Available online at www.sciencedirect.com
ScienceDirect
Food and Chemical Toxicology 45 (2007) S318–S361
www.elsevier.com/locate/foodchemtox

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

0278-6915/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.fct.2007.09.066

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

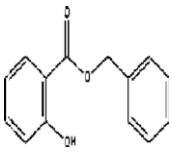
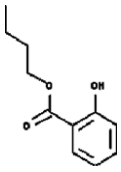
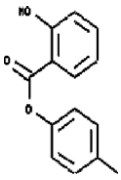
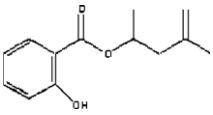
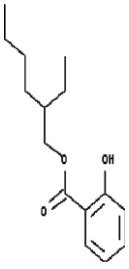
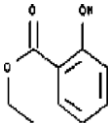
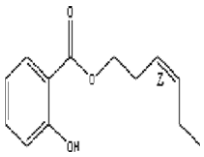
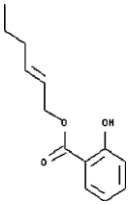
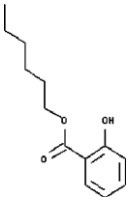
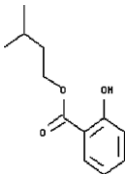
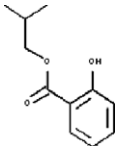
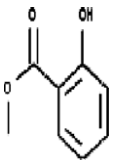
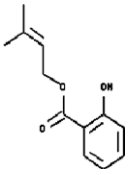
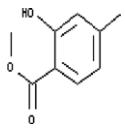
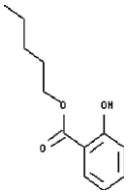
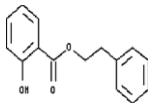
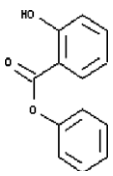
Material	Synonyms	Structure	Worldwide metric tons	Dermal systemic exposure in cosmetic products (mg/kg/day)	Maximum skin level (%)
Benzyl salicylate CAS# 118-58-1 Molecular weight: 228.25 Log K_{ow} (calculated): 4.31	Benzoic acid, 2-hydroxy-, phenylmethyl ester; Benzyl 2-hydroxybenzoate; Benzyl *o*-hydroxybenzoate; 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

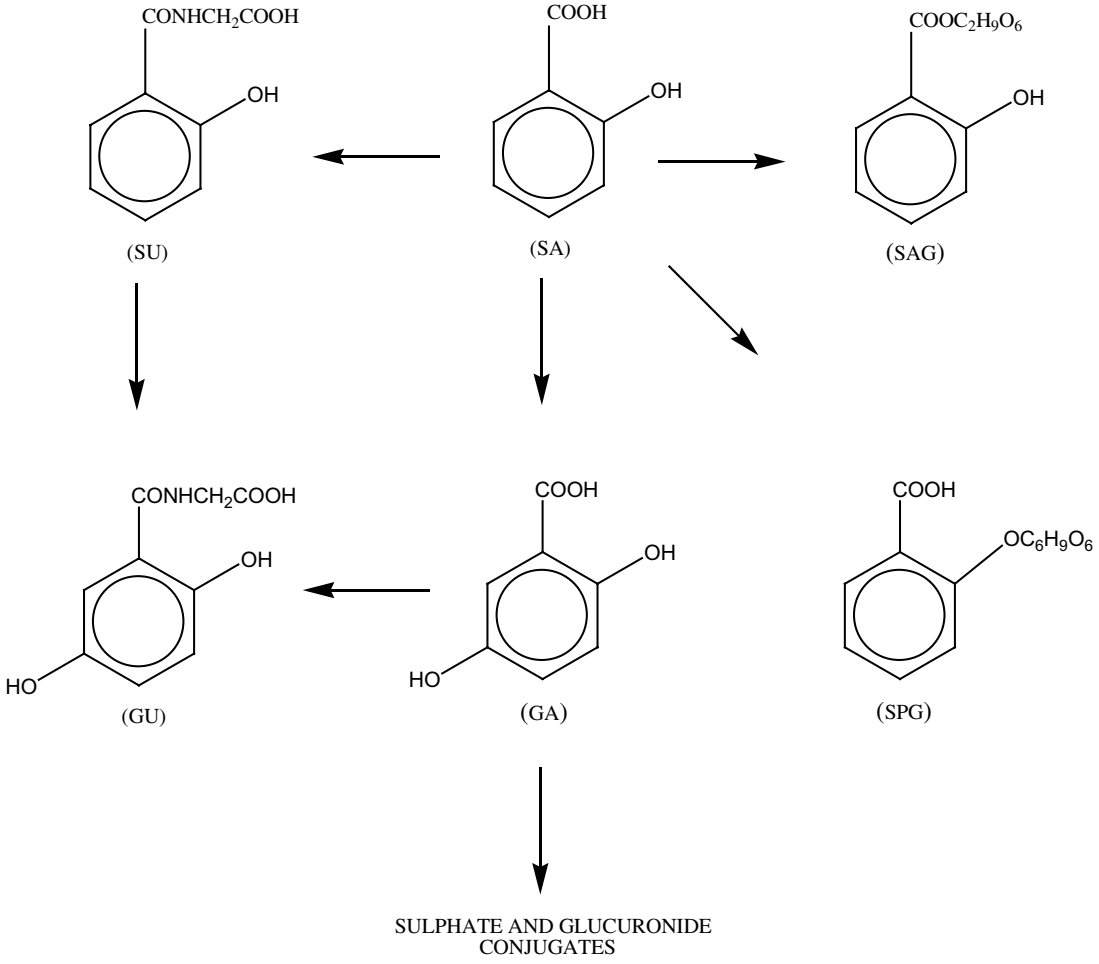


Fig. 1. Biotransformation of salicylic acid.

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)

(continued on next page)

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.

References

Abbott, D.D., Harrisson, J.W.E., 1978. Methyl salicylate: studies of osseous changes in the rat, reproduction in the rat and mouse, and liver and kidney effects in the dog. Unpublished report to the Flavor and Extract Manufactures Association, Washington, DC. Report number 26000.

Adams, T.B., Cohen, S.M., Doull, J., Feron, V.J., Goodman, J.I., Marnett, L.J., Munro, I.C., Portoghese, P.S., Smith, R.L., Waddell, W.J., Wagner, B.M., 2005. The FEMA GRAS assessment of hydroxy- and alkoxy-substituted benzyl derivatives used as flavor ingredients. Food and Chemical Toxicology 43, 1241–1271.

Alam, A.S., Gregoriades, D., Imondi, A.R., 1981. Bioavailability of magnesium salicylate. Journal of Pharmacy and Pharmacology 33, 792–793.

Alpen, E.L., Mandel, H.G., Rodwell, V.W., Smith, P.K., 1951. The metabolism of C^{14} carboxyl salicylic acid in the dog and in man. Journal of Pharmacology and Experimental Therapeutics 102, 150–155.

Alvarez, D.A., Stackelberg, P.E., Petty, J.D., Huckins, J.N., Furlong, E.T., Zaugg, S.D., Meyer, M.T., 2005. Removal of fragrance materials during U.S. and European wastewater treatment. Environmental Science and Technology 36, 2839–2847.

Anders, M.W., 1989. Biotransformation and bioactivation of xenobiotics by the kidney. In: Hutson, D.H., Caldwell, J., Paulson, G.D. (Eds.), Intermediary Xenobiotic Metabolism in Animals. Taylor and Francis, New York, pp. 81–97.

Bar, V.F., Griepentrog, F., 1967. Die Situation in der gesundheitlichen Beurteilung der Aromatisierungsmittel fur Lebensmittel. (Where we stand concerning the evaluation of flavoring substances from the viewpoint of health). Medizin Ernahr 8, 244–251.

BASF, A.G., Department of Toxicology, 1991. Report on the study of the oral toxicity of 2-ethylhexanol in rats after administration by gavage (aqueous emulsion) for 3 months. Unpublished report, project No. 31C0631/87077. On behalf of the Chemical Manufactures Association, Washington, USA.

Basketter, D.A., Gerberick, G.F., 1996. An interlaboratory evaluation of the Buehler test for the identification and classification of skin sensitizers. Contact Dermatitis 35, 146–151.

Basketter, D.A., Scholes, W., 1992. Comparison of the local lymph node assay with the guinea-pig maximization test for the detection of a range of contact allergens. Food and Chemical Toxicology 30, 65–69.

Basketter, D.A., York, M., McFadden, J.P., Robinson, M.K., 2004. Determination of skin irritation potential in the human 4-h patch test. Contact Dermatitis 51 (1), 1–4.

Bauer, K., Garbe, D., 1985. Common Flavor and Fragrance Materials. Verlagsgesellschaft mbH, D-6940, Weinheim, Germany [Cited In: FFHPVC, 2001].

Bertone, L.L., Monie, I.W., 1965. Teratogenic effect of methyl salicylate and hypoxia in combination. The Anatomical Record 151, 443.

Beutner, R., Calesnick, B., Powell, E., Bortin, L., 1943. On the absorption and excretion of methylsalicylate administered by inunction. Journal of Laboratory and Clinical Medicine 28, 1655–1663.

Boehnlein, J., Sakr, A., Lichtin, J.L., Bronaugh, R.L., 1994. Characterization of esterase and alcohol dehydrogenase activity in skin. Metabolism of retinyl palmitate to retinol (vitamin A) during percutaneous absorption. Pharmacological Reviews 11, 1155–1159.

Bonin, A.M., Arlauskas, A.P., Angus, D.S., Baker, R.S.U., Gallagher, C.H., Greenoak, G., Meher-Homji, K.M., Reeve, V., 1982. UV-absorbing and other sun-protecting substances: genotoxicity of 2-ethylhexyl *p*-methoxycinnamate. Mutation Research 105, 303–308.

Boyland, E., Huntsman-Mawson, E., 1938. CCLVII. Experiments on the chemotherapy of cancer. II. The effect of aldehydes and glucosides. Biochemical Journal 32, 1982–1987.

Bray, H.G., Ryman, B.E., Thorpe, W.V., 1947. The fate of certain organic acids and amides in the rabbit. Biochemical Journal 41, 212–218.

Bray, H.G., Ryman, B.E., Thorpe, W.V., 1948. The fate of certain organic acids and amides in the rabbit. 5. *o*- and *m*-hydroxybenzoic acids and amides. Biochemical Journal 43, 561–567.

Bray, H.G., Thorpe, W.V., White, K., 1952. Kinetic studies of the metabolism of foreign organic compounds. A mathematical model expressing the metabolic fate of phenols, benzoic acids and their precursors. Biochemical Journal 52, 423–430.

Brown, E.W., Scott, W.O., 1934a. The absorption of methyl salicylate by the human skin. The Journal of Pharmacology and Experimental Therapeutics 50, 32–50.

Brown, E.W., Scott, W.O., 1934b. The comparative absorption of certain salicylate esters by the human skin. The Journal of Pharmacology and Experimental Therapeutics 50, 373–385.

Buchbauer, G., Jirovetz, L., Jager, W., Plank, C., Dietrich, H., 1993. Fragrance compounds and essential oils with sedative effects upon inhalation. Journal of Pharmaceutical Science 82, 660–664.

Cadby, P., Troy, W.R., Vey, M.G.H., 2002. Consumer exposure to fragrance ingredients: providing estimates for safety evaluation. Regulatory Toxicology and Pharmacology 36, 246–252.

Carpenter, C.P., Smyth, H.F., 1946. Chemical burns of the rabbit cornea. American Journal of Ophthalmology 29, 1363–1372.

Cekanova, E., Larsson, K.S., Morck, E., Aberg, G., 1974. Interactions between salicylic acid and pyridyl-3-methanol: anti-inflammatory and teratogenic effects. Acta Pharmacologica et Toxicologica 35, 107–118.

Chapin, R.E., Sloane, R.A., 1997. Reproductive toxicology. Methyl salicylate. Environmental Health Perspectives 105, 199–395.

Cosmetic Ingredient Review Panel, Cosmetic, Toiletry and Fragrance Association (CIR), 2003. Safety assessment of salicylic acid, butyloctyl salicylate, calcium salicylate, C12–15 alkyl salicylate, capryloyl salicylic acid, hexyldodecyl salicylate, isocetyl salicylate, isodecyl salicylate, magnesium salicylate, MEA-salicylate, ethylhexyl salicylate, potassium salicylate, methyl salicylate, myristyl salicylate, sodium salicylate, TEA-salicylate, and tridecyl salicylate. International Journal of Toxicology 22, 1–108.

Clarke, N.E., Clarke, C.N., Mosher, R.E., 1958. Phenolic compounds in chemotherapy of rheumatic fever. American Journal of Medical Sciences 235, 7–22.

Collins, T.F.X., Hansen, W.H., Keeler, H.V., 1971. Effect of methyl salicylate on rat reproduction. Toxicology and Applied Pharmacology 18, 755–765.

Cross, S.E., Anderson, C., Roberts, M.S., 1998. Topical penetration of commercial salicylate esters and salts using human isolated skin and clinical microdialysis studies. British Journal of Clinical Pharmacology 46, 29–35.

Cross, S.E., Anderson, C., Thompson, M.J., Roberts, M.S., 1997. Is there tissue penetration after application of topical salicylate formulations?. Lancet 350 636.

Danon, A., Ben-Shimon, A., Ben-Zvi, Z., 1986. Effect of exercise and heat exposure on percutaneous absorption of methyl salicylate. European Journal of Clinical Pharmacology 31, 49–52.

Daston, G.P., Rehnberg, B.F., Carver, B., Rogers, E.H., Kavlock, R.J., 1988. Functional teratogens of the rat kidney. I. Colchicine, dinoseb, and methyl salicylate. Fundamental and Applied Toxicology 11, 381–400.

Davis, D.P., Daston, G.P., Odio, M.R., York, R.W., Kraus, A.L., 1994. Maternal reproductive effects of oral salicylic acid in Sprague–Dawley rats. The Toxicologist 14, 78.

Davison, C., Zimmerman, E.F., Smith, P.K., 1961. On the metabolism & toxicity of methyl salicylate. The Journal of Pharmacology and Experimental Therapeutics 132, 207–211.

Dirscherl, W., Wirtzfeld, A., 1964. Vanillic acid in human urine, its isolation, determination, and origin. Hoppe-Seyler's Zeitsschrift für physiologische Chemie 336, 81–90.

DiFrancesco, A.M., Chiu, P.C., Standley, L.J., Allen, H.E., Salvito, D.T., 2004. Dissipation of fragrance materials in sludge-amended soils. Environmental Science and Technology 38, 194–201.

Done, A.K., 1960. Salicylate intoxication. Significance of measurements of saliyclate in the blood in cases of acute ingestion. Pediatrics 26, 800–807.

Doull, J., Plzak, V., Brois, S.J., 1962. A survey of compounds for radiation protection. Armed Services Technical Information Agency, 1–124.

Duncan, E.J.S., Brown, A., Lundy, P., Sawyer, T.W., Hamilton, M., Hill, I., Conley, J.D., 2002. Site-specific percutaneous absorption of methyl salicylate and VX in domestic swine. Journal of Applied Toxicology 22, 141–148.

Drake, J.J., Gaunt, I.F., Butterworth, K.R., Hooson, J., Hardy, J., Gangolli, S.G., 1975. Short-term toxicity of isoamyl salicylate in rats. Food and Cosmetics Toxicology 13, 185–193.

Fishbeck, W.A., Langer, R.R., Kociba, R.J., 1975. Elevated urinary phenol levels not related to benzene exposure. American Industrial Hygiene Association Journal 36, 820–824.

Forbes, P.D., Urbach, F., Davies, R.E., 1977. Phototoxicity testing of Fragrance Raw Materials. Food and Cosmetics Toxicology 15, 55–60.

Ford, R.A., Domeyer, B., Easterday, O., Maier, K., Middleton, J., 2000. Criteria for development of a database for safety evaluation of fragrance ingredients. Regulatory Toxicology and Pharmacology 31, 166–181.

Fujii, T., Furukawa, S., Suzuki, S., 1972. Studies on compounded perfumes for toilet goods. On the non-irritative compounded perfumes for soaps. Yukugaku 21, 904–908.

Giri, A.K., Adhikari, N., Khan, K.A., 1996. Comparative genotoxicity of six salicylic acid derivatives in bone marrow. Mutation Research 370, 1–9.

Giroux, J., Granger, R., Monnier, P., 1954. Comparative toxicity of methyl diethylacetylsalicylate and methyl salicylate. Société Pharmacie Montpellier 14, 383–390.

Goldsmith, L.A., 1979. Salicylic acid. International Journal of Dermatology 18, 32–36.

Green, B.G., Shaffer, G.S., 1992. Psychophysical assessment of the chemical irritability of human skin. Journal of the Society of Cosmetic Chemists Japan 43, 131–147.

Hanzlik, P.J., Wetzel, N.C., 1920. The excretion of salicyl after the administration of methyl salicylate to animals. Journal of Pharmacology 14, 43–46.

Harrisson, J.W.E., Abbott, D.D., Packman, E.W., 1963. Salicylates and other hydroxybenzoates: effect upon osseous tissue of young rats. Federation Proceedings 22, 554.

Hausen, B.M., Wollenweber, E., 1988. Propolis allergy (III). Sensitization studies with minor constituents. Contact Dermatitis 19, 296–303.

Heymann, E., 1980. Carboxylesterases and amidases. In: Jakoby, W.B., Bend, J.R., Caldwell, J. (Eds.), Enzymatic Basis of Detoxication, second ed. Academic Press, New York, pp. 291–323.

Higo, N., Sato, S., Irie, T., Uekama, K., 1995. Percutaneous penetration and metabolism of salicylic acid derivatives across hairless mouse skin in diffusion cell in vitro. Scientific and Technical Pharmacy – Pharmaceutical Sciences 5, 302–308.

Howell, M.D., Manetz, T.S., Meade, B.J., 2000. Comparison of murine assays for the identification of chemical sensitizers. Toxicology Methods 10, 1–15.

Imokawa, G., Kawai, M., 1987. Differential hypermelanosis by allergic contact dermatitis. Journal of Investigative Dermatology 89, 540–546.

International Fragrance Association (IFRA), 2004. Volume of Use Survey.

International Fragrance Association (IFRA), 2007. Code of Practice. Standard on benzyl salicylate. Brussels.

Inder, R, 1999. Salicylic acid. INCHEM. October 1999. IPCS – International Programme on Chemical Safety. <http://www.inchem.org/documents/pims/chemical/pim412.htm>.

Infurna, R., Beyer, B., Twitty, L., Koehler, G., Daughtrey, W., 1990. Evaluation of the dermal absorption and teratogenic potential of methyl salicylate in a petroleum based grease. Teratology 41, 566.

Integrated Risk Information system (IRIS), 1998. Results of databank search on 23.10.98 for *n*-butyl alcohol. National Library of Medicine, Bethesda, MD, USA.

Ishidate Jr., M., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M., Matsuoka, A., 1984. Primary mutagenicity screening of food additives currently used in Japan. Food and Chemical Toxicology 22, 623–636.

Ishihara, M., Itoh, M., Nishimura, M., Kinoshita, M., Kantoh, H., Nogami, T., Yamada, K., 1986. Closed epicutaneous test. Skin Research 28, 230–240.

James, M.O., Smith, R.L., Williams, R.T., Reidenberg, M., 1972. The conjugation of phenylacetic acid in man, sub-human primates, and some non-primate species. Proceedings Royal Society London, B 182, 25–35.

James, M.O., Smith, R.L., Robert, L., 1973. Conjugation of phenylacetic acid in phenylketonurics. European Journal of Clinical Pharmacology 5, 243–246.

Janssen, K., Hollman, P.C.H., Reichmann, E., Venema, D.P., van Staveren, W.A., Katan, M.B., 1996. Urinary salicylate excretion in subjects eating a variety of diets shows that amounts of bioavailable salicylates in foods are low. American Journal of Clinical Nutrition 64, 743–747.

Joint Expert Committee on Food Additives (JECFA), 1996. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series: 37. Joint FAO/WHO Expert Committee on Food Additives World Health Organisation, Geneva 1996.

Joint Expert Committee on Food Additives (JECFA), 1998. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series: 40. Joint FAO/WHO Expert Committee on Food Additives. World Health Organisation, Geneva 1998.

Joint Expert Committee on Food Additives (JECFA)), 2001. Safety evaluation of certain food additives. WHO Food Additives Series: 48. Prepared by the Fifty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organization, Geneva 2001.

Jenner, P.M., Hagan, E.C., Taylor, J.M., Cook, E.L., Fitzhugh, O.G., 1964. Food flavorings and compounds of related structure. I. Acute oral toxicity. Food and Cosmetics Toxicology 2, 327–343.

Jiang, R., Roberts, M.S., Collins, D.M., Benson, H.A.E., 1997. Skin penetration of sunscreen agents from commercial products. Perspectives in Percutaneous Penetration 5B, 239–241.

Jimbo, Y., 1983. Penetration of fragrance compounds through human epidermis. Journal of Dermatology 10, 229–239.

Jones, P.S., Thigpen, D., Morrison, J.L., Richardson, A.P., 1956. *p*-Hydroxybenzoic acid esters as preservatives. III. The physiological disposition of *p*-hydroxybenzoic acid and its esters. Journal of the American Pharmaceutical Association 45, 268–273.

Juberg, D.R., David, R.M., Katz, G.V., Bernard, L.G., Gordon, D.R., Vlaovic, M.S., Topping, D.C., 1998. 2-Ethylhexanoic acid: subchronic oral toxicity studies in the rat and mouse. Food and Chemical Toxicology 36, 429–436.

Kashima, T., Oyake, Y., Okada, J., Ikeda, Y., 1993. Studies of new short-period method for delayed contact hypersensitivity assay in the guinea pig. Contact Dermatitis 28, 235–242.

Kavlock, R.J., Chernoff, N., Rogers, E., Whitehouse, D., Carver, B., Gray, J., Robinson, K., 1982. An analysis of fetotoxicity using biochemical endpoints of organ differentiation. Teratology 26, 183–194.

Kieckebusch, W., Lang, K., 1960. The tolerability of benzoic acid in chronic feeding experiments. Arzneimittel-Forschung 10, 1001–1003.

Kimber, I., Hilton, J., Dearman, R.J., Gerberick, G.F., Ryan, C.A., Basketter, D.A., Scholes, E.W., Ladies, G.S., Loveless, S.E., House, R.V., Guy, A., 1995. An international evaluation of the murine local lymph node assay and comparison of modified procedures. Toxicology 103, 63–73.

Kimber, I., Hilton, J., Botham, P.A., Basketter, D.A., Scholes, E.W., Miller, K., Robbins, M.C., Harrison, P.T.C., Gray, T.J.B., Waite, S.J., 1991. The murine local lymph node assay: Results of an inter-laboratory trial. Toxicology Letters 55, 203–213.

Kimber, I., Hilton, J., Dearman, R.J., Gerberick, G.F., Ryan, C.A., Basketter, D.A., Lea, L., House, R.V., Ladics, G.S., Loveless, S.E., Hastings, 1998. Assessment of the skin sensitization potential of topical medicaments using the local lymph node assay: an interlaboratory evaluation. Journal of Toxicology and Environmental Health 53, 563–579.

Kimmel, C.A., Wilson, J.G., Schumacher, H.J., 1971. Studies on metabolism and identification of the causative agent in aspirin teratogenesis in rats. Teratology 4, 15–24.

Klecak, G., 1979. The open epicutaneous test (OET), a predictive test procedure in the guinea pig for estimation of allergenic properties of simple chemical compounds, their mixtures and of finished cosmetic preparations. International Federation Societies Cosmetic Chemists, 9/18/79.

Klecak, G., Geleick, H., Frey, J.R., 1977. Screening of fragrance materials for allergenicity in the guinea pig. I. Comparison of four testing methods. Journal of the Society of Cosmetic Chemists Japan 28, 53–64.

Klecak, G., 1985. The Freund's complete adjuvant test and the open epicutaneous test. In: Andersen, K.E., Maibach, H.I. (Eds.), Current Problems in Dermatology, vol. 14. Karger, Basel, Switzerland, pp. 152–171.

Kociba, R.J., Kalnins, R.V., Wade, C.E., Garfield, E.L., Fishbeck, W.A., 1976. Elevated urinary phenol levels in dogs ingesting salol. Toxicology and Applied Pharmacology 37, 121.

Kolpin, D.W., Skopec, M., Meyer, M.T., Furlong, E.T., Zaugg, S.D., 2004. Urban contribution of pharmaceuticals and other organic wastewater contaminants to streams during differing flow conditions. Science of the Total Environment 328, 119–130.

Komsta, E., Chu, I., Secours, V.E., Valli, V.E., Villeneuve, D.C., 1988. Results of a short-term toxicity study for three organic chemicals found in Niagara River drinking water. Bulletin of Environmental Contamination and Toxicology 41, 515–522.

Kozuka, T., Hayashi, H., Hiroyama, H., Honoki, S., Kakishima, H., Katsumura, Y., Kawai, J., Nakagaki, E., Obata, K., Ozawa, N., Tatsumi, H., Fujimoto, K., Nakagawa, M., Yoshikawa, Y., Ikeda, Y., Ishii, I., Itoh, K., 1996. Allergenicity of fragrance materials: collaborative study of the Second Research Group of the Japanese Society for Cutaneous Health. Environmental Dermatology 3, 326–335.

Kuboyama, N., Fujii, A., 1992. Mutagenicity of analgesics, their derivatives, and anti-inflammatory drugs with S-9 mix of several animal species. Journal of Nihon University School of Dentistry 34, 183–195.

Ladics, G.S., Smith, C., Heaps, K.L., Loveless, S.E., 1995. Comparison of 125-idodeoxyuridine (^{125}IUdR) and [^{3}H]thymidine ([^{3}H]TdR) for assessing cell proliferation in the murine local lymph node assay. Toxicology Methods 5, 143–152.

Levy, G., Tsuchiya, T., 1972. Darvon poisoning with delayed salicylism: a case report. Pediatrics 49, 610–611.

The MAK-Collection for Occupational Health and Safety. Part I: MAK Value Documentations Volume 22. Greim, H. (Ed.), Wiley-VCH Verlag GmbH & Co. KGaA, Germany. http://www3.interscience.wiley.com/cgi-bin/mrwhome/104554790/HOME.

Marchand, B., Barbier, P., Ducombs, G., Foussereau, J., Martin, P., Benezra, C., 1982. Allergic contact dermatitis to various salols (phenyl salicylates). A structure–activity relationship study in man and in animal (guinea pig). Archives of Dermatological Research 272, 61–66.

Martin, D., Valdez, J., Boren, J., Mayersohn, M., 2004. Dermal absorption of camphor, menthol, and methyl salicylate in humans. Journal of Clinical Pharmacology 44, 1151–1157.

Maurer, T.H., Weirich, E.G., Hess, R., 1980. The optimization test in the guinea pig in relation to other predictive sensitization methods. Toxicology 15, 163–171.

McMahon, T.F., Diliberto, J.J., Birnbaum, L.S., 1990. Effects of age and dose on disposition and metabolism of salicylic acid in male Fischer F344 rats. Drug Metabolism and Disposition 18, 494–503.

Montelius, J., Wahlkvist, H., Boman, A., 1994. Experience with the murine local lymph node assay: inability to discriminate between allergens and irritants. Acta Dermato-Venereologica 74, 22–27.

Morra, P., Bartle, W.R., Walker, S.E., Lee, S.N., Bowles, S.K., Reeves, R.A., 1996. Serum concentrations of salicylic acid following topically applied salicylate derivatives. The Annals of Pharmacotherapy 30, 935–940.

Morrissey, R.E., Lamb IV, J.C., Morris, R.W., Chapin, R.E., Gulati, D.K., Heindel, J.J., 1989. Results and evaluations of 48 continuous breeding reproduction studies conducted in mice. Fundamental and Applied Toxicology 13, 747–777.

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B., Zeiger, E., 1986. Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. Environmental Mutagenesis 8, 1–119.

Motoyoshi, K., Toyoshima, Y., Sato, M., Yoshimura, M., 1979. Comparative studies on the irritancy of oils and synthetic perfumes to the skin of rabbit, guinea pig, rat, miniature swine and man. Cosmetics and Toiletries 94, 41–48.

Nagahama, M., Akiyama, N., Miki, T., 1966. Experimental production of malformations due to salicylates (acetyl salicylate and phenyl salicylate) in rats. Congenital Anomalies (Senten Ijo) 6, 20–31.

Nakayama, H., 1998. Fragrance hypersensitivity and its control. In: Frosch, P.J., Johansen, J.D., White, I.R. (Eds.), Fragrances. Beneficial and Adverse Effects. Springer-Verlag, Berlin Heidelberg, Germany, pp. 83–91.

National Toxicology Program (NTP), 1984a. Methyl salicylate: Reproduction and fertility assessment in CD-1 mice when administered by gavage. NTP-84-156; PB84-241140.

National Toxicology Program (NTP), 1984b. Methyl salicylate: Reproduction and fertility assessment in CD-1 mice when administered by gavage. NTP-85-022; PB85-164283.

Oda, Y., Hamono, Y., Inoue, K., Yamamoto, H., Niihara, T., Kunita, N., 1978. Mutagenicity of food flavors in bacteria. Shokuhin Eisei Hen 9, 177–181.

Ohsumi, T., Kuroki, K., Kimura, T., Murakami, Y., 1984. A study on acute toxicities of essential oils used in endodontic treatment. Journal Kyushu Dental Society 38, 1064–1071.

Overman, D.O., White, J.A., 1978. Comparative dose–response analysis of the teratogenic effects of methyl salicylate applied orally or topically to hamsters. The Anatomical Record 190, 499.

Overman, D.O., 1979. Percutaneous administration of teratogens: Effects of treatment with methyl salicylate and dimethyl sulfoxide in the hamster. Teratology 19, 42A.

Overman, D.O., White, J.A., 1983. Comparative teratogenic effects of methyl salicylate applied orally or topically to hamsters. Teratology 28, 421–426.

Owston, E., Lough, R., Opdyke, D.L., 1981. A 90-day study of phenylethyl alcohol in the rat. Food and Cosmetics Toxicology 19, 713–715.

Packman, E.W., Abbott, D.D., Wagner, B.M., Harrisson, J.W.E., 1961. Chronic oral toxicity of oil sweet birch (methyl salicylate). The Pharmacologist 3, 62.

Patrick, E., Maibach, H.I., Burkhalter, A., 1985. Mechanisms of chemically induced skin irritation. Toxicology and Applied Pharmacology 81, 476–490.

Patrick, E., Maibach, H.I., 1986. Interspecies differences in sensitivity to inflammatory mediators modify dose response and time course of chemically induced skin irritation. Journal of Investigative Dermatology 86, 499.

Patrick, E., Maibach, H.I., Burkhalter, A., 1987. Recent investigations of mechanisms of chemically induced skin irritation in laboratory mice. Journal of Investigative Dermatology 88, 24s–31s.

Pratzel, H.G., Schubert, E., Muhanna, N., 1990. Pharmacokinetic study of percutaneous absorption of salicylic acid from baths with salicylate methyl ester and salicylic acid. Zeitschrift für Rheumatologie 49, 185–191.

Pyun, J.S., 1970. Effect of methyl salicylate on developing rat embryos. Ch'oesin Uthak 13, 63–72.

RIFM (Research Institute for Fragrance Materials, Inc.), 1963. Eye irritation study of methyl salicylate in rabbits. Unpublished Report from IFF Incorporated, 29 November. Report number 12616 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1964. Repeated insult patch test of methyl salicylate in human subjects. Unpublished Report from IFF Incorporated, 3 April. Report number 12617 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1968a. Acute oral toxicity of octyl salicylate in rats. Unpublished Report from Felton International, 25 October. Report number 15430 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1968b. Sensitization and irritation studies of fragrance materials in human subjects. Unpublished report from Givaudan Incorporated, 18a November. Report number 15453 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1968c. Irritation study of 3-methyl-2-butenyl salicylate in rabbits. Unpublished report from IFF Incorporated, 11 October. Report number 15226 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1970a. Acute oral toxicity studies in rats. RIFM report number 2734, July 1 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1970b. Acute dermal toxicity study in rabbits. RIFM report number 2735, August 26 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1970c. Toxicological studies of two salicylates: isobutyl salicylate and isoamyl salicylate. Unpublished Report from Fritzsche, Dodge and Olcott, Incorporated, 31 August. Report number 13837 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1970d. Eye irritation study of 3-methyl-2-butenyl salicylate in rabbits. Unpublished report from IFF Incorporated, 7 April. Report number 15227 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1970e. The contact sensitizing potential of fragrance materials in humans. RIFM report number 1760, October 7 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1971. Irritation studies with octyl salicylate. Unpublished report from Felton International, 25 March. Report number 15431 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1973a. Acute toxicity studies on rats and rabbits. RIFM report number 2021, February 23 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1973b. Report on human maximization studies. RIFM report number 1803, November 26 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1973c. Report on human maximization studies. RIFM report number 1802, May 21 and October 11b (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1974a. Acute toxicity study in rats and rabbits. RIFM report number 2025 July 17 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1974b. Report on human maximization studies. RIFM report number 1801 August 19 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1975a. Acute toxicity studies. RIFM report number 2020, January 31 and February 3 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1975b. Acute toxicity studies. RIFM report number 2024, May 19 and June 10 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1975c. Report on human maximization studies. RIFM report number 1799, January 15a, March 27 and May 16a,b (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1975d. Report on human maximization studies. RIFM report number 1798, January 31 and March 28 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1975e. Sensitization test of benzyl salicylate in rabbits. Unpublished report from IFF Incorporated, 28 March. Report number 24192 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1975f. Phototoxicity and irritation studies of fragrance materials in the mouse and miniature swine. RIFM report number 2038, February 28 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1975g. Acute eye irritation study of benzyl salicylate in rabbits. Unpublished report from IFF Incorporated, 2 April. Report number 24191 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1975h. Repeated insult patch test of benzyl salicylate on human subjects. Unpublished report from IFF Incorporated, 18 June. Report number 24190 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1975i. Phototoxic potential of octyl salicylate on human skin. Unpublished Report from Felton International, September 5. Report number 15435 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1976a. Acute toxicity studies. RIFM report number 2019, May 12 and September 7 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1976b. Report on human maximization studies conducted with wintergreen oil. RIFM report number 1796, July 23 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1976c. Report on human maximization studies. RIFM report number 1797, March 9a and June 25 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1976d. Repeated insult patch test of octyl salicylate on human skin. Unpublished report from Felton International Incorporated, 17 February. Report number 15432 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1976e. Phototoxicity and irritation studies on wintergreen oil in miniature swine and mice. RIFM report number 2039, July 20 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1977a. Mutagenicity evaluation of octyl salicylate in rats. Unpublished Report from Felton International, 9 September. Report number 15434 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1977b. Report on human maximization studies. RIFM report number 1702, November 3 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1978a. Acute toxicity studies. RIFM report number 1699, February 1 and May 5 and May 8 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1978b. Report on human maximization studies. RIFM report number 1698, June 2 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1978c. Report on human maximization studies. RIFM report number 1787, February 28 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1978d. Draize eye irritation study of octyl salicylate in rabbits. Unpublished Report from Felton International, 20 April. Report number 15433 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1979. Report on human maximization studies. RIFM report number 1697, July 6 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1980a. Acute toxicity studies. RIFM report number 1774, May 28 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1980b. Report on human maximization studies. RIFM report number 1791, March 17 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1980c. Sensitization studies with benzyl salicylate in human subjects. RIFM report number 3305, January 21 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1981a. Acute dermal toxicity of 1,3-dimethyl-3-butenyl salicylate in rabbits. Unpublished report from Firmenich Incorporated, 10 April. Report number 39258 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1981b. Acute oral toxicity study of 1,3-dimethyl-3-butenyl salicylate in rats. Unpublished report from Firmenich Incorporated, 10 April. Report number 39257 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1981c. Repeated insult patch test/photosensitization study of 1,3-dimethyl-3-butenyl salicylate in human subjects. Unpublished report from Firmenich Incorporated, 20 May. Report number 39261 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1981d. Primary skin irritation of 1,3-dimethyl-3-butenyl salicylate in rabbits. Unpublished report from Firmenich Incorporated, 10 April. Report number 39259 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1981e. Guinea pig skin sensitisation test with hexyl salicylate. Unpublished report from Quest International, 12 November. Report number 46933 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1981f. Guinea pig skin sensitisation test with pentyl salicylate. Unpublished report from Quest International, 12 November. Report number 46934 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1981g. Guinea pig skin sensitisation test with phenethyl salicylate. Unpublished report from Quest International, 12 November. Report number 46935 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1981h. Primary eye irritation study of 1,3-dimethyl-3-butenyl salicylate in rabbits. Unpublished report from Firmenich Incorporated, 10 April. Report number 39260 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1982a. Acute toxicity studies in rats. Unpublished Report from Givaudan Incorporated, 13 and 27 September. Report number 1786 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1982b. Acute toxicity studies. RIFM report number 1689, April 30 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1982c. Report on human maximization studies. RIFM report number 1643, April 27 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1982d. Phototoxicity study with benzyl salicylate in guinea pigs. Unpublished report from Givaudan Incorporated, 9 November. Report number 33514 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1983a. Penetration studies "*in vitro*" on intact skin of naked rat and pig with benzyl salicylate. Unpublished report from Givaudan Incorporated, 31 August. Report number 33515 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1983b. Phototoxicity and photoallergy tests with benzyl salicylate in the guinea pig. Unpublished report from Rhodia Incorporated, 13 June. Report number 40988 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1983c. Phototoxicity test of benzyl salicylate in humans. Unpublished report from Givaudan Incorporated, 1 August. Report number 33513 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1983d. Phototoxicity study of fragrance materials in hairless mice. RIFM report number 2043, February 3 (RIFM, Woodcliff Lake, NJ).

RIFM (Research Institute for Fragrance Materials, Inc.), 1984. Primary irritation test in rabbits. RIFM report number 1795, June 1 (RIFM, Woodcliff Lake, NJ).

RIFM (Research Institute for Fragrance Materials, Inc.), 1985. Primary irritation test in rabbits. RIFM report number 3099, June 1 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1986a. Primary irritation test in rabbits. RIFM report number 5665, August 1 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1986b. Primary irritation test in rabbits. RIFM report number 5664, June 1 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1990. Acute oral toxicity (limit test) of pentyl salicylate in the rat. Unpublished report from Haarmann & Reimer GmbH, 12 April. Report number 35559 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1997a. Human irritation study with benzyl salicylate. Unpublished report from Takasago International Corporation, 9 December. Report number 31608 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1997b. Primary skin irritation study and phototoxicity study in guinea pigs with benzyl salicylate. Unpublished report from Takasago International Corporation, 9 December. Report number 31612 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1997c. Delayed contact hypersensitivity study in guinea pigs with benzyl salicylate. Unpublished report from Takasago International Corporation, 9 December. Report number 31605 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 1999. Toxicology studies of *cis*-3-hexenyl salicylate in the guinea pig. Unpublished report from Takasago International Corporation, 13 July. Report number 35055 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 2003. Topical photoallergy screening test of hexyl salicylate and beta-methyl naphthyl ketone in male albino hairless guinea pigs [CRL: IAF(HA)-hrBR (outbred)], including primary irritation, phototoxicity and contact hypersensitivity evaluations. RIFM report number 44882, June 9 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 2004a. Repeated insult patch test with fragrance materials. RIFM report number 45130, May 3 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 2004b. Evaluation of phototoxicity of hexyl salicylate in humans. RIFM report number 45136, March 16 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 2004c. Repeated insult patch test with fragrance materials. RIFM report number 45129, May 3 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 2005. Local Lymph Node Assay with benzyl salicylate. RIFM report number 47378, January 20 (RIFM, Woodcliff Lake, NJ, USA).

RIFM (Research Institute for Fragrance Materials, Inc.), 2006. Phenyl ethyl salicylate: local Lymph Node Assay. RIFM report number 2353, January 11 (RIFM, Woodcliff Lake, NJ, USA).

Riviere, J.E., Smith, C.E., Budsaba, K., Brooks, J.D., Olajos, E.J., Salem, H., Monteiro-Riviere, N.A., 2000. Use of methyl salicylate as a simulant to predict the percutaneous absorption of sulfur mustard. The Toxicologist 54, 151–152.

Riviere, J.E., Smith, C.E., Budsaba, K., Brooks, J.D., Olajos, E.J., Salem, H., Monteiro-Riviere, N.A., 2001. Use of methyl salicylate as a simulant to predict the percutaneous absorption of sulfur mustard. Journal of Applied Toxicology 21, 91–99.

Roberts, M.S., Favretto, W.A., Meyer, A., 1982. Topical bioavailability of methyl salicylate. Australian and New Zealand Journal of Medicine 12, 303–305.

Robinson, D., Williams, R.T., 1956. Glucuronides of salicylic acid. Biochemical Journal 62, 23.

Salvito, D.T., Senna, R.J., Federle, T.W., 2002. A Framework for prioritizing fragrance materials for aquatic risk assessment. Environmental Toxicology and Chemistry 21, 1301–1308.

Sammons, H.G., Williams, R.T., 1941. Studies in detoxication. The metabolism of vanillin and vanillic acid in the rabbit. The identification of glucurovanillin and the structure of glucurovanillic acid. Biochemical Journal 325, 1175–1188.

Sax, N., 1979. Dangerous Properties of Industrial Materials, fifth ed. Van Nostrand Reinhold, New York.

Schilling, K., Kayser, M., Deckardt, K., Kuttler, K., Klimisch, H.J., 1997. Subchronic toxicity studies of 3-methyl-1-butanol and 2-methyl-1-propanol in rats. Human and Experimental Toxicology 16, 722–726.

Sharp, D.W., 1978. The sensitization potential of some perfume ingredients tested using a modified Draize procedure. Toxicology 9, 261–271.

Shen, J., Wanwimolruk, S., Purves, R.D., McQueen, E.G., Roberts, M.S., 1991. Model representation of salicylate pharmacokinetics using unbound plasma salicylate concentrations and metabolite urinary excretion rates following a single oral dose. Journal of Pharmacokinetics and Biopharmaceutics 19, 575–595.

Short, C.R., Neff-Davis, C.A., Hsieh, L.C., Koritz, G.D., Malbrough, M.S., Barker, S.A., Davis, L.E., 1991. Pharmacokinetics and elimination of salicylic acid in rabbits. Journal of Veterinary Pharmacology and Therapeutics 14, 70–77.

Siddiqi, M., Ritschel, W.A., 1972. pH-Effects on salicylate absorption through the intact rat skin. Scientia Pharmaceutica 40, 181–189.

Simonich, S.L., Begley, W.M., Debaere, G., Eckhoff, W.S., 2000. Trace analysis of fragrance materials in wastewater and treated wastewater. Environmental Science and Technology 34, 959–965.

Simonich, S.L., Federle, T.W., Eckhoff, W.S., Rottiers, A., Webb, S., Sabaliunas, D., De Wolf, W., 2002. Removal of fragrance materials during U.S. and European wastewater treatment. Environmental Science and Technology 36, 2839–2847.

Stofberg, J., Grundschober, F., 1987. Consumption ratio and food predominance of flavoring materials. Perfumer Flavorist 12, 27.

Stoner, G.D., Shimkin, M.B., Kniazeff, A.J., Weisburger, J.H., Weisburger, E.K., Go, G.B., 1973. Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A mice. Cancer research 33, 3069–3085.

Strand, L.P., Scheline, R.R., 1975. The metabolism of vanillin and isovanillin in the rat. Xenobiotica 5, 49–63.

Strong, L.C., 1932a. The effect of oil of wintergreen on the incidence of spontaneous carcinoma in mice. IV. Effect on growth rate and survival time after onset of malignancy. American Journal of Medicine 4, 546–553.

Strong, L.C., 1932b. Possible effect of oil of Gaultheria in diet of mice susceptible to spontaneous carcinoma of the breast. I.A suggestion. Proceedings of the Society for Experimental Biology and Medicine 30, 386–390.

Szybalski, W., 1958. Special microbial systems. II. Observations on chemical mutaganesis in microorganisms. Annals New York Academy of Sciences 76, 475–489.

Tanaka, S., Kawashima, K., Nakaura, et al., 1973a. Studies on the teratogenicity of food additives (3). Teratogenic effect of dietary salicylic acid in rats. Shokuhin Eiseigaku Zasshi 14, 549–557.

Tanaka, S., Kawashima, K., Nakaura, et al., 1973b. Studies on the teratogenic effects of salicylic acid and aspirin in rats as related to fetal distribution. Congenital Anomalies (Senten Ijo) 13, 73–84.

Treffel, P., Gabard, B., 1996. Skin penetration and sun protection factor of ultraviolet filters from two vehicles. Pharmaceutical Research 13, 770–774.

Tsuchiya, S., Kondo, M., Okamoto, K., Takase, Y., 1982. Studies on contact hypersensitivity in the guinea pig. The cumulative contact enhancement test. Contact Dermatitis 8, 246–255.

Vree, T.B., van Ewijk-Beneken Kolmer, E.W.J., Verwey-Van Wissen, C.P.W.G.M., Hekster, Y.A., 1994a. Direct gradient reversed-phase high-performance liquid chromatographic determination of salicylic acid, with the corresponding glycine and glucuronide conjugates in humans plasma and urine. Journal of Chromatography, B: Biomedical Sciences and Applications 652, 161–170.

Vree, T.B., van Ewijk-Beneken Kolmer, E.W.J., Verwey-Van Wissen, C.P.W.G.M., Hekster, Y.A., 1994b. Effect of urinary pH on the

pharmacokinetics of salicylic acid, with its glycine and glucuronide conjugates in humans. International Journal of Clinical Pharmacology and Therapeutics 32, 550–558.

Walters, K.A., Brain, K.R., Howes, D., James, V.J., Kraus, A.L., Teetsel, L.M., Toulon, M., Watkinson, A.C., Gettings, S.D., 1997. Percutaneous penetration of octyl salicylate from representative sunscreen formulations through human skin in vitro. Food and Chemical Toxicology 35, 1219–1225.

Waltman, R., Tricomi, V., Shabanah, E.H., Arenas, R., 1973. The effect of anti-inflammatory drugs on parturition parameters in the rat. Prostaglandins 4, 93–106.

Warkany, J., Takacs, E., 1959. Experimental production of congenital malformations in rats by salicylate poisoning. American Journal of Pathology 35, 315.

Watkinson, A.C., Brain, K.R., Walters, K.A., Hadgraft, J., 1992. Prediction of the percutaneous penetration of ultra-violet filters used in sunscreen formulations. International Journal of Cosmetic Science 14, 265–275.

Webb, W.K., Hansen, W.H., 1963. Chronic and subacute toxicology and pathology of methyl salicylate in dogs, rats and rabbits. Toxicology and Applied Pharmacology 5, 576–587.

Webb, W.K., Hansen, W.H., 1962. Chronic and subacute toxicology and pathology of methyl salicylate in dogs, rats and rabbits. Federation Proceedings 21, 255.

Williams, R.T., 1959. The metabolism of aromatic alcohols, ethers, aldehydes, ketones and quinones. In: Detoxication Mechanisms. Chapman & Hall Ltd., London, pp. 318–320.

Wolowich, W.R., Hadley, C.M., Kelley, M.T., Walson, P.D., Casavant, M.J., 2003. Plasma salicylate from methyl salicylate cream compared to oil of wintergreen. Journal of Toxicology: Clinical Toxicology 41, 355–358.

Woo, D.C., Hoar, R.M., 1972. ''Apparent hydronephrosis'' as a normal aspect of renal development in late gestation of rats: the effect of methyl salicylate. Teratology 6, 191–196.

Yankell, S.L., 1972. The effects of various vehicles on absorption rates in skin. In: Montagna, W., Van Scott, E.J., Stoughton, R.B. (Eds.), Advances in Biology of Skin, Pharmacology and the Skin, vol. 12. Oregon Regional Primate Research Center, Oregon, pp. 511–522.

Yano, T., Kanetake, T., Saita, M., Noda, K., 1991. Effects of L-menthol and DL-camphor on the penetration and hydrolysis of methyl salicylate in hairless mouse skin. Journal of Pharmacobio-Dynamics 14, 663–669.

Yano, T., Nakagawa, A., Tsuji, M., Noda, K., 1986. Skin permeability of various non-steroidal anti-inflammatory drugs in man. Life Sciences 39, 1043–1050.

Yoshida, Y., Oyake, Y., Sakaguchi, H., Okuda, M., Suzuki, H., 2000. Comparison of the effect of allergen and irritant treatment on proliferation and subpopulation of the draining lymph node cells in mice and guinea pigs. The Toxicologist 54, 153.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., 1987. *Salmonella* mutagenicity tests: III. Results from the testing of 255 chemicals. Environmental Mutagenesis 9, 1–109.