate at 30% in petrolatum was applied under occlusion to the same site on the forearms of all subjects for five alternate day, 48-h periods. Patch test sites were pre-treated for 24 h with 5% aqueous SLS under occlusion for the initial patch only. Following a 10–14 day rest period, a challenge patch was applied to a fresh site for 48 h under occlusion. The challenge sites were pre-treated for 30 min with 2% aqueous SLS under occlusion on the left side of the back whereas the benzyl salicylate was applied without SLS on the right side. Reactions to challenge were read 48 and 72 h after patch removal. Benzyl salicylate produced no sensitization reactions (RIFM, 1975b).

4.4.2.2. Diagnostic studies (Table 8)

4.4.2.2.1. Closed patch tests were conducted on 313 patients with 0.05–0.5% benzyl salicylate in a base cream or in 99% ethanol. Patches consisted of a piece of 1.0 cm^2 lint with a 2.0 cm^2 cellophane disc placed on the lint and covered with a 4.0 cm^2 plaster. Patches were applied to the back, the forearm and the inside of the upper arm for 24 to 48 h. Reactions were read 30 min after patch removal. Erythema was observed in 5 out of 313 patients (Takenaka et al., 1986).

4.4.2.2.2. Patch testing was conducted using 394 subjects with contact dermatitis, cosmetic dermatitis, eczema, seborrhoic dermatitis and facial dermatitis. Test patches with 1%, 2%, or 5% benzyl salicylate in petrolatum were applied on the back of each subject with Finn Chambers® and Scanpor® tape. Test sites were read on Day 2 (day after application), Day 3 and Day 7 and scored according to JCDRG and ICDRG standards. Questionable reactions were observed at all concentrations, and a positive reaction (++) was observed at 5% (no further details provided) (Ueda, 1979, 1994).

4.4.2.2.3. The principle patch test results of the North American Contact Dermatitis Group for the period from July 1, 1975 to June 30, 1976 have been reported. A total of 183 patients were patch tested with fragrance allergens. Test materials were applied with A1 Test® strips or Finn Chambers® for 48 h in vertical rows affixed with 2-inch wide occlusive tape. Reactions were read at 48 and 96 h. Reactions to 2% benzyl salicylate (vehicle not reported) were observed in 2.1% of the 183 patients tested (Rudner, 1977, 1978).

4.4.2.2.4. Ferguson and Sharma (1984) reported the results of patch tests conducted on 241 patients (180 females and 61 males) from October 1981 to 1983. Patients were patch tested for sensitivity to fragrances in a perfume screening series. The Finn Chamber® technique was used.

Table 8
Summary of human diagnostic studies conducted on 100 or more patients

Method	Concentration	Results		References
		Reactions	Incidence (%)	
Closed patch test	0.05–0.5% in a base cream or 99% ethanol	5/313	1.6	Takenaka et al. (1986)
Patch test	1%, 2%, 5% in petrolatum	1/394	0.25	Ueda (1979, 1994)
Patch test	2% in an unspecified vehicle	4/183	2.1	Rudner (1977, 1978)
Patch test	2% in paraffin	6/241	2.5	Ferguson and Sharma (1984)
Patch test	2% in paraffin	1/457	0.22	Addo et al. (1982)
Patch test	2% in petrolatum	10/1825	0.5	deGroot et al. (2000)
Patch test	2% in petrolatum	1/89	1.12	Nethercott et al. (1989)
Patch test	2% in an unspecified vehicle	13/200	6.5	Asoh et al. (1985a)
Patch test	2% in petrolatum	5/157	3.18	Hayakawa (1986)
Patch test	2% in petrolatum	38/788	4.8	Sugai (1986)
Patch test	5% in an unspecified vehicle	30/756	4	Itoh et al. (1988)
Patch test	5% in an unspecified vehicle	12/155	7.74	Itoh (1982)
Patch test	0.2%, 1%, or 10% in ethanol	0/10538	N/A	Kohrman et al. (1983)
Patch test	1%	5/180	2.78	Ishihara et al. (1979)
	2%	9/180	5.0	
	5% in petrolatum	16/254	6.29	
Patch test	1%	6/394	1.52	Ueda (1979)
	2%	9/394	2.28	
	5% in petrolatum	23/394	5.84	
Patch test	5% in an unspecified vehicle	27/680	3.97	Itoh et al. (1986)
Patch test	5% in petrolatum	12/212	5.66	Hada (1983)
Patch test	2% in an unspecified vehicle	2/103	1.94	Fujimoto et al. (1997)
Patch test	5% in petrolatum	0/315	N/A	Heydorn et al. (2002)
Patch test	2% in petrolatum	1/386	0.26	Sugai (1996)
Patch test	0.1%	1/65	1.54	Kozuka et al. (1996)
	1% in petrolatum	3/201	1.49	
Patch test	5% in petrolatum	14/176	7.95	Shoji (1982)
Patch test	2% in petrolatum	3/102	2.94	Hausen (2001)
Patch test	1% in petrolatum	3/747	0.4	Wohrl et al. (2001)
Patch test	2% in petrolatum	7/706	0.99	Katoh et al. (1995)
Patch test	5% in petrolatum	2/658	0.3	Heydorn et al. (2003)
Patch test	2% in petrolatum	77/1255	6.1	Sugai (1982)
Patch test	0.2% in perfumed base cream	3/313	0.96	RIFM (1974)
Patch test	5% in an unspecified vehicle	24/522	4.6	Nishimura et al. (1984)
Patch test	5% in petrolatum	25/181	13.8	Hayakawa et al. (1983)
Patch test	1%	6/394	1.5	MJDRG (1984)
	2%	9/394	2.3	
	5% in petrolatum	23/394	5.8	
Patch test	5% in petrolatum	1/64	1.6	Haba et al. (1993)
Patch test	2% in petrolatum	4/482	0.83	Nagareda et al. (1996)
Patch test	2% in petrolatum	8/436	1.83	Nagareda et al. (1992)
Patch test	2%	5/167	3	Larsen et al. (1996)
	5% in petrolatum	8/167	4.8	
Patch test	1%	0/100	N/A	Frosch et al. (1995b)
	5% in petrolatum	1/100	1	
Patch test	5% in petrolatum	20/362	5.52	Ishihara et al. (1981)

Reactions to 2% benzyl salicylate in paraffin were observed in 6/241 patients and were characterized by erythema and edema.

4.4.2.2.5. Fifty patients with photosensitivity dermatitis with actinic reticuloid (PD/AR) syndrome, 32 subjects with polymorphous light eruption (PLE) and 457 with contact dermatitis (CD), were studied to determine the incidence of contact allergic sensitivity to some common fragrance materials. Each subject was patch tested to various fragrance materials using a standard closed patch test technique. A total of 10 mg of the test material supplied in paraffin was applied to standard Al-Test® strips which were then placed on the skin of the upper back, secured with Scanpor® adhesive tape, and removed at 48 h. Reactions were read at patch removal and then again at 72 h. Benzyl salicylate at 2% in yellow paraffin produced one reaction in (1/457) CD patients. No reactions were observed in PD/AR or PLE patients (Addo et al., 1982).

4.4.2.2.6. In a multicenter study conducted from September 1998 to April 1999, 1825 patients were patch tested with nine fragrance allergens and the fragrance-mix. The test procedures and concentrations were carried out according to internationally accepted criteria and published studies. Positive reactions to 2% benzyl salicylate in

petrolatum were observed in 10 (0.5%) patients (deGroot et al., 2000).

4.4.2.2.7. In a multicenter study conducted in North America from January 1980 to May 1987, 19 patients with eyelid dermatitis and 70 patients with dermatitis at other sites were patch tested with 2% benzyl salicylate in petrolatum. Benzyl salicylate was applied to Al-Test® strips or Finn Chambers®, which were applied to the upper back and secured to the skin with Scanpor® for a period of 48–72 h. Reactions were read at patch removal and re-examined in the majority of cases between 48 and 96 h after patch removal. Sites were scored according to the ICDRG scoring system. Positive reactions were observed in 5.3% of the 19 eyelid dermatitis patients. No other reactions were observed (Nethercott et al., 1989).

4.4.2.2.8. From January to August 1982, 31 fragrance materials were patch tested in order to determine their incidence of positive reactions. Benzyl salicylate at 2% (vehicle not provided) produced positive reactions in 13/200 subjects (Asoh et al., 1985a).

4.4.2.2.9. Hayakawa (1986) reported the incidence of positive patch tests conducted in 1984 by the Japan Patch Test Research Group. Forty-eight hour closed patch tests with cosmetic ingredients were conducted on patients with cosmetic dermatitis. Reactions were read 24 h after patch removal. Reactions to 2% benzyl salicylate in petrolatum were observed in 5/157 patients.

4.4.2.2.10. In patch tests conducted from 1981 to 1983, the incidence of positive reactions to 2% benzyl salicylate in petrolatum causing allergic contact dermatitis of the delayed type, based on the European and North American Standard Test series for patch testing, was 4.8% (38/788 reactions) (Sugai, 1986).

4.4.2.2.11. In a series of patch tests conducted from 1978 to 1986 with cosmetic ingredients in patients with eczema or dermatitis, 5% benzyl salicylate (vehicle not reported) produced reactions in 4.0% (30/756) patients (Itoh et al., 1988).

4.4.2.2.12. A total of 155 patients with cosmetic dermatitis and female facial melanosis were patch tested with various fragrance materials. The test samples were applied on the cloth disks of Torii's adhesive plaster and the plaster was applied to the upper back of the patient for 48 h. Reactions were assessed at 1 h, 24 h, 1 week and 2 weeks after removal. Positive responses to 5% benzyl salicylate in petrolatum were observed in 12 out of 155 patients (Itoh, 1982).

4.4.2.2.13. A 1979 survey conducted by the SDA (The Soap and Detergent Association) produced 10,538 patch test and repeate patch test results on 8430 subjects. Benzyl salicylate was tested in consumer products (maximum concentration used reported 0.2%), and in fragrance blends (maximum concentration reported was 1%), and was also tested alone (10%) in ethanol. No reactions were observed (Kohrman et al., 1983).

4.4.2.2.14. Benzyl salicylate was patch tested on dermatitis patients at 1%, 2% and 5% in petrolatum. At 1%, reactions were observed in 4/51 (7.8%) melanosis patients and 1/129 (0.8%) cosmetic dermatitis patients. At 2%, reactions were observed in 7/51 (13.7%) melanosis patients and 2/129 (1.6%) cosmetic dermatitis patients. At 5%, reactions were observed in 10/51 (19.6%) melanosis patients, 5/129 (3.9%) cosmetic dermatitis patients and 1/84 (1.2%) non-cosmetic dermatitis and/or eczema patients (Ishihara et al., 1979).

4.4.2.2.15. The Mid-Japan Contact Dermatitis Research Group conducted a study to determine the optimal patch testing concentration of benzyl salicylate. In the first series, 394 patients were patch tested with various fragrance materials including 1%, 2% and 5% benzyl salicylate in petrolatum. Six positive (6/394) reactions were observed at 1%, nine (9/394) at 2% and 23 (23/394) at 5%. In the second series, patch tests were conducted using 1%, 2% and 5% benzyl salicylate in petrolatum on 21 subjects using the same procedure as above. No reactions were observed at 1% and 5%, two (2/21) reactions were observed at 2% (Ueda, 1979).

4.4.2.2.16. Between 1978 and 1985, eczema and dermatitis patients were patch tested with various synthetic perfumes. A total of 680 patients were patch tested with benzyl salicylate at 5% (vehicle not provided). Positive reactions were observed in 27/680 patients (Itoh et al., 1986).

4.4.2.2.17. The Mid-Japan Contact Dermatitis Research Group conducted a study to determine the optimal patch testing concentration of benzyl salicylate. A total of 212 patients were patch tested with various fragrance materials including 5% benzyl salicylate in petrolatum. Positive reactions were observed in 12/212 patients (Hada, 1983).

4.4.2.2.18. From 1989 to 1992, 332 patients (25 male and 307 female) suspected of cosmetic contact dermatitis were patch tested with various cosmetics and their ingredients. Of these patients, 103 were patch tested with 2% benzyl salicylate (vehicle not provided). Positive reactions were observed in 2/103 patients (Fujimoto et al., 1997).

4.4.2.2.19. A total of 315 consecutive hand eczema patients were patch tested with various fragrance materials. No reactions were observed to 5% benzyl salicylate in petrolatum (Heydorn et al., 2002).

4.4.2.2.20. In 1994, patients with suspected contact dermatitis from cosmetic products were patch tested with cosmetic ingredients. One positive (1/386) reaction was observed to 2% benzyl salicylate in petrolatum (Sugai, 1996).

4.4.2.2.21. Patients with contact dermatitis were patch tested with 0.1% (65 patients) and 1% (201 patients) benzyl salicylate in petrolatum. Patch tests were conducted using Finn Chambers® and Scanpor® tape. Three reactions (3/201) were observed with 1% and one (1/65) was observed with 0.1% benzyl salicylate (Kozuka et al., 1996).

4.4.2.2.22. The Japan Contact Dermatitis Research Group conducted a study to determine the optimal patch testing concentration of benzyl salicylate. A total of 357 patients at 16 different centers were patch tested with various fragrance materials that included 5% benzyl salicylate

in petrolatum. Patch tests were conducted using Finn Chambers® and were secured with Scanpor® tape. Reactions were observed in 14/176 patients (Shoji, 1982).

4.4.2.2.23. Dermatitis Patients (2272) were tested with the standard series and 445 had a positive reaction to balsam Peru. Out of these (445) patients, 102 were patch tested with the balsam Peru series and propolis. Patch tests were applied to the backs for 24 h using Finn Chamber® and Scanpor® tape. Reactions were read after 24 and 72 h according to ICDRG rules. Reactions were observed in 3/102 patients to 2% benzyl salicylate in petrolatum (Hausen, 2001).

4.4.2.2.24. A total of 747 patients suspected of fragrance allergy were patch tested with a special fragrance series which was comprised of the eight constituents from the fragrance mix. Reactions were assessed at 72 h and scored according to criteria established by ICDRG. Three positive reactions (3/747) were observed with benzyl salicylate at 1% in petrolatum (Wohrl et al., 2001).

4.4.2.2.25. From January 1992 to June 1993, the incidence of positive reactions to various fragrance materials was investigated in patients with contact dermatitis. Patients were patch tested with 10 fragrance materials. Reactions were assessed according to rules established by ICDRG. Positive reactions were observed in 7/706 subjects with 2% benzyl salicylate in petrolatum (Katoh et al., 1995).

4.4.2.2.26. A total of 658 patients with hand eczema, who had reacted positively to fragrance materials in the European Standard series, were further patch tested to a selection of fragrances. A positive reaction was observed in 2/658 patients with 5% benzyl salicylate in petrolatum (Heydorn et al., 2003).

4.4.2.2.27. In a series of patch tests conducted in 1255 patients with contact dermatitis from 1973 to 1981, reactions to 2% benzyl salicylate in petrolatum were observed in 6.1% (77/1255) patients (Sugai, 1982).

4.4.2.2.28. A closed patch test was conducted on a group of Japanese male and female subjects, with 10% of the test population being characterized as eczema-prone and allergic persons. A 1.0 cm^2 patch was applied to the inside of the upper arm and flexor of the forearm then affixed with adhesive tape. Patches were applied for 24 h. The vehicle was a perfume based cream. Reactions were read 30 min after removal. Positive reactions to 0.2% benzyl salicylate were observed in 3/313 patients and questionable reactions were observed in 2/313 patients tested (RIFM, 1974).

4.4.2.2.29. A total of 212 patients with cosmetic dermatitis, 35 patients with facial melanosis, and 275 patients with non-cosmetic dermatitis or eczema were patch tested with 5% benzyl salicylate (vehicle not reported). In addition, 101 subjects used as controls were also tested with 5% benzyl salicylate. Reactions to 5% benzyl salicylate were observed in 8/212 cosmetic dermatitis patients, 7/35 facial melanosis patients, 9/275 non-cosmetic dermatitis and eczema patients and 1/101 controls (Nishimura et al., 1984).

4.4.2.2.30. From December 1981 to November 1982, 181 cases of dermatitis patients with melanosis faciei feminae were patch tested with their cosmetic products and 137 allergens. Positive reactions to 5% benzyl salicylate in petrolatum were observed in 25/181 patients (Hayakawa et al., 1983).

4.4.2.2.31. A total of 394 subjects most of whom suffered from various facial dermatoses were patch tested for 48 h under occlusion with benzyl salicylate at concentrations 1%, 2%, or 5% in petrolatum. Reactions were read 1 h after patch removal and again the next day. Reactions were assessed using ICDRG guidelines. At 1%, irritant reactions were observed in 4.6% of the subjects and allergic reactions were observed in 1.5% of the subjects. At 2%, irritant reactions were observed in 3.3% while allergic reactions were observed in 2.3% of the subjects. At 5%, irritation was observed in 4.8% of the subjects and allergic reactions were observed in 5.8% of the subjects (MJCDRG, 1984).

4.4.2.2.32. From 1990 to 1991, 64 patients with cosmetic dermatitis, 7 facial melanosis patients and 32 non-cosmetic dermatitis patients were patch tested with 5% benzyl salicylate in white petrolatum. A positive reaction to 5% benzyl salicylate was observed in 1/64 (1.6%) cosmetic dermatitis patient. No reactions were observed in facial melanosis patients and non-cosmetic dermatitis patients (Haba, 1990).

4.4.2.2.33. From September 1992 to August 1993, a series of patch tests to most allergenic ingredients of cosmetic and toiletry products were conducted. A total of 482 patients were tested with 2% benzyl salicylate in petrolatum. Positive reactions were observed in 4/482 patients (Nagareda et al., 1996).

4.4.2.2.34. Nagareda et al. (1992) reported the incidence of positive reactions to 17 ingredients derived from patch tests conducted during 1990 to 1991, on patients with contact dermatitis. Patch tests were conducted using Finn Chambers® and Scanpore® tape. Positive reactions were observed with 2% benzyl salicylate in petrolatum in 8/436 contact dermatitis patients.

4.4.2.2.35. Patch tests conducted on patients from 1971 to 1980 using 5% benzyl salicylate in petrolatum resulted in positive reactions in 11% of cosmetic dermatitis and 1% of non-cosmetic dermatitis patients from 1971 to 1974, in 25% cosmetic and 0% non-cosmetic dermatitis patients from 1975 to 1977, and in 11% cosmetic and 0% non-cosmetic dermatitis patients from 1978 to 1980 (Nakayama et al., 1984).

4.4.2.2.36. In a worldwide multicenter study to investigate fragrance sensitization in patients with suspected fragrance allergies, 167 patients were patch tested with benzyl salicylate which was applied to the upper back with Finn Chambers® and Scanpor® for a period of 48–72 h. Reactions were read at patch removal and then re-examined between 48 and 120 h after patch removal. Reactions were scored according to the North American Contact Dermatitis Group's modification of the ICDRG scoring criteria. Benzyl salicylate, at 2% in petrolatum, produced

irritant reactions in 3% of the patients and allergic reactions in 3% of the patients. At 5%, irritant reactions were observed in 3% of the patients and allergic reactions were observed in 4.8% of the patients (Larsen et al., 1996a).

4.4.2.2.37. Frosch et al. (1995b) reported the results of a multicenter study on patch tests with 48 fragrance materials. Benzyl salicylate was applied to the back with Finn Chambers® and Scanpor® for 2 days. Reactions were assessed as per ICDRG guidelines on days 2 and 3 or days 2 and 4. Benzyl salicylate at 1% and 5% in petrolatum was tested in 100 patients (64 females and 36 males). No reactions were observed with 1%. One (1/100) questionable reaction was observed with 5%.

4.4.2.2.38. Patch tests were conducted on patients with and without cosmetic dermatitis as well as patients with facial melanosis, from the period 1978 to 1980. The vehicle was not reported. Positive reactions were observed with 5% benzyl salicylate in 7.7% (12/155), 4.4% (7/159), and 2.1% (1/48) of subjects with prior histories of cosmetic dermatitis, eczema and dermatitis, or no prior condition, respectively (Ishihara et al., 1981).

4.4.3. Animal studies

4.4.3.1. Maximization (MAX) test (Table 9)

4.4.3.1.1. A guinea pig maximization test was conducted on 8 test and 8 control female albino Hartley-Dunkin guinea pigs weighing 435–490 g. Induction consisted of a two stage procedure. In the first stage, three intradermal injections (0.1 ml each) were administered to the clipped shoulder region of each animal. The injections consisted of Freunds Complete Adjuvant (FCA) plus distilled water (1:1); 10% w/v benzyl salicylate in FCA; and finally 10% w/v benzyl salicylate in FCA plus distilled water (1:1). The second stage was a 48-h topical application made seven days later to the same area on the shoulder. The shoulder was shaved again and treated with 10% sodium lauryl sulfate (SLS) in petrolatum. Two weeks after the topical application, the flank of each animal was shaved free of hair and divided into three sites (1.5 cm × 1.5 cm). The challenge test was performed by applying 0.02 ml of 5%, 10% and 20% benzyl salicylate in acetone to each site. The application sites were left uncovered. Reactions were graded per Draize at 24, 48 and 72 h after application. Sensitization was observed at all concentrations (RIFM, 1997c).

4.4.3.1.2. A guinea pig maximization test was conducted using benzyl salicylate at 10% for both induction and challenge phase. Sensitization was observed (Ishihara et al., 1986).

4.4.3.1.3. A Magnusson–Kligman guinea pig maximization test was conducted on 10 Hartley guinea pigs/dose using 1% benzyl salicylate in ethanol intradermally and 100% dermally. No sensitization reactions were observed (Tsuchiya et al., 1982).

4.4.3.1.4. Four week old female Hartley strain guinea pigs (20/group) weighing about 300 g were tested in a guinea pig maximization test. Benzyl salicylate at 10% in liquid paraffin and FCA was intradermally injected in the shoulder region of each animal. Five days after the intradermal injections 10% SLS in petrolatum was topically applied to the same region. Twenty-four hours later, 50% benzyl salicylate in white petrolatum was applied for 48 h with impermeable tape and adhesive bandage. Two weeks after the topical application, benzyl salicylate at 5%, 10% and 20% in white petrolatum was applied on the backs of the animals using mini-plasters (Torii Pharmaceutical Co.) for 24 h. The reactions were read 24 and 48 h after removal of the plaster. Two reactions were observed at 20%. Questionable reactions were observed in three (3/20) animals at 5%, five (5/20) animals at 10%, and four (4/20) animals at 20% (Kozuka et al., 1996).

4.4.3.1.5. A guinea pig maximization test was conducted using outbred Himalayan white-spotted male and female guinea pigs. Induction was via two intradermal injections of 0.1 ml of 5% benzyl salicylate in white petrolatum with and without FCA on day 0. On day 8, 25% benzyl salicylate in petrolatum was applied to a clipped area on the neck for 48 h under occlusion. Challenge conducted on day 21 was via a 24-h occluded patch. Reactions were read at 24 and 48 h after removing the patch. No sensitization reactions were observed (Klecak et al., 1977).

4.4.3.1.6. Another guinea pigs maximization test was conducted using 10 female Hartley albino guinea pigs. Induction consisted of intradermal injection of 10% benzyl salicylate in liquid paraffin and a 48-h occlusive patch with 30% benzyl salicylate in ethanol. The animals were challenge twice with benzyl salicylate at 0.003%, 0.01% and 0.03% in ethanol. The second challenge was conducted three weeks after the first challenge. Reactions were read at 24, 48 and 72 h. At first challenge, no reactions were observed at 24 h, but positive reactions were observed at 48 and 72 h at all doses. At the second challenge, positive reactions were observed with 0.03% at 24 h and all concentrations at 48 and 72 h (Kashima et al., 1993).

4.4.3.2. Other studies

4.4.3.2.1. A guinea pig open epicutaneous test (OET) was conducted on groups of 6–8 male and female guinea pigs weighting 300–450 g. Daily applications of 0.1 ml benzyl salicylate (undiluted or progressively diluted solutions) were made for 3 weeks to a clipped 8.0 cm^2 area on the flank of each guinea pig. The test sites were not covered and the reactions were read 24 h after each application. A total of 21 applications of benzyl salicylate in an unspecified vehicle were made for 21 days. Ten control animals were either left untreated or treated with 0.1 ml of the vehicle for 21 days. At the challenge phase, both the test and control animals were treated on days 21 and 35 on the contralateral flank with 30% benzyl salicylate. No sensitization was produced (Klecak, 1985).

4.4.3.2.2. An OET was conducted in guinea pigs. Induction consisted of 21 daily open applications to the shaved flank of 6–8 guinea pigs per group. Open challenge applications were made on days 21 and 35. Reactions were read at 24, 48 and 72 h. No reactions were observed with 10%

Table 9
Summary of animal sensitization studies

Method	Concentration	Species	Results	References
MAX	10% in FCA for induction; 5%, 10% and 20% in acetone for challenge	Guinea pigs	Sensitization observed	RIFM (1997c)
MAX	10% for induction and challenge	Guinea pigs	Sensitization observed	Ishihara et al. (1986)
MAX	1% in ethanol for induction; 100% for challenge	Guinea pigs	No sensitization	Tsuchiya et al. (1982)
MAX	10% in liquid paraffin for intradermal induction; 50% in white petrolatum for topical induction; 5%, 10% and 20% in white petrolatum for challenge	Guinea pigs	No sensitization at 5% and 10%; sensitization observed at 20%	Kozuka et al. (1996)
MAX	5% and 25% in white petrolatum	Guinea pigs	No sensitization	Klecak et al. (1977)
MAX	10% in liquid paraffin for intradermal induction; 30% in ethanol for topical induction; 0.003%, 0.01% and 0.03% in liquid paraffin for challenge	Guinea pigs	Sensitization observed	Kashima et al. (1993)
OET	30% for induction and challenge	Guinea pigs	No sensitization	Klecak (1985)
OET	10% for induction and challenge	Guinea pigs	No sensitization	Klecak (1979)
OET	0.03% and 30%	Guinea pigs	0.03% – minimum eliciting concentration 30% – minimum sensitization concentration	Klecak et al. (1977)
CET	30% – induction 1% – challenge	Guinea pigs	Sensitization observed (3/20)	Ishihara et al. (1986)
CCET	30% in ethanol for induction; 1%, 3% and 10% in ethanol for challenge	Guinea pigs	Sensitization observed	Kashima et al. (1993)
CCET	10%, 30% and 100% for induction application; 50% for challenge	Guinea pigs	No sensitization at 10%; Sensitization observed at 30 and 100%	Tsuchiya et al. (1982)
CCET	100% for induction application; 50% in ethanol for challenge application	Guinea pigs	Sensitization observed	Imokawa and Kawai (1987)
DCHA	30% for induction application: 1%, 3% and 10% for challenge	Guinea pigs	Sensitization observed	Kashima et al. (1993)
Modified FCAT	10% in FCA for intradermal induction; 10% in acetone for challenge	Guinea pigs	Sensitization observed	Hausen and Wollenweber (1988)
FCAT	50% in FCA for induction application; 0.1% for challenge application	Guinea pigs	No sensitization	Klecak et al. (1977)
Photoallergy (sensitization) test	10% for challenge	Guinea pigs	No sensitization	RIFM (1983a)
Optimization test	0.1% in saline or FCA/saline for induction 0.1% in saline and 10% in petrolatum for challenge	Guinea pigs	Sensitization observed	Maurer et al. (1980)
DRAIZE (Modified)	0.5% for intradermal induction; 0.5% for intradermal challenge and 2% fir dermal challenge	Guinea pigs	No sensitization	Sharp (1978)
DRAIZE (Modified)	0.1% in isotonic saline for intradermal induction; 0.1% in isotonic saline for challenge	Guinea pigs	No sensitization	Klecak et al. (1977)

benzyl salicylate (vehicle not provided; no further details provided) (Klecak, 1979).

4.4.3.2.3. Benzyl salicylate was tested for sensitization in an OET test in male and female outbred Himalayan guinea pigs (6/8-group) weighing 400–500 g. Animals received 21 daily open applications of 0.1 ml of undiluted and progressively diluted solutions of benzyl salicylate which were applied to an 8.0 cm^2 area on the clipped flank. Guinea pigs were challenged by an open application of 0.025 ml benzyl salicylate, in an unspecified vehicle, which was applied to a skin area measuring 2 cm^2 on the contralateral flank on days 21 and 35. Reactions were read 24, 48 and 72 h after application. A concentration of 0.03% was reported to be the minimum eliciting concentration and 30% was reported to be the minimum sensitizing concentration (Klecak et al., 1977).

4.4.3.2.4. A closed epicutaneous test (CET) was conducted using 20 guinea pigs. During the induction phase, benzyl salicylate at 30% (vehicle not provided) was applied under occlusion for 48 h on the shaved nape. The same procedure was repeated three times per week for two weeks. Following a 2-week rest period, the animals were challenge with 1% benzyl salicylate under occlusion for 48 h. Three (3/20) sensitization reactions were observed (Ishihara et al., 1986).

4.4.3.2.5. The cumulative contact enhancement test (CCET) was conducted using groups of 10 female Hartley albino guinea pigs weighing approximately 250 g. During

the induction phase, a 24-h occlusive patch containing 30% benzyl salicylate in ethanol was applied to each animal. The animals were challenged twice (2nd challenge was conducted 3 weeks after the 1st challenge) with 1%, 3% and 10% benzyl salicylate in ethanol. Sites were scored at 24, 48 and 72 h. At 1%, one positive (1/10) reaction was observed at 24 h, no reactions were observed at 48 or 72 h. At 3%, positive reactions (3/10) and (2/10) were observed at 24 and 48 h, respectively. At 10%, positive reactions were observed at 24, 48 and 72 h (Kashima et al., 1993).

4.4.3.2.6. Tsuchiya et al. (1982) conducted another CCET test on 6–10/group Pirbright and Hartley albino guinea pigs. A 0.2 ml aliquot of 3%, 10%, 30% (vehicle not reported) or 100% benzyl salicylate was applied to a lint patch which was then applied to the shaved back for 24 h under occlusion. The applications were repeated every other day over a period of 2 weeks. Eleven days after the final induction patch, a challenge was performed. A 0.01 ml aliquot of 50% benzyl salicylate was applied on a circular 2.0 cm in diameter cotton patch to a shaved part of each animal. Reactions were evaluated 24, 48 and 72 h. No sensitization was observed with induction concentration at 10% or 100% in Pirbright guinea pigs. Three (3/6) sensitization reactions were observed when the animals were induced with 30% benzyl salicylate and 1/10 reactions were observed when the animals were induced with 100% benzyl salicylate.

4.4.3.2.7. The cumulative contact enhancement test (CCET) was conducted using 30 tortoise shell guinea pigs weighing 250–300 g. Animals were shaved and a 24-h occluded patch with neat benzyl salicylate was applied. Patches were applied every third day for 2 weeks (maximum, 4 applications). An injection of FCA was intradermally administered before the third patch. An untreated group of five animals was used as a control. After an 11 day rest period, a 0.01 ml aliquot of 50% benzyl salicylate in ethanol was applied to a previously untreated site, once daily for 1–3 days. Reactions were evaluated over a period of 43 days. Sensitization reactions were observed in 13/30 animals (Imokawa and Kawai, 1987).

4.4.3.2.8. A delayed contact hypersensitivity assay was conducted in 10 female Hartley strain guinea pigs using the AP2 test method. Two induction applications were made 4 days apart and consisted of an intradermal injection with FCA and a 24 h occluded patch at the injection site with 30% benzyl salicylate in ethanol. Two open challenge applications were made on days 11 and 32 with 1%, 3% and 10% benzyl salicylate in ethanol. A third challenge application was made with 0.003%, 0.01% and 0.03% benzyl salicylate in ethanol on day 39 using a 24 h occluded patch. The reactions were evaluated at 24, 48 and 72 h after challenge. Sensitization was observed at all three challenges (Kashima et al., 1993).

4.4.3.2.9. Sensitization was evaluated in groups of 10 Pirbright guinea pigs weighing 280–350 g using a modified FCA method. Six intradermal injections (2 per day on 3 separate days) of 10% benzyl salicylate in FCA were made into the clipped, shaved shoulder area on days 1, 5 and 9 for a total of 4.5 mg of benzyl salicylate. Challenge was conducted 11 days after induction by applying 0.05 ml of 10% benzyl salicylate in acetone onto the clipped, shaved right flank. Reactions were read at 24, 48 and 72 h. Benzyl salicylate at 10% was a moderate sensitizer (Hausen and Wollenweber, 1988).

4.4.3.2.10. Sensitization was evaluated as a part of photoallergy study using 25 adult albino Dunkin-Hartley guinea pigs. Twenty-four hours prior to application all animals were clipped free of hair on the back and flanks. On day 1, a topical application of 0.5 ml of benzyl salicylate in absolute ethanol was applied to the middle of the anterior part of the back of the animals for 1 h 30 min. On day four of the study, four intradermal injections of 0.1 ml each of FCA diluted at 50% in isotonic saline were made on both sides of the occlusive patch. A second topical application of 0.5 ml benzyl salicylate in absolute ethanol was applied under an occlusive patch for 90 min. A 3rd and 4th topical application under an occlusive patch was made on days 7 and 9 of the study. The challenge was conducted 12 days after the 4th application. A challenge was conducted with 0.5 ml of 10% benzyl salicylate in absolute ethanol applied to a virgin area on the back of the animal for 1.5 h. Reactions were scored at 1, 6, 24 and 48 h. No sensitization reactions were observed (RIFM, 1983a).

4.4.3.2.11. The optimization test was conducted in 20 Pirbright White Strain guinea pigs (10/sex). During the induction period, the animals received one intracutaneous injection every other day of 0.1% benzyl salicylate in saline. During the second and third week, benzyl salicylate was incorporated at the same concentration in a mixture of FCA and physiological saline (adjuvant/saline, 1:1 v/v). A total of 10 injections were made. The animals were challenged with 0.1% benzyl salicylate in saline 14 days after the last induction injection using the same procedure. After a further rest period of 10 days, the animals were again challenged but with 10% benzyl salicylate in soft white petrolatum applied under occlusion for 24 h. Reactions sites were scored according to Draize scale, 24 h after removing the patch. One (1/20) reaction was observed after the intradermal challenge, and seven (7/20) reactions were observed after the epidermal challenge (Maurer et al., 1980).

4.4.3.2.12. The Freund's complete adjuvant test (FCAT) was conducted using outbred Himalayan white-spotted male and female guinea pigs weighing 400–500 g. Induction was via five intradermal injections of 0.1 ml of a 50:50 mixture of benzyl salicylate and FCA into the neck on days 0, 2, 4, 7 and 9. A 24-h closed patch challenge application was conducted on days 21 and 35 at sub-irritant concentration (0.1%). No reactions were observed (Klecak et al., 1977).

4.4.3.2.13. The sensitization potential of benzyl salicylate was measured in a guinea pig sensitization study using a modified Draize (Draize, 1959) procedure. Ten male and female inbred Hartley strain albino guinea pigs/group with an average weight of 350 g were shaved on both flanks. A

0.1 ml aliquot of 1.25% benzyl salicylate, at 2.5 times the ICC (injection challenge concentration: 0.5%), was injected intradermally at four sites which overlap the 2 axillary and 2 inguinal lymph nodes. The animals were challenged 14 days later by an intradermal injection of 0.1 ml benzyl salicylate into one flank and a topical open application of benzyl salicylate on the other flank at the respective injection challenge concentration of 0.5% and application challenge concentration at 2% (vehicle not provided). Reactions were scored 24 h after challenge treatments. A second challenge was carried out 7 days later. No sensitization was observed (Sharp, 1978).

4.4.3.2.14. Benzyl salicylate was tested in a guinea pig sensitization study using a modified Draize (Draize, 1959) procedure in male and female outbred Himalayan guinea pigs weighing 400–500 g. Induction consisted of ten intradermal injections on alternate days with a dose of 0.05 ml of 0.1% benzyl salicylate in isotonic saline. The animals were challenged on days 35 and 49 with an intradermal injection of 0.05 ml of 0.1% benzyl salicylate in saline. Control animals were also challenged intradermally on days 35 and 49 with benzyl salicylate. Sensitization was not observed (Klecak et al., 1977).

4.4.3.3. Local lymph node assay (LLNA) (Table 10)

4.4.3.3.1. A local lymph node assay was conducted on groups of four female mice. Approximately 25 μl of a 2.5%, 5%, 10%, 25% and 50% w/v preparation of benzyl salicylate in 1:3 EtOH:DEP was applied to the dorsal surface of each ear. The procedure was repeated daily for three consecutive days. Three days after the third application, all the animals were injected via the tail vein with 250 μl of phosphate buffered saline (PBS) containing 20 μCi of 2.0 Ci/mmol specific activity ^{3}H-methyl thymidine. Approximately 5 h later the animals were sacrificed. The draining auricular lymph nodes were removed from each animal and together with the nodes from the other animals in the group were placed in a container of PBS. A single cell suspension was prepared by mechanical disaggregation of lymph nodes through 200-mesh stainless steel gauze. The cell suspensions were then washed three times by centrifugation with approximately 10 ml of PBS. Approximately 3 ml of 5% w/v trichloroacetic acid (TCA) was added and, after overnight precipitation at 4 °C the samples were pelleted by centrifugation and the supernatant was discarded. The cells were then resuspended in approximately 1 ml of TCA. The lymph node suspensions were transferred to scintillation vials and 10 ml scintillant (Optiphase) was added prior to beta-scintillation counting using a Packard Tri-Carb Liquid Scintillation Counter. Under the conditions of the test, benzyl salicylate had the potential to be a skin sensitizer. The EC3 value was calculated to be 2.9% (725 μg/cm^2) (RIFM, 2005).

4.4.3.3.2. A local lymph node assay (LLNA) was conducted in 6–8 week old female CBA/JN mice (4/dose). A 25 μl aliquot of 10% benzyl salicylate in 4:1 acetone/olive oil was applied epidermally to the dorsal portion of the left and right ear lobe of each animal for three consecutive days. A control group was included, which received the vehicle only. On day 6, all mice were injected via the tail vein with 3 H-TdR (20 uCl/250 uL PBS). Five hours after the injection, animals were sacrificed and the draining auricular lymph nodes were excised and pooled for each group. A single cell suspension was prepared and using a quantification of 3 H-TdR incorporation was determined using a beta-scintillation counter. Stimulation Indices (S.I.) based on 1–4 experiments using the MNIC were calculated. Proliferating lymph node cell subpopulations were examined by flow cytometric analysis. The EC3 value was calculated to be 1.5% (375 μg/cm^2) (Yoshida et al., 2000).

4.5. Phototoxicity

4.5.1. In vivo human studies

4.5.1.1. A phototoxicity test was conducted on six female volunteers. A 0.025 ml/2cm^2 aliquot of 3% and 10% benzyl salicylate in 1:1 ethanol/acetone was applied to the left and right side on the back of each subject. The right test side was covered and served as an irritancy control site. Thirty minutes after the application, the test sites were exposed to non-erythrogenic UVA irradiation at 1, 2.5, 5, 10 and 20 J/cm^2. The light source was a bank of four ''blacklight'' fluorescent tubes with an emission spectrum of 320–400 mm housed in a reflector unit. Following irradiation, each test site was examined 4, 24, 48 and 72 h after application. No phototoxic responses were noted (RIFM, 1983b).

4.5.2. In vitro human studies

4.5.2.1. Photohaemolysis was studied using washed human red blood cells suspended in barbitone buffered saline, pH 7.4, at a dilution of 1:500. A 0.1 ml aliquots of 0.1% benzyl salicylate in ethyl alcohol was added to 99 ml of the red blood cell suspension. Aliquots of 5 ml, forming cell monolayers in petri dishes, were exposed to UV-A or UV-B. UV-A exposure was from batteries of four fluorescent tubes (Thorn: Ultraviolet non filter 20 W; irradiance approximately 1.2 mW/cm^2) and UV-B exposure consisted of approximately 1350 mJ/cm^2 from a battery of four Westinghouse FS20 sunlamp fluorescent tubes (irradiance approximately 1.5 mW/cm^2). Exposures for UV-A or

Table 10
Summary of local lymph node assays

EC3 value (%)	EC3 value (μg/cm^2)	Vehicle	References
2.9	725	DEP : EtOH	RIFM (2005)
1.5	375	Acetone:olive oil	Yoshida et al. (2000)

UV-B were for limited times up to 3 h. After the exposure the dishes were kept in the dark for 30 min and then the suspensions were centrifuged, the release hemoglobin in the supernatant being determined as cyanmethaemoglobin. No phototoxic effects were produced (Addo et al., 1982).

4.5.3. Animal studies

4.5.3.1. A phototoxicity study was conducted on groups of Skh:hairless mice (6/group). A 20 µl aliquot of 100% benzyl salicylate and 25% benzyl salicylate in methanol was applied to a 5 cm^2 site on the back of each animal. Thirty minutes later the animals were irradiated. One group was irradiated at a distance of 0.65 m or less by fluorescent blacklight (a bank of 6 Sylvania F40T12BL PUVA lamps with a broadband output of 350 nm) for 1 h to provide a measure dose of 200 RB units. The second group was irradiated at a distance of one meter from a simulated sunlight (Atlas Xenon light source, 6.5 KW long-arc xenon high-pressure burner with power supply, igniter and water cooling system) for 1 h providing a dose of 200 RB units. The treated areas were examined for presence or absence of erythema, scaling, edema, or fissuring at 4, 24, 48, 72 and 96 h after exposure. No phototoxic effects were observed (RIFM, 1983c).

4.5.3.2. In a phototoxicity study, open applications of 5%, 10% and 30% benzyl salicylate in acetone were made to clipped skin sites on five female albino Hartley–Dunkin guinea pigs. The application sites were irradiated with 13 J/cm^2 UV light (array of five National FL 20 S.BLB tubes (UV-A black light 300–400 nm, max 360 nm) at 10 cm for 60 min. Reactions were graded according to Draize at 24 and 48 h after application. No phototoxic effects were observed (RIFM, 1997b).

4.5.3.3. A phototoxicity study was conducted on Himalayan white spotted guinea pigs (10/dose). A 0.025-ml aliquot of benzyl salicylate at 1% or 3% in ethanol, with 2% DMSO added to enhance penetration, was applied to 2 cm^2 skin sites on the flanks. Thirty minutes after the application, the sites on the left flank were irradiated with 20 J/cm^2 UV light from a Westinghouse FS 40 "Black Lamp" (320–400 nm, energy 1×10^4 ergs/cm^2), at 10 cm from the animal. Sites on the right side were not irradiated and served as controls. Reactions were scored at 4, 24, and 48 h after the application. No reactions were observed with 1% benzyl salicylate. With 3%, phototoxic reactions were observed in 10/10 animals (RIFM, 1982).

4.5.3.4. Phototoxicity was evaluated during an associated photoallergy study. Twenty (10/sex) adult albino Dunkin Hartley guinea pigs weighing 300–400 g were used. The animals received a single application of 0.5 ml of 10% benzyl salicylate in absolute ethanol under an occlusive patch for 90 min on the anterior part of the back. Irradiation was carried out using a system of fluorescent lamps with continuous spectrum emission: 4000–3100, Mazadaflour black light fluorescent map: 3500–2850 Westinghouse sun fluorescent lamp. Radiation emitted by these lamps was principally in the UVA range (wavelength from 4000 to 3150 A) and in the UVB range (wavelength from 3150 to 2900 A). The two lamps used were placed 10 cm from the back of each animal and irradiated for 5 min. The total radiation dose was 12.5 J/cm^2 and the rage of UVB was 1%. Evaluation of the test sites was made at 6 and 24 h after irradiation. No phototoxic effects were observed (RIFM, 1983a).

4.6. Photoallergy

4.6.1. Diagnostic photopatch studies in humans

4.6.1.1. No photoallergic reactions were observed when a photopatch test was conducted in 482 patients with 2% benzyl salicylate in petrolatum (Nagareda et al., 1992, 1996).

4.6.1.2. Photopatch testing was conducted on 386 patients with suspected contact dermatitis from cosmetic and toiletry products. Benzyl salicylate at 2% in petrolatum produced no photoallergic reactions (Sugai, 1996).

4.6.1.3. A photopatch test was conducted in two volunteers with 10% benzyl salicylate in dimethylphthalate. No photoallergic reactions were observed (Galosi and Plewing, 1982).

4.6.1.4. Benzyl salicylate at 2% in petrolatum was photopatch tested in 706 patients with contact dermatitis. No photoallergic reactions were observed (Katoh et al., 1995).

4.6.2. Animal studies

4.6.2.1. A photoallergy study was conducted on 2 groups of adult albino Dunkin–Hartley guinea pigs: Group I consisted of 5 animals and Group II consisted of 20 animals. On day 1, 0.5 ml of benzyl salicylate at 10% in absolute ethanol was placed on a gauze pad, which was then applied to the middle of the anterior portion of the back for 90 min under occlusion. Group I and Group II animals were treated in the same manner, however, only Group II animals were irradiated with UV light at the patch removal. The posterior part of the back was covered with an aluminum paper sheet so that only the anterior portion of the back was irradiated. Irradiation was conducted with 2 fluorescent lamps for 5 min at a distance of 10 cm (energy was 0.43 J/cm^2). This was followed by additional irradiation from a Mazdafluor black light fluorescent lamp for 90 min at a distance of 5 cm (energy was 12.5 J/cm^2). The sites were examined at 6 and 24 h after the irradiation. On day 4, intradermal injections with 0.1 ml of Freund's Complete Adjuvant were made at each corner of the patch area. A 2nd occluded patch was applied for 90 min under the same conditions, but without the aluminum sheet and plaster. Irradiation was conducted after the patch removal with 2 UV lamps for 15 min at a distance of 5 cm (energy was 1.7 J/cm^2). This was followed by addi-

tional irradiation with a Mazdafluor black light fluorescent lamp for 40 min at a distance of 5 cm (energy was 5.4 J/cm^2). A 3rd and 4th application was made on days 7 and 9, respectively. Each occluded patch was applied for 90 min under the same conditions, but without the aluminum sheet and plaster. Irradiation was conducted after each patch removal with 2 UV lamps (15 min, distance of 5 cm). This was followed by additional irradiation with a Mazdafluor black light fluorescent lamp (40 min, distance of 5 cm). A 12-day rest period (day 10–day 21) was conducted after the 4th application. On day 23, a 90-min challenge application was made to a new test site on the posterior portion of the back, under the same conditions used for the 1st induction application. The anterior portion of the back was covered with an aluminum paper sheet. Irradiation was conducted with a Mazdafluor black light fluorescent lamp for 90 min, at a distance of 5 cm. The total energy was 9 J/cm^2. The irradiated animals were evaluated for reactions at 1, 6, 24, and 48 h after the irradiation. No photoallergic reactions were observed (RIFM, 1983a).

4.7. Absorption, distribution and metabolism

4.7.1. Skin absorption

4.7.1.1. In Vitro human studies

4.7.1.1.1. Penetration of benzyl salicylate through human epidermis was studied using a glass chamber. Human lower abdominal skin was excised from a cadaver; the subcutaneous tissue was removed and the epidermis separated from dermis. The upper surface of the epidermis was fixed to a glass tube which was then placed inside one arm of a U-shaped glass chamber. A 0.5 ml aliquot of saline was added to the chamber and was in complete contact with the bottom of the epidermis. A 0.2 ml aliquot of benzyl salicylate was applied to the top of the epidermis. To avoid evaporation, parafilm was placed over the mouth of the glass tube. The chamber was kept at 21 °C and 55% relative humidity for 72 h. The glass tube was removed from the glass chamber at 72 h and the saline was poured into a test tube. The U-shaped chamber and the bottom of the epidermis attached to the glass tube were both washed three times with saline which was also poured into the same test tube. The final volume in the tube of both original saline and the saline used for washing was approximately 10 ml. Saturated salt water and ether were added to the flask and mixed vigorously. The compound was extracted in ether then dehydrated, filtered and condensed. A 2 μl aliquot of the condensed sample was injected into a Shimazu GC-6A gas chromatograph. The experiment was repeated six times. The amount of benzyl salicylate that penetrated human skin was minimal; the percent penetration±S.E. through excised human skin was 0.031% ± 0.004% (Jimbo, 1983).

4.7.1.2. In Vitro animal studies

4.7.1.2.1. An *in vitro* skin absorption study was conducted on the excised intact skin of a naked rat. The connective and adipose tissue was removed and then the skin was separated into round flaps of approximately 20 cm^2. The skin specimen were marked with areas of 5.0 cm^2 each and benzyl salicylate ^{14}C at 1%, 3%, and 10% in ethanol was applied for 30 s at a dose of 120 μg, 360 μg or 1200 μg active substance/cm^2. The skin specimens were clamped into the penetration chambers with lower part of the prepared skin being in constant contact with the physiological salt solution. The unabsorbed benzyl salicylate was removed from the skin surface at 1, 6, 16 and 24 h after application. The amount of labeled material in the stratum corneum, stripped skin, and in the chamber liquid was also estimated. One hour after application of 1% benzyl salicylate the recovery as a percent of the applied dose was 70.3% as residual material, 7.9% was in the horny layer, 21.9% was in the stripped skin and minimal amounts (0.01 μg/cm^2) in chamber liquid. At 6 h, 60.4% was recovered as residual material, 2.8% in the horny layer, 20% in the stripped skin and 16.8% in the chamber liquid. At 16 h, 34.3% was recovered as residual material, 0.9% in the horny layer, 11.4% in the stripped skin and 53.4 in the chamber liquid. At 24 h, 27.7% was recovered as residual material, 0.4% in the horny layer, 9.3% in the stripped skin and 62.7% in the chamber liquid. One hour after 3% benzyl salicylate was applied to the skin, 62.1% was recovered as residual material, 9.6% in the horny skin, 28.3% in the stripped skin and minimal amounts (0.01 μg/cm^2) in the chamber liquid. At 6 h after application, 57.2% was recovered as residual material, 3.4% in the horny layer, 24.4% in the stripped skin and 15.0% in the chamber liquid. At 16 h after application, 32.6% was recovered as residual material, 1.5% in the horny layer, 16.5% in the stripped skin and 49.4% in the chamber liquid. At 24 h after application, 27.5% was recovered as residual material, 1.6% in the horny layer, 12.0 in the stripped skin and 58.8% in the chamber liquid. The recovery of 10% benzyl salicylate at 1 h after application was 77.5% as residual material, 5.3% in the horny layer, 17.2% in the stripped skin and minimal amounts (0.18 mg/cm^2) in the chamber liquid. At 6 h after application, 68.1% was recovered as residual material, 3.8% in the horny layer, 24.9% in the stripped skin and 3.2% the in chamber liquid. At 16 h after application, 42.3% was recovered as residual material, 5.2% in the horny layer, 25.8% in the stripped skin and 26.7% in the chamber liquid. At 24 h after application, 38% was recovered as residual material, 4.0% in the horny layer, 17.7% in the stripped skin and 40.3% in the chamber liquid (RIFM, 1983d).

4.7.1.2.2. As a part of the same experiment described above, *in vitro* absorption of 1%, 3% or 10% benzyl salicylate in ethanol was evaluated in intact pig skin. Absorption was evaluated at 1, 6, 16 and 24 h after application. The amount of labeled material in the stratum corneum, stripped skin, and in the chamber liquid was also estimated. With 1% benzyl salicylate, recovered radioactivity as a percent of the dose was 93.0% in skin wash, 5.3% in skin stripping, 1.7% in stripped skin and under 1% in receptor fluid at 1 h, 90.9% in skin wash, 5.5% in skin stripping,

3.3% in stripped skin and 0.3% in receptor fluid at 6 h, and 84.5% in skin wash, 7.2% in skin stripping, 4.7% in stripped skin and 3.5% in receptor fluid at 16 h. With 3% benzyl salicylate, recovered radioactivity as a percent of the dose was 92.1% in skin wash, 6.3% in skin stripping, 1.5% in stripped skin and under 1% in receptor fluid at 1 h, 90.7% in skin wash, 6.2% in skin stripping, 2.9% in stripped skin and 0.2% in receptor fluid at 6 h, and 88.7% in skin wash, 5.5% in skin stripping, 4.1% in stripped skin and 1.7% in receptor fluid at 16 h. With 10% benzyl salicylate, recovered radioactivity as a percent of the dose was 94.1% in skin wash, 4.8% in skin stripping, 1.1% in stripped skin and under 1% in receptor fluid at 1 h, 93.4% in skin wash, 5.0% in skin stripping, 1.6% in stripped skin and 0.1% in receptor fluid at 6 h, and 90.7% in skin wash, 4.7% in skin stripping, 3.8% in stripped skin and 0.9% in receptor fluid at 16 h (RIFM, 1983d).

4.8. Subchronic toxicity

No data available on this material.

4.9. Developmental toxicity

No data available on this material.

4.10. Mutagenicity and genotoxicity

4.10.1

A preincubation modification of the *Salmonella*/microsome test was conducted in the presence and absence of liver S9 from Aroclor-induced male Sprague–Dawley rats or male Syrian hamsters using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and/or TA97. Benzyl salicylate at doses of 3.3–333 μg/plate in DMSO did not produce any mutagenic effects (Zeiger et al., 1987).

4.11. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, D. McGinty, L. Jones, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on butyl salicylate

A. Lapczynski *, L. Jones, D. McGinty, S.P. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of butyl salicylate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Butyl salicylate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on butyl salicylate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Benzoic acid, 2-hydroxy-, butyl ester; *n*-butyl, *o*-hydroxybenzoate, butyl salicylate; *n*-butyl salicylate.
1.2 CAS Registry Number: 2052-14-4.
1.3 EINECS Number: 218-142-9.
1.4 Formula: $C_{11}H_{14}O_3$.
1.5 Molecular weight: 194.23.
1.6 COE: butyl salicylate was included by the Council of Europe in the list of substances granted B – information required – none listed – (COE No. 614) (Council of Europe, 2000).
1.7 JECFA: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 901) concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent (JECFA, 2001).
1.8 FEMA: Flavor and Extract Manufacturers' Association States: Generally Recognized as Safe as a flavor ingredient – GRAS 12 (3650) (FEMA, 1979).

2. Physical properties

2.1 Physical form: a colorless liquid.
2.2 Flash point: >200 °F; CC.
2.3 Boiling point: 268 °C.
2.4 log K_{OW} (calculated): 4.08.
2.5 Vapor pressure (calculated): 0.131 mm Hg 25 °C.
2.6 Specific gravity: 1.080.

3. Usage (Table 1)

Butyl salicylate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region <0.1 metric tonnes per annum.

The maximum skin level that results from the use of butyl salicylate in formulae that go into fine fragrances has not been reported. A default value of 0.2% is used, assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has not been reported. As such, a default value of 0.02% is used to calculate maximum daily exposure on the skin of 0.0005 mg/kg for high end users of these products.

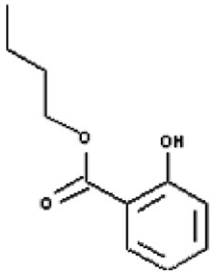

Fig. 1. Butyl Salicylate.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} of butyl salicylate was determined in rats (10/dose). Butyl salicylate was administered at dose levels of 1, 2, 2.5, 3, and 5 g/kg. One animal died at 1 g/kg; 6/10 deaths occurred at 2 g/kg; 9/10 deaths occurred at 2.5 g/kg and 10/10 deaths occurred at 3 and 5 g/kg. The LD_{50} was calculated to be 1.7 g/kg (95% C.I. 1.26–2.29) (RIFM, 1975a).

4.1.2. Dermal studies

4.1.2.1. The dermal LD_{50} of neat butyl salicylate in rabbits exceeded 5 g/kg based on no deaths in four animals tested at that dose (RIFM, 1975a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed when a 48-h closed patch test with 2% butyl salicylate in petrolatum was applied to the backs of five male volunteers (RIFM, 1975b).

4.2.2. Animal studies

4.2.2.1. Irritation was evaluated during the associated LD_{50} study described above. A single application of neat butyl salicylate produced no irritation (RIFM, 1975a).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test was carried out with 2% butyl salicylate in petrolatum on 9 male and 16 female volunteers. Application was under occlusion to the same site on the forearms of all subjects for five alternate-day 48-h

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing butyl salicylate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product	Ingredient/mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.02	0.0001
Face cream	0.80	2.00	1.000	0.003	0.02	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.02	0.0002
Fragrance cream	5.00	0.29	1.000	0.040	0.02	0.0002
Antiperspirant	0.50	1.00	1.000	0.010	0.02	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.02	0.0000
Bath products	17.00	0.29	0.001	0.020	0.02	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.02	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.02	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.02	0.0000
Total						0.0005

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60 kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	No. animals/dose	LD_{50}	References
Oral	Rat	10	1.7 g/kg	RIFM (1975a)
Dermal	Rabbit	4	>5 g/kg	RIFM (1975a)

periods. Patch test sites were pretreated for 24 h with 5% aqueous sodium lauryl sulfate under occlusion. Following a 10-day rest period, a challenge patch was applied to a fresh site for 48 h under occlusion. Reactions to challenge were read at patch removal and 24 h after patch removal. No reactions were observed (RIFM, 1975b).

4.4.2. Animal studies

No data available on this material.

4.5. Photoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution, and metabolism

4.6.1. Percutaneous absorption

4.6.1.1. Human studies

4.6.1.1.1. Brown and Scott (1934) studied the skin absorption of butyl salicylate in one volunteer. The combined surface of both hands was selected as the exposure area to butyl salicylate. To measure the skin absorption, the subject's urine was collected and analyzed for a period of 24 h after exposure. Before applying the chemical, hands were thoroughly scrubbed. Butyl salicylate (2 ml) at a temperature of 38 °C was applied every five minutes for 1 h. Hands were continuously massaged during application. One hour after the first application, hands were rubbed with paper towels so that no butyl salicylate was left on the skin surface. Water was avoided for a period of 4 h. Only a trace amount of butyl salicylate was found in the urine. In the second part of the same experiment, both hands were submerged in an enamel foot tub containing 5 L of water at a temperature of 43–44 °C for 5 min. Hands were removed from the warm water and fast dried. During a five-minute period, hands were massaged with 2 ml/min of butyl salicylate and returned to the water bath without wiping. This procedure was repeated for 1 h. An average of 62 mg of sodium salicylate was recovered in the 24-h urine (Brown and Scott, 1934).

4.6.1.1.2. The absorption of butyl salicylate was determined in 28 healthy male volunteers between the ages of 18 and 36 years. A 0.5 mg aliquot of butyl salicylate in 10 μl of acetone was applied to two 1.4 cm^2 areas of intact skin on the ventral forearm of each subject and the test sites were covered immediately with aluminum foil fixed with surgical tape. The test sites were demarcated with petrolatum prior to butyl salicylate application. The foil was removed from one site immediately after application and from the 2nd site after 4 h. Butyl salicylate was recovered from the foil and from the skin surface. The percentage absorption from 0 to 4 h was calculated as $17.1 \pm 5.3\%$ (mean ± S.E.) (Yano et al., 1986).

4.6.1.1.3. Watkinson et al. (1992) used a mathematical method to estimate the total body absorption of some salicylate esters including butyl salicylate. Rate constants were calculated form the relevant physicochemical properties. The applied dose of active ingredient used in the simulation was 40 $\mu g\,cm^{-2}$ based on the FDA recommendation (200 mg of product per 100 cm^2 of skin) and a value of 2%. The release rate from the formulation was fixed at 1 $\mu m\,cm^{-2}\,h^{-1}$. The simulations were conducted on a 12-h time scale. The estimated total body absorption of butyl salicylate per μg over 1.4 m^2 was 2.4 at 2 h, 57 at 6 h, and 380 at 12 h.

4.6.1.2. Animal studies

4.6.1.2.1. In vitro studies. In an *in vitro* study, hairless mouse skin (8–12 weeks old) was used in a glass flow-through diffusion cell. The dorsal skin was removed following sacrifice and clamped between the upper and lower halves of the diffusion cells. The area of skin exposed to the donor phase was 0.95 cm^2 and the vehicle was acetate

buffer at a pH of 4.0. One ml of butyl salicylate was topically applied to the exposed skin of the donor phase receptor cell chamber perfused with phosphate buffered saline at a rate 5 ml/h. Absorbed fractions in receptor fluid were automatically collected for 10 h and analyzed within 2 h. This prevented hydrolysis in the receptor cell. The steady state flux following topical application of butyl salicylate was 0.014 μmol/cm^2/h (Higo et al., 1995).

4.6.2. Pharmacokinetics

No data available on this material.

4.6.3. Metabolism

4.6.3.1. In vitro studies

4.6.3.1.1.. In conjunction with the above *in vitro* absorption study, the biotransformation of butyl salicylate was also determined. Hairless mouse skin was clamped between the donor and receptor cells of a glass flow-through type diffusion cell. The exposed skin to the donor phase (0.95 cm^2) received 1 ml of butyl salicylate and was pre-treated with l-menthol. The receptor cell chamber was perfused with phosphate buffered saline solution at a rate of 5 ml/h with hourly fractions collected automatically for 10 h and analyzed within 2 h. The absorbed butyl salicylate was 100% metabolized into salicylic acid (Higo et al., 1995).

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, L. Jones, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on *p*-cresyl salicylate

A. Lapczynski *, D. McGinty, L. Jones, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc. 50 Tice Boulevard, Woodcliff Lake, N.J. 07677, USA

Abstract

A toxicologic and dermatologic review of *p*-cresyl salicylate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; *p*-Cresyl salicylate

Contents

* Corresponding author. Tel. +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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doi:10.1016/j.fct.2007.09.055

In 2006, a complete literature search was conducted on *p*-cresyl salicylate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Benzoic acid, 2-hydroxy-, 4-methylphenyl ester; *p*-cresyl salicylate; *p*-Tolyl salicylate.
1.2 CAS Registry Number: 607-88-5.
1.3 EINECS Number: 210-144-8.
1.4 Formula: $C_{14}H_{12}O_3$.
1.5 Molecular Weight: 228.25.

2. Physical properties

2.1 Physical form: White rhomboid crystal.
2.2 Flash point: >200 °F; CC.
2.3 Log K_{ow} (calculated): 4.37.
2.4 Vapor pressure (calculated): <0.001 mm Hg 20 °C.
2.5 Water solubility (calculated): 22.02 mg/l @ 25 °C.
2.6 Melting point: 39 °C.

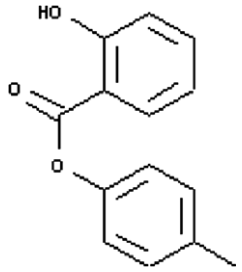

Fig. 1. *p*-Cresyl Salicylate.

3. Usage

p-Cresyl salicylate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of less than 0.01 metric tonnes per annum.

The maximum skin level that results from the use of *p*-cresyl salicylate in formulae that go into fine fragrances has been reported to be 0.001% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.0115% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.0003 mg/kg for high end users of these products (see Table 1).

4. Toxicological data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral toxicity of *p*-cresyl salicylate was calculated to be 1.3 g/kg (95% C.I. 0.99–1.79 g/kg). *p*-Cresyl salicylate was administered by gavage to male Wistar rats (10/dose) at dose levels of 0.76, 1.22, 1.95 and 5 g/kg in Mazola oil. The animals were observed for mortality and systemic effects 3–4 h after dosing and once daily for a 14-day period. Gross necropsy was conducted on all animals. At 0.76 g/kg, one (1/10) animal died; at 1.22 g/kg, 5/10 animals died; at 1.95 g/kg, 8/10 animals died and at 5.0 g/kg all 10/10 animals died. All deaths occurred by day 3. Most survivors were normal on day 14. Clinical signs that were observed included lethargy, tachypnea, diarrhea, ptosis, emaciation, piloeration and chromorhinorrhea. Necropsies were normal in survivors. Abnormalities in animals that died included staining at nose/mouth and anogenital area, congested or hemorrhagic lungs,

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing *p*-cresyl salicylate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.0115	0.0000
Face cream	0.80	2.00	1.000	0.003	0.0115	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.0115	0.0001
Fragrance cream	5.00	0.29	1.000	0.040	0.0115	0.0000
Antiperspirant	0.50	1.00	1.000	0.010	0.0115	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.0115	0.0000
Bath products	17.00	0.29	0.001	0.020	0.0115	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.0115	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.0115	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.0115	0.0000
Total						0.0003

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rats	10	1.3 g/kg	RIFM (1980a)
Dermal	Rabbits	10	>5.0 g/kg	RIFM (1980a)

dilated hearts, gastrointestinal distention. In one high dose rat, blood was found in the bladder (RIFM, 1980a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} of *p*-cresyl salicylate in New Zealand white rabbits was reported to be greater than 5 g/kg based on 2/10 deaths at that dose. A 50% (w/v) mixture of *p*-cresyl salicylate in Mazola Oil, was applied at a dose of 5.0 g/kg under occlusion to clipped intact or abraded skin on the rabbit's abdomen over a 200 cm^2 area (about 10% of body surface) for 24 h. Animals were observed for signs of toxicity and mortality over a period of 14 days. Gross necropsy was conducted on all animals. Clinical signs that were observed included yellow nasal discharge, diarrhea, few feces, and lethargy. Necropsy results revealed congested lungs and dilated hearts. Necropsy findings in survivors were normal (RIFM, 1980a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed after a 48 h closed patch test in which 4% *p*-cresyl salicylate in petrolatum was applied to the backs of 25 volunteers (RIFM, 1980b).

4.2.2. Animal studies

4.2.2.1. Irritation was evaluated during the associated LD_{50} study described above. Reactions were scored using the Draize system and included mild to well defined erythema and very slight to severe edema (RIFM, 1980a).

4.3. Mucous membrane (eye) irritation

No data available for this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out on 25 volunteers using 4% *p*-cresyl salicylate in petrolatum. A Webril® patch with 0.3 g of *p*-cresyl salicylate, covered with a 15 mm aluminum chamber was occlusively applied to the volar aspect of the forearm and held in place with Scanpor® tape. The dressing was applied to the same site for five 48-hour periods. Patch sites were pre-treated with 1% sodium lauryl sulfate (SLS). Following a 10-day rest period, a challenge patch was applied in the same manner to a fresh site for 48 h. A 10% aqueous solution of SLS was used during the challenge. Reactions were read at patch removal and 24 h later. No sensitization reactions were observed (RIFM, 1980b).

4.4.2. Animal studies

No data available on this material.

4.5. Phototoxicity and photoallergy

No data available for this material.

4.6. Absorption, distribution, metabolism

No data available for this material.

4.7. Subchronic toxicity

No data available for this material.

4.8. Reproductive and developmental toxicity

No data available for this material.

4.9. Mutagenicity and genotoxicity

No data available for this material.

4.10. Carcinogenicity

No data available for this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, D. McGinty, L. Jones, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Belsito, D., Bickers, D., Bruze, M., Calow, P., Greim, H., Hanifin, J.H., Rogers, A.E., Saurat, J.H., Sipes, I.G., Tagami, H., 2007. A toxicologic and dermatologic assessment of salicylates when used as fragrance ingredients. Food and Chemical Toxicology 45 (1S1), S318–S361.

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Review

Fragrance material review on 1,3-dimethyl-3-butenyl salicylate

D. McGinty *, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of 1,3-dimethyl-3-butenyl salicylate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; 1,3-Dimethyl-3-butenyl salicylate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: dmcginty@rifm.org (D. McGinty).

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doi:10.1016/j.fct.2007.09.032

In 2006, a complete literature search was conducted on 1,3-dimethyl-3-butenyl salicylate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Benzoic acid, 2-hydroxy-, 1,3-dimethyl-3-butenyl ester.
1.2 CAS registry number: 80118-10-1.
1.3 EINECS number: 279-400-4.
1.4 Formula: $C_{13}H_{16}O_3$.

2. Physical properties

2.1 Log K_{ow} (calculated): 4.91.

3. Usage (Table 1)

1,3-Dimethyl-3-butenyl salicylate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 0.1–1.0 metric tonnes per annum.

The maximum skin level that results from the use of 1,3-dimethyl-3-butenyl salicylate in formulae that go into fine fragrances has not been reported. A default value of 0.02% is used, assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has not been reported. As such, a default value of 0.02% is used to calculate a maximum daily exposure on the skin of 0.0005 mg/kg for high end users of these products.

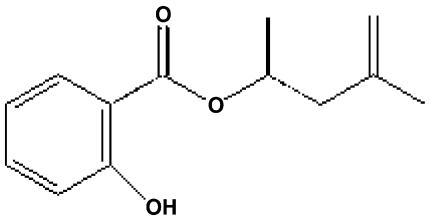

Fig. 1. 1,3-Dimethyl-3-butenyl salicylate.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} value in male and female albino Sherman-Wistar rats (5/sex) was reported to be greater than 5.0 g/kg based on 4/10 deaths at that dose. The rats were observed for mortality and/or systemic effects over a period of 14 days. A gross necropsy was conducted on all animals. Deaths occurred on days two and four. Clinical signs observed during the study included severe depression and ataxia. Gross observations at necropsy were normal (RIFM, 1981a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} value in male and female albino rabbits (3/sex) exceeded 2 g/kg based on no deaths in 6 rabbits tested at that dose. Neat 1,3-dimethyl-3-butenyl salicylate was applied to abraded skin for 24 h under occlusion. The animals were observed for mortality and/or clinical effects over a 14 day period. Gross necropsy was conducted on all animals. No clinical signs were

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing 1,3-dimethyl-3-butenyl salicylate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.02	0.0001
Face cream	0.80	2.00	1.000	0.003	0.02	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.02	0.0002
Fragrance cream	5.00	0.29	1.000	0.040	0.02	0.0002
Antiperspirant	0.50	1.00	1.000	0.010	0.02	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.02	0.0000
Bath products	17.00	0.29	0.001	0.020	0.02	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.02	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.02	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.02	0.0000
Total						0.0005

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60 kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	No. Animals/dose group	LD_{50}	References
Oral	Rat	10	>5.0 g/kg	RIFM (1981a)
Dermal	Rat	10	>2.0 g/kg	RIFM (1981b)

observed and gross observations at necropsy were normal (RIFM, 1981b).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. Irritation was evaluated during the induction phase of a repeated insult patch test (RIPT) which was conducted on 50 male and female volunteers (9 male/41 female). A 0.2 g sample of 1,3-dimethyl-3-butenyl salicylate at 10% in petrolatum was applied to the upper arm of each subject for 24 h under semi-occlusion. After a 24-h rest period, subjects were again patched at the same site. A total of nine applications were made over a 3-week period. No irritation was observed (RIFM, 1981c).

4.2.2. Animal studies

4.2.2.1. A primary skin irritation study was conducted in albino rabbits. A 0.5 ml aliquot of neat 1,3-dimethyl-3-butenyl salicylate was applied to the intact and abraded skin of six rabbits for 24 h under occlusion. Irritation was evaluated at patch removal and 72 h after patch removal. At 24 h, very slight erythema was observed in all 6/6 animals, and very slight edema was noted in 3/6 animals. No irritation was observed by 72 h. The primary irritation score was 0.63. Under the conditions of the test, 1.3-dimethyl-3-butenyl salicylate was not considered to be a primary skin irritant (RIFM, 1981d).

4.3. Mucous membrane (eye) irritation

4.3.1

A rabbit eye test was conducted in 6 healthy albino rabbits. A 0.1 ml aliquot of neat 1,3-dimethyl-3-butenyl salicylate was instilled into the right eye of each rabbit with no further treatment. The untreated left eye of each rabbit served as a control. Observations were made every 24 h for 3 days and then again on days 5 and 7. Reactions were scored according to Draize. No irritation was observed (RIFM, 1981e).

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A human repeated insult patch test was conducted on 50 male and female volunteers (9 male/41 female). A 0.2 g sample of 1,3-dimethyl-3-butenyl salicylate at 10% in petrolatum was applied to the upper arm of each subject under semi-occlusive patches for 24 h. Each subject received nine 24 h exposures, three times a week for three successive weeks. After a 14-day rest period, a semi-occlusive challenge patch was applied for 24 h to the same site as well as to a previously untreated skin site. Reactions to the challenge were scored at 24, 48, and 72 h after application. No reactions were observed (RIFM, 1981c).

4.4.2. Animal studies

No data available on this material.

4.5. Phototoxicity and photoallergy

4.5.1. Phototoxicity

4.5.1.1. Human studies

4.5.1.1.1. Phototoxicity was evaluated during the induction phase of a photosensitization study which was part of a larger repeated insult patch test study. A subset of 20 volunteers (4 male/16 female) was selected from the original group of 50 volunteers. These 20 subjects were treated simultaneously on the opposite arm with an additional 0.2 g sample of 1,3-dimethyl-3-butenyl salicylate at 10% in petrolatum. The test site was then irradiated for 15 min with UV using a Spectroline Model B-100, Black Light flood lamp (365 nm, 1680 μw/cm^2) at a distance of 15 in. After the UV exposure, the test site was covered with a semi-occlusive patch for 24 h. After patch removal the subjects were allowed to rest for 24 h. 1,3-Dimethyl-3-butenyl salicylate was then patched again at the same site. A total of nine applications were made over a 3-week period. Irradiation was applied at applications 1, 4, 7 and 9. Reactions were read at patch removal and again 24 h later. No phototoxic reactions were observed (RIFM, 1981c).

4.5.1.2. Animal studies. No data available on this material.

4.5.2. Photoallergy

4.5.2.1. Human studies

4.5.2.1.1. Twenty volunteers (4 male/16 female) from a repeated insult patch test were also tested for photoallergy. Each subject was treated simultaneously on the opposite arm with an additional 0.2 g sample of 1,3-dimethyl-3-butenyl salicylate at 10% in petrolatum. The test site was then irradiated for 15 min with UV using a Spectroline Model B-100, Black Light flood lamp (365 nm, 1680 μw/cm^2) at a distance of 15 in. After the UV exposure, the test site was covered with a semi-occlusive patch for 24 h. After patch removal the subjects were allowed to rest for 24 h. 1,3-Dimethyl-3-butenyl salicylate was then patched again at the same site. A total of nine applications were made over a 3-week period. Two weeks after the last induction patch, a 24 h semi-occluded challenge patch was applied to the same site as well as to a virgin site on the same arm. Irradiation was applied at applications 1, 4, 7, 9 and at the challenge application. Reactions were read at 24, 48 and 72 h after application. Photoallergy was not observed (RIFM, 1981c).

4.5.2.2. Animal studies. No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Repeated dose toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

D. McGinty, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on ethyl hexyl salicylate

A. Lapczynski *, D. McGinty, L. Jones, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of ethyl hexyl salicylate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Ethyl hexyl salicylate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on ethyl hexyl salicylate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Benzoic acid, 2-hydroxy-, 2-ethylhexyl ester; Dermoblock OS; Escalol 587; 2-Ethylhexyl 2-hydroxybenzoate; Ethylhexyl salicylate; Ethyl hexyl salicylate; 2-Ethylhexyl salicylate; Eusolex OS; Heliosol 2; Neo Heliopan; Type OS; Neotan L; Salicylic acid, 2-ethylhexyl ester; Trivent OS.
1.2 CAS Registry Number: 118-60-5.
1.3 EINECS Number: 204-263-4.
1.4 Formula: $C_{15}H_{22}O_3$.
1.5 Molecular weight: 250.34.

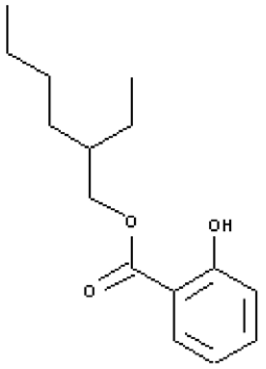

Fig. 1. Ethyl hexyl salicylate.

2. Physical properties

2.1 Physical description: a colorless liquid.
2.2 Flash point: >200 °C.
2.3 Log K_{ow} (calculated): 6.02.
2.4 Vapor pressure (calculated): 0.00000436 mm Hg 25 °C.
2.5 Water Solubility (calculated): 0.7171 mg/l @ 25 °C.

3. Usage (Table 1)

Ethyl hexyl salicylate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 0.1–1.0 metric tonnes per annum.

The maximum skin level that results from the use of ethyl hexyl salicylate in formulae that go into fine fragrances has not been reported. A default value of 0.02% is used, assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has not been reported. As such a default value of 0.02% is used to calculate a maximum daily exposure on the skin of 0.0005 mg/kg for high end users of these products.

4. Toxicological data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} of ethyl hexyl salicylate exceeded 5.0 g/kg based on 1/10 deaths at that dose. Animals received a single oral administration of ethyl hexyl salicylate at a dose level of 5.0 g/kg. Mortality and/or clinical signs were observed for 14 days. One animal died on

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing ethyl hexyl salicylate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.02	0.0001
Face cream	0.80	2.00	1.000	0.003	0.02	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.02	0.0002
Fragrance cream	5.00	0.29	1.000	0.040	0.02	0.0002
Antiperspirant	0.50	1.00	1.000	0.010	0.02	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.02	0.0000
Bath products	17.00	0.29	0.001	0.020	0.02	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.02	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.02	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.02	0.0000
Total						0.0005

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	No. animals/ dose group	LD_{50} (g/kg)	References
Oral	Rats	10	>5.0	RIFM (1974a)
Dermal	Rabbits	4	>5.0	RIFM (1974a)
i.p.	Mice	Not specified	0.2–0.3	Doull et al. (1962)

the 6th day of study. No clinical reactions were observed (RIFM, 1974a).

4.1.2. Dermal studies

4.1.2.1. The dermal LD_{50} in rabbits exceeded 5.0 g/kg based on no deaths in 4 animals tested at that dose. Neat ethyl hexyl salicylate was applied to intact or abraded skin for 24 h under occlusion. The animals were observed for mortality and/or clinical signs for a 14-day period. No clinical signs were observed (RIFM, 1974a).

4.1.3. Intraperitoneal injection

4.1.3.1. In a preliminary toxicity study that was conducted prior to testing compounds for possible radioprotective activity, groups of 10 adult CF_1 strain mice, weighing 20–25 g, were injected intraperitoneally with ethyl hexyl salicylate in propylene glycol. The animals were then observed over a 7-day period. The approximate LD_{50} was reported to be 0.2–0.3 g/kg (no further details reported) (Doull et al., 1962).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed after a 48-h closed patch test was conducted with 4% ethyl hexyl salicylate in petrolatum on the backs of 23 male volunteers (RIFM, 1974b).

4.2.2. Animal studies

4.2.2.1. Irritation was evaluated during the associated LD_{50} study described above. Mild erythema lasting 24 h was observed (RIFM, 1974a).

4.3. Mucous membrane (eye) irritation

No data available for this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 4% ethyl hexyl salicylate in petrolatum on 23 healthy males. Application was under occlusion to the same site on the forearms of all subjects for five alternate day, 48-h periods. Patch test sites were pre-treated for 24 h with 5% aqueous sodium lauryl sulfate under occlusion. Following a 10-day rest period, a challenge patch was applied to a fresh site for 48 h under occlusion. Challenge patch applications were preceded with a 30-min application of 2% aqueous sodium lauryl sulfate under occlusion to different sites. Reactions to challenge were read at patch removal and 24 h after patch removal. No sensitization reactions were observed (RIFM, 1974b).

4.4.2. Animal studies

No data available for this material.

4.5. Phototoxicity and photoallergy

No data available for this material.

4.6. Absorption, distribution, metabolism

4.6.1. In vivo human studies

4.6.1.1. The absorption of 3% ethyl hexyl salicylate in two different vehicles, petrolatum or an O/W emulsion–gel, was evaluated in four healthy volunteers. Areas (10 cm^2) were randomly allocated on the back, and ethyl hexyl salicylate in either petroleum or the emulsion–gel was applied at a dose of 2 mg/cm^2. At 30 min, and 2 and 6 h later, any ethyl hexyl salicylate that had not penetrated was removed with a paper towel. Fifteen strips (10×20 mm) were then made with cellux tape. Strips 1–5, 6–10, and 11–15 were pooled separately and extracted with methanol. The maximal stratum corneum levels of ethyl hexyl salicylate were obtained at 30 min, in strips 1–5, with 40–50% of the applied dose obtained when applied in the emulsion–gel and 10–15% when applied in petrolatum (Treffel and Gabard, 1996).

4.6.2. In vitro human studies

4.6.2.1. The *in vitro* penetration of ethyl hexyl salicylate was evaluated using skin samples (600 μm) obtained from two women undergoing abdominoplasty. Skin penetration was measured using static diffusion Franz cells with a 1.76 cm^2 surface area of exposed skin. The ethyl hexyl salicylate (3%) was applied at a dose of 2.26 ± 0.21 mg/cm^2 and 2.52 ± 0.4 mg/cm^2 in an emulsion–gel or petrolatum, respectively. The receptor fluid was physiological saline with albumine (1.5% w/v) maintained at 36.5 °C. Four application times were investigated 2 min, 0.5 h, 2 and 6 h. At 2 min, 0.94 ± 0.4% and 1.81 ± 0.7% of the applied dose was found in the epidermis when applied in the emulsion–gel or applied in petrolatum, respectively; and 0.46 ± 0.8% was found in the dermis when applied in petrolatum. At 0.5 h, 2.13 ± 0.7% and 0.60 ± 0.5% of the applied dose was found in the epidermis when applied in the emulsion–gel or petrolatum, respectively. At 2 h, 1.54 ± 0.3% and 1.97 ± 0.8 of the applied dose was found in the epidermis when applied in the emulsion–gel or petrolatum, respectively. At 6 h, 7.29 ± 1.8% and 1.96 ± 0.2% of the applied dose were found in the epidermis when applied in the emulsion–gel or petrolatum, respectively; and 0.51 ± 0.7% was found in the dermis when applied in the emulsion–gel. No ethyl hexyl salicylate was recovered in

the receptor fluid. The major amount of ethyl hexyl salicylate was recovered in the wash. The average total recovery of the applied dose at 2 min was 113 ± 20% for the emulsion–gel, and 54 ± 17% for petrolatum; at 0.5 h, the total recovery was 83 ± 11% for emulsion–gel and 40 ± 4% for petrolatum; at 2 h, the total recovery was 80 ± 4% for the emulsion–gel and 47 ± 6% for petrolatum; at 6 h, the total recovery was 68 ± 8% for the emulsion–gel and 54 ± 1 for petrolatum (Treffel and Gabard, 1996).

4.6.2.2. Watkinson et al. (1992) used a mathematical method to estimate the total body absorption of some salicylate esters including ethyl hexyl salicylate. Rate constants were calculated from the relevant physicochemical properties. The applied dose of the active ingredient used in the simulation was 40 μg cm^{-2} based on the FDA recommendation (200 mg of product per 100 cm^2 of skin) and a value of 2%. The release rate from the formulation was fixed at 1 μm cm^{-2} h^{-1}. The simulations were conducted on a 12-h time scale. The estimated total body absorption of ethyl hexyl salicylate per μg over 1.4 m^2 was 0.022 at 2 h, 0.5 at 6 h and 3.3 at 12 h.

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data are available on this material.

4.10. Carcinogenicity

No data are available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, D. McGinty, L. Jones, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on ethyl salicylate

A. Lapczynski *, D. McGinty, L. Jones, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicological and dermatological review of ethyl salicylate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Ethyl salicylate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on ethyl salicylate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1. Synonyms: Benzoic acid, 2-hydroxy-, ethyl ester; ethyl 2-hydroxybenzoate; ethyl *o*-hydroxybenzoate; ethyl salicylate; salicylic ether.
1.2. CAS Number: 118-61-6.
1.3. EINECS: 204-265-5.
1.4. Formula: $C_9H_{10}O_3$.
1.5. Molecular weight: 166.18.
1.6. COE: Ethyl salicylate was included by the Council of Europe in the list of substances granted B – information required – hydrolysis study (COE No. 432) (COE, 2000).
1.7. FEMA: Flavor and Extract Manufacturers' Association States: Generally recognized as safe as a flavor ingredient GRAS 3 (FEMA, 2458) (FEMA, 1965).
1.8. FDA: Ethyl salicylate was approved by the Food and Drug Administration as a flavor (21 CFR 172.515).
1.9. JECFA: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 900) concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent (JECFA, 2001).

2. Physical properties

2.1. Physical description: A clear colorless to very pale yellow liquid having a wintergreen-type odor.
2.2. Flash point: >212 °F; °CC.
2.3. Boiling point: 234 °C.
2.4. Log K_{OW} (calculated): 3.09.
2.5. Specific gravity 20 °C (I.A): 1.129–1.131.
2.6. Specific gravity 25 °C (I.A): 1.127–1.129.

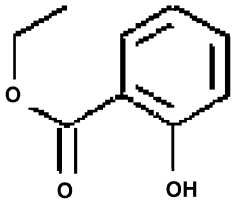

Fig. 1. Ethyl salicylate.

2.7. Vapor pressure (calculated): 0.05 mm Hg 20 °C.
2.8. Water solubility (calculated): 737.1 mg/l @ 25 °C.

3. Usage (Table 1)

Ethyl salicylate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 1–10 metric tones per annum.

The maximum skin level that results from the use of ethyl salicylate in formulae that go into fine fragrances has been reported to be 0.14% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.0064% (IFRA, 2002), which would result in a maximum daily exposure on the skin of 0.0002 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral toxicity of ethyl salicylate was determined in rats (10/dose). Ethyl salicylate was administered at dose levels of 0.34, 0.67, 1.31, 2.56, and 5.0 g/kg. The LD_{50} was calculated to be 1.32 g/kg (95% CI 1.01–1.63 g/kg). Animals were observed over a 14-day period. No deaths occurred at 0.34 and 0.67 g/kg; 5/10 animals died at 1.31 g/kg and 10/10 animals died at both 2.56 and 5.0 g/kg. Most deaths occurred on day one. Tremors, labored breathing and flaccidity were observed at 5.0 g/kg (RIFM, 1976a).

4.1.1.2. To determine the effect of ethyl salicylate in dogs, neat ethyl salicylate (5–20 cc) in soft gelatin capsules was given on successive days (up to nine days). Observations were made daily for mortality and clinical signs and bodyweights were monitored. Nausea, vomiting, diarrhea and a decrease in bodyweight were observed (no further details reported) (Houghton, 1905).

4.1.1.3. The minimum oral fatal dose of ethyl salicylate in guinea pigs was reported to be 0.0014 g/kg bodyweight (no further details reported) (Houghton, 1905).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg based on no deaths in 10 rabbits at that dose. Neat ethyl salicylate was applied to intact or abraded skin for 24 h under occlusion. Observations for mortality and clinical signs were conducted over a 14-day period (RIFM, 1976a).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing ethyl salicylate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/ day[b]
Body lotion	8.00	0.71	1.000	0.004	0.0064	0.0000
Face cream	0.80	2.00	1.000	0.003	0.0064	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.0064	0.0001
Fragrance cream	5.00	0.29	1.000	0.040	0.0064	0.0001
Antiperspirant	0.50	1.00	1.000	0.010	0.0064	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.0064	0.0000
Bath products	17.00	0.29	0.001	0.020	0.0064	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.0064	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.0064	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.0064	0.0000
Total						0.0002

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity data

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rats	10	1.32 g/kg	RIFM (1976a)
Dermal	Rabbits	10	>5.0 g/kg	RIFM (1976a)

4.1.3. Subcutaneous studies

4.1.3.1. The minimum subcutaneous fatal dose of ethyl salicylate in guinea pigs was reported to be 0.0015 g/kg of bodyweight (no further details reported) (Houghton, 1905).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed after a 48-hour closed patch with 12% ethyl salicylate in petrolatum was applied to the volar forearm or back of 25 adult volunteers (RIFM, 1976b).

4.2.2. Animal studies

4.2.2.1. Irritation was evaluated during the associated LD_{50} study described above. In rabbits, slight (3/10 rabbits) to moderate (7/10) erythema and moderate edema (9/10) were observed (RIFM, 1976a).

4.3. Mucous membrane (eye) irritation

No data are available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test was carried out with 12% ethyl salicylate in petrolatum on 25 adult volunteers. Application was under occlusion to the same site on the volar forearms or backs for five alternate day, 48-hour periods. Patch test sites were pre-treated for 24 h with 2.5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a 10-day rest period, a challenge patch was applied to a fresh site for 48 h under occlusion. Reactions to challenge were read at patch removal and 24 h after patch removal. No reactions were observed (RIFM, 1976b).

4.4.2. Animal studies

4.4.2.1. A closed epicutaneous test (CET) was conducted in guinea pigs. Induction consisted of six 48-hour closed patch applications on the nape using Torii's patch plaster and adhesive tape. Induction applications were made three times a week for two weeks with 30% ethyl salicylate. On day 28, the animals were challenged with 1% ethyl salicylate. Challenge application was a 48-hour closed patch on the clipped and shaved flank using Finn Chambers® and adhesive tape. Reactions were read at patch removal and 24 and 48 h after patch removal. No reactions were observed (Ishihara et al., 1986).

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

4.6.1. Absorption

4.6.1.1. Human studies

4.6.1.1.1. The percutaneous absorption of ethyl salicylate was studied in 2 male volunteers. Both hands of each worker were submerged in an enamel foot tub containing five liters of water at a temperature of 43–44 °C for five min. Next, the hands were rapidly dried and massaged for 5 min with 2 cc of ethyl salicylate applied every minute. After 5 min, the hands (which were not wiped) were submerged again and the procedure was repeated for an hour. The combined surface of both hands was selected as the exposure area. To measure the absorption of ethyl salicylate, urine was collected for a 24-hour period after

exposure and analyzed. The average excretion of sodium salicylate of one worker was 93 mg and the second worker was 102 mg (Brown and Scott, 1934).

4.6.1.1.2. The absorption of ethyl salicylate was determined in 28 healthy male volunteers between the ages of 18 and 36 years. A 0.5 mg aliquot of ethyl salicylate in 10 μl of acetone was applied to two 1.4 cm^2 areas of intact skin on the ventral forearm of each subject. The test sites were demarcated with petrolatum prior to ethyl salicylate application. The foil was removed from one site immediately after application and from the 2nd site after 4 h. Ethyl salicylate was recovered from the foil and from the skin surface. The percentage absorption from 0 to 4 h was reported to be 58.6% ± 6.6 (mean ± S.E.) (Yano et al., 1986).

4.6.1.2. Animal studies

4.6.1.2.1. In vivo animal studies

4.6.1.2.1.1. The effect of pH on the absorption of ethyl salicylate was determined in white male Sprague–Dawley rats. One hour prior to the test, the tails were washed with distilled water. The washed tails were immersed in a solution (ethyl salicylate, glycine buffer and 5% ethanol) in a perfusion container (19.5 × 2.5 cm) with a 68 ml capacity, and the container was sealed to prevent contamination and solvent evaporation. The container was immersed in a temperature controlled water bath. The test duration was approximately 45 min. Absorbance was continuously recorded at 240 nm wavelength to obtain an absorption rate constant and the total amount of ethyl salicylate absorbed at pH 2, pH 3, pH 6, pH 8 was measured. A standard curve was established and absorption of 1.97 and 1.53 was observed at pH 2 and 3, respectively. No absorption was observed at pH 6 or 8. The authors stated that the lack of absorption at pH 6 and 8 may have been due to hydrolysis, and the hydrolysis product, salicylic acid, is not absorbable at these high pH values (Siddiqi and Ritschel, 1972).

4.6.1.2.2. In vitro animal studies

4.6.1.2.2.1. In an *in vitro* study, hairless mouse skin (8–12 weeks old) was used in a glass flow-through diffusion cell. The dorsal skin was removed following sacrifice and clamped between the upper and lower halves of the diffusion cells. The area of skin exposed to the donor phase was 0.95 cm^2 and the vehicle was acetate buffer at a pH of 4.0. One ml of ethyl salicylate was topically applied to the exposed skin of the donor phase receptor cell chamber perfused with phosphate buffered saline at a rate 5 ml/h. Absorbed fractions in receptor fluid were automatically collected for 10 h and analyzed within 2 h. This prevented hydrolysis in the receptor cell. The steady state flux following topical application of ethyl salicylate was 0.72 ± 0.06 μmol/cm^2/h (Higo et al., 1995).

4.6.2. Pharmacokinetics

No data available on this material.

4.6.3. Metabolism

4.6.3.1. In vitro animal studies

4.6.3.1.1. In conjunction with the above *in vitro* absorption study, the biotransformation of ethyl salicylate was also determined. Hairless mouse skin was clamped between the donor and receptor cells of a glass flow-through type diffusion cell. The exposed skin to the donor phase (0.95 cm^2) received 1 ml of ethyl salicylate and was pretreated with l-menthol. The receptor cell chamber was perfused with phosphate buffered saline solution at a rate of 5 ml/h with hourly fractions collected automatically for 10 h and analyzed within 2 h. Approximately 25–30% of the absorbed ethyl salicylate was metabolized to salicylic acid (Higo et al., 1995).

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, D. McGinty, L. Jones, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on *cis*-3-hexenyl salicylate

A. Lapczynski *, D. McGinty, L. Jones, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of *cis*-3-hexenyl salicylate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; *cis*-3-Hexenyl salicylate

Contents

In 2006, a complete literature search was conducted on *cis*-3-hexenyl salicylate. On-line databases that were surveyed included Chemical Abstract Services and the

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Benzoic acid, 2-hydroxy-, 3-hexenyl ester, (Z)-; 3-hexenyl 2-hydroxybenzoate; (Z)-3-hexenyl salicylate; *cis*-3-hexenyl salicylate.
1.2 CAS Registry Number: 65405-77-8.
1.3 EINECS Number: 265-745-8.
1.4 Formula: $C_{13}H_{16}O_3$.
1.5 Molecular weight: 220.27.
1.6 COE: *cis*-3-Hexenyl salicylate was included by the Council of Europe in the list of substances granted waiting (COE No. 10685).

2. Physical properties

2.1 Physical description: A colorless oily liquid.
2.2 Flash point: >200 F; CC.
2.3 Boiling point: 145 °C @ 5 mm Hg.
2.4 Log K_{ow} (calculated): 4.84.
2.5 Vapor pressure (calculated): <0.001 mm Hg 20 °C.
2.6 Specific gravity: 1.06.

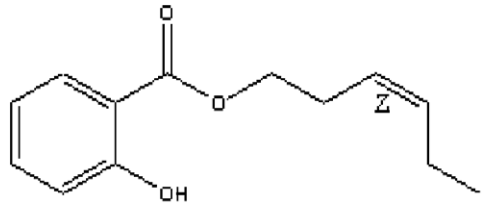

Fig. 1. *cis*-3-Hexenyl salicylate.

3. Usage (Table 1)

cis-3-Hexenyl salicylate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 100–1000 metric tonnes per annum.

The maximum skin level that results from the use of *cis*-3-hexenl salicylate in formulae that go into fine fragrances has been reported to be 2.02% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 3.9611% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.1009 mg/kg for high end users of these products.

4. Toxicological data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral toxicity of *cis*-3-hexenyl salicylate was evaluated in ten rats. The LD_{50} was approximately 5.0 g/kg based on five deaths at that dose. The animals were observed over a 14-day period for mortality and systemic effects. No clinical signs were reported (RIFM, 1975a).

4.1.2. Dermal studies

4.1.2.1. The dermal LD_{50} in rabbits exceeded 5.0 g/kg based on no deaths in ten animals tested at that dose. Neat *cis*-3-hexenyl salicylate was applied to intact or abraded skin for 24 h under occlusion. The animals were observed for mortality and systemic effects over a 14-day period. No clinical signs were reported (RIFM, 1975a).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing *cis*-3-hexenyl salicylate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	3.96	0.0150
Face cream	0.80	2.00	1.000	0.003	3.96	0.0032
Eau de toilette	0.75	1.00	1.000	0.080	3.96	0.0396
Fragrance cream	5.00	0.29	1.000	0.040	3.96	0.0383
Antiperspirant	0.50	1.00	1.000	0.010	3.96	0.0033
Shampoo	8.00	1.00	0.010	0.005	3.96	0.0003
Bath products	17.00	0.29	0.001	0.020	3.96	0.0001
Shower gel	5.00	1.07	0.010	0.012	3.96	0.0004
Toilet soap	0.80	6.00	0.010	0.015	3.96	0.0005
Hair spray	5.00	2.00	0.010	0.005	3.96	0.0003
Total						0.1009

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rats	10	~5 g/kg	RIFM (1975a)
Dermal	Rabbits	10	>5 g/kg	RIFM (1975a)

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, irritation was not observed after a 48-h closed patch test with 3% *cis*-3-hexenyl salicylate in petrolatum on the backs of five volunteers (RIFM, 1975b).

4.2.2. Animal studies (Table 3)

4.2.2.1. Irritation was evaluated as a part of a phototoxicity study which was conducted in 4 female Heartly albino guinea pigs. Hair on the back of each animal was clipped and shaved. Four hours later, open applications with 5, 10, 30 and 50% *cis*-3-hexenyl salicylate in acetone were made to 1.5 cm circular areas on the back of each animal. The test sites were scored 24 and 48 h after application. No irritation was observed (RIFM, 1999).

4.2.2.2. Irritation was evaluated during the associated LD_{50} study described above. Neat *cis*-3-hexenyl salicylate applied to the skin of rabbits for 24 h under occlusion produced slight (1/10 animals) to moderate (9/10 animals) erythema and slight (4/10 animals) to moderate (5/10 animals) edema (RIFM, 1975a).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test was carried out with 3% *cis*-3-hexenyl salicylate (2070 μg/cm^2) in petrolatum on 25 volunteers (8 male/17 female). Application was under occlusion to the same site on the forearms of all subjects for five alternate day, 48-h periods. Patch test sites were pre-treated for 24 h with 5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a 10-day rest period, a challenge patch was applied to a fresh site for 48 h under occlusion. Reactions to challenge were read at patch removal and 24 h after patch removal. No sensitization reactions were observed (RIFM, 1975b).

Table 3
Summary of animal irritation studies

Method	Dose (%)	Species	Results	References
Irritation evaluated as part of a phototoxicity study	5, 10, 30, or 50%	Guinea Pigs	0/4	RIFM (1999)
Irritation evaluated as part of a dermal LD_{50} study	100%	Rabbits	10/10 erythema 9/10 edema	RIFM (1975a)

4.4.2. Animal studies (Table 4)

4.4.2.1. A maximization test (Magnusson and Kligman, 1969) was conducted on five female Heartly albino guinea pigs, weighing 395–455 g/kg. The induction phase consisted of intradermal (i.d.) injections (10% *cis*-3-hexenyl salicylate in FCA and 10% in FCA plus physiological saline) followed approximately 1 week later by an occluded topical induction patch (10% in FCA). After a rest period, the animals were challenged with a topical application of 5, 10, 20 or 40% *cis*-3-hexenyl salicylate in propylene glycol plus acetone. Reactions were scored according to Draize at 24 and 48 h. No reactions were observed at 5, 10 and 20%. At 40%, two sensitization reactions were observed. *cis*-3-Hexenyl salicylate was considered a weak sensitizer at 40% (no further details reported) (RIFM, 1999).

4.4.2.2. An open epicutaneous test (OET) was conducted with 3% *cis*-3-hexenyl salicylate on groups of 6–8 male and female guinea pigs. An open application of a 0.1 ml aliquot of *cis*-3-hexenyl salicylate was made to a clipped 8 cm^2 area on the flank of each guinea pig once daily for 3 weeks. A total of 21 applications were made over a 3-week period. Open challenge applications were made on the contralateral flank days 21 and 35. No sensitization reactions were observed (no further details reported) (Klecak, 1985).

4.5. Phototoxicity and photoallergy

4.5.1. Animal studies

4.5.1.1. The phototoxic effects of *cis*-3-hexenyl salicylate were evaluated in five female Hartley albino guinea pigs weighing 365–380 g. Four hours after depilation, *cis*-3-hexenyl salicylate at concentrations of 5%, 10%, 30% and 50% in acetone was applied to a 1.5 cm circular area in the depilated area on both sides of the animal. Four applications were made on each side. Immediately after application, one side was covered with aluminum foil, while the other side was irradiated with UV from five Toshiba model FL-40 BLB lamps (320–400 nm) equipped with window glass filter to eliminate radiation below 320 nm. The distance between the light and the skin was 10 cm and the duration of exposure to was 70 min. Observations were made 24 and 48 h after irradiation. Skin reactions were

Table 4
Summary of guinea pig sensitization studies

Method	Induction concentration	Challenge concentration	Results	References
MAX	10% intradermal 10% dermal	5, 10, 20 or 40%	0/5	RIFM (1999)
OET	3%	3%	0/6–8	Klecak (1985)

scored according to Draize. No phototoxic reactions were observed (RIFM, 1999).

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxocity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, D. McGinty, L. Jones, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on *trans*-2-hexenyl salicylate

A. Lapczynski *, D. McGinty, L. Jones, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of *trans*-2-hexenyl salicylate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; *trans*-2-Hexenyl salicylate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on *trans*-2-hexenyl salicylate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: (E)-2-hexenyl salicylate; salicylic acid, 2-hexenyl ester, (e).
1.2 CAS Registry No.: 68133-77-7.
1.3 EINECS No.: 268-704-2.
1.4 Formula: $C_{13}H_{16}O_3$.
1.5 Molecular weight: 220.27.

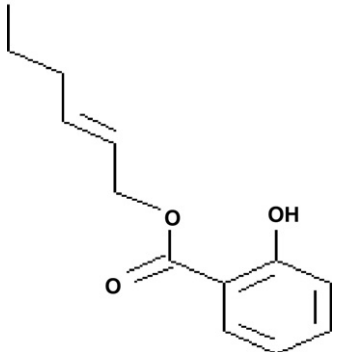

Fig. 1. *trans*-2-Hexenyl salicylate.

2. Physical properties

2.1 Log K_{ow} (calculated): 4.84.
2.2 Henry's Law (calculated): 0.0000165 atm m^3/mol 25 C.
2.3 Vapor pressure (calculated): 0.0000195 mm Hg 25 C.
2.4 Water solubility: 9.518 mg/l@ 25 C.

3. Usage (Table 1)

trans-2-Hexenyl salicylate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region <0.1 metric tonnes per annum.

The maximum skin level that results from the use of *trans*-2-hexenyl salicylate in formulae that go into fine fragrances has been reported to be 0.17% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 3.75% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.0955 mg/kg for high end users.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Groups of rats (10/dose) were dosed orally at levels of 2.47, 3.51, 5.0 and 7.12 g/kg. The LD_{50} was calculated to be 4.43 g/kg (95% C.I. 3.86–5.10 g/kg). Observations for morality and systemic effects were made over a 14-day period. At 2.47 g/kg, no deaths were observed; 1/10 animal died at 3.51 g/kg; 8/10 deaths were observed at 5.0 g/kg and

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing *trans*-2-hexenyl salicylate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/mixture [a]	Ingredient (mg/kg/day) [b]
Body lotion	8.00	0.71	1.000	0.004	3.75	0.0142
Face cream	0.80	2.00	1.000	0.003	3.75	0.0030
Eau de toilette	0.75	1.00	1.000	0.080	3.75	0.0375
Fragrance cream	5.00	0.29	1.000	0.040	3.75	0.0362
Antiperspirant	0.50	1.00	1.000	0.010	3.75	0.0031
Shampoo	8.00	1.00	0.010	0.005	3.75	0.0002
Bath products	17.00	0.29	0.001	0.020	3.75	0.0001
Shower gel	5.00	1.07	0.010	0.012	3.75	0.0004
Toilet soap	0.80	6.00	0.010	0.015	3.75	0.0004
Hair spray	5.00	2.00	0.010	0.005	3.75	0.0003
Total						0.0955

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	No. animals/ dose group	LD_{50}	References
Oral	Rat	10	4.43 g/kg	RIFM (1978a)
Dermal	Rabbit	10	>5.0 g/kg	RIFM (1978a)

7.12 g/kg. Clinical signs observed included lethargy and diarrhea at 3.51 g/kg; lethargy, ataxia, chromorhinorrhea, chromodacryorrhea, piloerection, bleeding from ears, head tilted to one side, and swinging head from side to side at 5.0 g/kg; diarrhea, prostration, convulsion, lethargy, ataxia, piloerection, and emaciation at 7.12 g/kg (RIFM, 1978a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg based on 0/10 deaths at that dose. Neat *trans*-2-hexenyl salicylate was applied at a dose level of 5.0 g/kg body weight to intact and abraded skin for 24 h under occlusion. Observations for mortality and/or systemic effects were made for 14 days. Clinical signs observed included diarrhea and clear gelatinous discharge from anus. Necropsy revealed brown anogenital exudates, bloated intestines, mottled livers and dark areas in the lungs (RIFM, 1978a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed following 48-h closed patch tests with 20% *trans*-2-hexenyl salicylate in petrolatum applied to normal sites on the backs of 33 healthy, male volunteers (RIFM, 1978b).

4.2.2. Animal studies

4.2.2.1 As part of the acute dermal LD_{50} study described above, the dermal reactions consisted of slight (6/10) to moderate erythema (4/10), and slight (6/10) to moderate (2/10) edema (RIFM, 1978a).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A human maximization test was carried out with 20% *trans*-2-hexenyl salicylate in petrolatum on 33 healthy, male volunteers. Application was under occlusion to the same site on the volar aspects of the forearms of all subjects for five alternate day, 48-h periods. Patch sites were pretreated for 24 h with 5% aqueous SLS under occlusion for the initial patch only. Following a 10–14 day rest period, challenge patch was applied under occlusion to fresh sites for 48 h. Challenge applications were preceded by 30-min application of 2% aqueous SLS under occlusion on the left side of the back whereas *trans*-2-hexenyl salicylate was applied without SLS treatment on the right side. Additional SLS controls were placed on the left and petrolatum on the right. At 20%, *trans*-2-hexenyl salicylate in petrolatum produced no sensitization (RIFM, 1978b).

4.5. Photoirritation and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, D. McGinty, L. Jones, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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IFRA (International Fragrance Association), 2002. Use Level Survey, April 2002.

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Review

Fragrance material review on hexyl salicylate

A. Lapczynski *, L. Jones, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of hexyl salicylate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Hexyl salicylate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on hexyl salicylate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Benzoic acid, 2-hydroxy-, hexyl ester, Hexyl *o*-hydroxybenzoate.
1.2 CAS Number: 6259–76–3.
1.3 EINECS: 228–408–6.
1.4 Formula: $C_{13}H_{18}O_3$.
1.5 Molecular Weight: 222.28.
1.6 COE: Ethyl salicylate was included by the Council of Europe in the list of substances granted waiting (COE No.10695).

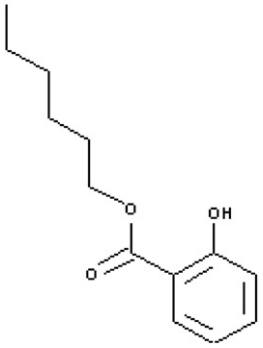

Fig. 1. Hexyl salicylate.

2. Physical properties

2.1 Physical Description: Colorless oily liquid.
2.2 Flash Point: >200°F; °CC.
2.3 Boiling Point: >200 °C.
2.4 Log K_{OW} (calculated): 5.06.
2.5 Vapor pressure :< 0.001 mm Hg 20 °C.
2.6 Water solubility (calculated): 6.084 mg/l @ 25 °C.

3. Usage (Table 1)

Hexyl salicylate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region greater than 1000 metric tonnes per annum.

The maximum skin level that results from the use of hexyl salicylate in formulae that go into fine fragrances has been reported to be 2.86% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 4.3473% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.11 mg/kg for high end users of these products (see Table 1).

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} of hexyl salicylate in rats exceeded 5.0 g/kg based on 1/10 deaths at that dose. Each animal received a single oral administration of hexyl salicylate. The rats were observed for mortality and/or systemic effects for 14 days. Urinary incontinence was observed at

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing hexyl salicylate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	4.35	0.0165
Face cream	0.80	2.00	1.000	0.003	4.35	0.0035
Eau de toilette	0.75	1.00	1.000	0.080	4.35	0.0435
Fragrance cream	5.00	0.29	1.000	0.040	4.35	0.0420
Antiperspirant	0.50	1.00	1.000	0.010	4.35	0.0036
Shampoo	8.00	1.00	0.010	0.005	4.35	0.0003
Bath products	17.00	0.29	0.001	0.020	4.35	0.0001
Shower gel	5.00	1.07	0.010	0.012	4.35	0.0005
Toilet soap	0.80	6.00	0.010	0.015	4.35	0.0005
Hair spray	5.00	2.00	0.010	0.005	4.35	0.0004
Total						0.1108

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60 kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	No. of animals/ dose group	LD_{50}	References
Oral	Rats	10	>5.0 g/kg	RIFM (1975a)
Dermal	Rabbits	10	> 5.0 g/kg	RIFM (1975a)

24 h. The LD_{50} was greater than 5 g/kg based on 1/10 deaths on day 4 at a dose of 5 g/kg (RIFM, 1975a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg based on 0/10 deaths at that dose. Ten rabbits received a single dermal application of neat hexyl salicylate at 5.0 g/kg. The rabbits were observed for mortality and clinical symptoms. No clinical signs were observed (RIFM, 1975a).

4.2. Skin irritation

4.2.1. Human studies (Table 3)

4.2.1.1. Irritation was evaluated as a part of a human repeated insult patch test study (HRIPT) which was conducted in 103 volunteers (29 male/74 female). A 0.3 ml aliquot of 30% hexyl salicylate in 3:1 DEP:EtOH was applied to 25 mm Hilltop Chambers® which were applied to the backs for 24 h under occlusion. A total of nine induction applications were made over a period of 3 weeks on a Monday, Wednesday, Friday schedule. No irritation was observed (RIFM, 2004a).

4.2.1.2. As a part of a maximization test, 3% hexyl salicylate was pre-tested on 22 male volunteers to determine whether SLS pre-treatment was necessary. Hexyl salicylate was applied under occlusion to normal sites on the backs for 48 h. No irritation was observed (RIFM, 1975b).

4.2.1.3. Primary irritation was evaluated in 30 volunteers. A 0.2 ml aliquot of neat hexyl salicylate was applied to 25 mm Hilltop Chambers® which were then applied to the upper arm for 4 h. Reactions were read at 24, 48 and 72 h after patch removal. No irritation was observed (Basketter et al., 2004).

Table 3
Summary of human irritation studies

Method	Dose (%)	Vehicle	Results	References
HRIPT (induction phase)	30	3:1 DEP:EtOH	3/103	RIFM(2004a)
Maximization pre-test	3	Petrolatum	No irritation	RIFM (1975b)
Primary irritation	100	N/A	No irritation	Basketter et al. (2004)
Irritation evaluated as a part of phototoxicity study	0.3, 3, 30	3:1 DEP:EtOH	No irritation	RIFM (2004b)

4.2.1.4. Irritation was assessed as part of an associated phototoxicity study conducted in 56 volunteers (15 male/41 female). Hexyl salicylate, in 0.3 ml aliquots, at 0.3%, 3%, or 30% in 3:1 DEP:EtOH was applied to 25 mm Hilltop Chambers® that were applied to the back of each subject for a 24-h period. Each subject received duplicate patches that were placed on both sides of the spine on naive sites. The test sites were evaluated at approximately 1, 24, 48, and 72 h after patch removal. No irritation was observed (RIFM, 2004b).

4.2.2. Animal studies (Table 4)

4.2.2.1. As a part of a modified Draize (Draize, 1959) sensitization study, a preliminary irritation screen was conducted to determine the injection challenge concentration (ICC). On the shaved flanks of four inbred Hartley strain albino guinea pigs with an average weight of 450 g, 0.1 ml aliquots of hexyl salicylate at a range of concentrations were given as intradermal injections. Reactions were read 24 h after injection. Hexyl salicylate at 0.1% produced a positive irritation reaction, and 0.1% (vehicle not reported) was selected as the ICC (Sharp, 1978).

4.2.2.2. As a part of a modified Draize sensitization study, a preliminary irritation screen was conducted to determine the application challenge concentration (ACC). On the shaved flanks of four inbred Hartley strain albino guinea pigs with an average weight of 450 g, 0.1 ml aliquots of hexyl salicylate at a range of concentrations were given as intradermal injections. Reactions were read 24 h after application. Hexyl salicylate at 5% (vehicle not reported) produced no irritation and was selected as the ACC (Sharp, 1978).

4.2.2.3. In a preliminary irritation screen conducted prior to a maximization test, 4 male albino Dunkin/Hartley strain guinea pigs weighing 482–544 g were treated topically with 8 mm diameter filter paper patches saturated with 10%, 25%, or 50% hexyl salicylate in acetone using 11 mm aluminum patch test cups. The cups were applied to shaved flanks and held in place with adhesive plaster wound around the animals' trunks. After 24 h, the patches were removed. Reactions were read 24 and 48 h after patch removal. No irritation was observed at 10%; very slight erythema was observed in three animals at 25% and 50% (RIFM, 1981).

4.2.2.4. In a preliminary irritation screen conducted prior to a maximization test, 4 male albino Dunkin/Hartley strain guinea pigs weighing 306–410 g were intradermally injected with 0.1 ml aliquots of 0.1%, 0.25%, 0.5%, 1.0%, and 2.0% hexyl salicylate in 0.01% DOBS/saline. After 24 h, the injection sites were examined for size (2 largest diameters), erythema, and edema. Very slight erythema was observed at 0.1%; slight erythema and edema were observed at 0.25%, 0.5%, 1% and 2% (RIFM, 1981).

Table 4
Summary of animal irritation studies

Method	Dose (%)	Species	Results	References
Preliminary intradermal irritation screen (for modified Draize test)	0.1% (ICC)	Guinea pigs	Slight but perceptible irritation ICC = 0.1% ACC = 5%	Sharp (1978)
Preliminary topical irritation screen (for modified Draize test)	5% (ACC)	Guinea pigs	No irritation was observed	Sharp (1978)
Preliminary topical irritation screen (for maximization test)	10%, 20%, 50% 25% 50%	Guinea pigs	No irritation at 10% Slight erythema at 25% and 50%	RIFM (1981)
Preliminary intradermal irritation screen (for maximization test)	0.1%, 0.25%, 0.5%, 1.0%, 2.0% in DOBS/saline	Guinea pigs	Very slight erythema observed at 0.1% Slight erythema and edema observed at 0.25–2%	RIFM (1981)
Irritation evaluated as part of a photoallergy study	1–50% in 3:1 DEP/EtOH, 100%	Guinea pigs	No irritation	RIFM (2003)
Irritation evaluated as part of phototoxicity study	100%	Miniature swine	No irritation	RIFM (1975b)
Primary irritation test	10%, 15%, 50% in DEP, 100%	Rabbits	Irritation observed at 50% and 100%	RIFM (1984); RIFM (1985); RIFM (1986a); RIFM (1986b)
Irritation evaluated as part of acute toxicity study	100%	Rabbits	Irritation observed	RIFM (1975a)
Irritation evaluated as part of phototoxicity study	100%	Mice	No irritation	RIFM (1975b)

4.2.2.5. Hexyl salicylate was evaluated for primary irritation in male hrBR outbred hairless albino guinea pigs as a part of a photoallergy screening study. The guinea pigs weighed 404–493 g and were assigned to 10 groups (5/group). A single application of 0.3 ml of hexyl salicylate at 1%, 5%, 10%, or 50% (in 3:1 DEP:EtOH) or 100% was applied to the dorsal skin of each animal using 25 mm Hilltop® Chambers. After 2 h (± 15 min), the chambers were removed and application sites were wiped with paper towels moistened with deionized water. Reactions were assessed 1 and 4 h later and at 1, 2, and 3 days after administration. No irritation was observed (RIFM, 2003).

4.2.2.6. As part of a phototoxicity study, hexyl salicylate was evaluated for irritation in two miniature swine. A 20 µl/5 cm^2 aliquot of neat hexyl salicylate was applied to the back of each animal. No irritation was observed (RIFM, 1975b).

4.2.2.7. Primary irritation was evaluated in a series of four tests which were conducted on either three or four healthy female New Zealand white rabbits. A 0.5 ml aliquot of 10%, 25%, 50% hexyl salicylate in DEP, or 100% hexyl salicylate was applied to a 2.5 cm^2 piece of surgical lint. The lint square was then placed onto a 6 cm^2 area of clipped, intact dorsal skin for 4 h under semi-occlusion. After a period of 4 h, the treated sites were cleansed by gentle swabbing with cotton wool soaked in warm water. Reactions were assessed at 1, 24, 48, 72 and 168 h after patch removal. No irritation was observed at 10% and 25%; irritation was observed with 50% and 100% hexyl salicylate (RIFM, 1984, 1985, 1986a, 1986b).

4.2.2.8. Hexyl salicylate was evaluated for irritation as part of the acute LD_{50} study described above. Ten rabbits received a single dermal application of neat hexyl salicylate at a dose of 5.0 g/kg. Moderate (7/10) to slight (3/10) edema and moderate (8/10) to slight (2/10) erythema were observed (RIFM, 1975a).

4.2.2.9. As part of a phototoxicity study, hexyl salicylate was evaluated for irritation in six hairless mice. A 20 µl/5 cm^2 aliquot of neat hexyl salicylate was applied to the back of each animal. No irritation was observed (RIFM, 1975b).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Dermal sensitization Quantitative Risk Assessment (QRA)

Significant developments have recently been incorporated in the way dermal sensitization risk assessments are conducted for fragrance ingredients. This new methodology represents a significant change over current risk assessment practices because it specifically addresses the elements of exposure-based risk assessment that are unique to the

induction of dermal sensitization, while being consistent with the principles of general toxicology risk assessment.

Full details of this risk assessment approach can be found in the "QRA Expert Group, Dermal Sensitization Quantitative Risk Assessment (QRA) for Fragrance Ingredients, Technical Dossier, revised June 22, 2006", and IFRA/RIFM Quantitative Risk Assessment (QRA) for Fragrance Ingredients Booklet, May 11, 2006", at http://www.rifm/org/pub/publications.asp and http://www.ifraorg.org/News.asp.

An exposure-based Quantitative Risk Assessment (QRA) methodology has been used to determine acceptable exposure limits for hexyl salicylate and a new IFRA Standard (IFRA, 2007) has been issued (see Tables 5 and 6).

4.4.2. Human studies

4.4.2.1. Induction studies (Table 7)

4.4.2.1.1. A repeated insult patch test (HRIPT) was conducted in 103 subjects (29 males and 74 females). During the induction phase a 0.3 ml aliquot of 30% hexyl salicylate in 3:1 DEP:EtOH was applied to Webril/adhesive patches (25 mm Hilltop® Chamber System) on the left side of the back of each subject. Patches remained in place and were kept dry for approximately 24 h and then removed. A series of nine induction applications were completed over a period of three weeks. A rest period of approximately 2 weeks followed the last induction. At the challenge phase, patches were applied as in the induction phase and kept in place for 24 h after which time they were removed and the challenge sites were scored. The test sites were also scored at 48, 72, and 96 h post-patching. No sensitization reactions were observed (RIFM, 2004a).

4.4.2.1.2. A maximization test (Magnusson and Kligman, 1969) was carried out with 3% hexyl salicylate in petrolatum on 22 adult volunteers. Application was under occlusion to the same site on the volar forearms or backs for five alternate-day 48-h periods. Patch test sites were pretreated for 24 h with 5% aqueous sodium lauryl sulfate under occlusion. Following a 10-day rest period, a challenge patch was applied to a fresh site for 48 h under occlusion. The challenge sites were pretreated for 30 min with 2% aqueous SLS under occlusion on the left side of the back whereas hexyl salicylate was applied without SLS on the right side. Reactions to challenge were read at patch removal and 24 h after patch removal. No reactions were produced (RIFM, 1975b).

4.4.2.2. Diagnostic studies

4.4.2.2.1. In a multicenter study, 218 fragrance sensitive patients with proven contact dermatitis were patch tested with various fragrance materials according to internationally accepted criteria. No reactions were observed with 5% hexyl salicylate in petrolatum (Larsen et al., 2002).

4.4.3. Animal studies (Table 8)

4.4.3.1. Hexyl salicylate was tested in a guinea pig sensitization study using a modified Draize procedure in 10 inbred

Table 5
IFRA Standard based on the QRA

Limits in the finished product	
For a description of the categories, refer to the QRA Information Booklet	
Category 1 – see Note (1) 1.0%	Category 7 2.7%
Category 2 1.3%s	Category 8 2.0 %
Category 3 5.3%	Category 9 5.0 %
Category 4 16.0%	Category 10 2.5 %
Category 5 8.4%	Category 11 – see Note (2)
Category 6 – see Note (1) 25.7%	

Note:
IFRA would recommend that any material used to impart perfume or flavour in products intended for human ingestion should consist of ingredients that are in compliance with appropriate regulations for foods and food flavourings in the countries of planned distribution and, where these are lacking, with the recommendations laid down in the Code of Practice of IOFI (International Organisation of the Flavor Industry). Further information about IOFI can be found on its website (www.iofiorg.org).
Category 11 includes all non-skin contact or incidental skin contact products. Due to negligible skin contact the concentration of a fragrance ingredient should not exceed the usual concentration of the fragrance compound in the finished product.
For example, hypothetically if the usual concentration of a fragrance compound in the final product, for example a candle, is at 5%, then any individual fragrance ingredient (in this case hexyl salicylate) must not exceed 5% in the candle.

Table 7
Summary of human sensitization studies

Test Method	Concentration	Results	References
HRIPT	30%	0/103	RIFM (2004a)
Maximization	3%	0/22	RIFM (1975b)

Table 6
Summary of the relevant sensitization data for the implementation of the QRA

LLNA weighted mean EC3 values (μg/cm^2) [no. studies]	Human data			LOEL[a] (induction) (μg/cm^2)	WoE NESIL (μg/cm^2)[c]
	NOEL – HRIPT (induction) (μg/cm^2)	Experimental NOEL – MAX (induction) (μg/cm^2)	Potency classification[b]		
45 [1]	35,433	2069	NA	Weak	35400

NOEL, no observed effect level; HRIPT, human repeat insult patch test; MAX, human maximization test; LOEL, lowest observed effect level; NA, not available.
[a] Data derived from HRIPT or HMT.
[b] Gerberick et al. (2001).
[c] WoE NESIL limited to two significant figure.

Table 8
Summary of animal sensitization studies

Test Method	Concentration	Results	References
Modified Draize test	0.25% (induction) 0.1% and 5% (challenge)	Sensitization observed	Sharp (1978)
Sensitization evaluated during photoallergy test	100% (induction) 50% and 100% (challenge)	No sensitization	RIFM (2003)
Maximization test	1% and 40% (induction) 10% (challenge)	No sensitization	RIFM (1981)

Hartley albino guinea pigs with initial weights of approximately 350 g each. Induction consisted of four intradermal injections with 0.1 ml of hexyl salicylate at 2.5 times the ICC (Injection Challenge Concentration = 0.1%) at four sites overlying the two auxillary and the two inguinal lymph nodes. The animals were challenged 14 days later with an intradermal injection in one flank and a topical application in the other flank using 0.1 ml hexyl salicylate at 0.1% (ICC) and 5% (ACC), respectively. A second challenge was conducted 7 days later. Sensitization reactions were observed after the second challenge (Sharp, 1978).

4.4.3.2. The sensitization potential of hexyl salicylate was evaluated during a photoallergy test using Crl:IAF(HA)-hrBR outbred albino hairless guinea pigs (5/group). During the induction phase, an intradermal injection with a 0.1 ml aliquot of a formulation of sterile water and Freund's complete adjuvant (FCA) (1:1 v/v) was made to a 2.5 cm^2 nuchal area of skin. The skin area was then tape-stripped five times. For the topical induction, a 0.3 ml aliquot of 100% hexyl salicylate in 3:1 DEP:EtOH was applied using 25 mm Hilltop® chamber patches for 2 h. After patch removal, the application sites were gently wiped with disposable paper towels moistened with osmosis membrane-processed deionized water. This procedure was repeated on days 3, 5, 8, 10 and 12 of the induction phase. On day 22, the animals were topically challenged with 50% in 3:1 DEP:EtOH and 100% using the same procedure. The test sites were observed at 1 and 4 h, and at 1, 2 and 3 days after hexyl salicylate application. No sensitization reactions were observed (RIFM, 2003).

4.4.3.3. A Magnusson–Kligman guinea pig maximization test was conducted on ten albino Dunkin/Hartley strain guinea pigs weighing 440–554 g. Induction consisted of intradermal injection followed one week later by a 48 h occluded patch. The six intradermal injections were made to a 2 × 4 cm clipped, shaved area in the dorsal shoulder region. There were two 0.1 ml injections of 1% hexyl salicylate in 0.01% DOBS/saline, two 0.1 ml injections of 1% hexyl salicylate in 50% Complete Freund's Adjuvant, and two 0.1 ml injections of 50% Complete Freund's Adjuvant. Seven days later, the site was clipped and shaved, and induction was supplemented topically with a 48 h occluded patch with 40% hexyl salicylate in acetone over the shoulder injection sites. Thirteen to 14 days after application of the shoulder patch, the guinea pigs were challenged on the clipped and shaved flank using an 8 mm diameter filter paper patch saturated with 10% hexyl salicylate in acetone which was applied for 24 h under occlusion. Reactions were assessed at 24 and 48 h after patch removal. Three additional challenge applications with 10% hexyl salicylate in acetone were made at weekly intervals on the contralateral flanks. No sensitization reactions were observed (RIFM, 1981).

4.5. Phototoxicity and photoallergy

4.5.1. Phototoxicity

4.5.1.1. Human studies

4.5.1.1.1. Phototoxicity of hexyl salicylate was evaluated in 56 subjects (41 females and 15 males) when used at concentrations of 0.3%, 3% and 30% in 3:1 DEP:EtOH. Hexyl salicylate was applied to a 25 mm Hilltop® Chamber which was applied to the back of each subject. Each subject received duplicate patches that were placed on both sides of the spine: three patches with hexyl salicylate and three control patches (vehicle control 3:1 DEP:EtOH and saline control). Patches remained in place for 24 h. Following 24 h, the patches on the left paraspinal region were removed and the skin sites were irradiated with 16 J/cm^2 of UVA irradiation for 10 min. Then the sites were irradiated with 0.75 MED UVB. A 150-W Berger Solar Ultraviolet Simulator was used as the ultraviolet radiation source in the study. Patches were removed from the non-irradiated test sites on the right paraspinal region after the UVA/UVB dosing was complete. The non-irradiated sites were used as controls to assess irritation potential of hexyl salicylate. Reactions were assessed at 1, 24, 48 and 72 h following UVA and UVB irradiation. No reactions were observed (RIFM, 2004a).

4.5.1.2. Animal studies

4.5.1.2.1. Neat hexyl salicylate was not phototoxic when tested in 12 (six used as control) Skh:hairless-1 mutant mice. Each animal received a single application of 20 μl of hexyl salicylate on a 2 cm^2 area of the back. Six mice treated with hexyl salicylate were exposed to a 6-kW long arc xenon lamp (distance = 1 m; intensity = 0.1667 W/m^2) for 40 min and four fluorescent blacklight lamps, type F40BL, with exposure for 1 h with an intensity of 3 W/m^2. The remaining six mice treated with hexyl salicylate served as a control for primary irritation reactions. The irradiation area was defined by 1-cm diameter hole punched in an aluminum foil adhesive tape, and the tape masked the skin surrounding the exposure area. One group of controls was treated with 8-methoxypsoralen (8-MOP) in methanol (0.01% w/v). The sites were assessed at 4, 24, 48, 72 and 96 h. No reactions were observed (RIFM, 1975c; Forbes et al., 1977).

4.5.1.2.2. Hexyl salicylate was evaluated for phototoxic potential. Two miniature swine were given a single application of neat hexyl salicylate (20 μl) on the back, to an area measuring approximately 5 cm^2. Irradiation was conducted using a 6-kW long-arc xenon lamp with exposure time of 40 min (distance = 1 m; intensity = 0.1667 W/m^2) and four fluorescent blacklight lamps, type F40BL, with exposure for 1 h (intensity = 3 W/m^2). Each irradiation area was defined by 1-cm diameter hole punched in an aluminum foil adhesive tape, and the tape masked the skin surrounding the exposure area. The reactions were graded at 4, 24, 48, 72 and 96 h after the irradiation exposure. The positive control was a 0.01% solution of 8-MOP in methanol, and the negative control was the vehicle alone. No phototoxicity was observed (RIFM, 1975c; Forbes et al., 1977).

4.5.1.2.3. The phototoxic potential of hexyl salicylate was evaluated in two groups (five/group) of Crl:IAF(HA)-hrBR outbred albino hairless guinea pigs. A 0.3 ml aliquot of hexyl salicylate at 0%, 5%, 10%, 50% and 100% in 3:1 DEP: EtOH was applied to 25 mm Hilltop Chambers® which were then applied to the dorsal skin along the midline of each guinea pig and occluded with dental dam. Two hours later the patches were removed and the application sites were gently wiped with disposable paper towels moistened with deionized water. The animals were exposed to UVR using a 6.5 kW long-arc xenon water-cooled lamp with a filter used to attenuate mid-range ultraviolet radiation (UVB). A dose of about 2.25 Minimal Erythema Doses (MED) was delivered for each exposure session (approximately 2.25 h). Observations were made immediately, 1 and 4 h later, and 1, 2 and 3 days after administration and UVR exposure. No phototoxic effects were observed (RIFM, 2003).

4.5.2. Photoallergy

4.5.2.1. Human studies. No data available on this material.

4.5.2.2. Animal studies

4.5.2.2.1. Photoallergy was evaluated in two groups of Crl:IAF(HA)-hrBR outbred albino hairless guinea pigs. A nuchal area of skin approximately 2.5 cm^2 was defined by intradermal injections (0.1 ml/corner) with a formulation of sterile water and Freund's complete adjuvant (1:1 v/v) in each animal. This skin area was then tape-stripped five times. A 0.3 ml aliquot of hexyl salicylate in 3:1 DEP:EtOH was applied to Hilltop® Chamber patches (25 mm diameter), then applied to the nuchal area, and occluded with a dental dam. After 2 h the patches were removed, and the application sites were gently wiped with disposable paper towels moistened with reverse osmosis, membrane-processed deionized water. The nuchal area of animals was exposed to UVR for approximately 2.25 h. The UVR source was a 6.5 kW long-arc xenon water-cooled lamp with a filter used to attenuate mid-range ultraviolet radiation (UVB). Exposures were monitored by a customized detector that records both intensity and UVR dose. A dose of about 2.25 instrumental Minimal Erythema Doses (MED) was delivered for each exposure session. Procedures were repeated once daily on days 3, 5, 8, 10 and 12 of the induction phase of the study. On day 22, using the induction procedure, hexyl salicylate at 50% and 100% was topically applied to each animal. Animals were exposed to UVR for 2.25 h after 2 h of patch application. The sites were scored 1 and 4 h after dosage administration and/or UVR exposure. Photoallergy was not observed (RIFM, 2003).

4.6. Absorption, distribution, and metabolism

4.6.1. Absorption studies

4.6.1.1. Watkinson (Watkinson et al., 1992) used a mathematical method to estimate total body absorption of some salicylate esters including hexyl salicylate. Rate constants were calculated from the relevant physicochemical properties. The applied dose of active ingredient used in the simulation was 40 μg cm^2 based on the FDA recommendation (200 mg of product per 100 cm^2 of skin) and a value of 2%. The release rate from the formulation was fixed at 1 μm cm^{-2} h^{-1}. The simulations were conducted on a 12-h time scale. The estimated total body absorption of hexyl salicylate per μg over 1.4 m^2 was 0.18 at 2 h, 4.1 at 6 h and 27 at 12 h.

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, L. Jones, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that

is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on isoamyl salicylate

A. Lapczynski *, L. Jones, D. McGinty, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of isoamyl salicylate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Isoamyl salicylate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on isoamyl salicylate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Amyl(iso) salicylate; Benzoic acid,2-hydroxy-, 3-methylbutyl ester; Isoamyl o-hydroxybenzoate; Isoamyl salicylate; Isopentyl salicylate; 3-Methylbutyl o-hydroxybenzoate; 3-Methylbutyl salicylate.
1.2 CAS Registry Number: 87-20-7.
1.3 EINECS Number: 201-730-4.
1.4 Formula: $C_{12}H_{16}O_3$.
1.5 Molecular Weight: 208.26.
1.6 COE: Isoamyl salicylate was included by the Council of Europe in the list of substances B-information required-hydrolysis study (COE No. 435).
1.7 FDA: Isoamyl salicylate was approved by the Food and Drug Administration as a flavor (21 CFR 172.515).
1.8 FEMA: Flavor and Extract Manufactures' Association states: Generally Recognized as Safe as ingredient – Gras 3 (2084).
1.9 JECFA: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 903) states that there is no safety concern at current levels of intake when used as a flavoring agent.

2. Physical properties

2.1 Physical Form: Colorless liquid.
2.2 Boiling Point: >200 °C.
2.3 Flash Point: >200 °F; CC.

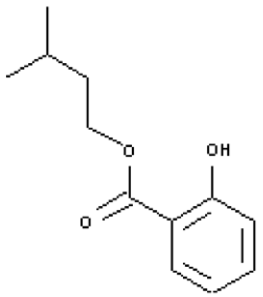

Fig. 1. Isoamyl salicylate.

2.4 Log K_{ow} (calculated): 4.49.
2.5 Specific Gravity: 1.049.
2.6 Vapor Pressure:1.45 mm Hg 20C.
2.7 Water solubility (calculated): 21.89 mg/l @ 25C.

3. Usage

Isoamyl salicylate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 100–1000 metric tonnes per annum (see Table 1).

The maximum skin level that results from the use of isoamyl salicylate in formulae that go into fine fragrances has been reported to be 2.19% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5%ile use level in formulae for use in cosmetics in general has been reported to be 4.09% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.1042 mg/kg for high end users.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. A dose range-finding study was conducted to determine the dose to be used in an associated oral LD_{50} study. Groups of 4 Sprague–Dawley rats (2/sex/dose) were fasted overnight, and then administered a single dose of 0.5, 0.8, 1.26, 2.0 or 5.0 g/kg isoamyl salicylate by gavage. The animals were observed for mortality for 7 days after the treatment. No deaths occurred at any dose level (RIFM, 1982).

4.1.1.2. The acute oral LD_{50} of isoamyl salicylate exceeded 5 g/kg based on 0/10 deaths at that dose. Ten Sprague–Dawley rats (5/sex) received a single oral (gavage) dose of 5 g/kg of isoamyl salicylate. Observations for mortality and/or systemic effects were made over a 14 day period. All animals were sacrificed at the end of the observation period and a necropsy was conducted. No deaths occurred and necropsy did not reveal any abnormalities (RIFM, 1982).

4.1.2. Dermal studies

No data available on this material.

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a 48-h closed patch test on the back with 20% isoamyl salicylate in vaselinum aldum or unguentum hydrophilicum, no irritation was observed in 29 male and female volunteers and in a 24–72 h closed patch test on

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing isoamyl salicylate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	4.09	0.0155
Face cream	0.80	2.00	1.000	0.003	4.09	0.0033
Eau de toilette	0.75	1.00	1.000	0.080	4.09	0.0409
Fragrance cream	5.00	0.29	1.000	0.040	4.09	0.0395
Antiperspirant	0.50	1.00	1.000	0.010	4.09	0.0034
Shampoo	8.00	1.00	0.010	0.005	4.09	0.0003
Bath products	17.00	0.29	0.001	0.020	4.09	0.0001
Shower gel	5.00	1.07	0.010	0.012	4.09	0.0004
Toilet soap	0.80	6.00	0.010	0.015	4.09	0.0005
Hair spray	5.00	2.00	0.010	0.005	4.09	0.0003
Total						0.1042

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60 kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rat	10	>5.0 g/kg	RIFM (1982)

the upper inner arm with 2% isoamyl salicylate in unguentum simplex or in unguentum hydrophilicum, no irritation was observed in 30 male and female volunteers (Fujii et al., 1972).

4.2.1.2. A 48-h semi-occluded patch test with 32% isoamyl salicylate in acetone was conducted on 50 male volunteers. A 0.05 ml aliquot of isoamyl salicylate was applied to a 15 mm patch which was then applied to the back of all the volunteers. After 48 h, the patches were removed and any residual isoamyl salicylate was swabbed with dry gauze. Reactions were read 30 min later and if needed, subsequent readings were performed at 72, 96 and 120 h. No irritation was observed (Motoyoshi et al., 1979).

4.2.2. Animal studies (Table 3)

4.2.2.1. Isoamyl salicylate was evaluated for irritation in 6 Pitman–Moore miniature swine. A single dermal application of a 0.05 g of neat isoamyl salicylate was applied to the clipped dorsal skin of each animal. The patches were secured by adhesive tape and the entire trunk of the animal was wrapped with rubberized cloth for 48-h exposure period. No irritation was observed (Motoyoshi et al., 1979).

4.2.2.2. Isoamyl salicylate was evaluated for irritation in 6 male Hartley guinea pigs weighing 350–500 g. Prior to application, hair on 2 areas measuring 3 cm^2 in the dorsal mid-lumbar region of each animal was clipped. Approximately 24 h later, 0.1 g of neat isoamyl salicylate was applied directly to the skin. The sites were evaluated 24 h later. After reading, the hair on the test areas was clipped again and isoamyl salicylate was applied 30 min later. A second set of readings and applications was made 48 h later. After the 72 h reading, all hair on dorsal surface of each animal was clipped and 40 mg/kg of Evans blue dissolved in physiological saline was injected intravenously into the each animal. Mild irritation was observed (Motoyoshi et al., 1979).

4.2.2.3. Isoamyl salicylate was evaluated for irritation in 6 albino angora rabbits with an average weight of 2.6 kg. A 0.1 g dose of neat isoamyl salicylate was applied directly to the clipped skin. A plastic collar 25 cm in diameter was wrapped around the neck of the animals. Reactions were assessed 24 h later. After reading, the hair was clipped again and the isoamyl salicylate was applied 30 min later. A second set of readings and applications was made 48 h later. After the 72 h reading, all hair on dorsal surface of each animal was clipped and 40 mg/kg of Evans blue dissolved in physiological saline was injected intravenously into the each animal. Severe irritation was observed (Motoyoshi et al., 1979).

4.2.2.4. Irritation was evaluated in rabbits. Isoamyl salicylate at concentrations of 15% and 100% was applied to the

Table 3
Summary of animal irritation studies

Method	Dose (%)	Species	Results	References
48-h occluded patch	100%	Miniature swine	No irritation	Motoyoshi et al. (1979)
Open application	100%	Guinea pigs	Irritation observed	Motoyoshi et al. (1979)
Open application	100%	Rabbits	Irritation observed	Motoyoshi et al. (1979)
Open application	15% and 100%	Rabbits	Irritation observed	RIFM (1970)

skin of 6 rabbits. Under the conditions of the test, isoamyl salicylate was not considered to be a primary irritant (RIFM, 1970).

4.3. Mucous membrane (eye) irritation (Table 4)

4.3.1.

An eye irritation study was conducted in which neat isoamyl salicylate or 15% (vehicle not reported) isoamyl salicylate was instilled into the lower eyelids of 6 rabbits. Irritation was observed in 1 rabbit with neat isoamyl salicylate; no irritation was observed with 15% isoamyl salicylate. Under the conditions of the test, isoamyl salicylate was not considered to be a primary eye irritant (RIFM, 1970).

4.4. Skin sensitization

4.4.1. Diagnostic studies (Table 5)

4.4.1.1. Patch tests were conducted on 179 cosmetic dermatitis patients with 50% isoamyl salicylate in petrolatum using Silver Patch Testers. Reactions were read at 48 and 72 h. One reaction was observed; however, it was reported that this may have been a false positive reaction due to Excited Skin Syndrome (de Groot et al., 1985).

4.4.1.2. Frosch et al. (1995) reported the results of a multicenter study on patch tests with 48 fragrance materials. The test materials were applied to the back with Finn Chambers® and Scanpor® for 2 days. Reactions were assessed as per ICDRG guidelines on days 2 and 3 or in some cases on days 2 and 4. Isoamyl salicylate at 1% and 5% in petrolatum was tested on 95 patients (35 male/60 female). No reactions were observed.

4.4.1.3. Positive reactions were observed in eight (8/216) dermatitis patients when 0.2% isoamyl salicylate in 99.9% ethanol or in a non-irritative cream base was applied for 24–48 h to the upper inside arm (Fujii et al., 1972).

Table 4
Summary of eye irritation studies in rabbits

Dose (%)	Vehicle	Results	References
15	N/A	No Irritation	RIFM (1970)
100	N/A	1/6	RIFM (1970)

Table 5
Summary of human diagnostic studies

Method	Concentration (%)	Results		References
		Reactions	Incidence (%)	
Patch test	50	1?/179	0.56	de Groot et al. (1985)
Patch test	1 and 5	0/95	0.0	Frosch et al. (1995)
Patch test	0.2	8/216	3.7	Fujii et al. (1972)

Table 6
Summary of animal sensitization studies

Test method	Concentration	Results	References
CET	30% (induction) 1% (challenge)	No sensitization	Ishihara et al. (1986)
Intradermal injection	0.1%	No sensitization	RIFM (1970)

4.4.2. Animal studies (Table 6)

4.4.2.1. Ishihara et al. (1986) conducted a Closed Epicutaneous Test (CET) using five guinea pigs. Induction consisted of six 48-h closed patch applications on the nape using Torii's patch plaster and adhesive tape. Induction applications were made 3 times per week for two weeks with 30% isoamyl salicylate. After a 2-week rest period, a 48-h occluded challenge application with 1% isoamyl salicylate was made to the clipped, shaved flank using Finn Chambers® and adhesive tape. Reactions were read at patch removal, 24, and 48 h after patch removal. No sensitization reactions were observed.

4.4.2.2. A sensitization test was conducted on male guinea pigs using a 0.1% suspension of isoamyl salicylate in 5% ethanol in distilled water. A total of 10 intradermal induction injections were made (0.05 ml for the 1st injection; 0.1 ml for 2nd to 10th injections) on alternate days, and after a 12-day rest period, the intradermal challenge injection was made. Readings were conducted 24 h after the injection. No sensitization was reported (RIFM, 1970).

4.5. Photoirritation and photoallergy

No data available on this material.

4.6. Absorption, distribution, and metabolism

4.6.1. Absorption

4.6.1.1. In vitro human studies

4.6.1.1.2. Lower abdominal skin was excised from a human cadaver during autopsy, kept at −20 °C, and thawed prior to usage. Subcutaneous tissue was removed and the epidermis was separated from the dermis. A static chamber system was used with the isoamyl salicylate placed on the top of the epidermis. Approximately 5 ml of saline was added to the chamber and was in complete contact with the bottom of the epidermis. A 0.2 ml aliquot of isoamyl salicylate was applied to the top of the epidermis. To avoid evaporation, Parafilm was placed over the mouth of the glass tube. The chamber was kept at 21 °C and 55% relative humidity for 72 h. The glass tube was removed from the glass chamber at 72 h. The saline was extracted in ether and analyzed by gas chromatography. The

experiment was repeated six times. The amount of isoamyl salicylate that penetrated the epidermis was 0.008% (Jimbo, 1983).

4.7. Subchronic toxicity

4.7.1. Oral studies

4.7.1.1. Groups of 30 rats (15/sex/dose) were administered isoamyl salicylate in the diet at 50, 500, or 5000 ppm (~equivalent to 4.7–4.8, 46–47, and 420–480 mg/kg body-weight/day, respectively) for 13 weeks. Additional groups of 5 rats/sex/groups were given diets containing 500 or 5000 ppm (~equivalent to 46–47 and 420–480 mg/kg body weight/day, respectively) for 2 or 6 weeks. Control animals received the diet alone. Body weights were recorded at days 1,2,6,9 and 13, then at weekly intervals up to day 91. Consumption of food and water was measured over a 24-h period preceding the day of weighing. Urine was collected during the last 2 days of treatment and was examined for appearance, microscopic components, and content of glucose, ketones, bile salts, and blood. The animals were sacrificed at the end of the treatment period after a 24-h fasting period. Necropsy was conducted and macroscopic abnormalities were noted. Major organs such as the brain, liver, heart, stomach, small intestine, cecum, spleen, kidneys, adrenal glands, gonads, pituitary glands, and thyroid were weighed. No effects were observed at 50 ppm, which was considered the no effect level (NOEL). At 500 ppm, a significant decrease in the erythrocyte counts of the 2-week treated females was observed. In the 13-week females, only a significant increase in the kidney weight was observed. No dose-related histological changes were produced. At 5000 ppm, one female rat died at week 6. Treated rats were visibly smaller than controls, and approximately 50% of the animals exhibited signs indicative of a respiratory infection from week 3. Lethargy, hypothermia, and rapid shallow respiration were observed, and upon histological examination, mucus and pus cells in the bronchioles were found. Compared to the controls, the weight of the males and females was lowered by 15% and 9%, respectively. In addition, a significant reduction in the food intake of males and females by 20 and 10%, respectively, was observed. Significant reduction in water intake was observed on the 1st day of treatment, but throughout the remainder of the study, the water intake of the males was similar to that of the controls, while the females showed an increase in water intake when compared to controls. The only hematological effect observed was a significant decrease in the erythrocyte counts of the 2-week treated females. Compared to the controls, both the male and female rats produced urine of a lower specific gravity following prolonged dehydration, and this difference was significant at week 6, but only in the females at week 13. In the 6-week males, a significant increase in the brain, spleen, cecum, and gonad weights with a significant decrease in the terminal bodyweight was observed. In the 6-week females, only a significant increase in the liver weight was observed. In the 13-week males, a significant increase in the brain and gonad weights was observed. In the 13-week females, a significant increase in the liver, kidneys, and small intestine weights with a significant decrease in the terminal body weight was observed. No dose-related histological changes were produced. Based upon these results, it was concluded that the NOAEL was 500 ppm since the only finding at this dose was of increased relative kidney weights in females that had no histopathological correlates (Drake et al., 1975).

4.7.1.2. A 98-day paired feeding study with 5000 ppm (~equivalent to 420–480 mg/kg body weight/day) isoamyl salicylate was conducted to aid in evaluation of the decreased food intake and weight gain in the top dose group of the associated 13-week feeding study. Groups of 10 Wistar rats were used. Animals were caged individually. Control rats were fed the quantity of food consumed by their littermate on the previous day. The rats were weighed at intervals. The rate of body-weight gain was similar in the test group and the pair fed group throughout the study. No effects were noted. The decreased weight gain in the associated study was therefore likely due to the unpalatability of the high dose diet (Drake et al., 1975).

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, L. Jones, D. McGinty, S. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on isobutyl salicylate

A. Lapczynski *, L. Jones, D. McGinty, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of isobutyl salicylate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Cinnamaldehyde

Contents

In 2006, a complete literature search was conducted on isobutyl salicylate. On-line databases that were surveyed included Chemical Abstract Services and the National

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Benzoic acid, 2-hydroxy-, 2-methylpropyl ester, isobutyl *o*-hydroxybenzoate, isobutyl salicylate, 2-methylpropyl *o*-hydroxybenzoate, 2-methyl-1-propyl salicylate.
1.2 CAS Registry Number: 87-19-4.
1.3 EINECS Number: 201-729-9.
1.4 Formula: $C_{11}H_{14}O_3$.
1.5 Molecular weight: 194.23.
1.6 COE: Isobutyl salicylate was included by the Council of Europe in the list of substances granted B-information required-hydrolysis study (COE No. 434) (COE, 2000).

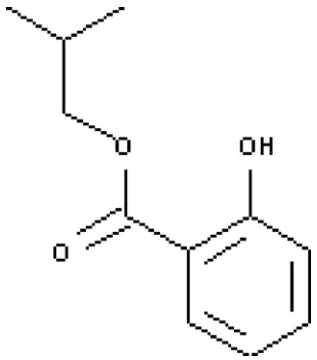

Fig. 1. Isobutyl salicylate.

1.7 FEMA: Flavor and Extract Manufacturers Association States: Generally recognized as safe as a flavor ingredient – GRAS 3 (2213) (FEMA, 1965).
1.8 FDA: Isobutyl salicylate was approved by FDA as flavor (21 CFR 172.515).
1.9 JECFA: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 902) concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent (JECFA, 2001).

2. Physical properties

2.1 Physical form: A colorless liquid having an orchid odor.
2.2 Flash point: >200 °F; CC.
2.3 Boiling point: 262 °C.
2.4 Log K_{ow} (calculated): 4.
2.5 Vapor pressure (calculated): 0.009 mm Hg 20 °C.
2.6 Specific gravity: 1.064.
2.7 Water Solubility (calculated): 67.83 mg/l @ 25 °C.

3. Usage (Table 1)

Isobutyl salicylate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 10–100 metric tonnes per annum.

The maximum skin level that results from the use of isobutyl salicylate in formulae that go into fine fragrances has been reported to be 0.81% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.168% (IFRA, 2002), which would result in a conservative calculated

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing isobutyl salicylate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product	Ingredient/mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.168	0.0006
Face cream	0.80	2.00	1.000	0.003	0.168	0.0001
Eau de toilette	0.75	1.00	1.000	0.080	0.168	0.0017
Fragrance cream	5.00	0.29	1.000	0.040	0.168	0.0016
Antiperspirant	0.50	1.00	1.000	0.010	0.168	0.0001
Shampoo	8.00	1.00	0.010	0.005	0.168	0.0000
Bath products	17.00	0.29	0.001	0.020	0.168	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.168	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.168	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.168	0.0000
Total						0.0043

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60 kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	No. animals /dose group	LD_{50}	References
Oral	Rats	10	1.56 g/kg	RIFM (1973a)
Dermal	Rabbits	8	>5.0 g/kg	RIFM (1973a)

maximum daily exposure on the skin of 0.0043 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral toxicity of isobutyl salicylate was determined in rats (10/dose). Isobutyl salicylate was administered at dose levels of 0.83, 1.04, 1.31, 2.05, or 5.0 g/kg. The animals were observed for mortality and systemic effects over a 14-day period. No deaths occurred at 0.83 g/kg; 3/10 deaths occurred at 1.04 g/kg; 5/10 deaths occurred at 1.31 g/kg, 8/10 deaths occurred at 2.05 g/kg and 9/10 deaths occurred at 5.0 g/kg. The LD_{50} was calculated to be 1.56 g/kg with 95% C.I. of 1.32–1.80 g/kg. No systemic effects were observed (RIFM, 1973a).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg based on 0/8 deaths at that dose. Eight rabbits received a single dermal application of neat isobutyl salicylate. The animals were observed for mortality and systemic effects over a 14-day period. No deaths occurred (RIFM, 1973a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed after a 48-h closed patch test with 10% isobutyl salicylate in petrolatum on the backs of five healthy males (RIFM, 1973b).

4.2.2. Animal studies

4.2.2.1. Irritation was evaluated during the acute dermal LD_{50} study described above. Dermal reactions consisted of slight (4/8) to moderate (2/8) erythema and slight (2/8) edema (RIFM, 1973a).

4.2.2.2. Isobutyl salicylate was not considered a primary skin irritant in rabbits when tested at 15% or 100% (no further details provided) (RIFM, 1970).

4.3. Mucous membrane (eye) irritation

4.3.1.

A rabbit eye irritation study was conducted on six albino rabbits with 15% isobutyl salicylate (vehicle not reported) or with neat isobutyl salicylate. A 0.1 ml aliquot of isobutyl salicylate was instilled into one eye of each rabbit. Irritation was observed in 1 rabbit with 15% isobutyl salicylate; no irritation was observed with neat isobutyl salicylate. It was concluded that isobutyl salicylate was not a primary eye irritant (RIFM, 1970).

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A human maximization test (Kligman, 1966; Kligman and Epstein, 1975) was carried out with 10% isobutyl salicylate in petrolatum on 25 healthy male volunteers. Isobutyl salicylate was applied under occlusion to the same site on the forearms of all subjects for five alternate-day 48-h periods. Patch sites were pretreated for 24 h with SLS under occlusion. Following a 10-day rest period, a challenge patch was applied to a fresh site for 48 hours under occlusion. Challenge test sites were pre-tested using 10% aqueous SLS under occlusion. The challenge sites were read at patch removal and 24 h after patch removal. No sensitization reactions were observed (RIFM, 1973b).

4.4.2. Animal studies (Table 3)

4.4.2.1. A sensitization test was conducted on white male guinea pigs. Isobutyl salicylate was first dissolved in alcohol and then suspended in distilled water to produce a suspension (0.1% suspension in 5% ETOH in distilled water). For the induction phase, 10 intradermal injections were made (0.05 ml for the 1st injection; 0.1 ml for 2nd to 10th injections) on alternate days. After a 12-day rest period, a challenge intradermal injection of 0.05 ml was administered. Readings were conducted 24 h after the injection. Isobutyl salicylate did not produce any sensitization reactions (RIFM, 1970).

4.4.2.2. Isobutyl salicylate was tested for sensitization using an open epicutaneous test in groups of 6–8 male and female guinea pigs weighing 300–450 g. During the induction period, an open application of a 0.1 ml aliquot of isobutyl salicylate (vehicle not reported) was applied daily for 21 days to an 8 cm^2 clipped area on the flank of each animal. Open challenge applications with 10% isobutyl salicylate (vehicle not reported) were conducted on days 21 and 35.

Table 3
Summary of guinea pig sensitization studies

Method	Induction concentration	Challenge concentration	Results	References
Intradermal injection	0.1%	0.1%	No sensitization	RIFM (1970)
OET	10%	10%	No sensitization	Klecak (1985, 1979)

No sensitization reactions were observed (Klecak, 1985, 1979).

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, L. Jones, D. McGinty, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on methyl salicylate

A. Lapczynski *, L. Jones, D. McGinty, S.P. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of methyl salicylate when used as a fragrance ingredient is presented.

Keywords: Fragrance; Review; Methyl salicylate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on methyl salicylate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Benzoic acid,2-hydroxy-,methyl ester; 2-carbomethoxyphenol; 2-hydroxybenzoic acid, methyl ester; methyl 2-hydroxybenozate; salicylic acid, methyl ester; synthetic sweet birch oil; synthetic teaberry oil; synthetic wintergreen oil.
1.2 CAS Registry Number: 119-36-8.
1.3 EINECS Number: 204-317-7.
1.4 Formula: $C_8H_8O_3$.
1.5 Molecular weight: 152.15.
1.6 COE: Methyl salicylate was included by the Council of Europe in the list of substances A – may be used in foodstuffs (COE No. 433).
1.7 FEMA: Flavor and Extract Manufacturers Association: generally recognized as safe as a flavor ingredient – GRAS 3 (2745).
1.8 JECFA: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 899) states that there are no safety concerns at current levels of intake when used as a flavoring agent. The 1967 ADI of 0–0.5 mg/kg bw was maintained at the fifty-seventh meeting (2001).
1.9 CIR: On the basis of the data included in their report, the Cosmetic Ingredient Review (CIR) Expert Panel of the Cosmetic, Toiletry and Fragrance Association (CTFA) concluded that methyl salicylate is safe as used when formulated to avoid skin irritation and when formulated to avoid increasing the skin's sun sensitivity, or, when increased sun sensitivity would be expected, directions for use include the daily use of sun protection.

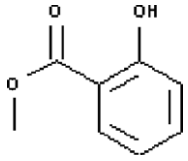

Fig. 1. Methyl salicylate.

2. Physical properties

2.1 Physical form: a clear colorless liquid having a characteristic wintergreen odor.
2.2 Boiling point: 222 °C.
2.3 Flash point : >212 °F; CC.
2.4 Log K_{ow} (calculated); 2.6.
2.5 Specific gravity: 1.18.
2.6 Vapor pressure (calculated): 0.09 mm Hg 20 °C.
2.7 Water solubility (calculated): 1875 mg/l @ 25 °C.

3. Usage

Methyl salicylate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 10–100 metric tonnes per annum.

The maximum skin level that results from the use of methyl salicylate in formulae that go into fine fragrances has been reported to be 0.29% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 0.13% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.0034 mg/kg for high end users (see Table 1).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing methyl salicylate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product	Ingredient/ mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.13	0.0005
Face cream	0.80	2.00	1.000	0.003	0.13	0.0001
Eau de toilette	0.75	1.00	1.000	0.080	0.13	0.0013
Fragrance cream	5.00	0.29	1.000	0.040	0.13	0.0013
Antiperspirant	0.50	1.00	1.000	0.010	0.13	0.0001
Shampoo	8.00	1.00	0.010	0.005	0.13	0.0000
Bath products	17.00	0.29	0.001	0.020	0.13	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.13	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.13	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.13	0.0000
Total						0.0034

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60 kg adult.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Male and female guinea pigs received a single oral dose (gavage) of methyl salicylate. The animals were observed for mortality and/or systemic effects over a period of 14 days. The LD_{50} was calculated to be 1.06 g/kg (95% CI 0.87–1.3 g/kg). Deaths occurred between 1 h and three days. Clinical signs included convulsions and gastro-intestinal irritation (Jenner et al., 1964).

4.1.1.2. Groups of 10 Osborne–Mendel rats (5/sex) were dosed orally with methyl salicylate. The rats were observed for mortality and/or systemic effects over a period of 14 days. The LD_{50} was calculated to be 0.89 g/kg (95% CI 0.72–1.10 g/kg). Deaths occurred between 4 and 18 h. Depression was observed (Jenner et al., 1964).

Table 2
Summary of acute toxicity studies

Route	Species	No. animals/ dose group	LD_{50}	References
Oral	Guinea pig	N/A	1.06 g/kg	Jenner et al. (1964)
Oral	Rat	10	0.89 g/kg	Jenner et al. (1964)
Oral	Rat	10	2.82 g/kg	RIFM (1982)
Oral	Rat	N/A	1.25 g/kg	Giroux et al. (1954)
Oral	Mice	10	1.39 g/kg	Ohsumi et al. (1984)
Oral	Mice	N/A	1.11 g/kg	Davison et al. (1961)
Oral	Mice	16	1.44 g/kg/day	NTP (1984a)
Dermal	Rabbit	10	> 5.0 g/kg	RIFM (1973a)
Intraperitoneal	Rat	3	0.75–1.0 g/kg	Giroux et al. (1954)
Intraperitoneal	Guinea pig	3	0.75–1.0 g/kg	Giroux et al. (1954)

4.1.1.3. The acute oral toxicity of methyl salicylate was determined in Sprague–Dawley rats (5/sex/dose). Methyl salicylate was administered at dose levels of 2.50, 3.15, 3.97, or 5.00 g/kg. Animals were observed for signs of toxicity and mortality over a 14-day period. Gross necropsy was carried out on all animals. One male and two females died at 2.50 g/kg; 3/5 males and 4/5 females died at 3.15 g/kg; 4/5 males and 5/5 females died at 3.97 g/kg and all animals died at 5.00 g/kg. Animals died within 48 h of dosing. Clinical signs included piloerection, shaggy coat, hunched posture, lethargy, oscillated movements and difficulty breathing. Necropsy of the animals that died showed severe congestion in the liver, stomach overload, black flakes in the stomach and slight reddening on the mucosal surface of the corpus and antrum of stomach. No significant necropsy findings were noted in the surviving animals. The LD_{50} for males and females was calculated to be 2.82 g/kg (95% CI 2.48–3.21 g/kg); the LD_{50} for males only was calculated to be 3.05 g/kg (95% CI 2.57–3.62 g/kg); and the LD_{50} for females only was calculated to be 2.64 g/kg (95% CI 2.24–3.11 g/kg) (RIFM, 1982).

4.1.1.4. The acute oral (gavage) toxicity of methyl salicylate was determined in rats. Methyl salicylate was administered in a 20% suspension (w/v) in a gum syrup and water mixture (1:3) at dose levels of 1, 1.25, 1.50, 2, 2.25, 2.50, or 3 g. Clinical signs observed included mydriasis, and convulsions. Necropsy findings included diffuse congestion in the digestive tract and hepatization of the lungs. The LD_{50} was reported to be approximately 1.25 g/kg (Giroux et al., 1954).

4.1.1.5. Methyl salicylate was tested as part of a study to determine possible gastric irritation produced by drugs. Rats weighing 130–150 g were divided into groups of 4–6

animals. A 0.5 g of methyl salicylate was administered directly into the stomach via gavage. One hour after administration, the rats were sacrificed and the stomach was removed and observed for the presence of bleeding or ulceration. Methyl salicylate produced some slight redness and irritation of the stomach mucosa. No bleeding or ulceration were observed (Strom and Jun, 1974).

4.1.1.6. The acute oral toxicity of methyl salicylate was determined in ddY male mice (10/dose). Methyl salicylate was administered at dose levels of 1.0, 1.2, 1.3, 1.5, or 1.7 g/kg. Mice were observed for a 7-day period. One animal died at 1.0 g/kg; 2/10 died at 1.2 g/kg; 4/10 died at 1.3 and 1.5 g/kg; 9/10 died at 1.7 g/kg. Most animals died on day 1. The LD_{50} was calculated to be 1.39 g/kg (95% CI 1.25–1.54 g/kg) (Ohsumi et al., 1984).

4.1.1.7. The acute oral toxicity of methyl salicylate was determined in male C3 H mice. The LD_{50} was calculated to be 1.11 g/kg (Davison et al., 1961).

4.1.1.8. The acute oral toxicity of methyl salicylate was determined in male and female CD-1 mice (8/sex/dose). Methyl salicylate was administered in corn oil by gavage once daily for 14 days at dose levels of 0.05, 0.1, 0.25, 0.50, and 1.00 g/kg. Two females died at 0.05 g/kg; 2 females and 3 males died at 0.10 g/kg; 1 female and 1 male died at 1.00. Clinical signs observed prior to death were piloerection and dehydration. The LD_{50} was calculated to be 1.44 g/kg/day (NTP, 1984a).

4.1.1.9. As a part of an associated reproductive toxicity study, a 2-week acute study was conducted using CD-1 mice (8/sex/dose). Methyl salicylate was administered by gavage at 0.05, 0.1, 0.25, 0.5 and 1 g/kg once a day for 14 days. The animals were observed for survival, body weights and clinical signs. The maximum tolerated dose (MTD) was determined for the associated study. No effects were observed at 0.05, 0.1, 0.25 and 0.5 g/kg. Two (2/8) animals died at 0.05 g/kg but the deaths were diagnosed as possible gavage trauma. Three (3/8) animals died at 1 g/kg (one death was diagnosed as possible gavage trauma) and the cause of death for the 2 remaining animals was diagnosed as pulmonary congestion or cardiac myodegeneration and tubular nephrosis. The dose of 0.5 g/kg was selected as the MTD (NTP, 1984b).

4.1.1.10. Methyl salicylate was evaluated as a part of a study investigating the development of acute myocardopathy in dogs. Healthy mongrel dogs with a mean weight of 12.5 kg in a fasting state were lightly anesthetized with pentobarbital sodium. Methyl salicylate was intragastrically administered at a dose of 0.7 g/kg. After 4–5 h, animals either died or were sacrificed. Increases in arterial concentrations of plasma salicylate, potassium and lactate were seen and a period of respiratory alkalosis was initially observed followed by metabolic acidosis after three hours. Microscopy studies revealed abnormalities in the mitochondria, swelling of cardiac muscles with separation of myofibrils and bulging of sarcolemma (Ojiambo, 1972).

4.1.2. Dermal studies

4.1.2.1. The dermal LD_{50} in rabbits exceeded 5 g/kg based on 1/10 deaths at that dose. A single dermal application of neat methyl salicylate at 5 g/kg was applied for 24 h under occlusion. Animals were observed for a 14-day period. No clinical signs were observed (RIFM, 1973a).

4.1.3. Intraperitoneal studies

4.1.3.1. The acute toxicity of methyl salicylate was determined in guinea pigs (3/dose). Methyl salicylate was administered by a single intraperitoneal injection at dose levels of 0.5, 0.75, or 1 g/kg in alcohol. No deaths occurred at 0.5 g/kg; 1/3 deaths occurred at 0.75 g/kg and 2/3 deaths occurred at 1 g/kg. Animals died within 12 h of dosing. The LD_{50} was concluded to be between 0.75 and 1 g/kg. Clinical signs included slight palsy, shaking, lateral decubitus, slower breathing or difficultly in breathing (Giroux et al., 1954).

4.1.3.2. The acute toxicity of methyl salicylate was determined in rats (3/dose). Methyl salicylate was administered by an intraperitoneal injection at dose levels of 0.5, 0.75, and 1 g/kg in alcohol. Clinical signs included drowsiness, nervousness, mydriasis, changes in breathing (rhythm and amplitude), paralysis of the hind legs and rapid and violent shaking. Necropsy revealed congested liver, kidneys, and lungs. The LD_{50} was concluded to be between 0.75 and 1 g/kg (Giroux et al., 1954).

4.1.3.3. In a preliminary screen conducted prior to a carcinogenesis assay, groups of 5 A/He mice received 6 intraperitoneal injections of methyl salicylate in tricaprylin over a 2-week period and were then observed for delayed toxicity over a 1–2 month period. The maximum tolerated dose (MTD) of methyl salicylate was determined to be 0.5 g/kg (Stoner et al., 1973).

4.1.4. Subcutaneous studies

4.1.4.1. A total of 6–9 male and female SD rats were used to determine the acute subcutaneous toxicity of methyl salicylate. Methyl salicylate was diluted in a saline solution (with Tween 80 as needed) and administered at a dose of 0.4 g/kg at a volume of 1.0 ml/100 g of body weight. Blood was collected for measurement of plasma and serum Ca levels 3 h after dosing. Plasma Ca levels were significantly decreased after administration of methyl salicylate (Saito et al., 1982).

4.1.4.2. As part of the same experiment described above methyl salicylate was applied to 6–9 ICR strain male mice. A dose of 0.4 g/kg methyl salicylate also resulted in a decrease in the plasma Ca (Saito et al., 1982).

4.2. Skin irritation

4.2.1. Human studies (Table 3)

4.2.1.1. In a pre-test for a human maximization study, no irritation was observed when a 48-h occluded patch test with 8% methyl salicylate in petrolatum was applied to the backs of 27 healthy male volunteers (RIFM, 1973b).

4.2.1.2. In a pre-test for a human maximization study, a patch test with 12% wintergreen oil (containing 80–99% methyl salicylate) in petrolatum was applied to the backs of 25 volunteers for 48 h under occlusion. No reactions were observed (RIFM, 1976a).

4.2.1.3. The irritation potential of methyl salicylate was evaluated in nine volunteers (3 male/6 female) between the ages of 22 and 32. A 25 ml aliquot of methyl salicylate in 80% ethanol and 20% deionized water was pipetted onto the skin on the forearm of each subject within a Teflon ring (1.2 cm ID, 1.6 cm OD) that was affixed to the skin with a bead of denture adhesive applied around the outside of its bottom edge. Immediately after methyl salicylate was applied, the ring was covered with a snug-fitting Teflon cap that prevented evaporation and left a headspace of only 0.21 cm^2. Each subject was tested six times. In each session, methyl salicylate was applied to one forearm and the vehicle to the other in random order. Subjects were tested no more frequently than every 48 hours. Irritation was observed with 30% and 60% methyl salicylate (Green and Shaffer, 1992).

4.2.2. Animal studies (Table 4)

4.2.2.1. The irritation potential of wintergreen oil (containing 80–99% methyl salicylate) was evaluated at nonirradiated sites in an associated phototoxicity study. An aliquot of 20 µl of neat methyl salicylate was applied to a 5 cm^2 area on the backs of 2 miniature swine. Flaking, hyperkeratosis and dry desquamation were observed (RIFM, 1976b).

4.2.2.2. Methyl salicylate was evaluated at various concentrations prior to an open epicutaneous test (OET). A 0.1 ml aliquot of methyl salicylate was applied to an area measuring 8 cm^2 on the clipped flank of 6–8 male and female outbred Himalayan white-spotted guinea pigs. The application site was left uncovered and reactions were read after 24 h. A total of 21 daily applications were made. The minimal irritating concentration after 21 applications was 3% (vehicle not specified) (Klecak et al., 1977).

4.2.2.3. Prior to an OET test, methyl salicylate at a range of concentrations was evaluated for irritation in 6–8 male and female outbred Himalayan white-spotted guinea pigs. A 0.025 ml aliquot was applied with a pipette to an area measuring 2 cm^2 on the clipped flank. The application site was left uncovered and reactions were read after 24 h. The concentration of 3% was the lowest concentration to produce mild erythema in at least 25% of the animals and this dose was selected as the minimal irritating concentration after 1 application (Klecak et al., 1977).

4.2.2.4. The primary irritation index of 1%, 3% or 6% methyl salicylate in four different vehicles (water, PEG 400, 70% ethanol and 70% ethanol plus emollients) was evaluated in rabbits (3/group). Methyl salicylate was applied to the shaved and depilated skin and Saran wrap was used to retard the evaporation of methyl salicylate. Irritation was evaluated according to Draize at 24 or 72 h after application. At 1%, no irritation was observed in water; mild irritation was observed in PEG 400 and in 70% ethanol, and moderate irritation in 70% ethanol and emollients. At 3% and 6% mild irritation was observed in water and PEG 400 and moderate irritation was observed in both ethanol vehicles. All moderate irritation consisted of necrosis and intradermal and subcutaneous hemorrhage (Yankell, 1972).

4.2.2.5. As a part of the acute dermal LD_{50} study conducted in rabbits, a single application of 5 g/kg neat methyl salicylate produced slight (2/9 rabbits) to moderate (7/9 rabbits) erythema and edema (RIFM, 1973a).

4.2.2.6. A primary irritation study was conducted to establish the concentration to be used for challenge in a mouse ear swelling test. Methyl salicylate was applied at a range of concentrations in 4:1 acetone to olive oil using a 4-day dosing protocol. The ear measurements were obtained before and after application of methyl salicylate. The minimal irritating concentration (MIC) was defined as the lowest concentration of methyl salicylate to produce a percent ear swelling significantly greater than the vehicle. The MIC was determined to be 20% (Howell et al., 2000).

4.2.2.7. The irritation potential of wintergreen oil (containing 80–99% methyl salicylate) was evaluated at nonirradiated sites in an associated phototoxicity study. An aliquot of 20 µl of neat methyl salicylate was applied to a 5 cm^2 area on the backs of 6 hairless mice. Flaking, hyperkeratosis and dry desquamation were observed (RIFM, 1976b).

Table 3
Summary of human irritation studies

Method	Dose (%)	Vehicle	Results	References
Maximization pre-test	8%	Petrolatum	No irritation	RIFM (1973b)
Maximization pre-test	12% (of a sample of wintergreen oil that contained 80-99% methyl salicylate)	Petrolatum	No irritation	RIFM (1976a)
Dermal application	30%, 60%	4:1 EtOH: deionized water	Irritation observed	Green and Shaffer (1992)

Table 4
Summary of animal irritation studies

Method	Dose	Species	Results	References
Irritation evaluation as part of a phototoxicity study	Wintergreen oil that contained 80-99% methyl salicylate was tested	Miniature swine	Irritation observed	RIFM (1976b)
Irritation evaluation as part of an OET	0.1–30%	Guinea pigs	3% was minimal irritating concentration	Klecak et al. (1977)
Primary irritation test	1%, 3%, or 6%	Rabbits	Irritation observed	Yankell (1972)
Irritation evaluation as part of an LD_{50} study	100%	Rabbits	Irritation observed	RIFM (1973a)
Primary irritation test	1–20%	Mice	Irritation observed	Howell et al. (2000)
Irritation evaluation as part of a phototoxicity study	80–99%	Mice	Irritation observed	RIFM (1976b)
Ear thickness technique	2.5–10 mg	Mice	Irritation observed	Patrick et al. (1985, 1987); Patrick and Maibach (1986)

4.2.2.8. Irritation of methyl salicylate was evaluated using the ear thickness technique in groups of 5–15 female ICR mice. Methyl salicylate at 2.5, 5.0, 7.5 or 10 mg in ethanol was applied as a solution of 5 μl to one ear and the solvent to the contralateral ear. Ear thickness measurements were taken before applying the solution to the ears and at selected time points after application. Inflammation produced by methyl salicylate reached maximum 15 min after application of 2.5, 5.0 and 7.5 mg and 30 min after application of 10 mg. Mean ear thickness was approximately 0.34, 0.39, 0.46 and 0.50 mm at 2.5, 5.0, 7.0 and 10 mg, respectively. The mean thickness in control groups was 0.22–0.29 mm. Based on these results, methyl salicylate was considered to be an irritant (Patrick et al., 1985, 1987; Patrick and Maibach, 1986).

4.2.2.9. As a part of the same experiment described above, one ear of 9-week-old ICR females was treated with 5 mg methyl salicylate in ethanol. Histological examination was conducted at the time of maximum ear thickness and at 1 h after application. Two non-serial cross sections of the ears of 3 animals were examined for each time period. Tissue was processed for light microscopy. The condition of blood vessels, degree of edema, location and type of cellular infiltrate, conditions of the epidermis, dermis, lymphatics and musculature of the ear were evaluated. Within 20 min, methyl salicylate produced rapid dilation of blood vessels, vessels at the margin of the ear became prominent, and moderate edema had developed. Within 1 h, tissue edema was regressing but vessels remained dilated (Patrick et al., 1985, 1987).

4.3. Mucous membrane (eye) irritation (Table 5)

4.3.1.

A rabbit eye irritation test was conducted in 5 healthy albino rabbits. A 0.005 ml aliquot of neat methyl salicylate was applied to the center of the cornea while the lids were retracted. One minute later the lids were released. The eyes were examined 18–24 h later in strong diffuse daylight and then stained with fluorescein. Methyl salicylate was assigned grade 3 – necrosis on 13–37% of the cornea visible after staining (Carpenter and Smyth, 1946).

4.3.2.

A rabbit eye test was conducted in 3 healthy albino rabbits. A 0.1 ml of 1.25% methyl salicylate in SDA 39C was instilled into the right eye of each rabbit with no further treatment. The untreated left eye served as control. Observations were made every 24 h for 4 days and then again on day 7 according to the Draize method. Intense conjunctival irritation accompanied by chemosis and considerable discharge was observed in all 3 rabbits. The treated eyes were normal on day 7 of observation (RIFM, 1963).

4.4. Skin sensitization

4.4.1. Human studies (Table 6)

4.4.1.1. Induction studies

4.4.1.1.1. A human maximization test was conducted on 25 healthy volunteers. Wintergreen oil (containing 80–99% methyl salicylate) in petrolatum was applied under occlusion, to the same site on the volar forearms of 25 subjects for 5 alternate-day 48-h periods. The patch sites were pretreated for 24 h with 5% aqueous SLS under occlusion for the initial patch only. Following a 10–14-day rest period, a challenge patch of methyl salicylate was applied to a fresh site for 48 h under occlusion. Prior to the challenge, 5% SLS was applied to the test sites for 30 min under occlusion on the left side of the back, while methyl salicylate was applied without SLS treatment on the right side. Additional SLS controls were placed on the left and petrolatum on the right, and labeled as the fifth site. No reactions were observed with 12% wintergreen oil (RIFM, 1976a) (see Table 6).

4.4.1.1.2. A maximization test was carried out on 27 healthy volunteers using 8% methyl salicylate in petrolatum.

Table 5
Summary of eye irritation studies in rabbits

Dose (%)	Vehicle	Results	References
100	N/A	Irritant effects	Carpenter and Smyth (1946)
1.25	SDA 39C	Irritant effects	RIFM (1963)

Table 6
Human studies for skin sensitization

Test method	Test concentration	Results	References
Maximization	12% in petrolatum (of a sample of wintergreen oil that contained 80–99% methyl salicylate)	0/25	RIFM (1976a)
Maximization	8% in petrolatum	0/27	RIFM (1973b)
HRIPT	1.25% (vehicle not provided)	0/39	RIFM (1964)

An occluded patch with methyl salicylate was applied to the same sites of the forearms of each subject for five alternate 48-h periods. Patch sites were pre- treated with 5% aqueous SLS under occlusion. Following a 10–14-day rest period a challenge patch was applied to a fresh site for 48 h under occlusion. An application of 10% aqueous solution of SLS under occlusion was applied 1 h prior to challenge. Reactions were read at patch removal and 24 h later. No sensitization reactions were observed (RIFM, 1973b).

4.4.1.1.3. A human repeated insult patch test (HRIPT) was conducted in 39 male and female volunteers (13 male/26 female) with 1.25% methyl salicylate. A 0.5 ml aliquot of methyl salicylate (vehicle not provided) was applied to a 1-inch square Webril patch fixed to the center of 1×3 in. strip of adhesive elastic bandage and placed on the upper arm of each subject. Patches were removed 24 h later. A total of nine applications were made over a three week period. The patches were applied to the same sites unless a reaction was observed. A challenge patch was applied to a fresh site on the Monday of the sixth week and removed 24 h later. Reactions were scored at 24 and 72 h after patch removal. No sensitization was observed (RIFM, 1964).

4.4.1.2. Diagnostic studies

4.4.1.2.1. A total of 4600 patients (2784 diagnosed with contact dermatitis, 189 diagnosed with dermatitis of the hands, 135 patients diagnosed with photoallergic and phototoxic reactions and 1491 healthy patients) were patch tested at the Allergy Department of Barcelona University between 1973 and 1977. The tests were comprised of the department's standard series in which the ICDRG series was included. Reactions to 2% methyl salicylate in petrolatum were observed in 6 patients (Romaguera and Grimalt, 1980).

4.4.1.2.2. The principle patch test results of the North American Contact Dermatitis Group for the period from July 1, 1975 to June 30, 1976 have been reported. A total of 183 patients were patch tested with fragrance allergens. Test materials were applied with A1 Test® strips or Finn Chambers® for 48 h in vertical rows affixed with 2-in. wide occlusive tape. Reactions were read at 48 and 96 h. Reactions to 2% methyl salicylate (vehicle not reported) were observed in 1.6% of the 183 patients tested (Rudner, 1977, 1978).

4.4.1.2.3. Ferguson and Sharma (1984) reported the results of patch tests conducted on 241 patients (61 male/180 female) from October 1981 to 1983. Patients were patch tested for sensitivity to fragrances in a perfume screening series. The Finn Chamber® technique was used. Reactions to 2% methyl salicylate in PMF (yellow soft paraffin) were observed in 3 female patients.

4.4.1.2.4. The North American Contact Dermatitis Research group reported the test results of a multi-center study conducted on eczema patients. During 1978–1980, 585 patients were tested with 2% methyl salicylate in petrolatum. Reactions were observed in 3% of the patients tested (Mitchell et al., 1982).

4.4.1.2.5. In a multicenter study conducted in North America from January 1980 to May 1987, 19 patients with eyelid dermatitis and 70 patients with dermatitis at other sites were patch tested with a routine screening tray. Methyl salicylate at 1% in petrolatum was applied to Al-Test strips® or Finn Chambers® which were applied to the upper back and secured to the skin with Scanpor® for a period of 48–72 h. Reactions were read at patch removal and re-examined in the majority of cases between 48 and 96 h after patch removal. Sites were scored according to the ICDRG scoring system. No reactions were observed in eyelid dermatitis patients; positive reactions (1.4%) were observed in 70 patients with dermatitis at other sites (Nethercott et al., 1989).

4.4.1.2.6. Closed patch tests were conducted on 197 patients with 0.05–0.5% methyl salicylate in a base cream or in 99% ethanol. Patches consisted of a piece of 1 cm^2 lint with a 2 cm^2 cellophane disc placed on the lint and then covered with a 4 cm^2 plaster. The patches were applied to the back, the forearm and the inside of the upper arm for 24–48 h. Reactions were read 30 min after patch removal. Erythema was observed in 4 out of 197 patients (Takenaka et al., 1986).

4.4.1.2.7. In a multicenter study conducted from September 1998 to April 1999, 1825 patients were patch tested with 9 fragrance allergens and fragrance mix. Test procedures were carried out according to internationally accepted criteria and published studies. Positive reactions to 2% methyl salicylate in petrolatum were observed in 7 patients (de Groot et al., 2000).

4.4.1.2.8. Fifty patients with photosensitivity dermatitis with actinic reticuloid (PD/AR) syndrome, 32 subjects with polymorphic eruption (PLE) and 457 with contact dermatitis (CD), were studied to determine increased incidence of contact allergic sensitivity to some common fragrance materials. Each subject was patch-tested to the various fragrance materials using a standard closed patch test technique. In paraffin, 10 mg of 2% methyl salicylate was applied to standard Al test® patches that were placed on the skin of the upper backs and secured with Scanpor® adhesive tape. Patches were removed at 48 h and the reactions were read at the time of removal and then at 72 h. Methyl salicylate at 2% in PMF produced one (1/50) reaction in a PD/AR patient. No reactions were observed in PLE or CD patients (Addo et al., 1982).

4.4.1.2.9. A total of 267 (82 male/194 female) health care employees that had contact dermatitis were studied to assess the prevalence of contact dermatitis in heath care personnel. Each subject was patch-tested with a GIRDCA standard series, a "health series" and a rubber series (when necessary). All the allergens were applied on the back with Van der Bend® square chambers and removed after 2 days. Readings were carried out at 2 and 3 days. No reactions were observed to 2% methyl salicylate in petrolatum (Stingeni et al., 1995).

4.4.2. Animal studies (Table 7)

4.4.2.1. Maximization studies

4.4.2.1.1. A guinea pig maximization test was conducted in 10 albino Dunkin/Hartley strain guinea pigs. Induction consisted of intradermal injections within a 2×4 cm clipped and shaved area of the dorsal shoulder region and a 48 h occluded patch 7 days later. The induction injections consisted of: two 0.1 ml injections of 1% methyl salicylate in 0.01% dodecyl benzene sulphonate (DOBS)/saline, two 0.1 ml injections of 1% test substance in 50% FCA, and two 0.1 ml injections of 50% FCAT. Seven days later, the animals received an occluded application of 40% methyl salicylate in acetone over the shoulder injection sites. The challenge was conducted 14 days later using an 8 mm diameter filter paper patch saturated with 10% methyl salicylate in acetone (11 mm aluminum patch test cup, held on the flank with adhesive plaster wound around the trunk). No sensitization reactions were observed (RIFM, 1981) (see Table 7).

4.4.2.1.2. The sensitization potential of methyl salicylate was determined in a maximization test (Magnusson and Kligman, 1969) using 9 or 10 Dunkin–Hartley albino guinea pigs. The test animals received 6 intradermal injections of 2.5% methyl salicylate in 0.01% DOBS and Freund's complete adjuvant in the shoulder region. Six to eight days later a 48-h occluded patch with 100% methyl salicylate was applied to the same sites. Control animals received the vehicle alone. Twelve to 14 days after induction, test and control animals were challenged on clipped and shaved flank with 10% methyl salicylate in acetone/polyethylene glycol 400 (70:30) with a 24 h occluded patch. Challenge sites were read 24 h later. No reactions were observed (Kimber et al., 1991; Basketter and Scholes, 1992).

4.4.2.1.3. A guinea pig maximization test (Magnusson and Kligman, 1969) was used to determine the sensitization of methyl salicylate. Induction consisted of two stages; intradermal injection followed eight days later by a 48-h occluded patch application. Male and female outbred Himalayan guinea pigs weighing 400–500 g were used. The intradermal injections consisted of 2 injections of 0.1 ml of 5% methyl salicylate, 2 injections of 0.1 ml of a 5% emulsion of methyl salicylate in FCA, and 2 injections of FCA alone. The topical induction concentration was 25% in petrolatum. On day 21, an occlusive patch of methyl salicylate in petrolatum was applied to the flank for 24 h. Reactions were read 24 and 48 h after patch removal. No sensitization was observed (Klecak et al., 1977).

4.4.2.2. Other studies

4.4.2.2.1. Methyl salicylate was tested in an open epicutaneous test (OET) in male and female outbred Himalayan

Table 7
Sensitization studies in animals

Method	Concentration	Species	Results	References
Maximization test	1% in FCA for intradermal induction; 40% in acetone for topical induction; 10% in acetone for challenge	Guinea pig	No sensitization	RIFM (1981)
Maximization test	2.5% in DOBS/ FCA for intradermal induction; 100% for topical induction; 10% in acetone/ polyethylene glycol for challenge	Guinea pig	No sensitization	Kimber et al. (1991); Basketter and Scholes (1992)
Maximization test	5% in FCA for intradermal induction; 25% in petrolatum for topical induction	Guinea pig	No sensitization	Klecak et al. (1977)
Open Epicutaneous test (OET)	1% and 30%	Guinea pig	1%-minimum eliciting concentration 30%-minimum sensitization concentration	Klecak et al. (1977)
OET	8%	Guinea pig	No sensitization	Klecak (1979, 1985)
Closed Epicutaneous test (CET)	30% for induction; 1% for challenge	Guinea pig	No sensitization	Ishihara et al. (1986)
Draize Test	0.1% in isotonic saline for induction; 0.1% in saline for challenge	Guinea pig	No sensitization	Klecak et al. (1977)
Freund's Complete Adjuvant Test (FCAT)	50% in FCA	Guinea pig	No sensitization	Klecak et al. (1977)
Optimization test	0.1% in saline or FCA/saline for induction; 0.1% in saline for intradermal challenge and 10% in petrolatum for topical challenge	Guinea pig	Sensitization observed in 2/20 animals after intradermal challenge; no sensitization observed after topical challenge	Maurer et al. (1980)

guinea pigs (6–8/group) weighing 400–500 g. Guinea pigs received 21 daily open applications of 0.1 ml of neat methyl salicylate and its progressively diluted solutions (vehicle not reported) to an 8 cm^2 area on the clipped flank. Guinea pigs were challenged by an open application with 0.025 ml of methyl salicylate at minimal irritating concentration and some lower non-irritating concentration (vehicle not specified) on days 21 and 35. Reactions were read 24, 48 and/or 72 h after application. The minimum eliciting concentration was reported to be 1% and the minimum sensitizing concentration was reported to be 30% (Klecak et al., 1977).

4.4.2.2.2. An open epicutaneous test was conducted with 8% methyl salicylate (vehicle not specified) in guinea pigs. Induction consisted of 21 daily open applications to the shaved flank of 6–8 guinea pigs/group. Open challenge applications were made on days 21 and 35. No reactions were observed (Klecak, 1979, 1985).

4.4.2.2.3. Ishihara et al. (1986) conducted a closed epicutaneous test (CET) in five guinea pigs with methyl salicylate (vehicle not provided). Induction consisted of six 48-h closed patch applications using Torii's patch plaster and adhesive tape. Induction applications were made 3 times a week for two weeks with 30% methyl salicylate. On day 28, the animals were challenged with 1% methyl salicylate. Challenge application was a 48-h closed patch on the clipped and shaved flank using Finn Chambers® and adhesive tape. Reactions were read at patch removal and at 24 and 48 h after patch removal. No sensitization reactions were observed.

4.4.2.2.4. Methyl salicylate was tested in a guinea pig sensitization study using a modified Draize procedure (Draize, 1959) in male and female outbred Himalayan guinea pigs weighing 400–500 g. Induction consisted of ten intradermal injections on alternate days with a dose of 0.05 ml of a 0.1% solution of methyl salicylate in isotonic saline starting on day 0. The animals were challenged on days 35 and 49 with an intradermal injection of 0.05 ml of a 0.1% solution of methyl salicylate in saline. Control animals were also challenged with an intradermal injection on days 35 and 49 with methyl salicylate. No sensitization was observed (Klecak et al., 1977).

4.4.2.2.5. Methyl salicylate was tested in a Freund's complete adjuvant test (FCAT) in male and female outbred Himalayan guinea pigs weighing 400–500 g. Guinea pigs received 5 intradermal injections of 0.1 ml of a 50:50 mixture of undiluted methyl salicylate and FCA on days 0, 2, 4, 7 and 9. Control animals received intradermal injections with FCA alone. Challenge was by a 24 h occluded patch with a subirritant concentration of methyl salicylate in petrolatum that was applied to the flank on days 21 and 35. Control animals were also challenged on days 21 and 35 with methyl salicylate. No sensitization was observed (Klecak et al., 1977).

4.4.2.2.6. Male and female Pirbright White Strain guinea pigs (10/sex) were tested in a guinea pig sensitization study using the Optimization test procedure. Induction consisted of a series of 10 intracutaneous injections that were made every other day over a three-week period. A 0.1% solution of methyl salicylate in saline was used during the first induction week and a 0.1% solution of methyl salicylate in a mixture of FCA and physiological saline (1:1) was used during the second and third induction weeks. An intradermal challenge was made 14 days after the last induction application with 0.1% methyl salicylate in saline. This was followed 10 days later by an occluded 24-h epidermal challenge patch that was applied to a 2 cm^2 area on the dorsum. The epidermal challenge concentration was 10% in petrolatum. Reactions were read 24 h after each challenge application. Reactions were observed in 2/20 guinea pigs after the intradermal challenge and in 0/20 guinea pigs after the epidermal challenge (Maurer et al., 1980).

4.4.2.3. Local lymph node assays

4.4.2.3.1. A guinea pig lymph node assay (GPLNA) was conducted on female Hartley albino guinea pigs. On day 1, 200 μl of 10% methyl salicylate in dimethyl sulfoxide (DMSO) was applied to the scapular region for 24 h under occlusion. On day six, superficial dorsal cervical lymph node cells were excised and lymph node cell suspensions were individually prepared and then cultured with methylthymidine (3HTdR). Quantification of 3HTdR incorporation was conducted on day 7. The stimulation index (SI) was 0.78. Under the conditions of the study, methyl salicylate was considered a non-sensitizer (Yoshida et al., 2000).

4.4.2.3.2. Sensitization was evaluated in a local lymph node assay (LLNA). Groups of five female CBA/Ca mice were tested with methyl salicylate at dose levels of 1%, 2.5%, 5%, 10% or 20% in acetone/olive oil (4:1). Each animal received a daily topical application of 25 μl of one concentration of methyl salicylate on the dorsal surface of each ear for three consecutive days. Control animals were treated with the vehicle alone. Five days after the first application all mice were injected intravenously through the tail vein with 250 μl phosphate buffered saline (PBS) containing 20 μCi 3H-methylthymidine (3H-TdR). All mice were sacrificed 5 h after the intravenous injection. Draining auricular lymph nodes were excised and were pooled for each experimental group. Single cell suspensions were then prepared, washed with PBS, suspended in trichloroacetic acid (TCA) and left for 12 h at 4 °C. The samples were then resuspended in TCA and then transferred to a scintillation cocktail. 3H-TdR incorporation was then measured by β-scintillation counting and stimulation indices were determined for each experimental group. For each concentration of methyl salicylate, a stimulation index (SI) relative to the concurrent vehicle-treated control was calculated. The calculated EC3 value from two laboratories for methyl salicylate was >20.0% (Kimber et al., 1991, 1995, 1998).

4.4.2.3.3. Using the same method as above, Yoshida (Yoshida et al., 2000) evaluated sensitization of methyl salicylate at 5% in acetone/olive oil (4:1). The SI value was 0.7, and methyl salicylate was not considered a sensitizer.

4.4.2.3.4. Sensitization was evaluated in a local lymph node assay (LLNA) using the same method as above. Groups of four female CBA/Ca mice were tested with methyl salicylate at dose levels of 1%, 5% or 25% in dimethylformamide (DMF). The SI for 1%, 5%, and 25% was 1.0, 1.2, and 3.0, respectively. When methyl salicylate was used at 5%, 10% and 25% in methyl ethyl ketone (MEK) the SI index was 2.3, 2.5 and 7.5, respectively (Montelius et al., 1994).

4.4.2.3.5. Sensitization was evaluated in a modified local lymph node assay (LLNA). Groups of five female CBA/JHsd mice were tested with methyl salicylate at dose range of 1.0–20.0% in acetone (4:1). Each animal received a daily topical application of 25 μl of one concentration of methyl salicylate on the dorsal surface of each ear for three consecutive days. Control animals were treated with the vehicle alone. Five days after the first application all mice received PBS injected intravenously through the tail vein with 250 μl phosphate buffered containing 20 μCi [^{125}I]-iododeoxy-uridine (^{125}I-UdR) and 10–5 M 5-fluoro-2'-deoxyuridine (FUdR) or 250 μl of PBS with 20 μCi of [^{3}H] TdR. All mice were sacrificed 5 h after the intravenous injection. Draining auricular lymph nodes were excised and were pooled for each individual mouse. Single cell suspensions were then prepared, washed with PBS, suspended in trichloroacetic acid (TCA) and left for approximately 18 h at 4 °C. The samples were then resuspended in TCA. [^{3}H]TdR incorporation was then measured by β-scintillation counting. All SI values were below 3. Under the conditions of the test methyl salicylate was considered to be a non-sensitizer (Ladics et al., 1995).

4.4.2.3.6. Groups of 4 CBA/Ca mice were used in a LLNA. Animals of both sexes were used, but single experiments were limited to one sex. Each animal was treated by a daily topical application of 25 μl of methyl salicylate on the dorsal surface of each ear for three consecutive days. Control animals received vehicle alone. Five days after the 1st topical application, all mice were injected intravenously through the tail vein with [^{3}H]methylthymidine (^{3}HTdr) in saline. After 5 h, the mice were sacrificed, draining auricular nodes were excised, lymph cells were isolated and [3HTdr] measured by liquid scintillation. Methyl salicylate at 25% in 4:1 acetone/olive oil was not considered a sensitizer (Basketter and Scholes, 1992).

4.5. Phototoxicity and photoallergy

4.5.1. In vivo animal studies

4.5.1.1. The phototoxicity of wintergreen oil (which contained 80–99% methyl salicylate) was evaluated in 2 miniature swine. A 20 μl aliquot of neat wintergreen oil was applied to a 5 cm^2 area on the back of each animal. Animals were exposed to UV from a fluorescent black light lamps F4OT12BL (filtered to limit exposure to long wave ultraviolet light only) at a dose of UVA 10 WM^{-2} for 1 h or from a Xenon XBF 6000 W (filtered to stimulate sea level sun light), ½ solar constant for 40 min. The negative control was methanol and the positive was 8-MOP in methanol. No phototoxicity was observed (RIFM, 1976b).

4.5.1.2. As a part of the same experiment described above, undiluted wintergreen oil was applied to the skin of 6 hairless mice miniature swine. No phototoxic reactions were observed (RIFM, 1976b).

4.5.2. In vitro human studies

4.5.2.1. An in vitro photohemolysis assay was conducted to predict the phototoxicity of several chemicals. In the photohemolysis assay, red blood cells (RBC) were obtained from volunteers. The cells were washed and suspended in buffered saline at a dilution of 1:500. A 1 ml aliquot of 0.1% methyl salicylate in ethyl alcohol was added to 99 ml of the RBC suspension. Aliquots of 5 ml, forming cell monolayers in Petri dishes, were exposed to UVA or UVB from batteries of fluorescent tubes for up to 3 h. After the exposure, the dishes were kept in the dark for 30 min and then the suspensions were centrifuged. Hemolysis of the red blood cells was then measured. Methyl salicylate produced no phototoxic effects (Addo et al., 1982).

4.6. Absorption, distribution and metabolism

4.6.1. Percutaneous absorption

4.6.1.1. Human studies. A study of absorption of methyl salicylate was conducted on 10 volunteers. The surfaces of both hands served as the area of application. An enameled foot-tub containing 5 l of the liquid was utilized for all immersion experiments. In a series of tests described below, methyl salicylate was applied in pure form, in aqueous suspensions, in solution in certain oils and in alcohol, and in ointment bases. At the conclusion of each experiment, any remaining methyl salicylate was removed from the skin. Urine was collected and analyzed for sodium salicylate for 24 h and then analyzed until no trace of sodium salicylate was excreted. The following results were obtained.

4.6.1.1.1. The average excretion of sodium salicylate with continuous immersion in methyl salicylate versus a 5-min period of immersion alternated with a 5-min period of draining at 43–44 °C was 90 and 95 mg, respectively. When a 5-min period of immersion was alternated with a 5-minute period of dipping and massage each minute at 43–44 °C, the average excretion was 138 mg. Doubling the immersion and massage period resulted in an average excretion of 232 mg. A 5-min period of immersion alternated with a 5-min period of dipping and massage each minute at room temperature resulted in the excretion of 121 mg. Immersion at room temperature and dipping and massaging at 43–44 °C resulted in an excretion of 125 mg. When the method was reversed and immersion was at 43–44 °C and dipping and massaging was at room temperature the excretion was 162 mg. The average excretion of 28 and 29 mg was observed when the hands were

dipped once, massaged 5 min and allowed to dry or dipped and massaged again at the end of 30 min, both times at room temperature. When the massage at 38 °C with 2 cm^3 portions was renewed every 5 min, the average excretion was 143 mg (Brown and Scott, 1934a). In his second report, Brown and Scott (1934b) reported that continuous massage of the hands with 2 cm^3 methyl salicylate applied every 5 min at 38 °C resulted in an average excretion of 145 and 138 mg for sodium salicylate and methyl salicylate, respectively.

4.6.1.1.2. As a part of the same experiment, a gauze compress was saturated with undiluted methyl salicylate, applied to the forearms and bandaged. The forearms were then placed in a cylindrical hot air oven at 97 °C. The average amount of sodium salicylate excreted in the urine was 278–292 mg (Brown and Scott, 1934a).

4.6.1.1.3. Faint traces of sodium salicylate excreted in urine were observed when 2.4 g of methyl salicylate was rubbed into a 5×7 or 9×10 in. area on the trunk for 20 and 30 min, respectively. When 2.0 g of methyl salicylate was rubbed into a 9×10 or 10×15 in. area on the trunk for 20 and 30 min, respectively, no sodium salicylate excretion was observed. The tests were conducted at room temperature using 2 volunteers (Brown and Scott, 1934a).

4.6.1.1.4. Excretion of sodium salicylate was evaluated when hands were subjected to 5 min of immersion in hot water at 43–44 °C; then rapidly dried and alternated with a 5-min period of dipping in methyl salicylate and draining each minute. The temperature in the room was 34 °C. The average sodium salicylate excretion was 171–232 g. With the same room temperature (34 °C), the average excretion was 254–293 g when hands were immersed for 5 min in water 43–44 °C; rapidly dried and alternated with a 5-min period of dipping and massage each minute (Brown and Scott, 1934a).

4.6.1.1.5. For a 5-min period of immersion in hot water (43–44 °C) after which hands were rapidly dried and alternated with a 5-min period of massage adding 2 cm^3 of methyl salicylate from pipette per minute at room temperature, an average sodium salicylate excretion of 299 mg was observed (Brown and Scott, 1934a). In another report (Brown and Scott, 1934b) an excretion of 299 mg sodium salicylate and 284 mg methyl salicylate was reported.

4.6.1.1.6. As a part of the same series of experiments, the excretion of sodium salicylate in an aqueous suspension was evaluated. Methyl salicylate at a range of concentrations (0.16–95%) was suspended in water (8–4750 ml methyl salicylate/250–5000 ml water). The mixture was agitated with three electric stirrers for 30 minutes prior to hand immersion at a temperature of 26–44 °C and continued throughout the experiment. The average sodium salicylate excretion ranged from 98 mg at 0.2% in 10 ml methyl salicylate/4990 ml water to 524 mg at 95% in 4750 ml of material/250 ml of water. Peak excretion of approximately 100% salicylate was reached after 1 h of immersion in a solution of 11.8% (suspension of 670 ml material/5000 ml water) (Brown and Scott, 1934a). The average methyl salicylate excretion was 300 mg at 0.16% (8 ml methyl salicylate/5000 ml water), 339 mg at 1.65% (84 ml/5000 ml), 397 at 3.8% (190 ml/5000 ml) and 429 mg at 5% (250 ml cm^3/4750 ml) (Brown and Scott, 1934b).

4.6.1.1.7. The influence of different vehicles and heat on methyl salicylate absorption was also evaluated. All solutions of methyl salicylate in different vehicles were prepared by weight. Continuous immersion of hands in a 50% solution of methyl salicylate and olive oil with temperatures at 43–44 °C resulted in an average sodium salicylate excretion of 125–132 mg; with a 5% solution of methyl salicylate only traces of sodium salicylate were excreted. When a 50% solution of methyl salicylate in lard was used, the average excretion at the same temperatures as above was 118–128 mg. With petrolatum, at 43–44 °C, and with a 50% solution, the average excretion was 154–160 mg; at 30–32 °C the excretion was 57 mg. No sodium salicylate excretion was detected with 5% methyl salicylate in petrolatum at 43–44 °C. Continuous immersions in a 50% solution of methyl salicylate in lanoline at 43–44 °C resulted in an excretion of 205–209 mg and at 30–32 °C an excretion of 85 mg. When the hands were immersed for a 5-min period in water 43–44 °C, rapidly dried, and alternated with 5 min periods with dipping and massage in a 50 % solution of methyl salicylate, the average excretion at 43–44 °C was 305–332 mg with lard, 180–217 mg with lanoline, 204 mg with petrolatum and 191–247 with Crisco. When the hands were massage with 2 ml portions of methyl salicylate solution (dose not reported) at 37–38 °C, the average absorption with lard as the vehicle was 32 mg, with petrolatum it was 87 mg and it was 70 mg with olive oil (Brown and Scott, 1934a, 1934b). Methyl salicylate was not detected with continuous immersion in 5% solution at temperatures 43–44 °C when petrolatum was used, and only traces were observed when oil was used. When the hands were continuously massage with 2 ml every 5 min at 38 °C, the average methyl salicylate excretion was 30 and 66 mg, with 50% solutions in lard and olive, respectively (Brown and Scott, 1934b).

4.6.1.1.8. Another test was conducted with 95% ethyl alcohol as the vehicle. At room temperature, hands were dipped once in a methyl salicylate solution, massaged 5 min, dried in the air and again dipped once in 30 min. The average excretion of sodium salicylate was 53 mg. When the same method was used with the exception that the hands were massaged five minutes after the second dipping, the average excretion was 37–42 mg. The average excretion with continuous immersion in 50% solution for one hour at 43–44 °C was 407–595 mg (Brown and Scott, 1934a).

4.6.1.1.9. The absorption from 20% ointments of methyl salicylate with lanoline, benzoinated lard and petrolatum was evaluated. Ointments of methyl salicylate were applied to the hands which were then immersed for a 5-min period in water at 43–44 °C, rapidly dried and alternated with a 5-min period of massage. All visible ointment was removed

before returning the hands to the hot water. The average excretion of sodium salicylate was 12 mg with lanoline, 13 mg with petrolatum and 19 mg with benzoinated lard (Brown and Scott, 1934a).

4.6.1.1.10. Thirty grams of a 20% ointment of methyl salicylate was spread on the left knee without limiting it to an exact area. Muslin bandage was then applied for 24 h. After the exposure period, all excess ointment was removed by washing with soap and water. The average excretion of sodium salicylate was 390–397 mg when lanoline was used as an ointment base. As a part of the same experiment 8 g of a 20% ointment of methyl salicylate was applied to a 45.6 square in. area of forearm that was then covered with oiled muslin sealed at the upper and lower lines with adhesive tape. A bandage was applied for 24 h and then the excess ointment was removed by washing with soap and water. When lanoline was used as the ointment base, the average excretion was 354–390 mg, with benzoinated lard the excretion was 351–356 mg and when petrolatum was used, the excretion was 340–343 mg (Brown and Scott, 1934a).

4.6.1.1.11. The percutaneous absorption of methyl salicylate was investigated in five volunteers. Ten grams of ointment consisting of 20% methyl salicylate and 80% anhydrous lanolin was rubbed into the skin on the chest, abdomen and thigh. Urine samples were collected after application, and at 1 and 2, 4, 8, 12 and 24 h. Each sample was tested qualitatively for the presence of salicylate by the addition of a ferric salt; if the urine sample had to be acidified, a small amount of hydrochloric acid was added. The average of salicylic acid excreted was 41.6 mg. When the same method was applied but the ointment consisted of 60% anhydrous lanolin, 20% methanol and 20% methyl salicylate, the average excretion in 22 volunteers was 55.1 mg (Beutner et al., 1943).

4.6.1.1.12. The same method as above was used to apply 10 g of ointment consisting of a 60% special absorbable base (35% glycerin monostereate, 4.2% phenolic resin, 3.5% acacia, 28% water, 28% alcohol and 1.3% glycerin), 20% methanol and 20% methyl salicylate. The average excretion of salicylic acid in 15 volunteers was 2% (Beutner et al., 1943).

4.6.1.1.13. A commercial formulation containing 20% methyl salicylate was applied to a 4 cm^2 area of forearm skin overlying microdialysis probes (cut of 20,000 Da) placed in the dermis and/or subcutaneous tissue of volunteers. Probes were perfused with normal saline (1–6 μl/min) and, after a 1 h equilibration, fractions were collected at 30- or 50-min intervals for 4–6 h, at which time blood samples were also taken. Salicylate levels were measured in the dialysis fluids and in plasma. Methyl salicylate was applied every 2–3 h for 24 h to determine steady-state concentrations. The mean dialysate levels were 3.4 μg/ml for the dermis and 2.4 μg/ml for the subcutaneous tissue. The level in plasma averaged 0.20 μg/ml. The dialysates contained salicylic acid with no unchanged methyl salicylate (Cross et al., 1997).

4.6.1.1.14. The effects of exercise, heat exposure or both on the percutaneous absorption of methyl salicylate were studied in six healthy male volunteers. The study was carried out under four experimental conditions: at rest and 22 °C; at rest and 40 °C; with exercise to 30% of VO_{2max} at 22 °C; and with exercise to 30% of VO_{2max} at 40 °C. On each occasion, 5 g of undiluted methyl salicylate was applied over the skin of the chest and back of each subject. Venous blood was collected from the cubital vein at 0, 1, 2, 3 and 5 h and urine was collected hourly for 8 h. Both plasma concentrations of total salicylate and urinary salicyluric acid indicated increased systemic availability of salicylate under the experimental conditions of exercise, heat exposure or both. Plasma salicylate peaked at 2 h (~15 μg/ml at control conditions – rest at 22 °C; ~ 20 μg/ml at exercise to 30% VO_{2max}; 25 μg/ml at rest at 40 °C and ~50 μg/ml at exercise at 40 °C). Significant elevation in salicyluric acid excretion was observed. Only 1% of the methyl salicylate applied was recovered in the 8-h urine collected at rest, and 2.6% was recovered after exercise and heat exposure (Danon et al., 1986).

4.6.1.1.15. A total of 28 healthy male volunteers with an average age of 29 were selected for a skin absorption study conducted according to the method of Feldmann and Maibach (1969). A 1.4 cm^2 area was demarcated by petrolatum at two skin sites on the intact skin of the ventral forearm of each subject. A 10 μl solution containing 0.5 mg of methyl salicylate was added drop wise by using a microsyringe. The solvent was evaporated by gentle blowing, and the areas were covered immediately with aluminum foil, the edges of which were fixed with a surgical tape for sealing. The dressings were removed immediately and 4 h after application. The percentage absorption of methyl salicylate through the skin four hours after application was calculated to be 92.9 ± 1.8% (Yano et al., 1986).

4.6.1.1.16. Three groups, each having the same number of male and female volunteers (12) were used in a skin absorption study. Methyl salicylate was used in the form of three 25% ointments made with three different vehicles: lard, petrolatum and hydrous wool fat. Ten grams of ointment were applied for 30 min four times a day to the inner surface of the right thigh, left thigh, right arm and left arm. The residue was completely removed after each application. Urine was collected and tested for the presence of drug during a period of 72 h. The average salicylic acid excreted in the urine was 0.291 g with lard as the vehicle, 0.279 g with petrolatum and 0.268 g with hydrous wool fat (Bliss, 1935).

4.6.1.1.17. Human in vivo microdialysis was performed in male and female volunteers using a topical commercial formulation containing 20% methyl salicylate. Microdialysis probes with a MW cutoff at 20,000 Da were introduced via a guide (16G 57 mm Jelco i.v. placement units) into the dermis or subcutaneous tissue through a 3 mm intradermal weal of lignocaine on swabbed (alcohol or chlorhexidine solution) ventral skin. Probes were taped in place, additionally secured with Opsite® semi permeable transparent

dressing and perfused with normal saline. A total of 17 probes (maximum of 2 probes per volunteer) were placed with at least 3 days washout. Following a 1 h probe equilibration, methyl salicylate was applied to a 16 cm^2 area of the skin over the probe tips but at least 10 mm from covered probe insertion points. Dialysate samples were collected at 30–60 min intervals for up to 360 min. Salicylate could be detected within 1 h of application and continued to rise rapidly over the first 30–90 min. The total recovery of salicylate into probes was 30.7 ± 3.5% (Cross et al., 1998).

4.6.1.1.18. Percutaneous uptake of methyl salicylate was investigated in 10 volunteers who took a 20 min bath by use of Leukona®-Rheumabad (Dr. Atzinger). Using a bathing concentration of 0.03 g/l of methyl salicylate, 2.3–8.7 mg of salicyluric acid was eliminated on the first day, and 0.47–1.48 mg on the second day. The calculated uptake of salicylic acid during a period of 20 min was about 6.76 mg (about 22%) (Pratzel et al., 1990).

4.6.1.1.19. Watkinson (Watkinson et al., 1992) used a mathematical method to estimate total body absorption of some salicylate esters including methyl salicylate. Rate constants were calculated form the relevant physicochemical properties. The applied dose of active ingredient used in the simulation was 40 μg cm^{-2} based on the FDA recommendation (200 mg of product per 100 cm^2 of skin) and a value of 2%. The release rate from the formulation was fixed at 1 μm cm^{-2} h^{-1}. The simulations were conducted on a 12-h time scale. The estimated total body absorption of methyl salicylate per μg over 1.4 m^2 was 91 at 2 h, 2000 at 6 h and 13,000 at 12 h.

4.6.1.2. In vitro human studies

4.6.1.2.1. In vitro skin absorption was evaluated in human breast skin from plastic surgical procedures. Thawed and hydrated full-thickness skin was cleared of any excess subcutaneous tissue, cut into approximately 15 mm^2 pieces and mounted, stratum corneum uppermost, in Franz-type glass diffusion cells, surface area 1.3 cm^2. Human epidermal membranes were also prepared using the heat separation method. Skin samples were allowed to equilibrate for 1 h after which 1 g of topically commercial formulation containing 20% methyl salicylate was placed on stratum corneum side of the skin. Receptor fluid was removed and replaced with fresh solution at 1, 2, 3, 4, 5, 6, 8, 22 and 24 h. The permeability as flux calculated from the cumulative amount versus time was 11.2 ± 0.7 μg cm^{-2} h^{-1} for full-thickness skin and 32.8 ± 2.0 μg cm^{-2} h^{-1} for epidermal membrane. The amount remaining in skin samples of methyl salicylate applied to full-thickness skin or epidermal membrane was 86.7 ± 28.7 μg/100 mg and 41.1 ± 44.1 μg/100 mg, respectively (Cross et al., 1998).

4.6.1.3. Animal studies

4.6.1.3.1. A skin absorption was conducted using the rabbit ear model. A semi-solid vehicle containing methyl salicylate was applied to one ear of a lop rabbit and blood samples were collected from the contralateral side. Plasma levels of the test material were determined by high performance liquid chromatography. With 10% methyl salicylate, the amount of salicylate absorbed decreased with increasing molecular weight of polyethylene glycol (PEG) (data from abstract only) (Davis et al., 1981).

4.6.1.3.2. The effect of pH on absorption was evaluated using white male Sprague–Dawley rats. One hour before the experiment, the tail of each rat was washed with distilled water. After this period, the rat's tail was immersed into a methyl salicylate buffer solution in a perfusion container (19.5 × 2.5 cm) of 68 ml capacity. To prevent any contamination and to prevent evaporation of the solvent, the container was sealed. The container was immersed into a thermostatically controlled water bath, where the water was circulated through the outside mantle of the flow cell. The absorbance was continuously recorded. Total absorption of methyl salicylate was 1.56 μg mm^{-2} h^{-1} at pH 2, 0.76 μg mm^{-2} h^{-1} at pH 3, 1.77 μg mm^{-2} h^{-1} at pH 6 and 1.57 μg mm^{-2} h^{-1} at pH 8 (Siddiqi and Ritschel, 1972).

4.6.1.3.3. Commercially marketed creams containing 10% to 28.3% methyl salicylate were applied to depilated abdominal skin of anesthetized male Wistar rats weighing 308 ± 17.0 g. The application duration was 2 h after which any remaining methyl salicylate was removed. Salicylate levels were measured in blood and in tissue samples taken from the application site and a contralateral site. Direct penetration of methyl salicylate to the muscle at the site of application was seen. Methyl salicylate was first absorbed into the bloodstream and subsequently distributed to both the deeper tissues on the treated site and the contralateral tissues (Megwa et al., 1995).

4.6.1.3.4. The skin absorption of methyl salicylate was evaluated in hairless mice. An aliquot of 5.2 mg methyl salicylate was applied to a 2 cm^2 plaster and then applied to the dorsal skin of each hairless mouse. At 1, 3 and 6 h after application, the mice were sacrificed and the plaster was carefully removed. The skin at the treated site was wiped with gauze dampened with warm water and then excised. The cutaneous levels of methyl salicylate and salicylic acid were 0.64 and 0.49 μM/g skin respectively, 1 h after application. At 6 h the levels were 0.29 and 22 μM/g skin, respectively (Yano et al., 1991).

4.6.1.3.5. The recovery of ^{14}C labeled methyl salicylate in ethanol was reported 1 and 6 h after epicutaneous administration to guinea pigs. The rate of the material recovered was reported for up to four recovery categories: residual, resorbed, in the skin and lost as evaporation. At 1 h, approximately 2% was in the skin, 14% was resorbed, 82% was lost by evaporation and 2% was residual; at 6 h, approximately 16% was resorbed, 82% was lost by evaporation and 2% was residual. When methyl salicylate was administered by intradermal injection, approximately 42% was in the skin, 54% was resorbed and 4% was lost by evaporation at 1 h; at 6 h, approximately 96% was resorbed and 4% was lost by evaporation (Data from graphs only) (Klecak, 1985).

4.6.1.3.6. Twenty-seven 10-week-old Yorkshire-Landrace cross barrow pigs were used in a skin absorption study. A circular plastic cup with two holes pierced through it to accept an 18-gauge needle was positioned over a piece of gauze cloth that was cut to a diameter slightly smaller than the cup and that was placed over the skin. Four sites were challenged including ear, epigastrium, perineum and inguinal crease with total area of exposure of 49.3, 132.4, 49.3 and 88.2 cm^2, respectively. Neat methyl salicylate was introduced into the cup through one of the holes at volumes of 848 μl for the ear, 2544 μl for the epigastrium, 848 μl for the perineum and 1696 μl for the inguinal crease. Arterial blood samples were taken every 10 min for the first 60 min and then every 15 min up to 360 min. The average dose absorbed through the skin at the ear region after 6 h was 11 $\mu g\ cm^{-2}$; at the perineum regions the average dose absorbed was 8 $\mu g\ cm^{-2}$ and through the epigastrium and inguinal crease regions the average dose absorbed was 3 $\mu g\ cm^{-2}$. The initial flux (permeation rate) of salicylic acid through the skin after application of neat methyl salicylate was 0.063 $\mu g\ cm^{-2}\ min^{-1}$ at the ear region, 0.025 $\mu g\ cm^{-2}\ min^{-1}$ at the epigastrium region, 0.044 $\mu g\ cm^{-2}\ min^{-1}$ at the perineum region and 0.012 $\mu g\ cm^{-2}\ min^{-1}$ at the inguinal crease region (Duncan et al., 2002).

4.6.1.4. In vitro animal studies

4.6.1.4.1. The percutaneous absorption of methyl salicylate through viable and nonviable hairless guinea pig skin was evaluated by Boehnlein et al. (1994). The experiment was conducted using flow-through diffusion cells. Skin viability was maintained in the diffusion cells by using Hepes buffered Hanks balanced salt solution (HHBSS) as receptor fluid. Nonviable skin was produced by perfusing the cells with distilled water instead of HHBSS. Radio labeled methyl salicylate was applied to skin in an acetone vehicle at doses of approximately 3, 5 and 20 $\mu g\ cm^{-2}$. After 24 h the surface of the skin was washed three times with soap and water to remove any unabsorbed methyl salicylate. Percutaneous absorption as a percent of the dose was 55% for viable male skin, 56% for viable female skin, 47% for nonviable male skin and 50% for nonviable female skin.

4.6.1.4.2. Percutaneous absorption of methyl salicylate was evaluated in the isolated perfused porcine skin flap (IPPSF). A dose of 400 $\mu g\ cm^{-2}$ of radio labeled ^{14}C methyl salicylate was applied non-occluded to a 7.5 cm^2 Stomadhesive® dosing template on the IPPSF. Skin flaps were allowed to equilibrate for 1 h prior to chemical application. A total of 16 flaps were dosed and terminated at 2, 4 and 8 h. Percutaneous absorption into IPPSF was 2.39% of the applied dose at 8 h. With the amount in skin and fat added, the penetration was 3.04% of the applied dose (Riviere et al., 2000, 2001).

4.6.1.4.3. The penetration of methyl salicylate was measured across full thickness hairless mouse skin using a glass flow-through diffusion cell. Fresh skin was clamped between the upper and lower halves of the diffusion cells. The area of skin exposed to the donor phase was 0.95 cm^2. Methyl salicylate was dissolved or suspended in acetate buffer. A 1% suspension was applied to the exposed skin surface in the donor phase of the diffusion cell. The receptor chamber (approximately 3 ml) was perfused with phosphate buffered saline at a rate of 5 ml/h. The steady state flux was $2.8 \pm 0.2\ \mu mol\ cm^{-2}\ h^{-1}$ (Higo et al., 1995).

4.6.2. Pharmacokinetics

4.6.2.1. Human studies

4.6.2.1.1. The rate of systemic methyl salicylate absorption was evaluated in male and female volunteers (6/sex). Each subject applied 5 g of an ointment, containing 12.5% methyl salicylate, to a 10 cm^2 area on the anterior aspect of the thigh. The site was then protected with a non-occlusive dressing consisting of ordinary gauze and Micropore tape. The applications were done twice daily for 4 days for a total of 8 applications. Blood samples were drawn on days 1 and 4, just before the morning application and at 1, 2, 3, 4, 6, 8, 12 and 24 h after application. A 24-hour urine collection was started immediately before each day morning application. Salicylic acid concentrations between 0.31 and 0.91 mg/l were detected in the serum within 1 h of the first application. Maximum concentrations between 2 and 6 mg/l were observed following the seventh application on day 4. The absorption rate constant increased significantly from the first to the seventh dose ($0.16–0.28\ h^{-1}$). Urinary recovery of total salicylate (salicylic acid and principal metabolites of salicylic acid) during the first 24 h averaged 175.2 mg. The fraction of methyl salicylate recovered in the urine increased significantly from 15.5% on day 1 to approximately 22% on the second, third and fourth day (Morra et al., 1996).

4.6.2.1.2. The rate of absorption of commercial products containing 12–50% methyl salicylate was evaluated by Roberts et al. (1982). Five grams (5 g) of product was applied to a 50 cm^2 area on the forearm of five subjects in a Latin Square design. A small portion of the product was rubbed into the area and the remaining product was then spread evenly over this site. The site was covered with a sheet of aluminum foil, greaseproof paper (70 cm^2) and with Elastoplast®. The product was left in place for 10 h and then removed with soap and warm water. All urine was collected at 6, 8, 10, and 12 h and up to 48 h. The skin permeability coefficients for methyl salicylate were 1.3–1.5 $cm^{-1}\ h^{-1}$ at 12%, 1.5–1.9 $cm^{-1}\ h^{-1}$ at 25% and 1.0 $cm^{-1}\ h^{-1}$ at 50%. The estimated steady-state salicylate concentrations ranged from 2.5 at 12% to 7.6 at 50%.

4.6.2.1.3. Four (1 male/3 female) adult human volunteers participated in a study that was conducted as an open label, 4-way crossover design with randomized treatment order. The subjects ingested 6.7 and 20 g of methyl salicylate cream (commercial Ben Gay 15% cream containing 900 or 2700 mg salicylate). Plasma was collected at 0, 20, 40, 60, 120, 240, 480, 720, and 1440 min for the determination of salicylate concentrations by TDx immunoassay. The time to reach maximum salicylate concentration (T_{max}) and the peak plasma salicylate concentration (C_p max)

were determined. The T_{max} for the low-dose cream (900 mg salicylate) was 2.4 h (1.5–4 h), and the C_p max was 42 mg/l (36–51 mg/l). The T_{max} for the high-dose cream was 7 h (4–12 h), and the C_p max was 145 mg/l (120–201 mg/l) (Wolowich et al., 2003).

4.6.2.1.4. As a part of the same experiment described above, four fasting adults ingested 1 ml of wintergreen oil (14.2 mg/kg mean). Plasma was collected for salicylate determination at 0, 20, 40, 60, 120, 240, 480, 720 and 1440 min. Time to reach maximum concentration was 2.4 h with the maximum concentration of 70 mg/l (Wolowich et al., 2003).

4.6.2.2. Animal studies

4.6.2.2.1. The absorption rate coefficient was evaluated using a recirculating perfusion method. Male rats with an average body weight of 270 g were fasted for 24 h prior to the experiment (water was *ad libitum*). The small intestine was exposed by a midline abdominal incision and cannulated at the immediately distal part and at the 20 cm distal part to the entrance of the bile duct with glass cannulae having inside diameter of 2.5 mm and outside diameter of 3.5 mm. The small intestine was first cleared by perfusion with 100 ml of 0.9% NaCl solution maintained at 37 °C and then the sample solution (50, 100 or 200 ml) at 37 °C was perfused by re-circulation from the proximal to the distal at a rate of 20 ml per minute. The average absorption rate coefficient was 1.43 (Nogami et al., 1968).

4.6.2.2.2. Groups of four female outbred Swiss mice with mean body weights of 28.5 g were used. Air was passed into the cage through a glass tube containing 1.5 ml methyl salicylate. Total methyl salicylate volume was 20–50 mg. Blood samples were taken from the animals after 0, 30, 60 and 90 min of inhalation exposure. Plasma was extracted and the sera were investigated by gas chromatographic-spectroscopic systems to identify and quantify the test materials. After 1 h of exposure only traces of methyl salicylate were found in blood samples (Buchbauer et al., 1993).

4.6.2.3. In vitro animal studies

4.6.2.3.1. As a part of the experiment described in Section 4.6.1.4.2, the rate of absorption was also evaluated. Radio labeled methyl salicylate showed a rapid absorptive flux profile that peaked at approximately 30 min at 0.016% dose/min (Riviere et al., 2000, 2001).

4.6.2.3.2. As a part of a percutaneous penetration study (Section 4.6.1.4.3) penetration parameters for methyl salicylate were calculated following topical application of a 1% suspension in acetate buffer to hairless mouse skin at 32 °C. The rate of absorption was 1.8 ± 0.2 μM cm^{-2} h^{-1} (Higo et al., 1995).

4.6.3. Metabolism

4.6.3.1. Human studies

4.6.3.1.1. Six young healthy adults (4 male/2 female) received a single dose of 0.42 ml methyl salicylate in 5 ml ethanol and 200 ml cold ginger ale. Subjects had fasted for at least 10 h prior. Blood was withdrawn by venipuncture 15 and 90 min later. Methyl salicylate blood levels after a single oral dose were 4.9 mg/l at 15 min and 2.8 mg/l at 90 min. The free salicylate levels were 7.9 mg/l at 15 min and 10.5 mg/l at 90 min (Davison et al., 1961).

4.6.3.2. Animal studies

4.6.3.2.1. A single dose of 300 mg/kg methyl salicylate in capsule form was administered orally to fasting male mongrel dogs weighing 12–15 kg. Blood was withdrawn from the cephalic vein at 1 and 4 h intervals, and plasma was analyzed. Hydrolysis was about 95% complete at both time intervals (no further details reported) (Davison et al., 1961).

4.6.3.2.2. Methyl salicylate was administered by gavage at a dose of 1.4, 1.72 and 2.98 g to three dogs. One additional dog received 1.4 g of methyl salicylate by intramuscular injection. The urine in each case was collected. Following the gastric administration of 1.4 g methyl salicylate, 0.542 g of sodium salicylate and 0.005 g of non-metabolized methyl salicylate (0.36%) were identified in the urine. Following the gastric administration of 1.72 g, 0.44 g of sodium salicylate and 0.003 g of non-metabolized methyl salicylate (0.2%) were identified in the urine. With administration of 2.93 g of methyl salicylate, 0.875 g of sodium salicylate and 0.016 g of non-metabolized methyl salicylate (0.52%) were identified in the urine. The duration of salicylic excretion was 3, 4 and 6 days (Hanzlik and Wetzel, 1920).

4.6.3.2.3. A single dose of 300 mg/kg methyl salicylate in 2% methylcellulose was administered by gavage to groups of 10 male Wistar rats weighing 200–350 g. Blood samples were obtained at 20 and 60 min after administration. Plasma and brain tissue were analyzed for methyl and free salicylate. Methyl salicylate was completely hydrolyzed within 20 min of a single oral dose to 10 rats. Brain levels of total salicylate were 8 mg/l at 20 min and 42 mg/l at 60 min (Davison et al., 1961).

4.6.3.2.4. In a study to evaluate teratogenic potential, undiluted methyl salicylate (2 g/kg per day) was dermally applied to groups of 12 or more pregnant rats, on gestational days 6–15. Urinalysis was conducted on each animal. Very high concentrations (toxic levels) of salicylic acid were found in the urine (no more details provided; data from abstract only) (Infurna et al., 1990).

4.6.3.3. In vitro animal studies

4.6.3.3.1. As a part of experiment described in Section 4.6.1.4.3, conversion of methyl salicylate to salicylic acid in hairless mouse skin following topical application of 1% methyl salicylate in acetate buffer to the skin was evaluated. Less than 5% of applied dose was metabolized to salicylic acid (Higo et al., 1995).

4.6.3.3.2. Metabolism of methyl salicylate in male and female hairless guinea pig skin was evaluated by Boehnlein et al. (1994), as a part of experiment described in Section

4.6.1.4.1. The differences between viable and non-viable skin and between the sexes of animals were significant. As a percentage of the dose absorbed, metabolism of 5 μg cm^{-2} methyl salicylate, by viable skin obtained from male guinea pigs, was 21% to salicyluric acid and 36% to salicylic acid. Viable skin from female guinea pigs led to 12% salicyluric acid and 12% salicylic acid. With nonviable skin obtained from males, metabolism was 38% to salicylic acid. With nonviable skin obtained from females, metabolism was 13% to salicylic acid.

4.7. Repeated dose studies

4.7.1. Subchronic toxicity

4.7.1.1. Dermal studies

4.7.1.1.1. Methyl salicylate was applied once daily to the clipped backs of male and female rabbits (3/dose) at dose levels of 0.5, 1.0, 2.0 and 4 ml/kg/day (~equivalent to 590, 1180, 2360 and 4720 mg/kg/day) for 6.5 hours a day, 5 days a week, up to 96 days. Microscopic examinations were made of select tissues from all animals but one high-dose rabbit which was discarded because of extreme autolysis. In addition, examinations were also made of Bouin-fixed liver, kidney and bone marrow stained with hematoxylin and eosin and of frozen sections of liver and kidney that were stained for fat. Additional formalin-fixed tissues from five animals were stained with hematoxylin and eosin. No effects were observed in animals that received 0.5 and 1 ml/kg/day of methyl salicylate. A slight sloughing of epidermal scales occurred in two of the three rabbits on 2.0 ml/kg/day. At 4 ml/kg/day all animals experienced anorexia, weight loss and depression, and all died by day 28. One animal in the high dose group had distinct microscopic lesions in the kidneys, skin, muscle, pancreas, bone marrow and liver (Webb and Hansen, 1962, 1963).

4.7.1.1.2. Three dogs, weighing 14–16 kg received dermal applications of methyl salicylate. A dose of 2000 mg/kg/day of methyl salicylate was applied to a previously shaved 12 × 10 cm area on their backs, twice a day (total dose each day, 5000 mg/kg) for 16 days. Liver and kidney functions were evaluated by urine and blood analysis. Clinical signs included markedly decreased diuresis, albumin in urine (0.12, 0.15 and 0.25 g/l), excess blood nitrogen (increased by 12% and 28%) and decreased alkaline reserve (average decrease by 23%). Ten days after the end of treatment animals showed only traces of albumin and a normal blood nitrogen level (Giroux et al., 1954).

4.7.1.2. Oral studies

4.7.1.2.1. An oral study was conducted using pure bred beagles (3/sex/dose). Methyl salicylate was administered by gelatin capsule at doses of 0, 150, 300, 500 and 800 mg/kg/day in divided doses following the morning and afternoon feeding. Body weights were determined weekly. Routine hematology, blood chemistries and urinalysis were conducted on animals of the 150 and 300 mg/kg/day groups and the control group. All surviving animals, with the exception of three dogs in the 300 mg/kg dose were sacrificed after 6.5–7.5 months. The remaining three animals from the 300 mg/kg group also received methyl salicylate for 6.5 months but the animals were sacrificed 6 weeks later. At the time of sacrifice, all animals were subjected to gross examination. During necropsy, weights of all principal organs were determined and all major tissues were taken for histological examination. All animals from the control group and animals receiving 150 and 300 mg/kg/day survived the test period. Five (5/6) animals receiving 800 mg/kg/day died during the first week and the sixth dog died in the second week. Two (2/6) dogs receiving 500 mg/kg/day survived the test period with one dog each dying at weeks 2, 3, 5 and 8. None of the dogs receiving 150 and 300 mg/kg/day exhibited any loss in weight during the test period. One (1/6) of the surviving dogs in the 500 mg/kg/day group showed a slight loss in body weights. Routine hematological examinations, blood chemistry and urinalysis on the animals of the 150 and 300 mg/kg/day groups performed in the fifth month were within normal limits and comparable to the values obtained on the control animals. Gross examinations of the sacrificed animals were negative. The relative organ weights in terms of grams per kilogram of body weight of the animals from all groups were comparable to control animals except for those of the liver and kidney. The mean relative liver and kidney weights of methyl salicylate animals were significantly in excess of those for the control group and appeared to be dose related. Histological examination revealed a general increase in liver cell size and an alteration in cytoplasmic granularity. These subtle changes were unaccompanied by alteration in tissues viability, growth pattern, fibrous tissue content, nodularity or other signs of hepatotoxicity. A NOAEL of 300 mg/kg/day can be derived from these data (Abbott and Harrisson, 1978).

4.7.1.2.2. A second study was conducted using purebred beagles. Methyl salicylate was administered by capsule in divided doses (morning and afternoon feedings) at dosages of 50, 100 and 167 mg/kg/day. The two lower dosage groups contained 8 dogs (4/sex) and the highest dosage group and the control group contained 12 dogs (6/sex). Food consumption and body weights were determined weekly. All test animals were subjected to routine hematology and blood chemistry examinations before treatment and then prior to sacrifice. Methyl salicylate was administered over a six-month period after which eight animals from each of the three dosage levels and from control groups were sacrificed. Daily administration to 4 remaining animals in the 167 mg/kg/day group was also terminated after six months, but these animals were sacrificed two months later (eight months from beginning of the test period). All sacrificed animals were subjected to comprehensive histological and macroscopic examinations with the weight of the liver and kidney determined. All animals survived the 6-month test period. The growth and body development of all dogs were normal. In the second month of the test period, many of the dogs showed signs of seborrhea

oleosum and pyoderma with the direct relationship in the severity of this condition to the dose of methyl salicylate. The addition of lard to the diets of all animals caused a remission of this skin condition. Routine hematology and blood chemistry examinations were in the range of normal and comparable to the values of the control group. The only gross observations attributed to methyl salicylate at the time of necropsy, were changes in the gastric mucosa. One animal from each of the three groups exhibited hyperemic foci of the pyloric mucosa. All kidney sections examined were within normal limits and detectable differences were not noted between test groups. There was an incidence of hepatic cellular infiltration in livers, but it was found to be within the normal range. The mean liver and kidney weights values for all three dosage groups were within the normal range (Abbott and Harrisson, 1978).

4.7.1.2.3. Methyl salicylate was given orally by capsule to dogs (1/sex/dose) at dose levels of 50, 100, 250, 500, 800 or 1200 mg/kg/day, 6 days a week for up to 59 days. Clinical observations and gross necropsy were conducted on all animals. Microscopic examinations were conducted on tissues from two animals in the 250 mg/kg and one in the 800 mg/kg groups. Two dogs that received 1200 mg/kg/day and one dog that received 800 mg/kg/day vomited 3–4 h following administration of each dose of methyl salicylate. Two dogs from the 500 mg/kg/day level exhibited diarrhea and weakness during the last 3–4 days and lost weight. All dogs that received 500 mg/kg/day or more died within a month of the experiment. A moderate to marked amount of fatty metamorphosis was observed in the livers of the two dogs from 1200 mg/kg/day group and of one dog from 800 mg/kg/day group. Animals receiving 50, 100 and 250 mg/kg/day of methyl salicylate showed no adverse effects during the experiment (Webb and Hansen, 1962, 1963).

4.7.1.2.4. Methyl salicylate was administered in diet to Sprague–Dawley rats (5/sex/group) at doses of 0.2%, 0.36%, 0.63%, 1.13% or 2% (~equivalent to 100, 180, 320, 560 or 1000 mg/kg/day) for 12 weeks. The test animals received methyl salicylate at 50% of the final dose during weeks 1 and 2 and at 75% of the final level during weeks 3 and 4 (these adjustments in the dietary levels of methyl salicylate during the initial 4 weeks was done to correct for the relatively large amount of food consumed by young animals in relation to their body weight). Body weights, food consumption and whole body roentgenograms were recorded. Whole body X-rays were taken during week 10. In some cases rats were sacrificed and their femurs and tibias examined for confirmation of the roentgenograms. Animals of both sexes receiving 0.2% and 0.36% in the diet and females in the 0.63% dose group were comparable to those of the control group. Males in the 0.63% dose group and male and females in the 1.13% and 2.0% groups exhibited decreased weight gain. Increased density at the metaphyses of the femur, humerus, tibia and radius in the animals of the highest dose groups (1.13% and 2%) were observed (Abbott and Harrisson, 1978).

4.7.1.2.5. To study the possible nutritional implications observed in the above study, groups of 10 male Spraque–Dawley rats received 0.6% or 2.0% (~equivalent to 300 or 1000 mg/kg/day) methyl salicylate *ad libitum* for six weeks. The pair fed groups received 0.6% methyl salicylate, 2% methyl *p*-OH benzoate or control diet. Survival and body weights were observed. At 0.6%, decreased growth was observed in the pair fed group and *ad libitum* group. No increases in mortality were observed when compared to controls in both groups. At 2%, 90% mortality and decreased weight gain were observed in the pair-fed and *ad libitum* groups (Abbott and Harrisson, 1978).

4.7.1.2.6. Groups of five male Sprague–Dawley rats received 0.6 and 2% (~equivalent to 300 or 1000 mg/kg/day) methyl salicylate in diet for 12 weeks. Survival and whole body roentgenograms were recorded. All of the animals in the high dose group died during the first 6 weeks. Also, bone lesions were observed in the whole body X-rays of the animals of this dose. No effects were observed in animals at the low dose (Abbott and Harrisson, 1978).

4.7.1.2.7. Another study was conducted by Abbott and Harrisson (1978) to evaluate the progression of bone change and to determine whether or not an intermediate level between 0.6% and 1.2% methyl salicylate in the diet would lead to an increase in cancellous bone. Groups of Sprague–Dawley rats (10/sex/dose) were administered methyl salicylate in the diet at dose levels of 0.6%, 0.9%, 1.2% and 2% (~equivalent to 300, 450, 600 and 1000 mg/kg/day) over a period of 11 weeks. Whole body X-rays were taken weekly, of 2 animals from each group. A week after the X-rays the animals were sacrificed and the femurs of some of the animals were subjected to histological examination. Bone lesions were observed in high dose animals at week 2. Animals in the 1.2% dose showed unequivocal signs of bone lesions starting at week 5. Bone lesions were not observed in animals in the two lowest dose groups and in the control group. Histology examination showed an increase in cancellous bone in the 2.0% methyl salicylate group after week 2 and in the 1.2% group after 8 weeks. No effects were observed in the 2 lowest dose groups (Abbott and Harrisson, 1978).

4.7.1.2.8. To investigate the effect of the addition of calcium to the diet, groups of Sprague–Dawley rats received a diet containing 1.2% (~equivalent to 600 mg/kg/day) methyl salicylate with or without 0.3% calcium carbonate added. Mortalities were 15% for the 1.2% with 0.3% calcium carbonate group and 90% for 1.2% without calcium carbonate group. The addition of calcium to the methyl salicylate had a positive effect in preventing the formation of bone lesions and in enhancing body growth and survival. In a subsequent study, 2% methyl salicylate (~equivalent to 1000 mg/kg/day) plus 0.33% calcium carbonate was fed to a group of 20 rats (10/sex) for an 11-week test period. X-rays taken between weeks 2 and 8 gave no evidence of bone lesions, and survival was 70% over the whole test period (Abbott and Harrisson, 1978).

4.7.1.2.9. Groups of 20 weanling Osborne–Mendel rats (10/sex) received diets containing 0.1 or 1.0% methyl salicylate (~equivalent to 50 or 500 mg/kg/day) for 17 weeks. The weight of each rat was recorded weekly. Organ weights and gross pathology was conducted on all animals. Histopathology was conducted on 4 animals/sex in the control and 1.0% groups. Significantly lower body weight gains were observed in both sexes in the high-dose group when compared to controls. Gross and microscopic findings were negative (Webb and Hansen, 1962, 1963).

4.7.1.2.10. A supplemental study was conducted on groups of 12 (3/sex/dose) Osborne–Mendel rats as a part of an associated chronic feeding study. Animals were fed diets containing 0 and 2.0% methyl salicylate (~equivalent to 1000 mg/kg/day) for up to 71 days. When a test rat died, a control rat of the same sex was killed. The entire carcass with head attached of each rat was fixed and X-rays of the entire carcass were taken of the animals which died on day 71 or were sacrificed on day 71 and the control animals. X-rays were taken of a front leg and a hind leg of all rats. Measurements taken from X-rays were used to construct growth curves for the femur, humerus, tibia and radius. Histopathology was conducted on select tissues from those dying or sacrificed at day 71. The control animals were healthy throughout the experiment. All treated animals died by day 71. One (1/3) treated male died at 11 days and 2 at 19 days whereas the females died at 31, 40 and 71 days. Rough hair coat and stunting of growth occurred in all six treated animals. Some animals experienced labored respirations. Four (4/6) animals had slight to moderate lung damage. Focal gastric hemorrhages in glandular stomach were observed in 3/6 animals. Increased bone density in growth areas of all bones was observed. In addition, increased life span of primary trabeculae was observed (Webb and Hansen, 1962, 1963).

4.7.1.2.11. Methyl salicylate at dose levels of 1.12 or 2% (~equivalent to 550 or 1000 mg/kg/day) was fed in a diet of rats for up to 10 weeks to investigate the increase of cancellous bone. Food intake and weight gain was decreased and mortality was increased at 2%. The increase of cancellous bone was observed at both doses (no further details, data from abstract only) (Harrisson et al., 1963).

4.7.1.3. Inhalation studies

4.7.1.3.1. Groups of male and female Alderly Park rats weighing an average of 200 g were exposed to dynamic atmospheres (atmospheres continuously generated and passed through the exposure chamber) containing methyl salicylate for up to 7 h a day, 5 days a week for up to 4 weeks. Food and water were available *ad libitum*. Animals were weighed each day, and their conditions and behavior were recorded throughout the exposure period. Urine was collected overnight after the last exposure day for biochemical testing. Blood was taken for hematological testing. Gross necropsy was conducted as well as microscopic examination of organs. Methyl salicylate (700 mg/m^3, 120 ppm) did not cause any adverse effects (Gage, 1970).

4.7.2. Chronic studies

4.7.2.1. Groups of beagles (2/sex/dose) were given 50, 150 or 350 mg/kg/day methyl salicylate orally by capsule, 6 days a week for 2 years. The animals were weighed weekly, and the dosages were recalculated at that time. Hematological examinations were made 3 times prior to the start of the experiment and at 2 weeks, 1, 3 and 6 months and 1 and 2 years after the start of the experiment. Necropsies were conducted on dogs dying or sacrificed during the study or at termination. Major organs were weighed and observations recorded. Microscopic examinations were made on three surviving high-dose dogs. Bone marrow smears, liver and kidney sections were stained for fat. One-high dose female died of infectious canine hepatitis after 33 days on the study. Her replacement died of canine distemper after 19 weeks on the study. No other mortalities were observed. All hematological examinations were negative. At 150 and 350 mg/kg/day, growth retardation and body weight loss was observed. Higher relative liver weights, grossly enlarged livers and larger hepatic cells were also observed at both doses. No effects were observed at 50 mg/kg/day (Webb and Hansen, 1962, 1963).

4.7.2.2. Groups of 50 (25/sex) weanling littermate Osborne–Mendel rats were fed diets containing 0.1%, 0.5%, 1.0% or 2.0% methyl salicylate (~equivalent to 50, 250, 500 or 1000 mg/kg/day) for 2 years. Animals were weighed weekly. Hematological examinations were made at 3, 11, 17 and 22 months on 10 animals per group. Organ weights and gross pathology was conducted on all animals. Histopathology examinations were conducted on 12 control animals, 6 animals in the 1.0% dose group and 5 animals in the 2.0% group. Gross examinations, lesions, leg bones and muscles examinations were conducted on 17 control animals, 25 animals in the 0.1% dose group, 24 animals in the 0.5% dose group, 12 animals in the 1.0% dose group and 7 animals in the 2.0% dose group. No effects were observed at the lowest dose. At 0.5%, gross pituitary lesions were observed in 10 treated animals and in 4 control animals. At 1%, significant growth inhibition and rough hair coats were observed; testes of the male rats and heart and kidneys of the female rats were larger than those of the control rats and a slightly increased amount of cancellous bone in the metaphysis was also observed at this dose. In the 2% dose group, 50% of the animals were dead after 8 weeks and 100% were dead after 49 weeks. Significant growth inhibition, rough hair coats and a high incidence of pneumonia were observed. Also, moderate to marked increased amount of cancellous bone in the metaphysis was observed at the highest dose (Webb and Hansen, 1962, 1963).

4.7.2.3. A 2-year study was conducted in albino rats (25/sex/dose) at dose levels of at 700 or 2100 ppm (~equivalent to 35 or 100 mg/kg/day) methyl salicylate. A control group was also included. A smaller group of animals received 0.06% "butter yellow" in the diet as a positive hepatic

carcinogen. Observations conducted included growth, survival, food usage, general condition, blood and urine studies, necropsy and histology. No effects were observed at 700 or 2100 ppm (no further details provided, data from abstract only) (Packman et al., 1961).

4.8. Mutagenicity and genotoxicity

4.8.1. Bacterial studies

4.8.1.1. The Rec-assay was conducted using *Bacillus subtilis* strains H 17 (rec+) and M 45 (rec−), and dimethyl sulfoxide (DMSO) as the vehicle. A dose of 23 μg methyl salicylate produced no effects (Oda et al., 1978).

4.8.1.2. The Rec-assay was conducted using *B. subtilis* strains H 17 (rec+) and M 45 (rec−), and dimethyl sulfoxide (DMSO) as the vehicle. No effects were observed with methyl salicylate up to 5000 μg/plate (Kuboyama and Fuji, 1992).

4.8.1.3. Doses of 1–333 μg/plate methyl salicylate in DMSO were not mutagenic when tested in a preincubation modification of the Ames test with *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, with and without rat and hamster S9 activation (Mortelmans et al., 1986).

4.8.1.4. In an Ames test (Ames et al., 1975) using *S. typhimurium* strains TA92, TA94, TA98, TA100, TA1535 and TA1537 with and without S9 activation, doses up to 10 mg/plate in DMSO were not mutagenic (Ishidate et al., 1984).

4.8.1.5. In an Ames test using preincubation method with *S. typhimurium* strains TA98 and TA100, positive effects were observed with 100 μg/plate and S9 obtained from hamster. No effects were observed when the S9 was obtained from rat, mouse or guinea pig (Kuboyama and Fuji, 1992).

4.8.2. Mammalian studies

4.8.2.1. Chromosomal aberration tests were carried out with a Chinese hamster fibroblast cell line. The cells were exposed to each sample at three different doses for 24 and 48 h without metabolic activation. Untreated cells and solvent treated cells served as negative controls. Methyl salicylate at maximum dose tested (25 mg/ml in DMSO) produced no effects (Ishidate et al., 1984).

4.9. Reproductive and developmental toxicity

4.9.1. Reproductive toxicity

4.9.1.1. A three-generation study was conducted on groups of 20 Osborne–Mendel rats, evenly divided by sex. Animals were fed methyl salicylate at 500, 1500, 3000 or 5000 ppm (∼equivalent to 25, 75, 150 or 250 mg/kg/day) mixed with food, for 100 days after which the animals were mated. Each generation was mated twice. Observations included fertility, survival, litter size, gross fetal abnormalities, and liver and kidney histopathology. There were no effects observed at 500 or 1500 ppm. In the animals treated with methyl salicylate at 3000 and 5000 ppm, decreases were seen in average litter size, average number of live born progeny, average number of survivors to day 4, and average number of survivors to weaning. Autopsy findings of third-generation weanlings were negative (Collins et al., 1971).

4.9.1.2. Abbott and Harrisson (1978) conducted a reproduction study on Wistar strain rats (25 rats/dose/sex). Rats were fed methyl salicylate at levels of 0.25% or 0.5% (∼equivalent to 125 or 250 mg/kg/day) in Purina Diet. The parent stocks (F_0) were maintained on their assigned diets for 60 days prior to mating. The F_0 stocks were mated twice to produce F_{1a} and F_{1b} litters. The F_{1a} were maintained through weaning; approximately 30 days after weaning the F_0 stock was remated. Thirty males and 30 females were randomly selected from the F_{1b} litters of each test and control group to serve as the parent stock for the F_{2a} and F_{2b} litters. The test diets were fed to all animals (parent and young) throughout the entire test period (from the initiation of the F_0 stock through the weaning of the F_{2b}). Reproduction parameters (total born, live born, live at 5 days and weaned at 21 days) and pup abnormalities were monitored. Decrease in litter size was observed at 0.25%; increased number of unsuccessful matings and increased number of deaths between birth and 5 days when compared to controls was observed at 0.5%. However, these findings were not statistically significant.

4.9.1.3. Parallel in design to the rat reproduction study, a mouse study was conducted. Mice (25/group/sex) of the parent stock (F_0) were maintained on dietary levels of 0.25% or 0.5% (∼equivalent to 125 or 250 mg/kg/day) methyl salicylate for a period of 30 days prior to the first mating. All parameters of the mouse study were the same as those cited above for the reproduction study. No effects were observed at either dose (Abbott and Harrisson, 1978).

4.9.1.4. The NTP Fertility Assessment by Continuous Breeding test was conducted using 20 F0 COBS CD-1 (ICR)BR outbred albino mice/sex/dose. Methyl salicylate was administered (gavage) daily at dose levels of 25, 50 and 100 mg/kg/day in corn oil during the 7-day premating, 98-day cohabitation, and 21-day segregation period. Reproductive parameters and histopathology were evaluated. Under the conditions of this study, methyl salicylate at daily oral dosage as high as 100 mg/kg/day was not a reproductive toxicant in either F_0 or F_1 breeding pairs of mice (NTP, 1984a; Morrissey et al., 1989; Chapin and Sloane, 1997).

4.9.1.5. Another NTP Fertility Assessment by Continuous Breeding test was conducted using 11-week-old 20 F0 COBS CD-1 (ICR)BR outbred albino mice per sex per

dose. Methyl salicylate at a dose levels of 100, 250 or 500 mg/kg/day in corn oil was administered by gavage. Dosing was daily, 7 days prior to mating, and throughout the 120 day mating trial. Observations included body weight (all animals were weighed once per week from day 0 to day 127), number of litters produced, number and percent of live pups per litter, mean body weight of live offspring, percent of infertile pairs and histopathology. Two animals died at 100 and 250 mg/kg/day doses and four died at 500 mg/kg/day; however, they were neither chemical nor dose related. No other effects were observed at 100 mg/kg/day. Reduced pup weights were observed at 0.25 g/kg/day. At the highest dose, decreased number of litters, pups per litter, live pups and mean live pup weight were observed when compared to controls. Crossover breeding could not determine which sex was affected (NTP, 1984b; Morrissey et al., 1989; Chapin and Sloane, 1997).

4.9.2. Developmental toxicity

4.9.2.1. Neat methyl salicylate was applied to the skin of 12 or more pregnant rats on gestation days 6–15 at a dose of 2000 mg/kg/day. However, due to maternal toxicity (25%) and severe dermal irritation the dose was reduced to 1000 mg/kg/day on gestation days 10–15. A 100% incidence of total resorptions was observed (data from abstract only) (Infurna et al., 1990).

4.9.2.2. Warkany and Takacs (1959) conducted a study using 116 female rats weighing 170–200 g. The rats were mated, and the day on which sperm was found in vaginal smears, was considered the first day of pregnancy. Pregnant females received single subcutaneous injections of methyl salicylate in doses ranging from 0.1 to 0.5 cm^3 (~equivalent to 118 to 590 mg/kg/day) on the 9th, 10th or 11th day of pregnancy. Maternal observations included weight, survival, and resorptions whereas fetal observations included external and skeletal malformations. Animals were sacrificed on gestation day 21. Most of the females lost about 15–20 g after treatment, but some recovered and regained the lost weight. Twenty-six females died and 47 resorbed their young after treatment. The remaining pregnant rats (43) regained the lost weight within 3 or 4 days and continued gaining until they were sacrificed. Of the 298 young obtained, 45 were externally abnormal. Of the 253 young that appeared externally normal, 75 showed skeletal anomalies. Observed malformations included craniorachischisis; gastroschisis; exencephaly; hydrocephalus; anomalies of vertebrae and ribs expressed in the cervical, thoracic and lumbar regions; harelip; oblique facial clefts and/or cleft palate.

4.9.2.3. Pregnant Long-Evans rats received subcutaneous injections of 0.1 ml (~equivalent to 118 mg/kg/day) methyl salicylate on the 10th and 11th day of gestation. Animals were sacrificed on day 20, and necropsies were undertaken on day 21. Of the animals receiving treatment on the 10th day, 27.3% underwent resorption and 31.4% living fetuses showed congenital malformations. Animals treated on the 11th day had 32.7% resorption and 18.2% living fetuses with abnormalities. In both groups, most abnormalities occurred in the cardiovascular, urogenital and skeletal system. Retarded fetal growth and abnormalities of the branchial arch arterial derivatives, cleft lip, cleft palate and hydroureter were also observed. Hydronephrosis and ectopic kidney were observed occasionally. Clubfoot and phocomelia of the hind limbs were commonly seen following injection on day 11th (Bertone and Monie, 1965).

4.9.2.4. Timed pregnant, 90-day old CD rats (5/dose) received intraperitoneal injections of methyl salicylate on the 9th and 10th gestation day at doses of 200 and 400 mg/kg/day. Dams were sacrificed on gestational day 21. The fetal brain, lungs, livers and kidneys were weighed and examined biochemically. The fetal body weight was significantly reduced in the high dose group. Dose-related reductions in brain weight, lung growth, liver growth and kidney growth were observed at both doses (Kavlock et al., 1982).

4.9.2.5. The effect of methyl salicylate on renal development in late gestation of rats was evaluated by Woo and Hoar (1972). Pregnant CD female rats received by intraperitoneal injection 0.05 or 0.1 g (~equivalent to 59 or 118 mg/kg/day) of methyl salicylate on days 10 and 11 of gestation. Control females received no treatment. Maternal body weights were recorded at weekly intervals. The young were investigated after cesarean section on gestation day 21 or postnatally at 1, 6, 12 or 24 days of age. They were counted, weighed, and examined for viability and external malformations. Kidneys were removed, weighed fresh or after fixation, sectioned transversely through the hilum and renal papilla and graded on a scale from zero (no papilla) to 4 plus (full size). Females given methyl salicylate gained less weigh, had fewer and smaller offspring, and had more resorptions and malformed young than controls. Reduced fetal kidney weight and lengthening of papilla suggested renal growth retardation. There was a marked reduction in mean body weight and mean kidney weight in 21-day treated fetuses, but recovery was rapid, and by day 6 there was little or no difference in kidney weight between control and treated group. A small number of kidneys (11/138) in the treated groups showed gross dilation of the renal pelvis and reduction of the renal parenchyma (apparent hydronephrosis or hypoplasia).

4.9.2.6. Pregnant female Sprague–Dawley rats received intraperitoneal injections of 250, 300, 375, 400 or 450 mg/kg/day methyl salicylate on gestation day 12; or 200, 250 or 300 mg/kg/day on gestation days 11–12; or 300, 350 or 375 mg/kg/day on gestation days 12–13 or 200, 250 or 300 mg/kg/day on gestation days 12–14. Controls were injected daily with 5 ml/kg 0.85% saline on gestation days 11–14. Dams were killed on gestation day 21. Number of live and dead fetuses and resorptions was counted. Live

fetuses were removed from the uterus, dissected free of their external membranes, weighed as a litter, fixed and examined for dilated renal pelvis and soft tissue anomalies. Degree of dilation was scored. Decrease in weight gain during pregnancy was noted in dams receiving 200 mg/kg/day or higher for 3 days (gestation days 12–14); in dams receiving 300 mg/kg/day or higher for 2 days (gestation days 11–12 or 12–13); and in dams receiving 400 mg/kg/day or higher on gestation day 12. A few maternal deaths were observed, generally at higher doses, but no significant dose-related pattern was detected. Malformations were observed in fetuses of dams receiving 350 mg/kg or greater on gestation day 12, and after 200 mg/kg or greater for more than 1 day of gestation. A dose-related reduction in fetal weight was observed, and some increase in the incidence of resorption. Ectopic kidneys located in the lower lumbar to sacral region were also observed. The overall incidence of dilated renal pelvis was less than 10% and was not dose related (Daston et al., 1988).

4.9.2.7. On the basis of the above results, Daston et al. (1988) dosed another group of rats with 200, 250 or 300 mg/kg/day methyl salicylate on gestation days 11–12 in order to study postnatal renal functions of the offspring. Average litter size and birth weight were not affected by the 200 mg/kg/day dose. There was increased mortality during the first 2 days after birth in the two higher dose groups (18% at 250 mg/kg/day and 67% at 300 mg/kg/day), however the surviving pups had no external abnormalities, and their weights were comparable to controls. A significant increase in kidney/body weight ratio was observed on day 15 in all groups exposed prenatally to methyl salicylate, but it cleared by 4 weeks of age. Body weight was decreased at 4 weeks of age in the 250 mg/kg/day group, but not in the 300 mg/kg/day. Renal defects were rarely observed postnatal.

4.9.2.8. LVG-strain pregnant hamsters were treated orally (gavage) on the 7th day of gestation with 1750 mg/kg body weight of methyl salicylate. Controls received an equivalent volume of saline solution. Blood samples after treatment were analyzed spectrophotometrically for salicylates. Most embryos were recovered on the 9th day of gestation. Some were allowed to continue their development, however since many embryos died between 9th and 12th day, the incidence of malformations observed at the 9th day was considered to be an indicator of teratogenic effects. Oral treatment resulted in an incidence of neural tube malformations in about 72% of fetuses. Blood salicylates levels rose rapidly after treatment to approximately 125 mg/100 ml in 2 h, and then returned to normal over a period of 12–24 h (Overman, 1979; Overman and White, 1978, 1983).

4.9.2.9. As a part of the same experiment described above methyl salicylate was applied to the skin of pregnant hamsters. The animals' backs were shaved prior to topical application of methyl salicylate at doses 3500 or 5250 mg/kg body weight on the 7th day of gestation. After 2 h the treated skin was thoroughly washed with running water. Blood samples after treatment were analyzed spectrophotometrically for salicylates. Topical application resulted in a more gradual increase in blood salicylate level, reaching a maximum 5–6 h after treatment. Teratogenic results were similar (6% at 3500 mg/kg and 53% at 5250 mg/kg) but less consistent than those following oral treatment. The study showed methyl salicylate to be teratogenic in hamsters when applied topically, although a very high dose is necessary to achieve the same blood level and teratogenic effects seen after oral treatment (Overman and White, 1978, 1983).

4.10. Carcinogenicity

4.10.1.

Methyl salicylate was examined for its ability to induce lung tumors in male and female A/He mice (15/sex/dose). The mice were 6–8 weeks old with an average initial weight of 18–20 g. Animals received intraperitoneal injections of methyl salicylate in tricaprylin 3 times a week for 8 weeks. Dose levels were set at the maximum tolerated dose (MTD) and a 1:5 dilution of the MTD. An MTD of 0.5 g/kg had been established in a preliminary toxicity screen. The total cumulative doses were 2.4 and 12 g/kg. An untreated control group of 50 mice per sex was also included. The experiments were terminated 24 weeks after the first injection. Treated and control animals were sacrificed and a gross and microscopic examinations of the lungs were carried out. Liver, kidney, spleen, thymus, intestine, and salivary and endocrine glands were also examined for abnormalities and necropsy. Three (3/15) male animals and two (2/15) female animals died in the high dose group; two (2/15) male animals and one (1/15) female animal died at low dose. Methyl salicylate was not considered carcinogenic, as no significant difference from control animals was observed (Stoner et al., 1973).

4.10.2.

The antitumor activity of wintergreen oil (99% methyl salicylate) was evaluated in 32 mice of the A strain that had developed spontaneous carcinomas of the mammary gland. Methyl salicylate was added to the basic diet in three proportions: 1 drop of oil to 1 g of diet (1:1), 2 drops to 1 g of food (2:1) and 3 drop to 1 g (3:1). All animals were kept under the same conditions. The growth rate of the tumor, food intake, total body weight and the survival time after the discovery of the tumor was evaluated. Daily administration of methyl salicylate after the onset of cancer had no detectable effect on malignancy. Authors conclude that in order to influence tumors, natural oil of wintergreen must be administered to young animals before tumors arise (Strong, 1932a).

4.10.3.

The effect of wintergreen oil on the occurrence of spontaneous carcinomas of the breast was studied in 45 female

D strain mice with an average age of 11.7 months. For 41 days, one drop of oil was added to 10 g of oat diet, then for 26 days one drop of oil was added to 50 g of diet and finally one drop to 40 g has been continued to the end of the experiment (until they reached about 23 months of age). Ten (10/45) mice on the test diet developed spontaneous tumors. Their ages averaged 18.0 months, whereas the average for the mice on the control diet was 12.1 months. Thirty-five (35/45) mice died of other causes than cancer at ages beyond the time the control mice had normally developed carcinoma (average 19 months) (Strong, 1932b).

4.10.4.

The effects of wintergreen oil (sample from *Betula lenta*) on the growth of grafted tumors were evaluated in groups of 5 Strong A or Little Dilute Brown (dba) mice with grafted Crocker Sarcoma 180 tumors. Methyl salicylate was administered by gavage, 5 or 6 times per week beginning at grafting at a dose level of 10 mg/day. Treatment continued for 50 days. The tumors were measured 3 times weekly and their growth compared with that of control tumors that had been grafted onto the same strain of mice at the same time from the same tumor. Animals were also observed to see if methyl salicylate had any effect on the prolongation of life. No effects were observed (Boyland and Huntsman-Mawson, 1938).

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, L. Jones, D. McGinty, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on 3-methyl-2-butenyl salicylate

A. Lapczynski *, L. Jones, D. McGinty, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of 3-methyl-2-butenyl salicylate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; 3-Methyl-2-butenyl salicylate

Contents

In 2006, a complete literature search was conducted on 3-methyl-2-butenyl salicylate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Benzoic acid, 2-hydroxy-,3-methyl-2-butenyl ester, 3-methyl-2-butenyl salicylate, prenyl salicylate.
1.2 CAS Registry number: 68555-58-8.
1.3 EINECS number: 271-434-8.
1.4 Formula: $C_{12}H_{14}O_3$.
1.5 Molecular weight: 206.24.

2. Physical properties

2.1 Physical description: A colorless liquid.
2.2 Flash point: >200 F; CC.
2.3 Boiling point: 125 °C @ 2 mm Hg.
2.4 $\log K_{ow}$ (calculated): 4.41.
2.5 Vapor pressure (calculated): 0.000074 mm Hg 25 °C.
2.6 Water solubility (calculated): 26.51 mg/l @ 25 °C.
2.7 Specific gravity: 1.09.

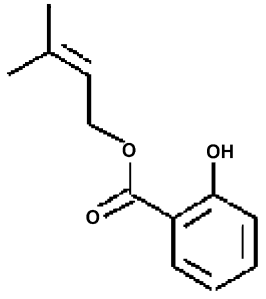

Fig. 1. 3-Methyl-2-butenyl salicylate.

3. Usage (Table 1)

3-Methyl-2-butenyl salicylate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 1–10 metric tonnes per annum.

The maximum skin level that results from the use of 3-methyl-2-butenyl salicylate in formulae that go into fine fragrances has not been reported. A default value of 0.02% is used, assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has not been reported. As such, a default value of 0.02% is used to calculate a maximum daily exposure on the skin of 0.0005 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral toxicity of 3-methyl-2-butenyl salicylate was determined in 10 rats/group. 3-Methyl-2-butenyl salicylate was administered at 1.22, 1.95, 3.12, and 5.0 g/kg. The animals were observed over a 14-day period and a gross necropsy was conducted on all animals. No deaths occurred at 1.22 and 1.9 g/kg doses; 5/10 animals died at 3.12 g/kg; 10/10 animals died at 5 g/kg. Clinical signs that were observed included chromorhinorrhea, lethargy, diarrhea, emaciation, piloerection, ataxia, chromodacryorrhea, and convulsions. Necropsy was normal in 10/10 animals at 1.2 g/kg. At 1.95 g/kg, necropsy revealed mottled kidneys in one animal. At 3.12 and 5.0 g/kg, necropsy observations

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing 3-methyl-2-butenyl salicylate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product	Ingredient/mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.02	0.0001
Face cream	0.80	2.00	1.000	0.003	0.02	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.02	0.0002
Fragrance cream	5.00	0.29	1.000	0.040	0.02	0.0002
Antiperspirant	0.50	1.00	1.000	0.010	0.02	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.02	0.0000
Bath products	17.00	0.29	0.001	0.020	0.02	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.02	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.02	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.02	0.0000
Total						0.0005

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60 kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	Number of animals/ dose group	LD_{50} (g/kg)	References
Oral	Rat	10	3.2	RIFM (1978a)
Dermal	Rabbits	10	>5	RIFM (1978a)

included exudates from the nose/mouth and anogenital regions, intestines with red or yellow areas, dark liver and lungs and small and mottled spleens. The LD_{50} was calculated to be 3.2 g/kg (95% C.I. 2.6–3.9 g/kg) (RIFM, 1978a).

4.1.2. Dermal studies

4.1.2.1. The dermal LD_{50} in rabbits was reported to be greater than 5 g/kg based on 0/10 deaths. Neat 3-methyl-2-butenyl salicylate was applied to intact or abraded skin for 24 h under occlusion. Animals were observed over a 14-day period. Gross necropsy was conducted on all animals. Clinical signs observed during the study included diarrhea, slight emaciation, yellow discharge from nose and swinging head from side to side. Necropsy was normal in 5/10 animals. Bloated intestines and dark lungs and livers were observed in 5/10 animals (RIFM, 1978a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1 In a pre-test for a human maximization study, no irritation was observed after a 48 h closed patch test with 20% 3-methyl-2-butenyl salicylate in petrolatum applied to the back or forearms of 25 healthy male and female (5 male/20 female) volunteers (RIFM, 1978b).

4.2.2. Animal studies

4.2.2.1. Irritation was evaluated during the associated LD_{50} study described above. Neat 3-methyl-2-butenyl salicylate produced slight (2/10 rabbits) to moderate (8/10 rabbits) erythema and slight (4/10 rabbits) to moderate (6/10 rabbits) edema (RIFM, 1978a).

4.3. Mucous membrane (eye) irritation

4.3.1.

An eye irritation test was conducted on three normal, healthy, albino rabbits. A 0.1 ml aliquot of 5% 3-methyl-2-butenyl salicylate in 75% ethanol was instilled into the right eye of each of animal without further treatment. The left eye of each animal was used as a control. Both eyes were examined every 24 h for 4 days and again on the seventh day. Reactions were scored according to Draize. No irritation was observed (RIFM, 1970).

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A maximization test was carried out with 20% 3-methyl-2-butenyl salicylate in petrolatum on 25 healthy male and female volunteers (5 male/20 female). The application was under occlusion to the same site on the forearms of all subjects for five alternate 48-h periods. Patch sites were pretreated for 24 h with 2.5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a 10-day rest period, a challenge patch was applied to a fresh site for 48 h under occlusion. Before the challenge application, 5–10% SLS was applied to the test site for 1 h. Reactions to challenge were read at patch removal and 24 h after patch was removal. No sensitization reactions were observed (RIFM, 1978b).

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution, and metabolism

No data available on this material.

4.7. Subchronic toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, L. Jones, D. McGinty, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the

manufacturers of fragrances and consumer products containing fragrances.

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Belsito, D., Bickers, D., Bruze, M., Calow, P., Greim, H., Hanifin, J.H., Rogers, A.E., Saurat, J.H., Sipes, I.G., Tagami, H., 2007. A toxicologic and dermatologic assessment of salicylates when used as fragrance ingredients. Food and Chemical Toxicology 45 (1S1), S318–S361.

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Review

Fragrance material review on methyl 4-methylsalicylate

D. McGinty *, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of methyl 4-methylsalicylate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Methyl 4-methylsalicylate

Contents

In 2006, a complete literature search was conducted on methyl 4-methylsalicylate. On-line databases that were surveyed included chemical abstract services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Benzoic acid, 2-hydroxy-4-methyl-, methyl ester; methyl 2-hydroxy-4-methylbenzoate.

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: dmcginty@rifm.org (D. McGinty).

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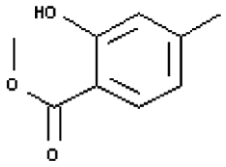

Fig. 1. Methyl 4-methylsalicylate.

1.2 CAS registry number: 4670-56-8.
1.3 EINECS number: 225-117-6.
1.4 Formula: $C_9H_{10}O_3$.
1.5 Molecular weight: 166.76.

2. Physical properties

2.1 Log K_{ow} (calculated): 3.15.
2.2 Vapor pressure (calculated): 0.0459 mm Hg 25C.

3. Usage (Table 1)

Methyl 4-methylsalicylate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of less than 0.01 metric tonnes per annum.

The maximum skin level that results from the use of methyl 4-methylsalicylate in formulae that go into fine fragrances has not been reported. A default value of 0.02% is used, assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has not been reported. As such, a default value of 0.02% is used to calculate a maximum daily exposure on the skin of 0.0005 mg/kg for high end users of these products.

4. Toxicology data

4.1. Acute toxicity

No data available on this material.

4.2. Skin irritation

No data available on this material.

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

No data available on this material.

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

No data available on this material.

4.7. Repeated dose toxicity

No data available on this material.

4.8. Reproductive and developmental toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing methyl 4-methylsalicylate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	0.02	0.0001
Face cream	0.80	2.00	1.000	0.003	0.02	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.02	0.0002
Fragrance cream	5.00	0.29	1.000	0.040	0.02	0.0002
Antiperspirant	0.50	1.00	1.000	0.010	0.02	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.02	0.0000
Bath products	17.00	0.29	0.001	0.020	0.02	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.02	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.02	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.02	0.0000
Total						0.0005

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60 kg adult.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

D. McGinty, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufactures of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufactures of fragrances and consumer products containing fragrances.

Reference

Belsito, D., Bickers, D., Bruze, M., Calow, P., Greim, H., Hanifin, J.H., Rogers, A.E., Saurat, J.H., Sipes, I.G., Tagami, H., 2007. A Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients. Food and Chemical Toxicology 45 (1S1), S318–S361.

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Review

Fragrance material review on pentyl salicylate

A. Lapczynski *, D. McGinty, L. Jones, S.P. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of pentyl salicylate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Pentyl salicylate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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doi:10.1016/j.fct.2007.09.057

In 2006, a complete literature search was conducted on pentyl salicylate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Amyl salicylate; benzoic acid, 2-hydroxy-, pentyl ester; 2-hydroxybenzoic acid, pentyl ester; pentyl 2-hydroxybenzoate; salicylic acid, pentyl ester.
1.2 CAS Registry Number: 2050-08-0.
1.3 EINECS Number: 218-080-2.
1.4 Formula: $C_{12}H_{16}O_3$.
1.5 Molecular weight: 208.26.
1.6 COE: Pentyl salicylate was included by the Council of Europe in the list of substances – information required – none listed (COE No. 613) (Council of Europe, 2000).

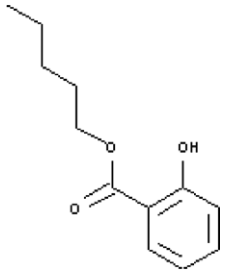

Fig. 1. Pentyl salicylate.

2. Physical properties

2.1 Log K_{ow} (calculate): 4.57.
2.2 Vapor pressure (calculated): 0.003 mm Hg 20 °C.
2.3 Water solubility (calculated):18.94 mg/l @ 25 °C.
2.4 Henrys law (calculated): 0.0000141 atm m^3/mol 25 °C.

3. Usage

Pentyl salicylate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 100–1000 metric tonnes per annum.

The maximum skin level that results from the use of pentyl salicylate in formulae that go into fine fragrances has been reported to be 2.98% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 6.93% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.1766 mg/kg for high end users (see Table 1).

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. Healthy male Wistar albino rats (10/dose) were dosed orally with pentyl salicylate at 3.1, 4.0, 5.0 or 6.3 g/kg body weight. The rats were observed for mortality and/or systemic effects 3–4 h after dosing and daily thereafter for 14 days. At 3.1 and 4.1 g/kg, 4 out of 10 animals died; 7/10 animals died at 5 g/kg and all animals died at

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing pentyl salicylate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	6.93	0.0262
Face cream	0.80	2.00	1.000	0.003	6.93	0.0055
Eau de toilette	0.75	1.00	1.000	0.080	6.93	0.0693
Fragrance cream	5.00	0.29	1.000	0.040	6.93	0.0670
Antiperspirant	0.50	1.00	1.000	0.010	6.93	0.0058
Shampoo	8.00	1.00	0.010	0.005	6.93	0.0005
Bath products	17.00	0.29	0.001	0.020	6.93	0.0001
Shower gel	5.00	1.07	0.010	0.012	6.93	0.0007
Toilet soap	0.80	6.00	0.010	0.015	6.93	0.0008
Hair spray	5.00	2.00	0.010	0.005	6.93	0.0006
Total						0.1766

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rat	10	4.1 g/kg	RIFM (1982a)
Oral	Rat	10	2.0 g/kg	RIFM (1990)
Dermal	Rabbit	10	>5 g/kg	RIFM (1982a)

6.3 g/kg. Lethargy, chromorhinorrhea, ptosis, chromodacryorrhea, yellow nasal discharge, and brown staining were observed during the course of the study. Necropsy observations were normal in surviving animals. Necropsy of the animals that died revealed abnormalities of the liver, gastrointestinal tract, kidneys and lungs. The LD_{50} was calculated to be 4.1 g/kg (95% CI 3.3–5.0 g/kg) (RIFM, 1982a).

4.1.1.2. The acute oral LD_{50} of pentyl salicylate was reported to be 2.0 g/kg based on 5/10 deaths at that dose. Male and female Sprague–Dawley rats (5/sex) were given a single oral dose of the 2.0 g/kg pentyl salicylate in arachis oil BP. The animals were observed 1 and 4 h after dosing and then once daily for 14 days. Five animals were found dead one to three days after treatment. Signs of toxicity included hunched posture, lethargy and pilo-erection. Reduced gain in bodyweight and bodyweight loss were noted during the study. Observations at necropsy revealed hemorrhage or abnormally red lungs, dark or pale liver, pale spleen, hemorrhage of glandular gastric epithelium, sloughing of non-glandular gastric epithelium and hemorrhage of the small intestine (RIFM, 1990).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits exceeded 5.0 g/kg based on 0/10 deaths at that dose. Ten rabbits (5/sex) received a single dermal application of neat pentyl salicylate which was applied to intact or abraded skin for 24 h under occlusion. The rabbits were observed for mortality and/or systemic effects over a period of 14 days. Gross necropsy was conducted on all animals. Lethargy, chromorhinorrhea, ptosis, chromodacryorrhea, yellow nasal discharge, and moderate irritation were observed in 3 or more rabbits during the course of the study. A decrease in body weight was noted in 2 rabbits. Necropsy observations revealed abnormalities in the lung and intestines in 1–2 rabbits and alopecia in 2 rabbits (RIFM, 1982a).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. As a part of maximization test, 10% pentyl salicylate in petrolatum was pre-tested for irritation on 27 male and female volunteers. Pentyl salicylate was applied to normal sites on the backs for 48 h under occlusion. No irritation was observed (RIFM, 1982b).

4.2.2. Animal studies

4.2.2.1. As a part of modified Draize sensitization study, a preliminary irritation screen was conducted to determine the injection challenge concentration (ICC). Four inbred Hartley strain albino guinea pigs with an average weight of 450 g were given intradermal (i.d.) injections on the shaved flanks with 0.1 ml aliquots of pentyl salicylate at a range of concentrations. Reactions were read 24 h after application. Pentyl salicylate at concentration of 0.05% produced irritation and was selected as the ICC (Sharp, 1978).

4.2.2.2. As a part of modified Draize sensitization study, a preliminary irritation screen was conducted to determine the application challenge concentration (ACC). Four inbred Hartley strain albino guinea pigs with an average weight of 450 g received dermal applications on the shaved flanks with 0.1 ml aliquots of pentyl salicylate at a range of concentrations. Reactions were read 24 h after application. Pentyl salicylate at 10% (vehicle not reported) produced no irritation and was selected as the ACC (Sharp, 1978).

4.2.2.3. Prior to an open epicutaneous test (OET), pentyl salicylate, at a range of concentrations, was evaluated for irritation in 6–8 male and female Himalayan white-spotted guinea pigs. A 0.025 ml aliquot was applied with a pipette to an area measuring 2 cm^2 on the clipped flank. The application site was left uncovered and reactions were read after 24 h. Pentyl salicylate at 10% (vehicle not specified) was the lowest concentration to produce mild erythema in at least 25% of the animals and this dose was selected as the minimum irritating concentration after one application (Klecak et al., 1977).

4.2.2.4. Pentyl salicylate was evaluated for irritation, at several dose levels, during the induction phase of an open epicutaneous test (OET). A 0.1 ml aliquot of pentyl salicylate was applied to an area measuring 8 cm^2 on the clipped flank of 6–8 male and female outbred Himalayan white-spotted guinea pigs. The application site was left uncovered and reactions were read after 24 h. A total of 21 daily applications were made. The minimum irritating concentration after 21 applications was 3% (vehicle not specified) (Klecak et al., 1977).

4.2.2.5. In a preliminary irritation study conducted prior to a guinea pig maximization study, 4 male albino Dunkin/Hartley strain guinea pigs weighing 360–428 g were intradermally injected with 0.1 ml aliquots of 0.1%, 0.25%, 0.5%, 1.0% and 2.0% pentyl salicylate in 0.01% DOBS/saline. After 24 h, the injection sites were examined for size, erythema and edema. The concentration which produced irritation was 1.0 % and it was selected as an induction challenge concentration (RIFM, 1981).

4.2.2.6. As a part of the same preliminary irritation study (Section 4.2.2.5), four guinea pigs were topically treated

with 8 mm diameter filter paper patches saturated with 10%, 25%, or 50% pentyl salicylate in acetone using 11 mm aluminum patch test cups. The cups were applied to shaved flanks for 24 h under occlsuion. Reactions were assessed for irritation at 24 and 48 h after patch removal. No irritation was observed. For the topical induction concentration, 40% pentyl salicylate was selected and for the challenge application 10% pentyl salicylate was selected (RIFM, 1981).

4.2.2.7. Pentyl salicylate was evaluated for irritation as a part of an acute LD_{50} study conducted in ten healthy albino rabbits weighing 2.2–3.0 kg. A dose of 5.0 g/kg of neat pentyl salicylate was applied via occluded patches to the clipped abdomen of each animal for 24 h. Reactions were read at patch removal, 7 days later, and 14 days later. Well-defined to moderate erythema and very slight to moderate edema were observed on day 1; very slight to severe erythema and very slight to moderate edema were observed on days 7 and 14 (RIFM, 1982a).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies (Table 3)

4.4.1.1. Induction studies

4.4.1.1.1. A human maximization test was conducted on 27 healthy male and female volunteers. Pentyl salicylate was applied on the volar aspects of the forearms of all subjects for 5 alternate days, 48 h periods. Patch sites were pretreated for 24 h with 5% aqueous Sodium Lauryl Sulfate (SLS) under occlusion for the initial patch only. Following a 10–14 day rest period, challenge patches were applied under occlusion to fresh sites for 48 h. Challenge applications were preceded by 30 min applications of 5% aqueous SLS. Pentyl salicylate at 10% in petrolatum produced one reaction. However, the results clinically appeared to be due to irritation. The subject refused retesting (RIFM, 1982b).

4.4.1.1.2. A maximization test was carried out with 20 healthy, male volunteers. Five applications of 10% pentyl salicylate were applied for 48 h periods to the volar forearm sites of each volunteer. Prior to the fourth and fifth application, test sites were pretreated with 24-h occlusive applications of 5% SLS. Following a 10 day rest period the volunteers were challenged with pentyl salicylate applied under occlusion to the backs following one hour pretreatment with 10% aqueous SLS. Reactions were read at 48 and 72 h. No sensitization was observed (RIFM, 1970).

4.4.1.1.3. A maximization test was carried out on 26 healthy Japanese–American volunteers. All volunteers were pretreated for 24 h with 3% SLS. Pentyl salicylate at 10% in petrolatum was applied under occlusion to same sites on the volar aspects of the forearms for 48 h periods for 5 alternate days. The initial patch was preceded by 24 h pretreatment with 5% aqueous SLS under occlusion. Following a 10–14 day rest period challenge patches of all materials were applied under occlusion to fresh sites for 48 h. Challenge applications were preceded by 30-min applications of 5% aqueous SLS under occlusion on the left side and without SLS treatment on the right side. No sensitization reactions were observed (RIFM, 1979).

4.4.1.2. Diagnostic studies (Table 4)

4.4.1.2.1. Frosch et al. (1995) reported the results of a multicenter study on patch tests with 48 fragrance materials. Pentyl salicylate was applied to the backs with Finn Chambers® and Scanpor® for 2 days. Reactions were assessed per ICDRG guidelines on days 2 and 3 or in some cases on days 2 and 4. Pentyl salicylate at 5% in petrolatum was tested in 100 patients (48 females and 52 males). Questionable reactions were observed in two (2/100) patients. When 1% pentyl salicylate was tested one (1/100) reaction was observed and one questionable (1/100) reaction was also observed.

4.4.1.2.2. Closed patch tests were conducted on 155 patients with 0.05–0.5% pentyl salicylate in a base cream or in 99% ethanol. Patches consisted of a piece of 1 cm^2 lint with a 2 cm^2 cellophane disc placed on the lint and then covered with a 4 cm^2 plaster. Patches were applied to the back, the forearm and the inside of the upper arm for 24–48 h. Reactions were read 30 min after patch removal. Erythema was observed in 8 out of 316 patients (Takenaka et al., 1986).

Table 3
Summary of human skin sensitization studies

Method	Concentration	Results		References
		Reaction	Incidence	
MAX	10% (6900 μg/cm^2)	1 questionable reaction/27	3.7%	RIFM (1982b)
MAX	10% (6900 μg/cm2)	0/20	0%	RIFM (1970)
MAX	10% (6900 μg/cm^2)	0/26	0%	RIFM (1979)

Table 4
Summary of diagnostic patch tests in studies with 100 or more patients

Method	Concentration	Results		References
		Reaction	Incidence	
Patch test	5% in petrolatum	2/100	2%	Frosch et al. (1995)
Patch test	1% in petrolatum	1/100	1%	Frosch et al. (1995)
Patch test	0.05–0.5% in base cream or 99% ethanol	8/316	2.53%	Takenaka et al. (1986)
Patch test	5% as a part of the FM standard series 1	3/1855	0.16%	Frosch et al. (2002)

4.4.1.2.3 In a multicenter study, a total of 1855 consecutive dermatitis patients at six different dermatology centers were patch tested with a standard series, FM 1 and various other fragrance materials. In most centers test materials were applied on patients back for 2 days using Finn Chambers® on Scanpor®. Reactions were assessed on day 2 and day 4 in most centers. Three reactions and five questionable reactions were observed with 5% pentyl salicylate (Frosch et al., 2002).

4.4.2. Animal studies (Table 5)

4.4.2.1. Pentyl salicylate was tested for sensitization in an open epicutaneous test (OET) in male and female outbred Himalayan guinea pigs (6–8/group) weighing 400–500 g. Guinea pigs received 21 daily open applications of 0.1 ml of progressively diluted solutions of pentyl salicylate (vehicle not specified), which were applied, to an 8 cm^2 area on the clipped flank. The applications were repeated daily for 21 days using the same site. Guinea pigs were challenged by an open application with 0.025 ml of various concentrations of pentyl salicylate (vehicle not specified) that was applied with a pipette to a skin area measuring 2 cm^2 on the contralateral flank on days 21 and 35. Reactions were read 24, 48 and/or 72 h after application. Six to eight untreated controls were also treated with pentyl salicylate on days 21 and 35. The concentration of 30% was the minimum sensitization concentration and 3% was the minimum eliciting concentration (Klecak et al., 1977).

4.4.2.2. A guinea pig open epicutaneous test (OET) was conducted on groups of 6–8 male and female guinea pigs weighting 300–450 g. Daily applications were made for 3 weeks to a clipped 8-cm^2 area on the flank of each guinea pig. The test sites were not covered and the reactions were read 24 h after each application. A total of 21 applications of 0.1 ml pentyl salicylate in an unspecified vehicle were made for 21 days. The 10 controls were either left untreated or treated with 0.1 ml of the vehicle for 21 days. At the challenge phase, both the test and control animals were treated on days 21 and 35 on the contralateral flank with the test material. Pentyl salicylate at 10% produced no sensitization reactions (Klecak, 1979, 1985).

Table 5
Summary of guinea pig sensitization studies

Method	Induction concentration	Challenge concentration	Results	References
OET	100, 30, 10, 1, 0.3, 0.1, and 0.03	100, 30, 10, 1, 0.3, 0.1, and 0.03	30% determined as minimum sensitization concentration	Klecak et al. (1977)
OET	10%	10%	No sensitization	Klecak (1979, 1985)
DRAIZE (modified)	0.1% i.d.	0.1% i.d.	No sensitization	Klecak et al. (1977)
DRAIZE (modified)	0.05% i.d.	0.05% i.d. 10% dermal	No sensitization	Sharp (1978)
MAX	5% i.d. 25% dermal	Sub-irritant	No sensitization	Klecak et al. (1977)
MAX	1% i.d. 40% dermal	10%	No sensitization	RIFM (1981)
FCAT	50% i.d.	Sub-irritant	No sensitization	Klecak et al. (1977)
Optimization test	0.1% i.d.	0.1% i.d. 10% dermal	No sensitization	Maurer et al. (1980)

4.4.2.3. Pentyl salicylate was tested in a guinea pig sensitization study using a modified Draize procedure in male and female outbred Himalayan guinea pigs weighing 400–500 g. Induction consisted of ten intradermal injections on alternate days with 0.05 ml of 0.1% pentyl salicylate in isotonic saline. The animals were challenged on days 35 and 49 with an intradermal injection of 0.05 ml of 0.1% pentyl salicylate in saline. Control animals were also challenged intradermally on days 35 and 49 with pentyl salicylate. Sensitization was not observed (Klecak et al., 1977).

4.4.2.4. Pentyl salicylate was tested in a guinea pig sensitization study using a modified Draize procedure in ten inbred Hartley albino guinea pigs with initial weight of 350 g. Induction consisted of four intradermal injections with a 0.1 ml aliquot of 0.05% pentyl salicylate at 4 sites overlying the two auxillary and the two inguinal lymph nodes. The animals were challenged 14 days later with an intradermal injection in one flank and a topical application in the other flank with 0.1 ml aliquot of pentyl salicylate at 0.05% and 10%, respectively. Reactions were scored 24 h later. A second challenge was carried out 7 days later. No sensitization reactions were observed (Sharp, 1978).

4.4.2.5. A guinea pig maximization test was conducted using outbred Himalayan white-spotted male and female guinea pigs. Induction was via two intradermal injections of 0.1 ml of 5% pentyl salicylate with and without FCA on day 0. In addition, on day 8, 25% pentyl salicylate in petrolatum was applied to a clipped area on the neck for 48 h under occlusion. Challenge on day 21 was via a 24 h closed patch at a sub-irritant concentration. Reactions were read at 24 and 48 h after removing the patch. No sensitization reactions were observed (Klecak et al., 1977).

4.4.2.6. A guinea pig maximization test was conducted in ten albino Dunkin/Hartley guinea pigs. Six intradermal injections were made within a 2×4 cm clipped and shaved area of the dorsal shoulder region. They consisted of: two 0.1 ml injections of 1% pentyl salicylate in 0.01% DOBS/saline, two 0.1 ml injections of 1% pentyl salicylate in 50% FCA, and two 0.1 ml injections of 50% FCA. Seven days later, the site was clipped and shaved and induction was supplemented by a 48 h occluded application to the injection site with 40% pentyl salicylate in acetone. After

a rest period of 13–14 days, the guinea pigs were challenged on the clipped and shaved flank using an 8 mm diameter filter paper patch saturated with 10% pentyl salicylate in acetone which was applied for 24 h under occlusion. The animals were re-challenged at weekly intervals. All challenge applications were conducted with 10% pentyl salicylate in acetone. Reactions were read at 24 and 48 h after patch removal. No reactions were observed (RIFM, 1981).

4.4.2.7. A Freund's complete adjuvant test (FCAT) was conducted using outbred Himalayan white-spotted male and female guinea pigs. Induction was via intradermal injection of 0.1 ml of a 50:50 mixture of pentyl salicylate and FCA into the neck on days 0, 2, 4, 7 and 9. The control animals were similarly treated with 5×0.05 ml of FCA alone. The animals were challenged on days 21 and 35 via a 24 h closed patch at a sub-irritant concentration. No sensitization reactions were observed (Klecak et al., 1977).

4.4.2.8. An optimization test was conducted using 20 Pirbright White strain guinea pigs (10/sex). During the induction period, the animals received one injection into the skin every other day with 0.1% pentyl salicylate. During the second and third week of the induction, pentyl salicylate was incorporated at the same concentration in a mixture of FCA and physiological saline (adjuvant/saline, 1:1 v/v). The animals were challenged by intradermal injection with 0.1% pentyl salicylate 14 days after the last induction injection. After a further rest period of 10 days, the animals were challenged with 10% pentyl salicylate in petrolatum which was applied under occlusion for 24 h. No reactions were observed (Maurer et al., 1980).

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, distribution and metabolism

4.6.1. Absorption

4.6.1.1. In vivo studies

4.6.1.1.1. A study of the absorption of pentyl salicylate was conducted on one volunteer. The surfaces of both hands served as the area of application. An enameled foot-tub containing 5 l of the liquid was utilized for all immersion experiments. Hands were immersed for a 5 min period in water at 43–44 °C, rapidly dried and alternated with a 5-min period of massage applying 2 cc of pentyl salicylate per minute. Hands were not wiped before returning to the hot water. Urine was collected for 24 h and analyzed; after 24-h urine continued to be analyzed until no trace of sodium salicylate was excreted. The average excretion was 43 and 33 mg for pentyl salicylate and sodium salicylate, respectively (Brown and Scott, 1934).

4.6.1.1.2. Watkinson et al. (1992) used a mathematical method to estimate the total body absorption of some salicylate esters including pentyl salicylate. Rate constants were calculated from the relevant physicochemical properties. The applied dose of the active ingredient used in the simulation was 40 μg cm^{-2} based on the FDA recommendation (200 mg of product per 100 cm^2 of skin) and a value of 2%. The release rate from the formulation was fixed at 1 μm cm^{-2} h^{-1}. The simulations were conducted on a 12 h time scale. The estimated total body absorption of pentyl salicylate per μg over 1.4 m^2 was 0.63 at 2 h, 14 at 6 h and 96 at 12 h.

4.7. Subchronic toxicity

No data available on this material.

4.8. Developmental and reproductive toxicity

No data available on this material.

4.9. Mutagenicity and genotoxicity

No data available on this material.

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, D. McGinty, L. Jones, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on phenethyl salicylate

A. Lapczynski *, D. McGinty, L. Jones, S. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of phenethyl salicylate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Phenethyl salicylate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on phenethyl salicylate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used As Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Benzoic acid, 2-hydroxy-,2-phenylethyl ester; benzylcarbinyl 2-hydroxybenzoate; benzylcarbinyl salicylate; phenethyl salicylate; 2-phenylethyl 2-hydroxybenzoate, phenylethyl salicylate; 2-phenylethyl salicylate.
1.2 CAS Registry number: 87-22-9.
1.3 EINECS number: 201-732-5.
1.4 Formula: $C_{15}H_{14}O_3$.
1.5 Molecular weight: 242.28.
1.6 COE: Phenethyl salicylate was included by the Council of Europe in the list of substances granted B-information required – hydrolysis study (COE No. 437).
1.7 FDA: Phenethyl salicylate was approved by the Food and Drug Administration as a flavor (21 CFR 172.515).

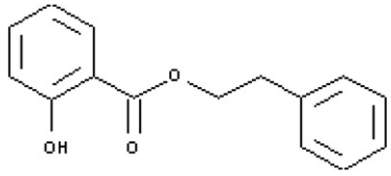

Fig. 1. Phenethyl salicylate.

1.8 FEMA Flavor and Extract Manufacturers' Association States: Generally recognized as Safe as a flavor ingredient – GRAS 3 (2868).
1.9 JECFA: The Joint FAO/WHO Expert Committee on Food Additives (JECFA No. 905) concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent.

2. Physical properties

2.1 Physical description: White crystalline substance.
2.2 Flash point: >200 F; CC.
2.3 log K_{ow} (calculated): 4.8.
2.4 Vapor pressure : <0.001 mm Hg 20 °C.
2.5 Water solubility (calculated): 7.856 mg/l @ 25 °C.
2.6 Melting point: 41 °C.

3. Usage

Phenethyl salicylate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of 1–10 metric tonnes per annum.

The maximum skin level that results from the use of phenethyl salicylate in formulae that go into fine fragrances has been reported to be 1.49% (IFRA, 2002), assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5 percentile use level in formulae for use in cosmetics in general has been reported to be 1.8827% (IFRA, 2002), which would result in a conservative calculated maximum daily exposure on the skin of 0.0480 mg/kg for high end users of these products (see Table 1).

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing phenethyl salicylate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/ product	Ingredient/ mixture[a]	Ingredient mg/kg/day[b]
Body lotion	8.00	0.71	1.000	0.004	1.8827	0.0071
Face cream	0.80	2.00	1.000	0.003	1.8827	0.0015
Eau de toilette	0.75	1.00	1.000	0.080	1.8827	0.0188
Fragrance cream	5.00	0.29	1.000	0.040	1.8827	0.0182
Antiperspirant	0.50	1.00	1.000	0.010	1.8827	0.0016
Shampoo	8.00	1.00	0.010	0.005	1.8827	0.0001
Bath products	17.00	0.29	0.001	0.020	1.8827	0.0000
Shower gel	5.00	1.07	0.010	0.012	1.8827	0.0002
Toilet soap	0.80	6.00	0.010	0.015	1.8827	0.0002
Hair spray	5.00	2.00	0.010	0.005	1.8827	0.0002
Total						0.0480

[a] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60-kg adult.

Table 2
Summary of acute toxicity studies

Route	Species	Number of animals/ dose group	LD_{50} (g/kg)	References
Oral	Rats	10	>5	RIFM (1973)
Dermal	Rabbits	9	>5	RIFM (1973)

4. Toxicological data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} of phenethyl salicylate was determined in 10 rats. The rats were observed for mortality and/or systemic effects for 14 days. Slight lethargy was observed during the course of the study. The LD_{50} exceeded 5 g/kg based on one (1/10) death at that dose (RIFM, 1973).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} in rabbits was reported to be greater than 5 g/kg based on 0/9 deaths. Nine rabbits received a single dermal application of neat phenethyl salicylate at a dose of 5 g/kg. The rabbits were observed for mortality and/or systemic effects for 14 days. No clinical signs were observed (RIFM, 1973).

4.1.3. Intraperitoneal studies

4.1.3.1. An acute intraperitoneal study was conducted using two rabbits. Each rabbit was injected with 5 ml of a suspension of 0.5% phenethyl salicylate in 0.5% Tween 80 in water. The rabbits were observed for clinical signs and/or mortality for seven days. No effects were observed (Gupta and Ranga Rao, 1979).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. Phenethyl salicylate was evaluated for irritation as a part of maximization study. Phenethyl salicylate was applied at concentration of 8% to normal sites on the backs of five healthy male volunteers for 48 h under occlusion. No irritation was observed (RIFM, 1973a).

4.2.2. Animal studies

4.2.2.1. Prior to the induction of an open epicutaneous test (OET), phenethyl salicylate at a range of concentrations was evaluated for irritation in 6–8 male and female Himalayan white-spotted guinea pigs. A 0.025 ml aliquot of phenethyl salicylate was applied to a 2 cm^2 area on the clipped flank. The application site was left uncovered and reactions were read after 24 h. A concentration of 0.1% was the lowest concentration to produce mild irritation after a single application and was selected as the minimal irritating concentration (Klecak et al., 1977).

4.2.2.2. As a part of the induction phase of the same OET test, a 0.1 ml aliquot of neat phenethyl salicylate and progressively diluted solutions of phenethyl salicylate were applied to a 8 cm^2 area on the clipped flanks of 6–8 guinea pigs/group. The applications were repeated daily for 21 days, using the same skin site. The sites were left uncovered and the reactions were read 24 h after each application. The minimum irritating concentration was 0.1% (Klecak et al., 1977).

4.2.2.3. Prior to a guinea pig sensitization test, a preliminary irritation study was conducted in four male albino Dunkin/Hartley guinea pigs. The animals were intradermally injected with 0.1 ml aliquots of 0.25% or 0.5% phenethyl salicylate in 6% acetone/20% PEG400/0.01% Tween 80/saline. Reactions were read 24 h after injection. Very slight erythema was observed at 0.25% and 0.5%. A concentration of 0.5% was selected for the intradermal induction (RIFM, 1981).

4.2.2.4. A preliminary irritation test was conducted in four male albino Dunkin/Hartley guinea pigs prior to a guinea pig maximization study. The animals received a single dermal application of 10%, 25% or 50% phenethyl salicylate in acetone using 8 mm saturated filter paper and 11 mm aluminum patch test cups which were applied to the shaved flank for 24 h. Reactions were assessed for irritation at 24 and 48 h after patch removal. Barely perceptible erythema was observed at all concentrations. A concentration of 50% phenethyl salicylate was selected for the topical induction application and 10% was selected for the challenge application (RIFM, 1981).

4.3. Mucous membrane (eye) irritation

No data available for this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A human maximization test was conducted on 25 healthy male volunteers. Phenethyl salicylate at 8% in petrolatum was applied under occlusion to the same site on the volar forearms of each volunteer for five alternate-day 48-h periods. Patch sites were pretreated for 24 h with 5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a 10-day rest period, challenge patch was applied to a fresh site for 48 h under occlusion. Challenge sites were pretreated for 1 h with 10% SLS under occlusion. Reactions were read at patch removal and 24 h later. No reactions were observed (RIFM, 1973a).

4.4.2. Animal studies (Table 3)

4.4.2.1. Kligman (1966) guinea pig maximization test was conducted using 10 albino Dunkin/Hartley guinea pigs. Induction consisted of intradermal injections followed 1

Table 3
Summary of guinea pig sensitization studies

Method	Induction concentration	Challenge concentration	Results	References
MAX	0.5% i.d., 50% dermal	10%	Sensitization observed	RIFM (1981)
MAX	5% i.d., 25% dermal	Subirritant	Sensitization observed	Klecak et al. (1977)
CET	30%	1%	No sensitization reactions	Ishihara et al. (1986)
FCAT	50%	Subirritant	Sensitization observed	Klecak et al. (1977)
DRAIZE (modified)	0.1% i.d.	0.1% i.d	No sensitization reactions	Klecak et al. (1977)
OET	NA	8%	No sensitization reactions	Klecak (1979)
OET	NA	8%	Sensitization observed	Klecak (1985)
OET	100%, 30%, 10%, 3% or 1%	100%, 30%, 10%, 3% or 1%	Sensitization observed	Klecak et al. (1977)

week later by a 48 h occluded patch. Three pairs of intradermal injections were made as follows: two 0.1 ml injections of 0.5% phenethyl salicylate in 6% acetone/20% PEG400/0.01% Tween 80/saline; two 0.1 ml injections of 0.5% phenethyl salicylate in 50% FCA; and two 0.1 ml injections of 50% FCA. Seven days later, an occluded patch with 50% phenethyl salicylate in acetone was applied over the shoulder injection sites for 48 h. After a 14-day rest period, the animals were challenged with 10% phenethyl salicylate in acetone applied for 24 h under occlusion using an 11 mm aluminum patch test cup. Reactions were read 24 and 48 h after patch removal. A 2nd challenge was conducted 1 week after the primary challenge. Sensitization reactions were observed after both challenges (RIFM, 1981). Cross-challenge applications with 10% benzyl salicylate and 10% phenyl salicylate were then made at weekly intervals. Cross-reactions were observed after each challenge (RIFM, 1981).

4.4.2.2. A guinea pig maximization test was conducted using outbred Himalayan white-spotted male and female guinea pigs. Induction was via two intradermal injections of 0.1 ml of 5% phenethyl salicylate with and without FCA on day 0 followed 8 days later by a 48 h occluded application with 25% phenethyl salicylate in petrolatum to a clipped area on the neck. Challenge was on day 21 via a 24 h occluded patch at a subirritant concentration. Reactions were read at 24 and 48 h after removing the patch. Sensitization was observed (Klecak et al., 1977).

4.4.2.3. Ishihara et al. (1986) conducted a closed epicutaneous test (CET) in eight guinea pigs. Induction consisted of six, 48 h occluded patch applications which were made to the shaved nape using Torii's patch plaster and adhesive tape. The applications were made three times a week for 2 weeks with 30% phenethyl salicylate. On day 28, a 48 occluded challenge application with 1% phenethyl salicylate was made to the shaved flank. Reactions were read at patch removal and 24 and 48 h after patch removal. No sensitization reactions were observed.

4.4.2.4. A Freund's complete adjuvant test (FCAT) was conducted using outbred Himalayan white-spotted male and female guinea pigs. Induction was via intradermal injection of 0.1 ml of a 50:50 mixture of phenethyl salicylate and FCA into the neck on days 0, 2, 4, 7 and 9. Challenges on days 21 and 35 were conducted via a 24 h occluded patch at a subirritant concentration. Sensitization was observed (Klecak et al., 1977).

4.4.2.5. A modified Draize test was conducted in male and female outbred Himalayan guinea pigs. Induction consisted of ten intradermal injections on alternate days with a 0.05 ml aliquot of 0.1% phenethyl salicylate in isotonic saline. The animals were challenge on days 35 and 49 with an intradermal injection of 0.05 ml of 0.1% phenethyl salicylate in saline. No sensitization was observed (Klecak et al., 1977).

4.4.2.6. A guinea pig open epicutaneous test (OET) was conducted on groups of 6–8 male and female guinea pigs. Open applications with a 0.1 ml aliquot of phenyl salicylate were made once daily to a 8 cm^2 area on the clipped flank. Reactions were read 24 h after each application. A total of 21 applications were made over the 3-week period. Open challenge applications with 8% phenethyl salicylate were made on days 21 and 35. No sensitization reactions were observed (Klecak, 1979); however, Klecak (1985) reported sensitization reactions with 8% phenethyl salicylate when tested in another OET using the same method as above.

4.4.2.7. An OET was conducted in male and female outbred Himalayan guinea pigs (6–8 per group). Guinea pigs received 21 daily open applications to an 8 cm^2 area on the clipped flank with neat phenethyl salicylate and phenethyl salicylate at concentrations of 0.03–30% (vehicle not specified). Reactions were read 24 h after each application. Guinea pigs were challenged by an open application with 0.025 ml of phenethyl salicylate applied to a skin area measuring 2 cm^2 on the contralateral flank on days 21 and 35. Sensitization was observed. The minimum sensitizing concentration was 30% and the minimum eliciting concentration was 0.03% (Klecak et al., 1977).

4.4.3. Local lymph node assay (LLNA)

4.4.3.1. An LLNA was conducted in 25 female CBA/Ca female mice (four per dose). Each animal received a daily topical application of 25 μl of 1.0%, 2.5%, 5.0%, 10% or 25% test material in EtOH:DEP (3:1) on the dorsal surface of each ear for three consecutive days. Control animals

were treated with the vehicle alone. Three days after the third topical application all mice were injected intravenously through the tail vein with 250 μl sterile saline (PBS) containing 20 μCi 3H-methylthymidine (3H-thymidine). All mice were sacrificed 5 h after the intravenous injection. Draining auricular lymph nodes were excised and were pooled for each experimental group. Single cell suspensions were then prepared, washed with PBS, suspended in trichloroacetic acid (TCA) and left overnight at 4 °C. The samples were then resuspended in TCA and then transferred to a scintillation cocktail. Incorporation of 3H-TdR was then measured by β-scintillation counting and stimulation indices were determined for each experimental group. The EC3 value was calculated to be 2.1% (525 μg/cm^2). Under the conditions of the study, phenethyl salicylate was considered to be a sensitizer (RIFM, 2006).

4.5. Phototoxicity and photoallergy

No data available for this material.

4.6. Absorption, distribution, metabolism

No data available for this material.

4.7. Subchronic toxicity

No data available for this material.

4.8. Reproductive and developmental toxicity

No data available for this material.

4.9. Mutagenicity and genotoxicity

No data available for this material.

4.10. Carcinogenicity

No data available for this material.

This individual Fragrance Material Review is not intended as a stand alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, D. McGinty, L. Jones, S.P. Bhatia, C.S. Letizia and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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Review

Fragrance material review on phenyl salicylate

A. Lapczynski *, L. Jones, D. McGinty, S.P. Bhatia, C.S. Letizia, A.M. Api

Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA

Abstract

A toxicologic and dermatologic review of phenyl salicylate when used as a fragrance ingredient is presented.

Keywords: Review; Fragrance; Phenyl salicylate

Contents

* Corresponding author. Tel.: +1 201 689 8089; fax: +1 201 689 8090.
E-mail address: alapczynski@rifm.org (A. Lapczynski).

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In 2006, a complete literature search was conducted on phenyl salicylate. On-line databases that were surveyed included Chemical Abstract Services and the National Library of Medicine. In addition, fragrance companies were asked to submit pertinent test data. All relevant references are included in this document.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

1. Identification (Fig. 1)

1.1 Synonyms: Benzoic acid, 2-hydroxy-, phenyl ester; 2-hydroxybenzoic acid, phenyl ester; 2-phenoxycarbonylphenol; phenyl-2-hydroxybenzoage; phenyl salicylate; salol.
1.2 CAS registry number: 118-55-5.
1.3 EINECS number: 204-259-2.
1.4 Formula: $C_{13}H_{10}O_3$.
1.5 Molecular weight: 214.22.
1.6 FEMA: Flavor and Extract Manufacturers Association – Generally Recognized as Safe as an ingredient – GRAS 19 (3960).
1.7 JECFA: The Joint FAO/WHO Expert Committee on Food Additives concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent (736).

2. Physical properties

2.1 Physical form: White granular crystal.
2.2 Boiling point: 172 °C @ 12 mm Hg.
2.3 Flash point > 200 °F; CC.
2.4 Log K_{ow} (calculated) 3.82.
2.5 Vapor pressure (calculated) 0.0000627 mm Hg 25 C.

3. Usage

Phenyl salicylate is a fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its use worldwide is in the region of less than 0.1 metric tonnes per annum.

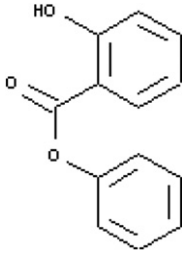

Fig. 1. Phenyl salicylate.

The maximum skin level that results from the use of phenyl salicylate in formulae that go into fine fragrances has not been reported. A default value of 0.02% is used, assuming use of the fragrance oil at levels up to 20% in the final product. The 97.5‰ use level in formulae for use in cosmetics in general has not been reported. As such, a default value of 0.02% is used to calculate a maximum daily exposure on the skin of 0.0005 mg/kg for high end users of these products (see Table 1).

4. Toxicology data

4.1. Acute toxicity (Table 2)

4.1.1. Oral studies

4.1.1.1. The acute oral LD_{50} of phenyl salicylate was evaluated in rats (10/dose). The animals received a single oral administration of phenyl salicylate at 1.67, 2.5, 3.3, or 5.0 g/kg. The animals were observed for mortality and/or systemic effects for 14 days. No deaths were observed at 1.67 kg/kg. Three (3/10) animals died at 2.5 g/kg and 8/10 animals died at both 3.3 and 5.0 g/kg. The acute LD_{50} was calculated to be 3.0 g/kg (95% CI 2.52–3.57 g/kg) (RIFM, 1975b).

4.1.2. Dermal studies

4.1.2.1. The acute dermal LD_{50} of phenyl salicylate in rabbits exceeded 5 g/kg based on 0/4 deaths at that dose. Four rabbits received a single dermal application of 5 g/kg of neat phenyl salicylate. The rabbits were observed for mortality and/or systemic effects. No clinical signs were observed (RIFM, 1975b).

4.2. Skin irritation

4.2.1. Human studies

4.2.1.1. In a pre-test for a human maximization test, a 48 hour closed patch test was conducted on five healthy volunteers with 6% phenyl salicylate in petrolatum which were applied to the backs. No irritation was observed (RIFM, 1975a).

4.2.2. Animal studies

4.2.2.1. Irritation was evaluated as part of the dermal LD_{50} study described above. No irritation was observed with a single dose of 5 g/kg of neat test material (RIFM, 1975b).

4.3. Mucous membrane (eye) irritation

No data available on this material.

4.4. Skin sensitization

4.4.1. Human studies

4.4.1.1. A human maximization test was conducted with 6% phenyl salicylate in petrolatum under occlusion to the

Table 1
Calculation of the total human skin exposure from the use of multiple cosmetic products containing phenyl salicylate

Type of cosmetic product	Grams applied	Applications per day	Retention factor	Mixture/product	Ingredient/mixture[a]	Ingredient (mg/kg/day)[b]
Body lotion	8.00	0.71	1.000	0.004	0.02	0.0001
Face cream	0.80	2.00	1.000	0.003	0.02	0.0000
Eau de toilette	0.75	1.00	1.000	0.080	0.02	0.0002
Fragrance cream	5.00	0.29	1.000	0.040	0.02	0.0002
Antiperspirant	0.50	1.00	1.000	0.010	0.02	0.0000
Shampoo	8.00	1.00	0.010	0.005	0.02	0.0000
Bath products	17.00	0.29	0.001	0.020	0.02	0.0000
Shower gel	5.00	1.07	0.010	0.012	0.02	0.0000
Toilet soap	0.80	6.00	0.010	0.015	0.02	0.0000
Hair spray	5.00	2.00	0.010	0.005	0.02	0.0000
Total						0.0005

[a] Upper 97.5‰ levels of the fragrance ingredient in the fragrance mixture used in these products.
[b] Based on a 60 kg adult.

Table 2
Summary of acute toxicity data

Route	Species	No. animals/dose group	LD_{50}	References
Oral	Rat	10	3.0 g/kg	RIFM (1975b)
Dermal	Rabbit	4	>5.0 g/kg	RIFM (1975b)

Table 3
Summary of animal sensitization studies

Test method	Concentration	Results	References
Buehler test	25%	No sensitization	Basketter and Gerberick (1996)
FCAT	5% (induction) 0.3% and 1.0% (challenge)	8/9 reactions at 0.3% 9/9 reactions at 1.0%	Marchand et al. (1982)
CET	30% (induction)1% (challenge)	No sensitization	Ishihara et al. (1986)

same site on the volar forearms of 25 healthy male and female volunteers for five alternate day 48-hour periods. Patch sites were pretreated for 24 h with 5% aqueous sodium lauryl sulfate under occlusion. After a rest period, a challenge patch with 6% phenyl salicylate in petrolatum was applied. Challenge sites were read on patch removal and 24 h later. No sensitization reactions were observed (RIFM, 1975a).

4.4.1.2. One hundred fifty women with cosmetic dermatitis were patch tested with the European standard series and a cosmetic series according to procedures recommended by the ICDRG. No reactions to 1% phenyl salicylate in petrolatum were observed (de Groot et al., 1988d).

4.4.1.3. Over a 3-year period, a total of 173 volunteers who were suspected of occupational dermatosis due to exposure to plastics and glues were patch tested with a plastic and glue series. Each patch was applied for two days under occlusion. Reactions were read on days 2, 3, and 4–6 and were scored according to ICDRG recommendations. No reactions were observed to 1% phenyl salicylate in petrolatum (Kanerva et al., 1997).

4.4.2. Animal studies (Table 3)

4.4.2.1. A Buehler guinea pig test (Buehler, 1965) was conducted in 20 animals. Three 6-hour occluded induction patches were applied to the same clipped (shaved) induction site on the dorsal surface of each animal, one patch per week for three weeks. Following a 10–14 day rest period, a 6-hour occluded challenge application with 25% phenyl salicylate was made to a naive site. Reactions were read 24 and 48 h after patch removal. No reactions were observed (Basketter and Gerberick, 1996).

4.4.2.2. A Freund's complete adjuvant test (FCAT) was conducted on female Hartley albino guinea pigs. For induction, a 0.1 ml aliquot of an emulsion of 5% phenyl salicylate in a 1:1 FCA:saline solution was intradermally injected in the postnuchal area. A total of five intradermal induction injections were made on alternate days. Four control animals were injected on alternate days with a 0.1 ml aliquot of a 1:1 FCA/saline mixture. After a 2-week rest period, an open challenge application of a 25 µl of 0.3% or 1% phenyl salicylate in olive oil:ethanol (1:9) was made to a 2 cm^2 area on the shaved flank. Reactions were read at 24 h. At 0.3%, 8/9 reactions were observed; at 1%, 9/9 reactions were observed (Marchand et al., 1982).

4.4.2.3. A guinea pig closed epicutaneous test (CET) was conducted by Ishihara et al. (1986). Induction consisted of an occluded application with 30% phenyl salicylate (vehicle not provided) applied to the shaved nape of each animal for 48 h. The same procedure was repeated three times per week for two weeks. Following a 2-week rest period, a challenge patch with 1% phenyl salicylate was applied to the flank for 48 h under occlusion. No reactions were observed.

4.5. Phototoxicity and photoallergy

No data available on this material.

4.6. Absorption, pharmacokinetics, and metabolism

4.6.1. Percutaneous absorption

4.6.1.1. The effect of pH on the absorption of phenyl salicylate was determined in white male Sprague-Dawley rats. One hour prior to the test, the tails were washed with distilled water. The washed tails were immersed in a solution (phenyl salicylate, glycine buffer and 5% ethanol) in a perfusion container (19.5 × 2.5 cm) with a 68 ml capacity, and the container was sealed to prevent contamination and solvent evaporation. The container was immersed in a temperature controlled water bath. The test duration was approximately 45 min. Absorbance was continuously recorded at 240 nm wave length to obtain an absorption rate constant and the total amount of phenyl salicylate absorbed at pH 2, pH 3, pH 6, pH 8 was measured. A standard curve was established and absorption of 2.53, 2.90, 2.32 and 2.18 μg/mm^2/hr was observed at pH 2, 3, 6 and 8, respectively (Siddiqi and Ritschel, 1972).

4.6.2. Metabolism

4.6.2.1. The metabolism of phenyl salicylate was evaluated in one volunteer. Capsules containing 1 ounce of phenyl salicylate were ingested once an hour for 8 h. Fractionated urine specimens were analyzed for total phenol from the start of phenyl salicylate ingestion and for three days following the intake of phenyl salicylate. The total urinary phenol level peaked at 472 ppm during the second eight-hour collection period. The total phenol levels progressively subsided to a level of 8 ppm 60 h after the start of phenyl salicylate ingestion. Free phenol peaked at 25 ppm during the second collection period. The base line for the subject's free phenol urinary excretion was between 0.5 and 1.0 ppm. No unchanged phenyl salicylate was detected (Fishbeck et al., 1975).

4.6.2.2. The ability of human plasma derived arylesterase to hydrolyse a group of esters including phenyl salicylate was evaluated by Augustinsson and Ekedahl (1962). The Warburg manometric technique was used for esterase determination at pH 7.4 during which the initial substrate concentration was 8 mM. Tween 20 at a concentration of 0.05% was used for solubilization. Phenyl salicylate was not hydrolyzed by arylesterase.

4.7. Subchronic toxicity

4.7.1.

A 51-day study was conducted on three beagle dogs. The dogs received daily administration of phenyl salicylate by capsule. An initial dose of 500 mg/kg/day had to be reduced to 250 and then to 125 mg/kg/day as doses of 250 and 500 mg/kg/day were not tolerated by the dogs. Clinical observations were evaluated before and during the study and a gross necropsy was conducted at the completion of the study. At regular intervals during the study, complete blood count, blood sugars, liver and kidney functions were determined. At 125 and 250 mg/kg decreased activity, body weight, and appetite were observed. The urine and feces were darkened and there were transient increases in the percentage of nonsegmented neutrophilic leukocytes in peripheral blood. Serum glutamic pyruvic transaminase and glutamic oxaloacetic transaminase activities were elevated. Following the reduction of the dose to 125 mg/kg/day, all affected parameters returned to normal. No gross or microscopic abnormalities were noted at necropsy (Fishbeck et al., 1975; Kociba et al., 1976).

4.8. Developmental toxicity

4.8.1.

In a study to evaluate the developmental toxicity of aspirin or phenyl salicylate, pregnant female Wistar-Funahashi strain rats weighing 130–200 g received by oral administration, phenyl salicylate in a suspension of 0.5% C.M.C. at dose levels of 0.1, 0.2, 0.3 and 0.4 g/kg on each day from the 7th to 9th and 7th to 12th day of gestation. While malformations were observed with phenyl salicylate, the incidence was much less than those produced by aspirin and the varieties of the malformations that were produced by phenyl salicylate were extremely small as compared with those produced by aspirin (Nagahama et al., 1966).

4.9. Mutagenicity and genotoxicity

4.9.1. Bacterial studies

4.9.1.1. A preincubation modification of the *Salmonella*/microsome test was conducted in the presence and absence of liver S9 using *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537. Two sets of tests were conducted with phenyl salicylate in DMSO. Results were questionable at 1–100 μg/plate in one set of tests and negative at 3.3–333.3 μg/plate in a second set of tests (Zeiger et al., 1987).

4.9.1.2 An assay that employed streptomycin dependent mutants of *Escherichia coli* was conducted with phenyl salicylate at doses of 1–100 μg/plate in DMSO. No mutagenic effects were observed (Szybalski, 1958).

4.10. Carcinogenicity

No data available on this material.

This individual Fragrance Material Review is not intended as a stand-alone document. Please refer to the Toxicologic and Dermatologic Assessment of Salicylates

When Used as Fragrance Ingredients (Belsito et al., 2007) for an overall assessment of this material.

Conflict of interest statement

A. Lapczynski, L. Jones, D. McGinty, S.P. Bhatia, C.S. Letizia, and A.M. Api are employees of the Research Institute for Fragrance Materials, an independent research institute supported by the manufacturers of fragrances and consumer products containing fragrances. This research was supported by the Research Institute for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances.

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