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Review

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

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Abstract

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

Keywords: Safety; Review; Fragrance; Ionones

Contents

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1. Chemical identity, regulatory status and exposure

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

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 The Fragrance Material Reviews on each individual ionone contain a comprehensive summary of published and unpublished reports and comprehensive bibliographies.

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

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

Table 1
Material identity

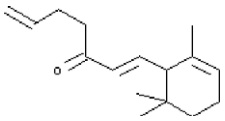
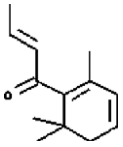
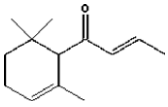
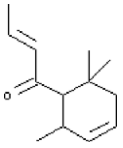
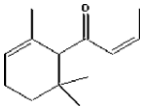
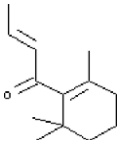
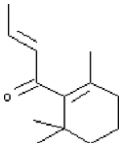
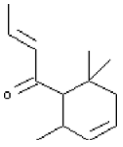
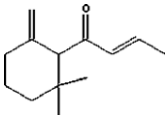
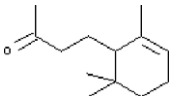
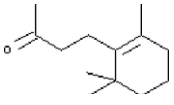
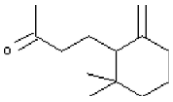
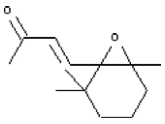
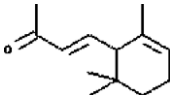
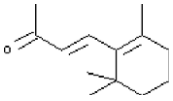
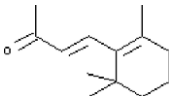
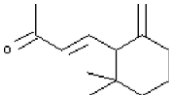
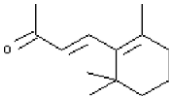
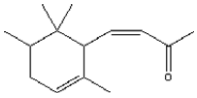
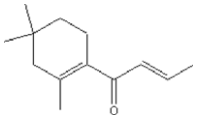
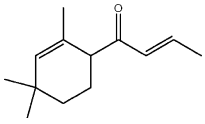
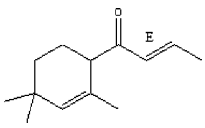
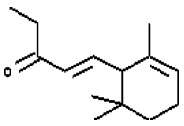
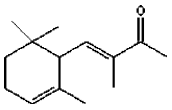
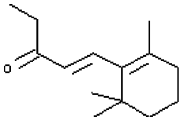
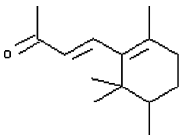
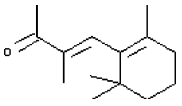
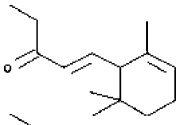
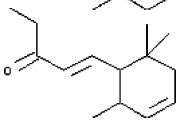
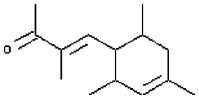
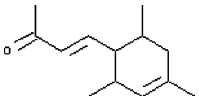
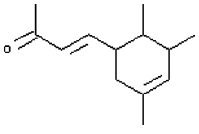
Compound	Structure	Synonyms
Allyl α-ionone CAS# 79-78-7 Molecular weight 232.37 log K_{ow} (calculated) 5.63		Allyl cyclocitrylideneacetone α-Allylionone α-Cyclocitrylidenemethyl butenyl ketone Cetone V 1,6-Heptadien-3-one 1-(2,6,6-trimethyl-2-cyclohexen-1-yl)- 1-(2,6,6-Trimethyl-2-cyclohexene-1-yl)-1,6-heptadien-3-one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

Table 1 (*continued*)

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

(*continued on next page*)

Table 1 (*continued*)

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

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

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

Table 2
Volume of use and dermal exposure

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

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

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

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

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

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

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

1.1. Estimated consumer exposure

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

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

2. Absorption, distribution and metabolism, and potential for enzyme induction

2.1. Absorption

In the scientific literature there are no definitive data from which to quantify the *in vivo* absorption of ionones and/or rose ketones following dermal exposure. By analogy with fragrance ketones and aldehydes for which *in vivo* absorption data are available, dermal absorption of ionones/rose ketones is likely to be significant. All are lipophilic substances with oil/water partition coefficient ($\log K_{ow}$) values in the range of 3.85–5.20. In light of these data, and the lack of specific information on any of the individual ionones/rose ketones, a dermal absorption rate of 100% was conservatively assumed for the purposes of human health risk assessment. The assumption of 100% dermal bioavailability is considered especially conservative given that in an *in vitro* dermal penetration/permeability study, only 0.7% or undetectable amounts of methyl ionone (mixture of isomers) were recovered in the fluid beneath the skin preparations of rats and pigs, respectively, 6 h after application of a 3000 μg dose (600 $\mu g/cm^2$ over 5 cm^2 of skin) (RIFM, 1984a). In this study, approximately 50% (rat) and 10% (pig) of methyl ionone ^{14}C penetrated into, but not through the epidermis and dermis, while another 30% was lost to evaporation.

There also are no oral pharmacokinetic studies available from which the bioavailability of this class of compounds can be quantitatively determined. Based on metabolic studies on α-ionone (Prelog et al., 1951) and β-ionone (Bielig and Hayasida, 1940; Ide and Toki, 1970) in which ionone-specific metabolites were recovered in the urine of treated rabbits, and in the urine of dogs treated orally with β-ionone (Prelog and Meier, 1950), oral absorption of these compounds does occur to some extent. These studies, however, were not designed as pharmacokinetic investigations suitable to determine oral absorption. Given that a certain, but unquantifiable amount, of orally ingested α- and β-ionone is absorbed, it is prudent to assume that the other 34 structurally related ionones and rose ketones assessed in this report would also be bioavailable *via* the oral route. As a result, rose ketones were assumed to be 100% bioavailable for the purposes of human health risk assessment. Given the *in vitro* skin penetration data (RIFM, 1984a) on methyl ionone (mixed isomers), bioavailability by the oral route is likely to be considerably greater than by the dermal route. However, the magnitude of this potential difference cannot be quantified or extrapolated to all chemicals included in this assessment.

2.2. Distribution and pharmacokinetics

Data available describing the distribution and pharmacokinetics of ionones/rose ketones following absorption are limited to a single study in mice reporting the presence of β-ionone at trace levels (<0.1 ng/ml) in the blood 30–90 min following a 1-h inhalation exposure to 0.00001 ppm (Buchbauer et al., 1993).

2.3. Metabolism

All the compounds discussed in this group are simple molecular modifications of the basic ionone and damascene structures, which are in essence cyclohexene derivatives carrying a butanone side chain. Therefore ionone and damascone can be regarded as being archetypal for the group as a whole. Furthermore, it is anticipated that compounds in this group will show a high degree of metabolic homology, bearing in mind that, in general, the same functional groups will be involved in biotransformation reactions. The α- and β-ionones are structural positional isomers as are also the α- and β-damascones. The only structural differences between the ionones and the rose ketones are the position of the allylic double bond and of the ketone in the butanone side chain (see Table 1 for structure and CAS numbering system).

The ionones and rose ketones, because of their highly lipophilic nature would be expected to be extensively metabolized *in vivo* and eliminated as transformation products. This appears to be the case as in several studies involving the administration of α- or β-ionone to rabbits and dogs. Little unchanged compound was recovered from the urine compared to the relatively large amounts of transformation products that could be isolated (Bielig and Hayasida, 1940; Prelog and Meier, 1950; Ide and Toki, 1970). Based upon the molecular structures of the ionones and rose ketones several metabolic options might be predicted:

1. hydroxylation/oxygenation of the cyclohexene ring;
2. reduction of the buteneone group to a secondary alcohol;
3. oxidation of the angular methyl groups;
4. reduction of the double bond in the exocyclic alkenyl side chain to form dihydro derivatives;
5. conjugation of the hydroxylated metabolites with glucuronic acid;
6. conjugation with glutathione.

Finally there could be various combinations of these pathways to produce an array of metabolites.

Overall, while the empirical metabolic data are limited to studies primarily on β-ionone, it should be noted that the ionones and rose ketones are close structural analogues, both having a cyclohexa(e)ne ring with an allylic side chain containing a ketone moiety. Differences in the structures are related to the presence of an additional ketone group [e.g., 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-butane-1,3-dione], unsaturation of the cyclohexene ring (e.g., dihydro-γ-ionone), unsaturation of the allylic side chain, differences in the points of methylation of the cyclohexene ring, the position of the double bond in the allylic side chain (i.e., the ionones *versus* the rose ketones), and various combinations of the above. While these differences would be expected to lead to the production of compound-specific metabolites without a common terminal metabolite, some generalizations can be made. As reported by JECFA (1999), α-ionone, dihydro-α-ionone, methyl-α-ionone, α-irone, α-*iso*-methylionone, and allyl-α-ionone would likely share a common metabolic pathway, with differences in rates of metabolism only. Likewise, β-ionone, dihydro-β-ionone, and methyl-β-ionone, could be expected to be metabolized in a very similar manner. For the other compounds, while common pathways cannot be clearly established, similar metabolic processes would be expected to occur and could include various combinations of hydroxylation/oxygenation of the cyclohexene ring, reduction of the butenone group to a secondary alcohol, oxidation of the angular methyl groups, reduction of the double bond in the exocyclic alkenyl side chain to form dihydro derivatives, and conjugation of the hydroxylated metabolites with glucuronic acid.

Other metabolic routes such as epoxidation may potentially be available to certain ionones and rose ketones, but no metabolites indicative of this pathway have been reported. It should be noted that the rose ketones, which are more likely to undergo epoxidation have not been subjected to metabolic study. For most ionones and rose ketones, the endocyclic unsaturated bond is structurally hindered by methyl substituents which likely impede epoxidation reactions at this site. Similarly, based on *in vitro* studies with two archetypal α,β-unsaturated ketones included in the chemicals under assessment, namely 4-(2,6,6-trimethylcyclohex-1-enyl)-2-buten-4-one and 1-(2,6,6-trimethylcyclohexa-1,3-dienyl)-2-buten-1-one, reactivity with glutathione, and hence the potential for electrophilic reactions with biological molecules, was concluded to be minimal (Portoghese et al., 1989). The authors concluded that these compounds exhibit low reactivity towards glutathione because the electrophilic centers are sterically hindered by directly attached substituents (methyl groups) and neighboring groups. Reactivity with other nucleophilic centers (e.g., guanine components of nucleotides) would be expected to be dramatically less than with glutathione. As a result, the metabolism of the α,β-unsaturated ketone in the side chain of the rose ketones is not expected to produce reactive intermediates of greater toxicity than similar metabolism of the more sterically hindered α,β-unsaturated ketone side chain of the ''ionone'' series.

Three rose ketones (*trans*,*trans*-δ-damascones; δ-damascone; damascone) as well as dehydrodihydroionone, have an additional and unhindered double bond in the cyclic ring structure that could provide a potential site for epoxidation to occur. Similarly, for methyl-δ-ionone, the cyclohexene ring contains a point of unsaturation less hindered by the presence of methyl groups, possibly increasing the likelihood of epoxidation. Epoxidation of these specific chemicals could produce products with higher reactivities/toxicities than other members of this class.

In summary, empirical metabolic data on ionone isomers demonstrate the activity of various metabolic pathways leading to polar metabolites, both in free and conjugated forms. The primary differences in the chemical structure of members of this class of compounds that could affect metabolism, and potentially the toxicity of metabolites, are the position of the double bond in the allylic side chain (ionones *versus* rose ketones) and the potential for epoxidation depending upon the number and position of the double bonds in the cyclohexene ring. Since the allylic side chain of the rose ketones does not appear to have strong electrophilic activity, based on *in vitro* data (Portoghese et al., 1989), the damascone metabolites are unlikely to be of greater toxicity than those of the ionones. However, based on metabolic considerations, unique epoxide metabolites could be generated for each of *trans*,*trans*-δ-damascones; δ-damascone; 1-(2,6,6-trimethyl-3-cyclohexa-1-e-dienyl)-2-buten-1-one); dehydrodihydroionone, and methyl-δ-ionone. Thus, these compounds may have greater toxic potential than other members of this class.

The most complete *in vivo* metabolic data are from animal studies; there are no human data for these compounds. The most extensive data are for β-ionone with a limited amount of data for the α isomer; the metabolic data available can be viewed as being representative for the class as a whole. Following administration of β-ionone to a male rabbit (oral gavage, 1 g/day for seven days), Ide and Toki (1970) isolated from the urine and characterized the following transformation products (numbered on the CAS system): 3-oxo-β-ionone, 3-oxo-β-ionol, dihydro-3-oxo-β-ionol and 3-hydroxy-β-ionol together with the glucuronides of 3-oxo-β-ionol and dihydro-3-oxo-β-ionol. Only a small amount of unchanged β-ionone (circa 1% of dose) was recovered from the urine of the dosed animal.

In an earlier study, Bielig and Hayasida (1940) isolated β-ionol and dihydro-β-ionol as reduction products from the urine of dogs fed β-ionone; three additional hydroxylated metabolites were detected but not characterized. Prelog and Meier (1950) confirmed these findings and identified 3-oxo-β-ionol and 3-hydroxy-β-ionol or 3-hydroxy-β-ionone. In the single metabolic study of α-ionone in mammals, Prelog et al. (1951) isolated a trans-

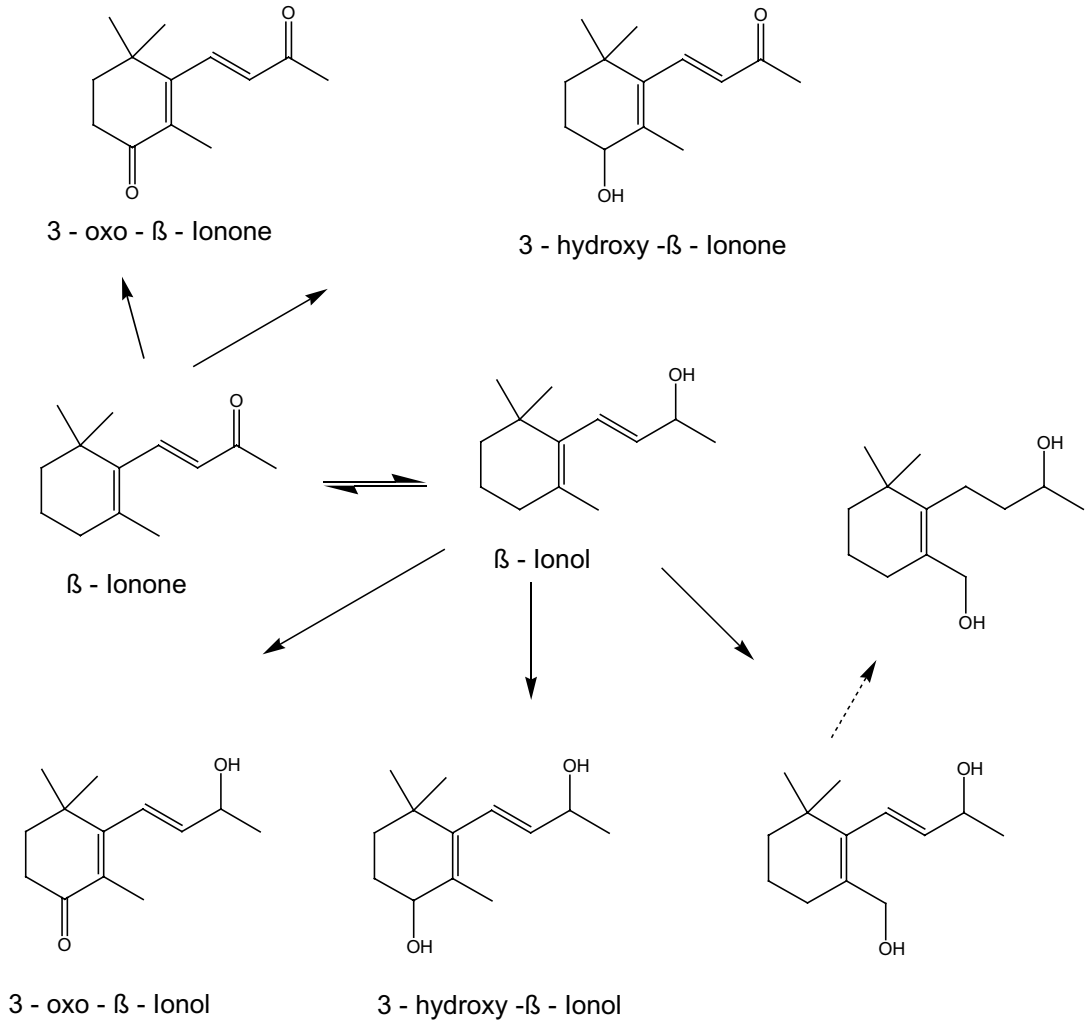


Fig. 1. Major pathways of metabolism of β-ionone in mammals.

formation product in urine of rabbits which appeared to be an oxidation product, tentatively identified as 4-oxo-tetrahydro-ionone (Fig. 1).

There is no available information on the metabolic fate of the ionones and rose ketones in humans, but one might reasonably presume that it would be similar to that seen in mammals such as the rabbit and dog, i.e., oxidative and reductive transformation followed by conjugation. Support for this view comes from the pattern of metabolism of other compounds containing the ionone structure. For example, the retinoids, such as 13-*cis*-retinoic acid (isotretinoid) contain the ionone ring structure. 13-*cis*-Retinoic acid undergoes extensive metabolism in humans by oxidation and conjugation, including oxidation of the ionone nucleus to give the 4-oxo-13-*cis*-retinoic acid metabolite (Vane et al., 1990; Kraft et al., 1991). This position of oxidation is analogous to the 3-oxo metabolites of β-ionone as numbered using the CAS system of nomenclature. In summary, the available evidence indicates that the ionones and rose ketones are extensively metabolized *in vivo* by pathways involving oxidation, reduction and conjugation. These metabolites do not raise issues of toxicological concern.

3. Toxicological studies

3.1. Acute toxicity

Overall, the acute oral and dermal toxicity of ionones is low to moderate based on the lowest reported oral LD_{50} of 1500–1800 mg/kg body weight for α-1-(2,6,6,-trimethyl-3-cyclohexen-1-yl)-2-buten-1-one (RIFM, 1979a; Piccirillo et al., 1979). Many of the ionones have oral LD_{50} values of >2000 mg/kg body weight, the normal limit dose in this assay. Acute dermal LD_{50} values exceeded 2000 mg/kg. Parenteral administrations of β-ionone and ionone (60% α- and 40% β-isomers) yielded LD_{50} values of 700 mg/kg

and 2277 mg/kg, respectively. The subcutaneous LD_{50} of ionone (60% α- and 40% β-isomers) in mice was 2605 mg/kg. Further data on the acute toxicity of ionones and rose ketones are presented in Tables 3a (oral), 3b (dermal), and 3c (other routes of exposure).

3.2. Subchronic toxicity

The results of subchronic studies with ionones are summarized in Table 4 and described below.

3.2.1. Dermal studies

Of the 30 ionones/rose ketones assessed, only α-*iso*-methyl ionone (RIFM, 1980a, 1981a) has been subjected to 90-day subchronic dermal toxicity testing (2 rat studies).

In the first study (RIFM, 1980a), Sprague–Dawley rats (15/sex/dose) were administered 50, 170, 580, or 2000 mg/kg body weight/day of neat α-*iso*-methyl ionone (no dosing vehicle) *via* clipped skin for a period of 90 days. Clinical, laboratory and gross and histopathological evaluations were conducted.

On the skin at the application site there was a dose-dependent increase in the severity of erythema, and eschar formation. Since erythema and eschar formation occurred in all treatment groups, a NOAEL for this effect could not be established.

Body weight gains were significantly reduced in females in the highest dose group and in males treated at 580 and 2000 mg/kg body weight/day. Total food consumption throughout the study was significantly increased in females treated at the 2 highest dose levels and there was a significant decrease in food efficiency and food intake in both sexes in the 2 highest dose groups. The body weight changes may not represent a direct, test-material-related effect since many of these animals manifested severe skin lesions.

There were hematological changes in the 2 highest dose groups and reduced serum glucose in the high-dose animals, all largely attributable to the inflammation and infection at the site of application.

Table 3a
Acute oral toxicity studies

Material	Species	No. of animals/ dose/group	LD_{50}[a] (mg/kg)	Reference
Allyl-α-ionone	Mice	5–10	9500	RIFM (1955)
Dihydro-α-ionone	Rats	10	>5000	RIFM (1976d)
Damascenone	Rats	5	>2000	RIFM (1986a)
α-Damascone	Rats	10 (5/sex)	1800 for males; 1500 for females; 1670 combined	RIFM (1979a)
δ-Damascone	Mice	10 (5/sex)	1821 (95% C.I. 1354–2414)	RIFM (1978f), Moran et al. (1980)
γ-Damascone	Rats	10 (5/sex)	>2000	RIFM (1987a)
trans-β-Damascone	Rats	10 (5/sex)	>2000	RIFM (1986b)
trans-β-Damascone	Rats	10 (5/sex)	2920 (95% C.I. 2655–3212)	RIFM (1969), Posternak and Vodoz (1975)
Dihydro-β-ionone	Rats	6 (3/sex)	>2000	RIFM (1999a)
Dihydromethyl-α-ionone[b]	Rats	10	>5000	RIFM (1976f)
1,3-Dimethyl-α-ionone[b]	Rats	10 (5/sex)	>5000	RIFM (1984g)
1,3-Dimethyl-α-ionone[b]	Rats	10	>5000	RIFM (1976e)
α-Ionone	Mice	10	6657 ± 652	RIFM (1967a)
α-Ionone	Mice	10	7000	RIFM, 1980i
β-Ionone	Mice	5	5331 ± 755	RIFM (1967a)
β-Ionone	Mice	10	2000	RIFM (1980i)
β-Ionone	Rats	10	3290	RIFM (1980i)
Ionone	Mice	10 (5/sex)	10,000	RIFM (1980j)
Ionone	Rats	10 (5/sex)	4590 (95% C.I. 3880–5400)	Jenner et al. (1964), Bár and Griepentrog (1967)
α-Irone	Rats	10 (5/sex)	>5000	RIFM (1972c)
α-Irone	Mice	10	7410 ± 519	RIFM (1967b)
Isodamascone	Rats	10 (5/sex)	6300	RIFM (1979s)
Iso-β-ionone[b]	Rats	10	>5000	RIFM (1980e)
α-*iso*-Methylionone	Mice	10	8714 ± 252	RIFM (1967a)
α-*iso*-Methylionone	Mice	10 (5/sex)	~10,000	RIFM (1980k)
α-*iso*-Methylionone	Rats	10	>5000	RIFM (1973)
Methyl ionone	Rats	10	>5000	RIFM (1973)
Methyl ionone	Mice	10 (5/sex)	Between 5000 and 1000	RIFM (1980l)
4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one	Rats	10	5200 (95% C.I. 3800–7200)	RIFM (1978e)

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

Table 3b
Acute dermal toxicity studies

Material	Species	No. of animals/dose/group	LD_{50}[a] (mg/kg)	Reference
Allyl α-ionone	Rabbits	6	>5000	RIFM (1971c)
Damascenone	Rabbits	6 (3/sex)	>2000	RIFM (1979aa)
α-Damascone	Rabbits	6 (3/sex)	>2000	RIFM (1979z)
α-Damascone	Rat	10 (5/sex)	2900 (95% C.I. 2164–3886)	RIFM (1979cc)
γ-Damascone	Rabbits	10 (5/sex)	>2000	RIFM (1987b)
trans-β-Damascone	Rabbits	6 (3/sex)	>2000	RIFM (1979t)
Dihydro-α-ionone	Rabbits	10	>5000	RIFM (1976d)
Dihydromethyl-α-ionone[b]	Rabbits	10	>5000	RIFM (1976f)
1,3-Dimethyl-α-ionone[b]	Rabbits	10 (5/sex)	>2000	RIFM (1984b)
1,3-Dimethyl-α-ionone[b]	Rabbits	10	>5000	RIFM (1976e)
α-Irone	Rabbits	3	>5000	RIFM (1972c)
Iso-β-ionone[b]	Rabbits	10	>5000	RIFM (1980e)
α-*iso*-Methylionone	Rabbits	8	>5000	RIFM (1973)
Methyl ionone	Rabbits	8	>5000	RIFM (1973)
4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one	Rabbits	10	>5000	RIFM (1978e)

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

Table 3c
Acute miscellaneous toxicity studies

Material	Dose route	Species	No. of animals/dose group	LD_{50}[a] (mg/kg)	Reference
Ionone	s.c. injection	Mice	10	2605 (95% C.I. 2113–3198)	Wenzel and Ross (1957)
Ionone	i.p. injection	Mice	620	2277	Sporn et al. (1963)
β-Ionone	i.p. injection	Mice	10	700	RIFM (1980i)

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

A significant increase in serum BUN was reported in males in the top 2 dose groups. Urinalysis showed a significant increase in the incidence of albuminuria in males in the 3 highest dose groups. In the high-dose males, abundant eosinophilic globules were observed in the kidney epithelium at necropsy.

At necropsy there was a significant increase in the absolute and relative liver weights in both sexes at all dose levels. Increases, most of which attained statistical significance, in the absolute and relative weights of the kidneys were reported in all but the lowest dose groups of each sex. The absolute adrenal weights were significantly increased in the 2 highest dose groups of both sexes.

The interpretation of the data is complicated by the severe skin damage at the application site, especially in the 2 highest dose groups. Depressed body weight gains and increased neutrophil count are probably attributable to infection and inflammation. Azotemia and proteinuria likely are a result of chronic severe tissue damage and infection. The liver weight increase probably resulted from induction of microsomal mixed-function oxidases. Increased adrenal weights probably reflect the response to stress caused by tissue damage and infection.

Severe tissue destruction and infection in the skin may have combined to elicit increased kidney weight at higher doses and epithelial eosinophilic globules in the convoluted tubules of the outer cortex. To determine if these effects were specific to male rat nephropathy, a review of the histopathology of kidneys from rats in this study was conducted. This lesion occurred in a dose-responsive fashion in males only and was seen also in male control rats. It was accompanied by interstitial nephritis in control and treated rats. The findings suggest an endogenous disease process which was exacerbated by the application of the irritating test material and marked skin necrosis. On the basis of the review of the kidney histopathology data and considering the dermal inflammation and infection in these animals, the results of this study are concluded to show a systemic NOAEL of topical α-*iso*-methyl ionone of 50 mg/kg (RIFM, 1980a).

In a subsequent 90-day study (RIFM, 1981a), α-*iso*-methyl ionone was applied dermally daily to groups of 5 male and 5 female Sprague–Dawley rats at a daily dose of 10 mg/kg as a 1% solution in phenethyl alcohol (PEA) (RIFM, 1981a). No dermal reaction to treatment was noted at any time during the study. The hematology, clinical chemistry, and urinalysis parameters evaluated were comparable to the controls. A slight, but significant increase in serum alkaline phosphatase activity was reported in males. The relationship of this finding to treatment was considered questionable. There was no evidence of a treatment-related effect on body weight gain, necropsy observations, organ weights, or on the results of the microscopic examination. As a result, the NOAEL for the skin appeared to be 10 mg/kg, the only dose tested, but the inclusion of only 5 animals per sex and of only one dose precludes statistical analyses of the data. The lack of dermal reactions in this study (RIFM, 1981a) contrasts to

Table 4
Subchronic toxicity studies

Material	Method	Dose[a]	Species	Results	Reference
trans-β-Damascone	Oral (diet) 90-day toxicity study	2.26 mg/kg	16 CF/Gif rats sex/dose	No adverse toxic effects	RIFM (1969), Posternak and Vodoz (1975)
α-Ionone	Oral (diet) 90-day study	10 and 100 mg/kg/day	Sprague–Dawley rats (15/sex/dose)	10 mg/kg: Increase liver weight, decrease erythrocyte and packed cell volume 100 mg/kg: Reduced weight gain, food consumption and serum glucose concentration, increased water intake, mild renal changes; increase in neutrophil and decrease lymphocytes Increased hepatic p450 content and activity of drug metabolizing enzymes. Increased liver weight most likely resulted from enzyme induction	RIFM (1983a)
α-Ionone	Oral (diet) 90-day study	Males 11.8 mg/kg, females 11.1 mg/kg	15 FDRL rats sex/dose	No adverse toxic effects	Oser et al. (1965)
α-Ionone	Oral (diet) 90-day study	10.6 mg/kg	Unspecified number of rats	No adverse toxic effects	Bár and Griepentrog (1967)
β-Ionone	Oral (diet) 90-day study	10 and 100 mg/kg	60 Sprague–Dawley rats (15/sex/dose)	Higher relative liver weights in male; relative brain, liver, kidney and serum weights were also significantly higher in females. Males exhibited a significant decrease in serum alkaline phosphate activity and females exhibited a significant increase and decrease in serum urea and glucose concentration, respectively	RIFM (1983a)
β-Ionone	Oral (diet) 90-day study	Males 11.6 mg/kg, females 13.1 mg/kg	15 FDRL rats sex/dose	No adverse toxic effects	Oser et al. (1965)
β-Ionone	Oral (gavage) 12 weeks study	11.4 mg/kg	Rats	No adverse toxic effects	Bár and Griepentrog (1967)
Ionone (mixed isomers)	Oral (diet) 8 weeks study	10 mg on alternate days	32 young white rats/8/group	No adverse toxic effects	Sporn and Dinu (1964)
Ionone (mixture of 60% α, 40% β)	Oral (diet) 17 weeks study	50, 125 and 500 mg/kg/day	Osborne–Mendel rats 10/sex	1000 ppm: Very slight swelling of parenchymal cells 2500 ppm: Slight swelling of parenchymal cells 10,000 ppm: Moderate swelling of parenchymal cells	Hagan et al. (1967), Bár and Griepentrog (1967)
Ionone (mixed isomers)	Oral (diet) 8 weeks study	10 mg on alternate days	56 white rats	Significant increase of deoxyribonucleic acid content and a significant decrease of aspartate-glutamic transaminase	Sporn and Dinu (1964)
Ionone (mixed isomers)	Oral (diet) 7 weeks study	3 mg in oil every 2nd day for 7 weeks	72 young white rats. 10 rats/group	No adverse toxic effects	Sporn et al. (1963)
α-Irone	Oral (diet) 90-day study	5.2 and 5.9 mg/kg/day	FDRL strain rats (15/sex)	No adverse toxic effects in males Females exhibited increase: food consumption, hematocirt hemoglobin, lymphocytes	Oser et al. (1965)
α-*iso*-Methylionone	Oral (gavage) 90-day study	5, 30 and 500 mg/kg/day	Crl:CD (SD) IGS BR rats (10/sex/dose)	NOAEL of 30 mg/kg	RIFM (2006a)
α-*iso*-Methylionone	Oral (gavage) 90-day study	3.4 mg/kg	Rats	No adverse toxic effects	Bár and Griepentrog (1967), Oser et al. (1965)
α-*iso*-Methylionone	Dermal 90-day toxicity study	10 mg/kg (1%) in phenethyl alcohol	10 Sprague–Dawley albino rats (5/sex)	A slight increase in alkaline phosphatase values in males, but the relationship to treatment was questionable. Small group sizes limit the interpretation of the study	RIFM (1981a)

(*continued on next page*)

Table 4 (*continued*)

Material	Method	Dose[a]	Species	Results	Reference
α-*iso*-Methylionone	Dermal 90-day toxicity study	50, 170, 580 and 2000 mg/kg	15 Sprague–Dawley rats/sex/dose	50 mg/kg: Dose related increase in liver weight and changes in urinalysis parameters at this dose 170 mg/kg: Changes in hematology parameters in both sexes. BUN levels increased with dose in males. Urine albumin levels were significantly increased in male groups at termination. Increases in the absolute and relative weights in the liver and kidneys in both sexes 580 and 2000 mg/kg: Reduced body weight gain in females and in males. Food consumption elevations in females, lower efficiency food utilization in both male and females. Serum glucose levels were depressed in males at week 7 and in both sexes at termination. BUN levels increased with dose in males. Urine albumin levels were significantly increased in male groups at termination. Increases in the absolute and relative weights in the liver and kidneys in both sexes Moderate to severe erythema and eschar formation was observed in all test groups and increased with increasing levels of test material. Severe tissue destruction and infection on doses above 50 mg/kg may have combined to elicit increased kidney weight at higher doses	RIFM (1980a)

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

the severe skin effects in the earlier study (RIFM, 1980a) as expected with the application of a far lower and less concentrated solution (1% in PEA).

It is concluded that there are currently no entirely adequate studies available to assess the subchronic dermal toxicity of any individual ionones/rose ketones or the group as a whole. Of those dermal studies available, a systemic NOAEL from the RIFM (1980a) study was established at 50 mg/kg body weight/day.

3.2.2. Oral studies

A 90-day repeated dose oral subchronic toxicity study was conducted with α-*iso*-methylionone. Ten rats/sex/dose were gavaged once daily for 90 days with α-*iso*-methylionone at dosages of 0, 5, 30, and 500 mg/kg/day. There were no unscheduled deaths, treatment-related changes in behavioral or functional performance parameters or in sensory reactivity. There were no adverse effects on bodyweight, food consumption, or water consumption. There were no treatment-related ocular or hematological changes observed. No macroscopic abnormalities were detected at necropsy. At the high dose only, increased salivation, red/brown stained fur, episodes of noisy respiration and hunched posture were evident in a number of animals throughout the treatment period. Blood chemistry analyses showed statistically significant increases in total protein, albumin and cholesterol. Increase in absolute and relative liver and kidney weights and histopathological changes in liver, kidneys, thyroid and bone marrow was observed. Due to the histopathological observations in the high-dose rats, examination of sections of liver, kidneys, thyroid, and bone marrow from all animals in the low and intermediate dose groups was performed and showed liver hepatocyte enlargement in animals treated with 500 mg/kg/day, globular accumulation of eosinophilic material in kidney tubular epithelium of males treated with 30 and 500 mg/kg/day, higher incidence of follicular cell hypertrophy in thyroid and adipose infiltration of the bone marrow in males pretreated with 500 mg/kg/day. The NOEL was established to be 30 mg/kg/day for females and 5 mg/kg/day for males. The kidney changes identified histopathologically were consistent with well documented changes that are peculiar to the male rat in response to treatment with some hydrocarbons, therefore, for the purposes of hazard evaluation the NOAEL for males, was established as 30 mg/kg/day (RIFM, 2006a).

Subchronic oral toxicity studies have been conducted on α- and β-ionone (Oser et al., 1965; RIFM, 1983a; Bár and Griepentrog, 1967), ionones (mixed isomers) (Sporn et al., 1963; Sporn and Dinu, 1964; Hagan et al., 1967; Bár and Griepentrog, 1967), α-*iso*-methylionone (Oser et al., 1965; Bár and Griepentrog, 1967), α-irone (Oser et al., 1965), and β-damascone (RIFM, 1969). Oser et al. (1965) tested

a number of chemicals with relatively few details reported of the methods used and results obtained. However, this study is useful since several ionones were tested allowing for a comparison, albeit limited in detail, of the toxicity of various members of this class of compounds. The oral studies reported in RIFM (1983a) on α- and β-ionone and in RIFM (1969) on β-damascone are considered to have utilized the appropriate study design and protocols and to have reported the results to an extent to allow independent evaluation of the data. There are no oral subchronic studies available on the 3 rose ketones [*trans*, *trans*-δ-damascone, δ-damascone; damascone and 2 ionones (dehydrodihydroionone and methyl-δ-ionone)].

In the Oser et al. (1965) study, toxicological tests were conducted on several ionones. Rats of the FDRL strain were fed diets containing, in cottonseed oil, α-ionone (11.8 and 11.1 mg/kg/day in males and females, respectively), β-ionone (11.6 and 13.1 mg/kg body weight/day in males and females, respectively), α-*iso*-methylionone (3.6 and 4.1 mg/kg body weight/day in males and females, respectively), or α-irone (5.2 and 5.9 mg/kg body weight/day in males and females, respectively) for a period of 90 days. There were no adverse effects on body weight gain and food consumption for any of the 4 ionones tested. For α-ionone and β-ionone, no effects were observed in measured hematology and blood chemistry parameters. Male rats receiving α-*iso*-methylionone had a slightly reduced (more than 2 standard deviations, no other statistical data cited) hemoglobin level. However, the hematocrit and erythrocyte counts were within the control ranges. This group also had a mean BUN level below (more than 2 standard deviations, no other statistical data cited) that of the controls. No evidence of adverse toxic effects was observed in the males treated with α-irone. Females treated with α-irone exhibited an increased efficiency of food utilization (13.7 g body weight gain/100 g food eaten) as compared to controls (13.0). Females were also reported to have a slightly increased (more than 2 standard deviations, no other statistical data cited) hematocrit, hemoglobin, and lymphocyte count. Liver and kidney weights were not affected by any of the 4 ionones tested, and there was no adverse effect on the gross or microscopic appearance of major organs at necropsy. The NOAEL values for this study were identified as 11.1 mg/kg α-ionone, 11.6 mg/kg β-ionone, 3.6 mg/kg α-*iso*-methylionone, and 5.2 mg/kg α-irone body weight/day. In all cases, the NOAEL values represent the only dose tested and are the lower of the doses reported (for males or females). These data, while limited in scope, indicate that at these low oral doses, the ionones are non-toxic and well tolerated and that, at least within the ionone series, major differences in toxicity are not expected.

Hagan et al. (1967) administered ionone (60% α- and 40% β-isomers) to groups of 10 male and 10 female Osborne–Mendel rats at dietary concentrations of 1000, 2500, or 10,000 ppm for 17 weeks, equivalent to approximately 50, 125, and 500 mg/kg body weight/day. There were no reported effects on body weight gain, clinical signs, or on any of the measured hematological parameters. At necropsy, no macroscopic changes were observed. Histopathology examination of the liver, the only organ analyzed revealed slight to moderate swelling of hepatocytes in high-dose animals, slight swelling in mid-dose animals, and very slight swelling of hepatocytes in the low-dose group. Due to the limited reporting of the methodology and the results, the study is difficult to assess in terms of establishing a NOAEL value. The hepatocellular swelling is presumably related to microsomal enzyme induction and not an "adverse" effect.

α-Ionone and β-ionone were tested separately in groups of 15 male and female Sprague–Dawley rats *via* dietary administration to provide daily doses of 10 or 100 mg/kg body weight for a period of 90 days (RIFM, 1983a). There were no mortalities, and no abnormal clinical signs in rats treated with either α- or β-ionone. No significant effects on mean body weights occurred in treated males. Sporadic, statistically significant, lower body weights were reported at various times in the last 6 weeks of the study in females treated with α-ionone, 100 mg/kg body weight/day. Food intake of both sexes treated with either α- or β-ionone, 100 mg/kg body weight/day, was significantly reduced, with the effect greater in females. It is possible that the lower mean body weights and reduced food consumption at the high dose may have been related to an unpalatable diet.

At 6 weeks, erythrocyte counts and packed cell volumes showed a significant decrease in males treated with 100 mg β-ionone/kg body weight/day, and decreased erythrocyte counts were reported in 10 mg/kg body weight/day α-ionone treated males. Males given 100 mg α-ionone/kg body weight/day showed a slight, but significant, increase in neutrophil counts and a decrease in lymphocytes. No effects of α- or β-ionone, at either 10 or 100 mg/kg body weight/day, were reported on hematological parameters in females. No hematological changes were observed in either sex after 13 weeks of treatment. The earlier abnormalities were considered to be of no toxicological significance.

The serum chemistry analyses were normal except for significantly lower alkaline phosphatase values in males and lower glucose concentrations in females dosed with 100 mg of either α- or β-ionone/kg body weight/day. Given the small magnitude of these changes, they were considered to be of no biological significance.

In high-dose animals treated with either α- or β-ionone, mild changes in urinary parameters were considered not to be of biological significance. Relative kidney weights were significantly increased in males but not females treated with the high dose of α-ionone. Males treated with the β-isomer (100 mg/kg body weight/day) showed significantly increased absolute and relative liver weights and females showed increased relative liver and brain weights at the high dose of α- and β-ionone. Relative liver weights were significantly increased also in females given 10 mg/kg body weight/day α-ionone.

Necropsy revealed no effect of treatment on gross or microscopic pathology except for statistically significant "desquamation" of the thyroid epithelium in 3 females treated with 100 mg/kg body weight/day of α-ionone. There were no gross or histopathological correlates to increased absolute and/or relative kidney and/or liver weights. As a result, the only biologically significant finding at the low dose of either ionone was an increase in relative liver weight in females treated with α-ionone, a finding likely associated with enzyme induction. Thus, 10 mg/kg body weight/day is considered to be a NOAEL value for both α- and β-ionone.

One of the rose ketones, β-damascone, has been tested in a study in which groups of 16 male and female CF/Gif rats were administered the compound in feed at a dose of 2.26 mg/kg body weight/day for a period of 13 weeks (RIFM, 1969). There were no mortalities and no abnormal clinical signs during the course of the study. Feed consumption increased for both males (+5.8%) and females (+9.5%), with the increase statistically significant in females. There was, therefore, a moderate decrease in feed efficiency in both sexes (−9.04% in the males and −9.60% in the females). The study authors ascribed no toxicological significance to the feed consumption and utilization data.

The absolute weights of the liver and kidneys in females were increased (7.8%, respectively), and relative weights of the liver and kidneys were reported to be significantly increased in both sexes. There was no effect of treatment on gross or histopathological appearance of tissues including the liver and kidney. The authors considered the test article to be well tolerated and not to produce any changes of toxicological significance. Given the lack of histopathological changes in the liver and kidneys and the relatively minor increases in relative weights of these organs and decreases in food utilization efficiency, the single dose of 2.26 mg/kg body weight/day in this 13 week study is considered to approximate a NOAEL value.

3.2.3. Summary of subchronic toxicity studies

Dermal subchronic toxicity studies have been conducted on α-*iso*-methyl ionone (RIFM, 1980a, 1981a), and several subchronic oral toxicity studies have been conducted on certain of the ionones (Sporn et al., 1963; Sporn and Dinu, 1964; Oser et al., 1965; Bár and Griepentrog, 1967; RIFM, 1983a) and one of the rose ketones (RIFM, 1969). Not one material has been subject to both subchronic oral and subchronic dermal toxicity testing. The 90-day dermal toxicity study (the most appropriate route) of α-*iso*-methyl ionone (RIFM, 1980a) was significantly compromised by severe effects of the test chemical on the skin. A systemic NOAEL of 50 mg/kg body weight/day was identified (given the likelihood that many of the systemic "effects" observed were secondary to infection/inflammation associated with the severe necrosis and ulceration of the skin). In the second, limited dermal study, of α-*iso*-methyl ionone (RIFM, 1981a), the NOAEL was 10 mg/kg, the only dose tested. It is concluded that there are currently no adequate studies available to assess the subchronic dermal toxicity of any individual ionones/rose ketones or the group as a whole; the available data do not indicate a high order of toxicity in the absence of severe effects on the skin.

The oral subchronic toxicity studies, of which one performed on α- and β-ionone (RIFM, 1983a) and another on the β-damascone (RIFM, 1969) and a more recent one on α-*iso*-methylionone (RIFM, 2006a), are considered the most useful to characterize the toxicity of this group of chemicals. They demonstrate a low order of toxicity. In each of these studies, the most notable findings were of modestly, but significantly, increased absolute and/or relative liver weights. Since this group of chemicals is known to induce microsomal enzymes, an effect well established to be associated with generalized increases in liver weight, and noting the absence of histological effects on the liver in these studies, these findings are likely of minimal toxicological significance. In the absence of other evidence of overt toxicity, 10 mg/kg body weight/day is considered to represent the NOAEL for α- and β-ionone (RIFM, 1983a), and 2.26 mg/kg body weight/day (the only dose tested) is considered a provisional NOAEL value for β-damascone (RIFM, 1969) and 30 mg/kg/body weight/day is the NOAEL for α-*iso*-methylionone (RIFM, 2006a). Similar results were obtained by Oser et al. (1965) for α- and β-ionone. No toxicity was also observed for α-*iso*-methylionone at 3.6 mg//kg body weight and for α-irone at 5.2 mg/kg body weight (Oser et al., 1965).

From the available data, there do not appear to be large differences in the toxicity of the ionones by the oral route of exposure. Comparison with dermal exposure is hindered by the lack of appropriate comparative studies and by direct dermal toxicity. There are no dermal or subchronic oral toxicity studies available on the ionones and rose ketones that may be subject to metabolism by epoxidation and, hence, have a higher potential for the generation of toxic metabolites. In conclusion, keeping in mind the inadequacies of the studies available, a dermal NOAEL value of 10 mg/kg body weight (RIFM, 1981a) and a systemic NOAEL of 50 mg/kg body weight/day associated with dermal exposure (RIFM, 1980a) and a systemic NOAEL of 30 mg/kg body weight/day associated with oral exposure can be used for quantitative human health risk assessment of the use of the ionones as fragrance compounds.

3.3. Chronic toxicity

No chronic toxicity data are available on any of the 36 ionones evaluated.

3.4. Mutagenicity and genotoxicity

Of the 30 ionones, several have been tested in various *in vitro* and *in vivo* test systems. In a number of studies, results for a large number of compounds have been insufficiently described so that interpretation of the data is

difficult. Studies that did not report the concentration/dose of the test material are not included in this safety assessment. Results of those studies that provide sufficient details for evaluation are summarized in Tables 5–7 and are described below.

3.4.1. Bacterial studies

In the Ames assay using *Salmonella typhimurium* with and without metabolic activation, allyl-α-ionone (Wild et al., 1983), dihydro-β-ionone (RIFM, 2000a), 4-(1,2-epoxy-2,6,6-trimethylcyclohexyl)-3-buten-2-one (RIFM, 1988a) α-ionone (Kasamaki et al., 1982), β-ionone (Florin et al., 1980; Mortelmans et al., 1986), ionone mixed isomers (RIFM, 1980b, 2004a), α-*iso*-methyl ionone (RIFM, 1980c), methyl ionone (RIFM, 1999b), methyl-α-ionone (Wild et al., 1983), methyl-δ-ionone (Wild et al., 1983), and methyl ionone mixed isomers (RIFM, 1980d) have all been reported to be without mutagenic activity. Similarly, the rose ketones damascone and α-damascone did not show any mutagenic activity in the bacterial reverse mutation test with *S. typhimurium* and *Escherichia coli* WP2 *uvrA* (RIFM, 2000b, 2003).

Only in a non-standard Ames test in which the TA1535 strain contained a plasmid carrying the fused gene *umuC'-lac2*, was one of the ionones (β-ionone) reported to have mutagenic activity (Ono et al., 1991). The utility of this assay for predicting mutagenic potential is questionable given that a number of chemicals considered "non-mutagenic" were also reportedly positive in this assay. Ionone was reported to be marginally genotoxic in the Rec assay (Yoo, 1986).

Overall, the ionones and rose ketones are concluded to be without mutagenic potential in bacteria in *in vitro* studies.

3.4.2. Mammalian studies

Clastogenic potential of methyl ionone was tested in a chromosome aberration assay using Chinese hamster ovary (CHO) cells. At the highest dose of 50 μg/ml, the cell growth inhibition was about 60–68% in all treatment groups. Methyl ionone did not produce any significant structural or numerical chromosome aberrations after 4-h treatment without S9, but in the presence of S9 there was an increase in structural chromosome aberrations at the highest dose tested of 50 μg/ml. However, since the increase in the percentage of structurally aberrant cells was within the range of historical control values, the chromosome aberrations observed in the presence of S9 after 4-h treatment with methyl ionone were not considered biologically significant. Structural chromosome aberrations were reported after 20-h exposure at concentrations of 12.5 and 25 μg/ml in the absence of S9. There were no increases in numerical chromosome aberrations in this group. Based on these results, it was concluded that methyl ionone was positive in the absence of S9 and negative in the presence of S9 for the induction of structural chromosome aberrations in CHO cells. Methyl ionone was negative in both the absence and presence of S9 for the induction of numerical chromosome aberrations in CHO cells (RIFM, 2000c).

In order to evaluate the biological significance of the positive *in vitro* chromosome aberration assay, an erythrocyte micronucleus test was performed with methyl ionone in mice. After a preceding toxicity test, groups of 5 male and female mice were dosed with 462.5, 925, or 1850 mg methyl ionone/kg body weight by a single intraperitoneal injection. In male mice, 24 h after treatment with 925 mg/kg, a significant increase in micronucleated polychromatic erythrocytes (0.8 ± 0.45/1000 polychromatic erythrocytes (PCE), mean ± SD) relative to the control values (0.1 ± 0.22/1000) was observed. No such effect was reported in females. No effects on the incidence of micronucleated PCE were reported in either sex at the highest dose tested of 1850 mg/kg body weight. The historical control value for micronucleated PCEs in the performing laboratory during 1996–1998 was 0.65 ± 0.76 in males and 0.7 ± 0.80 in females, with a range of between 0 and 7/1000 cells in both genders. Considering the historical controls, the lack of a dose–response of the effects, and the negative data after 48 h at the highest dose tested, it is concluded that methyl ionone is negative in the micronucleus test (RIFM, 2000d).

A mouse micronucleus test was also performed with α-ionone in order to evaluate the biological significance of the positive *in vitro* chromosome aberration assay. α-Ionone, at doses of 300, 600, or 1200 mg/kg in corn oil was administered by intraperitoneal injection to male and female ICR mice (5/sex/dose). Reductions (up to 21%) in the ratio of polychromatic erythrocytes to total erythrocytes were observed in some of the α-ionone treated groups relative to the respective vehicle controls. These reductions suggest the bioavailability of α-ionone to the bone marrow. There were no statistically significant increases in the incidence of micronucleated polychromatic erythrocytes in α-ionone treated groups relative to their respective vehicle control in either male or female mice, regardless of dose level or bone marrow collection time. α-Ionone was concluded to be negative in the mouse micronucleus assay (RIFM, 2006b).

In an older mouse micronucleus assay, Wild et al. (1983) tested allyl-α-ionone (2 doses of 464, 696, or 928 mg/kg by i.p. injection) and methyl-α-ionone (single dose of 825, 1444, or 2063 mg/kg by i.p. injection). These two ionones did not show evidence of genotoxic activity in this assay.

Based on the foregoing data, it is concluded that the ionones tested, including α-ionone (Kasamaki et al., 1982) and methyl ionone (RIFM, 2000c), may have weak clastogenic activity in mammalian cells *in vitro*. However, these responses do not appear to be translated to *in vivo* exposures, based on results from mouse micronucleus assays (Wild et al., 1983; RIFM, 2000d).

Table 5
Mutagenicity and genotoxicity: bacterial studies

Material	Test system	Species	Concentration	Results	Reference
Allyl α-ionone	Ames with and without S9 activation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3600 μg/plate	Negative	Wild et al. (1983)
Damascenone	Bacterial reverse mutation assay with and without S9 activation using plate incorporation method	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537 and *E. coli* WP2 uvrA	Up to 5000 μg/plate	Negative	RIFM (2000b)
α-Damascone	Ames with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535 and TA1537 and *E. coli* WP2uvrA	Up to 125 μg/plate for TA98 and TA1537, 250 μg/plate for TA1535 and TA100, and 5000 μg/plate for *E. coli*	Negative	RIFM (2003)
γ-Damascone	Ames with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	Up to 5000 μg/plate	Negative	RIFM (1986c)
Dihydro-β-ionone	Pre-incubation assay with S9 activation	*S. typhimurium* TA102	Up to 1000 μg/plate	Negative	RIFM (2000a)
Dihydro-β-ionone	Direct incorporation test with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	Up to 1000 μg/plate	Negative	RIFM (2000a)
4-(1,2-Epoxy-2,6, 6-trimethylcyclohexyl)-3-buten-2-one	Ames with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535, TA1537	5–500 μg/plate	Negative	RIFM (1988a)
α-Ionone	Rec-assay	*Bacillus subtilis* in strains H17 (rec+) and M45 (rec−)	19 μg/disk	Negative	Oda et al. (1978)
α-Ionone	Ames with and without S9 activation	*S. typhimurium* TA98 and TA100	0.01–50 μg/plate	Negative	Kasamaki et al. (1982)
β-Ionone	Ames with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 μM/plate	Negative	Florin et al. (1980)
β-Ionone	Ames test preincubation assay with and without S9 activation	*S. typhimurium* TA1535, TA98, TA100 and in either TA97 or TA1537	1–180 μg/plate	Negative	Mortelmans et al. (1986)
Ionone	Ames test with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0.001–1 μl/plate	Negative	RIFM (1980b)
Ionone	Reverse mutation assay	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	Up to 5000 μg/plate	Negative	RIFM (2004a)
Ionone	Umu-test	*S. typhimurium* TA1535/ pSK1002	100 μg/ml	Positive	Ono et al. (1991)
Ionone	Spore plate rec-assay	*B. subtilis* H17 & M45	20 μl/ plate	Positive	Yoo (1986)
Ionone	Antimutagenic test	*E. coli* WP2 uvrA (trp-)	10–40 mg/ml	Negative	Yoo (1986)
Ionone	Mutation test	*E. coli* WP2 uvrA (trp-)	2.5–20.0 mg/plate	Negative	Yoo (1986)
Methyl-α-ionone	Ames with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538,	3600 μg/plate	Negative	Wild et al. (1983)
Methyl-δ-ionone[a]	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Up to 3600 μg/plate	Negative	Wild et al. (1983)
α-*iso*-Methylionone	Ames test with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	9.3–9300 μl/plate	Negative	RIFM (1980c)
Methyl ionone	Bacterial reverse mutation assay with and without S9 using the plate incorporation method	*S. typhimurium* TA98, TA100, TA1535 and TA1537 and *E. coli* WP2uvrA	Up to 5000 μg/plate	Negative	RIFM (1999b)
Methyl ionone	Ames test with and without S9 activation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	Up to 10 μg/plate	Negative	RIFM (1980d)

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

3.4.3. Summary of genotoxicity data

The mutagenicity and genotoxicity data are summarized in Tables 5 and 6. In the standard *Salmonella*/microsome assays, as well as in *E. coli* mutation assays, the different ionones tested were negative. Only in a non-standard Ames test in which the TA1535 was used, β-ionone reported to have mutagenic activity. The utility of this assay for predicting mutagenic potential is

Table 6
Mutagenicity and genotoxicity: mammalian studies

Material	Test system	Species	Dose or concentration	Results	Reference
Allyl α-ionone	*In vivo* micronucleus test	Male and female NMRI mice	464, 696, and 928 mg/kg	Negative	Wild et al. (1983)
α-Ionone	*In vitro* chromosome aberration assay	CH cell line B241	25 mM	Positive	Kasamaki et al. (1982)
α-Ionone	*In vivo* micronucleus test	Male and female ICR mice	300, 600, or 1200 mg/kg by i.p. injection	Negative	RIFM (2006b)
Methyl-α-ionone	*In vivo* micronucleus test	male and female NMRI mice	825, 1444, and 2063 mg/kg	Negative	Wild et al. (1983)
Methyl ionone	*In vitro* chromosome aberration assay with and with out S9 activation	Chinese Hamster Ovary (CHO)	12.5–175 μg/ ml	Positive in the absence of S9 and negative in the presence of S9 for the induction of structural chromosome aberrations; negative with and without S9 for induction of numerical chromosome aberrations	RIFM (2000c)
Methyl ionone	*In vivo* micronucleus test	Male and female ICR mice	462.5, 925, or 1850 mg/kg by i.p. injection	Negative	RIFM (2000d)

Table 6a
Mutagenicity and genotoxicity: insect studies

Material	Test system	Species	Dose or concentration (mM)	Results	Reference
Allyl-α-ionone	Basc test	*Drosophila melanogaster*	25	Negative	Wild et al. (1983)
Methyl-α-ionone	Basc test	*Drosophila melanogaster*	20	Negative	Wild et al. (1983)

Table 7
Carcinogenicity studies

Material	Method	Dose[a]	Species	Results	Reference
β-Ionone	Tumor inhibiting or tumor enhancing activity in a tumor promoting system of 7,12-dimethyl benz[*a*]anthracene (DMBA). Mice were initiated once with 0.125 mg DMBA in 0.25 ml acetone applied to their backs. Three weeks later the mice received application of 0.25 ml of 0.04% β-ionone in acetone or application of a mixture of β-ionone and 0.006% croton resin five times weekly for 18 weeks	~3 mg/kg body weight	30 ICR Swiss mice	No evidence of carcinogenic activity	Shamberger (1974)
β-Ionone	Chemo preventive activity of β-ionone against DMBA mammary tumor genesis was examined by administering a diet containing 36 mmol β-ionone/ kg to rats for 2 weeks prior to and following treatment with DMBA at 35 mg/kg body weight	~350 mg/kg body weight/day	27 female Sprague–Dawley rats	No evidence of carcinogenic activity	Yu et al. (1993, 1995)
β-Ionone	Following implantation of B16 melanoma cells, animals were palpated for the presence of tumors and initiated on dietary treatment with β-ionone following detection of tumors	~50 mg/kg body weight/day	12 C57 Bl female mice	No evidence of carcinogenic activity	He et al. (1997)
α-Irone	Mice received intraperitoneal injections of α-irone in re-distilled tricaprylin 3 times weekly for 8 weeks. Twenty-four weeks after the first injection, animals were sacrificed	1950 and 9600 mg/ kg bodyweight	A/He mice (15/sex/dose)	No evidence of carcinogenic activity	Stoner et al. (1973)

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

questionable given that a number of chemicals considered "non-mutagenic" were also reportedly positive in this assay. Ionone was reported to be marginally genotoxic in the Rec assay.

In *in vitro* chromosome aberration tests, methyl ionone increases structural aberrations when incubated with CHO cells in the absence of S9 for 20 h. After 4 h incubation, methyl ionone was negative with and without S9.

In the *in vivo* mouse micronucleus test single intraperitoneal doses of methyl ionone produced equivocal effects which, following careful evaluation, are not considered to represent genotoxic activity of methyl ionone. In all cases in this mouse micronucleus assay, there was no apparent dose–response relationship; absolute incidence rates for structural chromosome aberrations were within historical control values; the positive controls produced much higher frequencies of structural and numerical aberrations, and, at the highest dose tested, there were no differences in the incidence of either structural or numerical aberrations between treated groups (both sexes) and controls. In addition, a recent mouse micronucleus test with α-ionone at doses as high as 1200 mg/kg did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in either male or female ICR mice and it was concluded that α-ionone was negative in the mouse micronucleus assay. Also, another mouse micronucleus assay previously reported in the literature (Wild et al., 1983) failed to detect any indication of a genotoxic effect of either methyl-α-ionone or allyl-α-ionone. Given the foregoing, it is concluded that the ionones do not possess significant *in vivo* mutagenic or genotoxic potential under intended conditions of use as fragrance ingredients.

3.5. Carcinogenicity

No standard 2-year rodent bioassays investigating the carcinogenic potential of any of the 36 ionones are available. One dermal tumor promotion study of β-ionone is available (Shamberger, 1974). There are 2 oral studies conducted on β-ionone to assess its potential to inhibit tumor formation and/or growth (Yu et al., 1995; He et al., 1997). Details of these and other studies are provided in Table 7 and in the following sections.

3.5.1. Tumor initiation and promotion studies

β-Ionone has been tested in a tumor promoting system in ICR Swiss mice initiated once with 0.125 mg 7,12-dimethylbenz[*a*]anthracene (DMBA) (about 4 mg/kg body weight) in 0.25 ml acetone applied to their backs (Shamberger, 1974). After 3 weeks the mice received applications of 0.25 ml of 0.04% β-ionone in acetone (about 3 mg/kg body weight) or of a mixture of β-ionone and 0.006% croton resin five times weekly for 18 weeks. A group given DMBA and tumor promoter (croton resin) served as positive control. β-Ionone treatment did not have any effect on the incidence of tumors, but the number of papillomas per mouse was slightly but not statistically decreased (90% of control). No tumors were seen with DMBA or β-ionone alone (Shamberger, 1974). There was no evidence of tumor initiation or promotion activity of β-ionone.

In an intraperitoneal study (Stoner et al., 1973), α-irone, injected 3 times a week in tricaprylin for 8 weeks (to groups of 15 A/He mice of each sex to provide total doses of either 1950 or 9600 mg/kg body weight), was reported to produce a significant increase in lung tumors in comparison to controls. A repeat of this study was conducted due to concerns about the quality of the tricaprylin vehicle. Use of re-distilled tricaprylin and a similar dosing regimen resulted in no differences in the incidence of lung tumors between α-irone treated and control mice.

3.5.2. Anti-carcinogenic effects

The anti-tumor effect of β-ionone was examined by Yu et al. (1995) in female Sprague–Dawley rats fed diets containing 36 mmol/kg (approximately 6922 ppm in the diet, equivalent to a dietary dose of about 350 mg/kg body weight/day) of β-ionone dissolved in corn oil. At the end of 2 weeks feeding, tumors were induced by single gastric intubation of DMBA, 65 mg/kg body weight in sesame oil. The dietary regimen continued for 22 weeks. β-Ionone significantly reduced the number of animals with mammary tumors (adenocarcinomas, adenomas, fibroadenomas) which were seen in 91% of control rats and in 41% of β-ionone fed rats. Tumor latency was significantly increased in β-ionone fed rats, 74 days in control rats and >151 days in β-ionone fed rats. Tumor multiplicity was significantly reduced by β-ionone.

He et al. (1997) reported that following the injection of B 16 melanoma cells into weanling C57Bl female mice, addition of 2 mmol/kg β-ionone (diluted in vitamin E-stripped corn oil) (dose equivalent to approximately 385 ppm in the diet, or about 50 mg/kg body weight/day) to the diet increased the median duration of survival compared to controls (not administered β-ionone) by about 50%, and mean duration of survival by about 30%.

Overall, the Yu et al. (1995) study indicates that β-ionone may possess anti-carcinogenic activity in the face of exposure to a potent carcinogen. The model used by He et al. (1997) tests the potential to block establishment and growth of injected malignant cells and is not relevant to toxicity.

3.5.3. Summary of the carcinogenicity data

In summary, on the basis of the negative results obtained in a dermal initiation–promotion study (Shamberger, 1974), a study design of relevance to human exposure to ionones through their use as fragrance ingredients, there is no evidence to indicate that β-ionone has tumor initiating or promoting potential. The intraperitoneal study on α-irone by Stoner et al. (1973) that produced initial tumor inducing or promoting results was confounded by the quality of the dosing regimen vehicle. In the repeat study that used re-distilled tricaprylin as vehicle, no evidence of lung tumor induction or promotion was elicited (Stoner et al., 1973). It is therefore concluded that the ionones are unlikely to possess tumor initiation or promotion potential. This conclusion is supported by the result of a study demonstrating that β-ionone may actually possess anti-carcinogenic activity (Yu et al., 1995).

3.6. Reproductive and developmental toxicity

Several reproductive and/or developmental toxicity studies have been conducted on ionones, most notably on α- and β-ionone (Sporn et al., 1963; Willhite, 1986; Verrett et al., 1980; Gomes-Carneiro et al., 2003) and α-*iso*-methylionone (RIFM, 2005). There are no adequate reproductive/developmental toxicity studies available on any of the rose ketones. The developmental studies in rats reported by RIFM (2005) were conducted according to standardized protocols and represent the most appropriate studies from which to assess developmental toxicity. Also, Sporn et al. (1963) presented data on a reproductive toxicity study in which females were monitored through 3 consecutive reproductive cycles and in which reproductive performance of an F1 generation was also studied (this study was not conducted to modern standards). Willhite (1986) studied teratogenesis by administration of a single dose of β-ionone on day 8 of pregnancy in timed-pregnant hamsters (this study did not evaluate the entire period of organogenesis). The Sporn et al. (1963), Willhite (1986), and the RIFM (2005) studies provide the most useful data to assess reproductive/developmental toxicity and teratogenic potential of the ionones. Since the studies of Gomes-Carneiro et al. (2003) (evaluation of the inhibition of cyclophosphamide-induced teratogenesis in rats by β-ionone) and of Verrett et al. (1980) (teratogenicity in the White Leghorn chickens) utilized non-standard protocols, or protocols that have not been validated for the purposes of human risk assessment, they are not discussed further. In any case, no adverse effects were reported in either the Gomes-Carneiro et al. (2003) or the Verrett et al. (1980).

Prior to the 2005 RIFM developmental toxicity study, a preliminary dose-range finding study was conducted. Forty presumed pregnant female rats were dosed *via* gavage on days 7 through 17 of gestation with α-*iso*-methylionone at dosages of 1.25, 2.5, 5, or 10 mg/kg. All female rats were pregnant and survived to the scheduled sacrifice. No fetal effects were observed (RIFM, 2005).

Based on the above results, the developmental toxicity of α-*iso*-methylionone was investigated in 100 (25/group) presumed pregnant female rats dosed, *via* gavage, on days 7 through 17 of gestation with α-*iso*-methylionone at dosages of 0, 3, 10, or 30 mg/kg/day. All female rats survived to the scheduled sacrifice. Pregnancy occurred in 21–25 rats in each dosage group. There were no abnormal clinical observations or necropsy observations in the female rats that were determined to be test article related. No fetal effects were observed that were determined to be test article related. Based on these data, the maternal and developmental NOAEL of α-*iso*-methylionone is greater than 30 mg/kg/day (RIFM, 2005).

Sporn et al. (1963) studied the effects of ionone on reproduction in rats given 2 mg ionone in 0.1 ml oil solution on alternate days for 8 months (equivalent to a dose of approximately 8–10 mg/kg body weight/day). The female rats were studied through 3 reproduction cycles for number of pregnancies, weight, number of offspring, live pups, weight of pups at birth and after 7 and 21 days, and the viability of the pups after birth. The F1 generation (offspring) were allowed to reach maturity and treated with 15 mg/kg of ionone prior to being subject to reproductive toxicity testing. Their offspring, the F2 generation were evaluated for reproduction parameters. Ionone had no adverse effect on any of the parameters measured. Based on these data, no effects were observed for ionone at approximately 10 mg/kg body weight/day Sporn et al. (1963) study.

The teratogenic potency of β-ionone was evaluated in pregnant hamsters administered a single intubation dose of 48, 240, or 480 mg/kg body weight on day 8 of pregnancy (Willhite, 1986). The animals were sacrificed on day 14 of pregnancy. There was no significant effect of β-ionone on maternal weight gain, number of litters, incidence of abnormal litters, number of implantation sites and resorptions, number of dead or abnormal fetuses, or the types of malformations if present. A NOAEL of 480 mg/kg body weight was identified in this study; however, the utility of this study is limited by the use of a single dose only at day 8 of gestation.

In summary, a limited number of ionones (α- and β-ionone and α-*iso*-methylionone) have been subject to reproductive, developmental, and teratogenicity testing. No rose ketones have been evaluated. The reproductive toxicity studies on α-*iso*-methylionone (RIFM, 2005) indicate that these compounds are unlikely to be reproductive toxicants. Supporting data on ionone in the form of a one-dose teratogenicity study (Willhite, 1986) and a limited 2-generation, 3-reproductive cycle, study in rats (Sporn et al., 1963) also show no evidence of adverse effects on reproductive parameters. From the RIFM (2005) studies, a reproductive, maternal and fetal, NOAEL of at least 30 mg/kg body weight/day, the highest dose tested, can be established. This is in reasonable agreement with a 90-day oral subchronic toxicity study on each of α- and β-ionone in which the low dose of 10 mg/kg body weight/day approximated the NOAEL and no overt toxicity was reported even at the high dose of 100 mg/kg body weight/day (RIFM, 1983a).

3.7. Skin irritation

3.7.1. Human studies

Approximately 459 male and female volunteers were tested. Minimal irritation was observed with α-*iso*-methylionone at 60%, and moderate irritation was observed with α-ionone at a concentration of 32% which may be attributed to the use of acetone as a vehicle. No irritation was observed with any other ionone (allyl-α-ionone, dihydro-β-ionone, ionone, α-irone, α-iso methyl ionone and methyl ionone) when tested at concentrations ranging from 2% to 100%.

Mild to marked cumulative irritation was observed with *cis*-β-damascone at concentrations as low as 0.05%, α-isodamascone at 2% and 0.1% α-damascone. No other

Table 8
Skin irritation studies in humans

Material	Method	Concentration	Subjects	Results	Reference
Allyl α-ionone	HRIPT induction phase	2% in petrolatum	50 male and female volunteers	No irritation	RIFM (1971b)
Allyl α-ionone	Maximization pretest	10% in petrolatum	5 volunteers	No irritation	RIFM (1972a)
Damascenone	HRIPT induction phase	0.5% in specially denaturated alcohol (SDA 39C)	15 male and female volunteers	No irritation	RIFM (1978c)
Damascenone	HRIPT induction phase	0.05% in alcohol SDA 39C	29 male and female volunteers	No irritation	RIFM (1978c)
Damascenone	HRIPT induction phase	3% in triacteoin	50 male and female volunteers	No irritation	RIFM (1979b)
α-Damascone	HRIPT induction phase	1% in petrolatum	54 male and female volunteers	No irritation	RIFM (1979o)
α-Damascone	HRIPT induction phase	0.1% in isopropyl alcohol	51 volunteers	Irritation observed in 3/51	RIFM (1979d)
α-Damascone	HRIPT induction phase	0.5% in DEP	107 male and female volunteers	No irritation	RIFM (2001a)
α-Damascone	Maximization pretest	0.2% in petrolatum	25 male and female volunteers	No irritation	RIFM (1985c)
δ-Damascone	HRIPT induction phase	1% in ethanol	15 volunteers	No irritation	RIFM (1978b)
δ-Damascone	HRIPT induction phase	0.1% in ethanol	30 volunteers	No irritation	RIFM (1978b)
cis-β-Damascone	HRIPT induction phase	0.05% in alcohol SDA 39C	53 male and female volunteers	No irritation	RIFM (1980h)
cis-β-Damascone	HRIPT induction phase	0.5% in ethanol (Panel I) 0.05% in ethanol (Panel II)	18 volunteers (Panel I) and 32 volunteers (Panel II)	0.5%: Irritation observed in 3/18 0.05%: Irritation observed in 2/32	RIFM (1979c)
trans-β-Damascone	HRIPT induction phase	1% in white petrolatum	54 male and female volunteers	No irritation	RIFM (1979p)
trans-β-Damascone	HRIPT induction phase	0.5% in DEP	104 male and female volunteers	No irritation	RIFM (2000e)
trans-β-Damascone	Maximization pretest	0.2% in white petrolatum	23 male and female volunteers	No irritation	RIFM (1985b)
Dihydro-α-ionone	Maximization pretest	12% in petrolatum	25 male and female volunteers	No irritation	RIFM (1976a)
Dihydromethyl-α-ionone[a]	Maximization pretest	4% in petrolatum	25 male and female volunteers	No irritation	RIFM (1976b)
1,3-Dimethyl-α-ionone[a]	Maximization pretest	10% in petrolatum	27 male and female volunteers	No irritation	RIFM (1985a)
1,3-Dimethyl-α-ionone[a]	Maximization pretest	4% in petrolatum	26 volunteers	No irritation	RIFM (1976c)
α-Ionone	48-h closed patch test	32% in acetone	50 male volunteers	Moderate irritation (no further details reported)	Motoyoshi et al. (1979)
Ionone	24-h closed patch test	100% (vehicle not specified)	11 male and female volunteers	No irritation	Katz (1946)
α-Irone	Maximization pretest	10% in petrolatum	5 male volunteers	No irritation	RIFM (1972b)
Isodamascone	HRIPT induction phase	1% in DEP	65 male and female volunteers	No irritation	RIFM (1995b)
α-Isodamascone	HRIPT induction phase	0.2% in DEP	103 male and female volunteers	No irritation	RIFM (1995a)
α-Isodamascone	HRIPT induction phase	2% in DEP	22 female volunteers	Irritation observed in 1/22	RIFM (1994)
Iso-β-ionone[a]	Maximization pretest	12% in petrolatum	25 male and female volunteers	No irritation	RIFM (1980f)
α-*iso*-Methylionone	HRIPT Induction phase	10% in alcohol	28 volunteers	No irritation	RIFM (1962)
α-*iso*-Methylionone	HRIPT induction phase	2% in dimethyl phthalate (DEP)	8 volunteers	No irritation	RIFM (1968)
α-*iso*-Methylionone	HRIPT induction phase	60% in 3:1 DEP:Ethanol (EtOH)	12 male and female volunteers	No irritation	RIFM (2002c)

Table 8 (*continued*)

Material	Method	Concentration	Subjects	Results	Reference
α-*iso*-Methylionone	HRIPT induction phase	60% in 3:1 DEP:EtOH	106 male and female volunteers	Irritation observed in 1/106	RIFM (2004b)
α-*iso*-Methylionone	HRIPT induction phase	60% in 3:1 EtOH:DEP	12 male and female volunteers	No irritation	RIFM (2002c)
α-*iso*-Methylionone	HRIPT induction phase	60% in 3:1 EtOH:DEP	23 male and female volunteers	No irritation	RIFM (2004c)
Methyl ionone	24-h closed patch test	100%	16 male and female volunteers	No irritation	Katz (1946)
Methyl ionone	24-h closed patch test	5% in vaselinum aldum or unguentum hydrophilicum	19 male and female volunteers	No irritation	Fujii et al. (1972)
4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one	Maximization pretest	20% in petrolatum	28 healthy male volunteers	No irritation	RIFM (1978a)

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

irritation reactions were observed with rose ketones. The variability of results may be due to different concentrations being tested in different vehicle. See Table 8 for details of individual studies.

3.7.2. Animal studies

Mixed results were observed when ionones were evaluated for irritation. Dihydro-α-ionone, dihydro-β-ionone, 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one, α-ionone, β-ionone, ionone, α-*iso*-methyl ionone and methyl ionone were tested in guinea pigs, rabbits or rats.

No irritation or slight to well defined irritation reactions lasting 24 h were observed at a concentration of 5% with α- or β-ionone in rabbits. Irritant reactions were produced by most of the ionones when used at concentrations of 100%. No other concentrations were tested.

With guinea pigs, dihydro-β-ionone did not produce irritation reactions at 1%, but discrete irritation was observed at concentrations of 5% or higher. No irritation reactions were observed when α- or β-ionone and methyl ionone were used at concentrations up to 25%.

No irritation or very slight irritation reactions were observed with ionones at 10% in rats. Irritant reactions were observed at concentrations of 30% or higher, with severe cumulative irritation produced by neat α-*iso*-methylionone in 90-day study.

cis-β-Damascone and α-damascone produced irritation in guinea pigs at 1.5% and 1.8%, respectively, when tested as a part of a delayed contact hypersensitivity study. γ-Damascone produced irritation reactions in guinea pigs at 20% and 50%, when tested prior to a Buehler sensitization study. No other irritation reactions were observed with other rose ketones when tested at concentrations ranging from 0.0025% to 50%. For details of individual studies see Table 9.

3.8. Mucous membrane (eye) irritation

The potential for the ionones to induce eye irritation has been evaluated only in a limited manner. Irritation reactions were observed only with α-*iso*-methylionone at 12.5%.

Rose ketones tested (damascone, α-isodamascone, isodamascone, *cis*-β-damascone and α-damascone) have shown no evidence of eye irritation at concentrations of 0.5–100% (see Table 10).

3.9. Skin sensitization

3.9.1. Human studies

Both ionones and rose ketones were evaluated for the potential to induce sensitization. For details of individual studies, see Table 11 and the corresponding Fragrance Material Reviews.

Seven ionones (allyl-α-ionone, dihydro-α-ionone, ionone, α-irone, α-*iso*-methylionone, methyl ionone and 4-(2,4,6-trimethyl-3-cyclohexen-1-yl)-3-buten-2-one) were evaluated in maximization and human repeated insult patch tests (HRIPT) at concentrations ranging from 2% to 60% in 524 volunteers. No sensitization reactions were observed.

Patch tests conducted by Frosch et al. (1995) and deGroot (1985, 1988) on α-ionone, ionone (mixed isomers), α-irone and α-*iso*-methyl ionone did not produce sensitization reactions at concentrations up to 1%.

The rose ketones have been tested in 2 maximization tests, and 18 HRIPT studies in 992 volunteers. Sensitization reactions were observed when eight different isomers (damascone, *trans*-β-damascone, α-isodamascone, *cis*-β-damascone, α-damascone, isodamascone, and γ-damascone) were evaluated at concentrations ranging from 0.5% to 20%. No effects were observed at concentrations of 0.2% or lower.

α-Damascone and *cis*-β-damascone were also evaluated in patch tests. No sensitization was observed with α-damascone at 3% or *cis*-β-damascone at 2%.

There have been a few cases of positive patch testes on dermatological patients but with no consistent pattern.

3.9.2. Cross sensitization

Cross sensitization reactions have been reported in humans who were induced with 1% γ-damascone and

Table 9
Skin irritation studies in animals

Material	Method	Concentration	Species	Results	Reference
Damascenone	Primary irritation test (24-h closed patch test)	0.5% in alcohol SDA 39C	3 Albino rabbits	No irritation	RIFM (1978d)
Damascenone	Primary irritation test	50% in triacetoin	6 Albino rabbits	No irritation	RIFM (1979e)
Damascenone	Buehler pretest	10% in propylene glycol	11 male Hartley guinea pigs	No irritation	RIFM (1971a)
Damascenone	24-h closed patch test	0.0625%, 0.125%, 0.25%, and 0.5% in distilled water	4 Hartley–Dunkin guinea pigs	No irritation	RIFM (1979h)
Damascenone	24-h closed patch test	0.375%, 0.75%, 1.5%, and 3% in distilled water	4 Hartley–Dunkin guinea pigs	No irritation	RIFM (1979h)
α-Damascone	Primary irritation test (24-h closed patch test)	0.5% in alcohol SDA 39C	6 Albino rabbits	No irritation	RIFM, 1979
α-Damascone	Primary irritation test (24-h closed patch test)	50% in alcohol SDA 39C	6 Albino New Zealand rabbits	No irritation	RIFM (1979bb)
α-Damascone	Primary irritation test (24-h closed patch test)	100% (vehicle not specified)	6 Albino rabbits	No irritation	RIFM (1979g)
α-Damascone	Maximization pretest	1.25%, 2.5%, 5%, and 10% in distilled water	4 Hartley–Dunkin guinea pigs	No irritation	RIFM (1980g)
α-Damascone	Maximization pretest	0.125%, 0.25%, 0.5%, 1.0% in distilled water	4 Hartley–Dunkin guinea pigs	No irritation	RIFM (1980g)
α-Damascone	Buehler pretest	0.6% and 1.8% in 80% alcohol (ethanol)	Hartley guinea pigs	0.6%: No irritation 1.8%: Irritation observed in 4/4	RIFM (1983c)
α-Damascone	Buehler pretest	10% in propylene glycol	11 Hartley guinea pigs	No irritation	RIFM (1971a)
γ-Damascone	Irritation evaluated during an associated LD_{50} study	100%	10 Albino New Zealand rabbits (5/sex)	Irritation observed in 10/10	RIFM (1987b)
γ-Damascone	4-h semi-occlusive patch test	40%, 55%, 75% in ethanol or 100%	4 Albino New Zealand rabbits	40%, 55%, and 75%: No irritation 100%: Irritation observed in 4/4	RIFM (1986d) RIFM (1986e)
γ-Damascone	Buehler pretest	10%, 20%, 50% in ethanol or 100%	4 Dunkin–Hartley guinea pigs	10%: No irritation 20%, 50%, and 100%: Irritation observed in 2/4	RIFM (1986f)
cis-β-Damascone	Irritation evaluated as part of delayed contact hypersensitivity study	1.5% in 80% ethanol	20 Hartley guinea pigs (10/sex)	Irritation observed in 18/20	RIFM (1992b)
cis-β-Damascone	Primary irritation test	0.5% in alcohol SDA 39 C	6 Albino New Zealand rabbits	No irritation	RIFM (1979x)
cis-β-Damascone	Buehler pretest	1.5% in 80% ethanol	4 Hartley guinea pigs 2/sex	No irritation	RIFM (1983b)
trans-β-Damascone	Primary irritation test (24-h closed patch test)	50% in triethyl citrate	6 Albino rabbits	No irritation	RIFM (1979f)
trans-β-Damascone	Maximization pretest	0.625%, 1.25%, 2.5%, and 5% in distilled water	4 Hartley–Dunkin guinea pigs	No irritation	RIFM (1979i)
trans-β-Damascone	Maximization pretest	0.125%, 0.25%, 0.5%, and 1% in distilled water	4 Hartley–Dunkin guinea pigs	No irritation	RIFM (1979i)
Dihydro-α-ionone	Irritation evaluated during an associated LD_{50} study	100%	10 rabbits	Irritation observed in 10/10	RIFM (1976d)
Dihydro-β-ionone	Maximization pretest	25%, 50%, 75%, and 100%	2 male Himalayan guinea pigs	25%: Irritation observed in 2/2 50%: Irritation observed in 2/2 75%: Irritation observed in 2/2 100%: Irritation observed in 2/2	RIFM (1999c)

Table 9 (*continued*)

Material	Method	Concentration	Species	Results	Reference
Dihydro-β-ionone	Maximization pretest	1%, 5%, 10%, and 15% in PEG (polyethylene gylocol) 400	2 Himalayan spotted guinea pigs	1%: No irritation 5%: Irritation observed in 1/2 10%: Irritation observed in 2/2 15%: Irritation observed in 2/2	RIFM (1999c)
Dihydro-β-ionone	Maximization pretest	100%	10 Himalayan spotted guinea pigs	Irritation observed in 10/10	RIFM (1999c)
Dihydro-β-ionone	Primary skin irritation study 4-h semi-occluded patch test	100%	3 New Zealand white rabbits	No irritation	RIFM (1999d)
1,3-Dimethyl-α-ionone[a]	Primary skin irritation study 4-h semi-occluded patch test	100%	6 Albino rabbits	Irritation observed in 6/6	RIFM (1984c)
1,3-Dimethyl-α-ionone[a]	Irritation evaluated during an associated LD_{50} study	100%	10 Albino rabbits	Irritation observed in 10/10	RIFM (1984b)
1,3-Dimethyl-α-ionone[a]	Irritation evaluated during an associated LD_{50} study	100%	10 rabbits	Irritation observed in 10/10	RIFM (1976e)
1,3-Dimethyl-α-ionone[a]	Irritation evaluated during an associated maximization study	0.1% in peanut oil	10 Hartley guinea pigs	Irritation observed in 7/10	RIFM (1984f)
α-Ionone	Primary irritation test (24-h closed patch test)	100% and 5% in DEP	3 rabbits/dose	5%: Irritation observed in 1/3 100%: Irritation observed in 3/3	RIFM (1967a)
α-Ionone	Draize pretest	30% (vehicle not specified)	4 Hartley albino guinea pigs	No irritation	Sharp (1978)
α-Ionone	24-h closed patch test	100%	6 Albino Angora rabbits	Irritation observed (no further details reported)	Motoyoshi et al. (1979)
α-Ionone	24-h closed patch test	100%	6 male Hartley guinea pigs	Irritation observed (no further details reported)	Motoyoshi et al. (1979)
α-Ionone	48-h closed patch test	100%	6 Pitman–Moore miniature swine	No irritation	Motoyoshi et al. (1979)
β-Ionone	24-h closed patch test	5% and 100% in DEP	3 rabbits	5%: Irritation observed in 2/3 100%: Irritation observed in 3/3	RIFM (1967b)
β-Ionone	Irritation evaluated during an associated phototoxicity study	5%, 10%, 30%, and 50% in acetone	5 Hartley Albino guinea pigs	No irritation	RIFM (1999e)
Ionone	4-h semi-occluded patch test	100%	8 New Zealand white albino rabbits	Irritation observed in 8/8	RIFM (1979u)
Ionone	Irritation evaluated during an associated phototoxicity study	10%, 30%, 100% in ethanol	5 Albino Wistar rats	Irritation observed in 5/5	RIFM (1981b)
α-Irone	Irritation evaluated during an associated LD_{50} study	100%	6 Albino rabbits	Irritation observed (no further details reported)	RIFM (1972c)
Isodamascone	Maximization pretest	25%, 50%, and 100% in peanut oil	Pilbright white guinea pigs (2/dose)	No irritation observed at 25% and 50% 100%: Irritation observed in 2/2	RIFM (1991)
Iso-β-ionone[a]	Irritation evaluated during an associated LD_{50} study	100%	10 New Zealand white rabbits	Irritation observed in 10/10	RIFM (1980e)
α-*iso*-Methylionone	Irritation evaluated during an associated LD_{50} study	100%	8 rabbits	Irritation observed in 6/8	RIFM (1973)
α-*iso*-Methylionone	Primary irritation test	100%	3 New Zealand white Albino rabbits	Irritation observed in 3/3	RIFM (1984d)
α-*iso*-Methylionone	Primary irritation test	100%	4 New Zealand white Albino rabbits	No irritation	RIFM (1985d)
α-*iso*-Methylionone	Irritation was evaluated during associated 90 day study	1% in phenethyl alcohol	Sprague–Dawley Albino rats (5/sex)	No irritation	RIFM (1981a)
α-*iso*-Methylionone	Irritation was evaluated during associated 90 day study	100%	15 Sprague–Dawley rats	Irritation observed in 15/15	RIFM (1980a)

(*continued on next page*)

Table 9 (*continued*)

Material	Method	Concentration	Species	Results	Reference
α-*iso*-Methylionone	Primary irritation test (24-h closed patch test)	100% and 5% in DEP	3 rabbits/group	5%: No irritation 100%: Irritation observed in 2/3	RIFM (1967a)
α-*iso*-Methylionone	4-h semi-occluded patch test	100%	8 New Zealand white Albino rabbits	Irritation observed in 8/8	RIFM (1979v)
α-*iso*-Methylionone	Irritation evaluated during an associated phototoxicity study	10%, 30%, and 100% in ethanol	5 Albino Wistar rats	10%: No irritation 30%: Irritation observed in 5/5 100%: Irritation observed in 5/5	RIFM (1981c)
Methyl ionone	4-h semi-occlusive patch test	100%	8 New Zealand White rabbits	Irritation observed in 8/8	RIFM (1979w)
Methyl ionone	Irritation evaluated during an associated phototoxicity study	30% in ethanol	4 Albino Wistar rats Colworth colony	Irritation observed in 4/4	RIFM (1982e)
Methyl ionone	Irritation evaluated during an associated LD_{50} study	100%	8 rabbits	Irritation observed in 6/8	RIFM (1973)
Methyl-β-ionone	4-h semi-occlusive patch	100%	3 Albino rabbits	Irritation observed in 3/3	RIFM (1988b)
Methyl-β-ionone	Buehler pretest	100%, 50%, 25%, and 12.5% in ethanol	4 female Dunkin–Hartley Albino guinea pigs	12.5% and 25%: No irritation 50%: Irritation observed in 2/4 100%: Irritation observed in 4/4	RIFM (1989)
Methyl-β-ionone	Buehler pretest	50%, 20%, 10%, and 5% in light liquid paraffin	4 female Dunkin–Hartley Albino guinea pigs	No irritation	RIFM (1989)
4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one	Irritation evaluated during an associated LD_{50} study	100%	10 rabbits	Irritation observed in 10/10	RIFM (1978e)

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

Table 10
Mucous membrane (eye) irritation studies (in rabbits)

Material	Concentration	Results	Reference
1,3-Dimethyl-α-ionone[a]	100%	No irritation	RIFM (1984e)
Damascenone	0.5% in propylene glycol	No irritation	RIFM (1978d)
Damascenone	50% in triacetoin	No irritation	RIFM (1979j)
α-Damascone	100% (vehicle not specified)	No irritation	RIFM (1979y)
α-Damascone	0.5% in propylene glycol	No irritation	RIFM (1979n)
cis-β-Damascone	0.5% in propylene glycol	No irritation	RIFM (1979aa)
trans-β-Damascone	50% in triethyl citrate	No irritation	RIFM (1979k)
α-Ionone	5% and 100% in DEP	No irritation	RIFM (1967a)
β-Ionone	5% and 100% in DEP	No irritation	RIFM (1967b)
Isodamascone	1.5% in petrolatum	No irritation	RIFM (1979l)
α-*iso*-Methylionone	12.5% (vehicle not specified)	Intense conjunctival irritation observed in 3/3	RIFM (1963)

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

then cross challenged with 0.1% α-damascone or *cis*-β-damascone. When the mixture of α- and β-damascone was evaluated, sensitization reactions were observed at 0.2%.

3.9.3. Animal studies

Ionones and rose ketones were evaluated for sensitization in guinea pigs using various test methods including Magnusson–Kligman maximization test, Buehler delayed hypersensitivity test, Freund's Complete Adjuvant Test, Open Epicutaneous Test and modified Draize test.

Methyl-β-ionone at a concentration of 12.5% produced sensitization reactions in Buehler delayed hypersensitivity test, but when animals were rechallenged with 5% methyl-β-ionone, no sensitization was observed. No sensitization reactions were observed when other ionones (dihydro-α-ionone, dihydro-β-ionone, α-ionone, β-ionone, ionone, α-irone, and methyl ionone) were tested at concentrations ranging from 0.1% to 50%.

Sensitization reactions were observed when damascone, *trans*-β-damascone, *cis*-β-damascone and α-damascone were tested at concentrations ranging from 0.5% to 10%. No sensitization was observed with isodamascone at 50%.

Table 11
Skin sensitization studies in humans

Material	Method	Concentration	Subjects	Results	Reference
Allyl α-ionone	MAX[a]	10% in petrolatum	25 male volunteers	No reactions (0/25)	RIFM (1972a)
Allyl α-ionone	HRIPT[b]	2% in petrolatum	50 male and female volunteers	No reactions (0/50)	RIFM (1971a)
Damascenone	HRIPT	0.5% induction 0.05% challenge in alcohol	14 volunteers	2/14 reactions plus one questionable reaction	RIFM (1978c)
Damascenone	HRIPT	0.05% in alcohol SDA 39C	23 volunteers	No reactions (0/23)	RIFM (1978c)
Damascenone	HRIPT	3.0% in triacetoin	50 male and female volunteers	1/50 reactions	RIFM (1979b)
α-Damascone	HRIPT	1% in white petrolatum	54 volunteers	No reactions (0/54)	RIFM (1979o)
α-Damascone	MAX	0.2% in petrolatum	25 male and female volunteers	No reaction (0/25)	RIFM (1985c)
α-Damascone	HRIPT	10% in petrolatum	50 male and female volunteers	Study aborted because of strong reactions during induction	RIFM (1992a)
α-Damascone	HRIPT	0.1% in alcohol	51 volunteers	No reactions (0/51)	RIFM (1979d)
α-Damascone	HRIPT	0.5% in DEP	107 male and female volunteers	No reactions (0/107)	RIFM (2001a)
δ-Damascone	HRIPT	1% in SDA-39C alcohol	54 male and female volunteers	7/54 reactions	RIFM (1982a)
δ-Damascone	HRIPT	1% in ethanol	15 volunteers	2/15 reactions plus 2 questionable reactions	RIFM (1978b)
δ-Damascone	HRIPT	0.1% in ethanol	24 volunteers	No reactions (0/24)	RIFM (1978b)
cis-β-Damascone	HRIPT	0.05% in alcohol SDA 39C	53 male and female volunteers	No reactions (0/53)	RIFM (1980h)
cis-β-Damascone	HRIPT	0.5% induction 0.05% challenge in ethanol	17 volunteers	6/17 reactions	RIFM (1979c)
cis-β-Damascone	HRIPT	0.05% in ethanol	28 volunteers	0/28	RIFM (1979c)
cis-β-Damascone	HRIPT	5% in white petrolatum	50 male and female volunteers	Study aborted because of strong reactions during induction	RIFM (1992a)
trans-β-Damascone	HRIPT	0.5% in DEP	104 male and female volunteers	No reactions (0/104)	RIFM (2000e)
trans-β-Damascone	HRIPT	1% in white petrolatum	54 volunteers	No reactions (0/54)	RIFM (1979p)
trans-β-Damascone	MAX	0.2% in petrolatum	23 male and female volunteers	No reactions (0/23)	RIFM (1985b)
Dihydro-α-ionone	MAX	12% in petrolatum	25 male and female volunteers	No reactions (0/25)	RIFM (1976a)
Dihydromethyl-α-ionone[c]	MAX	4% in petrolatum	25 male and female volunteers	No reactions (0/25)	RIFM (1976b)
1,3-Dimethyl-α-ionone[c]	MAX	10% in petrolatum	27 male and female volunteers	No reactions (0/27)	RIFM (1985a)
1,3-Dimethyl-α-ionone[c]	MAX	4% in petrolatum	26 male and female volunteers	No reactions (0/26)	RIFM (1976c)
Ionone	MAX	8% (vehicle not specified)	25 male and female volunteers	No reactions (0/25)	Greif (1967)
α-Irone	MAX	10% in petrolatum	25 male volunteers	No reactions (0/25)	RIFM (1972b)
Isodamascone	HRIPT	1% in DEP	65 males and females volunteers	No reaction (0/65)	RIFM (1995b)
α-Isodamascone	HRIPT	0.2% in DEP	103 male and female volunteers	No reactions (0/103)	RIFM (1995a)
α-Isodamascone	HRIPT	2% in DEP	22 female volunteers	2/22 reactions	RIFM (1994)
Iso-β-ionone[c]	MAX	12% in petrolatum	25 male and female volunteers	No reactions (0/25)	RIFM (1980f)

(*continued on next page*)

Table 11 (*continued*)

Material	Method	Concentration	Subjects	Results	Reference
Methyl ionone	MAX	10% (vehicle not specified)	25 volunteers	No reactions (0/25)	Greif (1967)
α-*iso*-Methylionone	HRIPT	2% in dimethyl phthalate	52 male and female volunteers	No reactions (0/52)	RIFM (1968)
α-*iso*-Methylionone	HRIPT	10% in alcohol	28 volunteers	No reactions (0/28)	RIFM (1962)
α-*iso*-Methylionone	HRIPT	12.5% in unspecified vehicle	37 male and female volunteers	No reactions (0/37)	RIFM (1964)
α-*iso*-Methylionone	HRIPT	60% in 3:1 EtOH:DEP	23 male and female volunteers	No reactions (0/23)	RIFM (2004c)
α-*iso*-Methylionone	HRIPT	60% in 3:1 DEP:EtOH	106 male and female volunteers	No reactions (0/106)	RIFM (2004b)
4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one	MAX	20% in petrolatum	28 male volunteers	No reactions (0/28)	RIFM (1978a)

[a] Human maximization test (MAX).
[b] Human repeated insult patch test (HRIPT).
[c] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

Sensitization was also evaluated in mice using 8 Local Lymph Node Assays conducted with both ionones and rose ketones. With ionones, dihydro-γ-ionone was not considered a sensitizer at concentrations up to 30% (EC3 value not calculable), but α-*iso*-methylionone was considered a likely sensitizer at concentrations of 25% or higher (EC3 value 21.8%).

The following rose ketones: damascone, *trans*-β-damascone and γ-damascone produced evidence indicative of weak to moderate skin sensitization potential at concentrations ranging from 0.25% to 30% and EC3 values between 1.22% and 9.6%. For details of individual studies see Table 12.

3.10. Phototoxicity and photoallergenicity

UV spectra have been obtained for 10 materials (dihydro-α-ionone; β-ionone; ionone; α-*iso*-methylionone; methyl ionone; damascenone; 4-(2,6,-trimethyl-3-cyclohexene-1-yl)-3-buten-2-one; α-isodamascone; *cis*-β-damascone; γ-damascone) with the UVB light absorbed at a range of 220–400 nm. The results of phototoxicity–photoallergenicity are summarized in Tables 13–17 and described below.

3.10.1. Human studies

Phototoxicity and photoallergy (using exposure to 365 nm wavelength light at an intensity of 1680 μW/cm^2) have been investigated as a part of HRIPT tests. Sensitization, but not photosensitization, was reported in 1/20 subjects exposed to damascone (RIFM, 1979q). No evidence of photosensitization, skin sensitization or irritation, was reported with either of α-damascenone or *cis*-β-damascone (RIFM, 1992a).

3.10.2. Animal studies

Hartley guinea pigs treated with β-ionone at concentrations of 5%, 10%, 30%, and 50% in acetone showed no evidence of phototoxicity (RIFM, 1999e). In 20 Pirbright white guinea pigs no phototoxic or photoallergenic effects were observed with 1.5% isodamascone (RIFM, 1979r).

3.11. Environmental data

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

However, there are environmental data in the RIFM/FEMA Database for materials within the ionone group. These include biodegradation, bioconcentration, acute *Daphnia* and fish studies, and algal population growth inhibition data. Due to the limited availability of data and the apparent consistency in the ecotoxicity data, the ionone and rose ketone groups are discussed collectively and not as two separate groups. Data are available for 7 materials. Overall, these materials appear to be readily biodegradable; the acute toxicities range from 1 to 20 mg/L. The one bioconcentration study indicates limited bioconcentration with a maximum BCF of 56 reported at 1 μg/L (RIFM, 1985e).

In addition, three papers describe the fate of some of the ionone compounds in the environment. In a study by Difrancesco et al. (2004), α-*iso*-methylionone was spiked into wastewater treatment plant sludge amended to soil in a

Table 12
Skin sensitization studies in animals

Material	Method	Concentration	Species	Results	Reference
Damascenone	Buehler test	10% in propylene glycol	11 Male Hartley guinea pigs	No reactions	RIFM (1971a)
Damascenone	Maximization test	1.5% in distilled water	10 Hartley–Dunkin guinea pigs	1/10 reactions	RIFM (1979h)
Damascenone	Maximization test	3% in distilled water	10 Hartley–Dunkin guinea pigs	2/10 reactions	RIFM (1979h)
Damascenone	Maximization test	0.25% in distilled water	10 Hartley–Dunkin guinea pigs	1/10 reactions	RIFM (1979h)
Damascenone	Maximization test	0.5% in distilled water	10 Hartley–Dunkin guinea pigs	1/10 reactions	RIFM (1979h)
Damascenone	LLNA	0.25%, 0.5%, 1.0%, 2.5%, 5.0% in 4:1 acetone/olive oil	CBA/J Hsd female mice (5/dose)	EC3 = 1.24%	RIFM (2001b)
Damascenone	LLNA	0.25%, 5%, 1.0%, 2.5%, 5.0% in 4:1 acetone/olive oil	CBA/J Hsd female mice (5/dose)	EC3 = 1.22%	RIFM (2002d)
α-Damascone	Maximization test	0.6% in 80% ethanol (primary challenge concentration) 1.8% in 80% ethanol (rechallenge concentration)	19 Hartley guinea pigs	Primary challenge 0.6%: 1/19 reactions Rechallenge 1.8%: 9/18 reactions	RIFM (1983c)
α-Damascone	Maximization test	5% and 10% in distilled water	10 Hartley–Dunkin guinea pigs	5%: No reactions 10%: 3/10 reactions	RIFM (1980g)
α-Damascone	Maximization test	0.5% and 1% in distilled water	10 Hartley–Dunkin guinea pigs	0.5%: No reactions 1%: 3/10 reactions	RIFM (1980g)
α-Damascone	Buehler test	10% in propylene glycol	11 Male Hartley guinea pigs	No reactions	RIFM (1971a)
α-Damascone	Maximization test	2%, 5%, and 10% in petrolatum	20 Hartley Guinea pigs	2% and 5%: No reactions 10%: 2/20 reactions	Kozuka et al. (1996)
α-Damascone	LLNA	0.1, 0.25, 0.5, 1.0, 2.5, 5.0 in 4:1 acetone/olive oil	CBA/J Hsd female mice (5/dose)	EC3 = 3.3%	RIFM (2001d)
δ-Damascone	LLNA	0.25%, 0.5%, 1%, 2.5%, or 5% in 4:1 acetone/olive oil	Female CBA/J mice	EC3 = 0.9%	RIFM (2002a)
δ-Damascone	LLNA	0.25%, 0.5%, 1%, 2.5%, or 5% in 4:1 acetone/olive oil	Female CBA/J mice	EC3 = 5.19%	RIFM (2002b)
δ-Damascone	LLNA	7.5%, 15% or 30% in 3:1 DEP: EtOH	Female CBA/J mice (5/dose)	EC3 = 9.6%	RIFM (2004)
γ-Damascone	Buehler test	5% or 10% in ethanol	20 Hartley guinea pigs	5%: 1/10 reaction 10%: 2/10 reactions	RIFM (1986f)
γ-Damascone	LLNA	0.25%, 0.5%, 1%, 2.5%, or 5% in 4:1 acetone/olive oil	Female CBA/J mice	EC3 = 4.6%	RIFM (2001e)
cis-β-Damascone	Delayed hypersensitivity test	1.5% in 80% ethanol	20 Hartley guinea pigs (10/sex)	1/20 reactions	RIFM (1992b)
cis-β-Damascone	Maximization test	2%, 5%, and 10% in petrolatum	19 Hartley female guinea pigs	2%: 17/19 reactions 5%: 18/19 reactions 10%: 18/19 reactions	Kozuka et al. (1996)
cis-β-Damascone	Buehler test	1.5% in 80% ethanol	20 Hartley guinea pigs	1/20 reactions	RIFM (1983b)
trans-β-Damascone	Maximization test	0.5% and 1.0% in distilled water	10 Hartley–Dunkin guinea pigs	0.5%: No reactions 5%: 1/10 reactions	RIFM (1979i)
trans-β-Damascone	Maximization test	2.5% and 5% in distilled water	10 Hartley–Dunkin guinea pigs	2.5%: 1/10 reactions 5%: 2/10 reactions	RIFM (1979i)
trans-β-Damascone	LLNA	0.1%, 0.25%, 0.5%, 1%, 2.5%, or 5% in 4:1 acetone/olive oil	6 female CBA/J Hsd mice	EC3 = 2.4%	RIFM (2001c)
Dihydro-α-ionone	Open epicutaneous test (OET)	12% (unspecified vehicle)	6–8 male and female guinea pigs	No reactions	Klecak (1985)
Dihydro-β-ionone	Maximization test	1% in PEG 400	10 male Himalayan spotted guinea pigs	No reactions (0/10)	RIFM (1999c)
Dihydro-γ-ionone	LLNA	7.5%, 15%, 30% in 3:1 DEP:Ethanol	25 female CBA/J mice	7.5%: SI = 1.39 15%: SI = 1.52 30%: SI = 1.76	RIFM, 2004e

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Table 12 (*continued*)

Material	Method	Concentration	Species	Results	Reference
1,3-Dimethyl-α-ionone[a]	Maximization test	0.1% in peanut oil	10 Hartley Albino guinea pigs	3/10 reactions	RIFM (1984f)
α-Ionone	Modified Draize test	ACC (application challenge concentration) dose was 30% ICC (intradermal challenge concentration) dose was 0.1%	10 Hartley Albino guinea pigs	No reactions (0/10)	Sharp (1978)
β-Ionone	Maximization test	5%, 10%, 20%, and 40% in acetone	5 Hartley guinea pigs	No reactions (0/5)	RIFM (1999e)
Ionone	OET	100%	6–8 Himalayan white-spotted pigs	No reactions	Klecak et al. (1977)
Ionone	Draize test	0.1% in isotonic saline	6–8 Himalayan white-spotted pigs	No reactions	Klecak et al. (1977)
Ionone	Maximization test	5% in isotonic saline	6–8 Himalayan white-spotted pigs	No reactions	Klecak et al. (1977)
Ionone	Freund's complete adjuvant test (FCAT)	50% in Freund's Complete Adjuvant	6–8 Himalayan white-spotted pigs	No reactions	Klecak et al. (1977)
Ionone	OET	8% (vehicle not specified)	6–8 Himalayan white-spotted pigs	No reactions	Klecak (1979, 1985)
Ionone	Maximization test	10% (vehicle not specified)	Hartley guinea pigs	No reactions	Ishihara et al. (1986)
α-Irone	OET	10% (vehicle not specified)	6–8 guinea pigs per group	No reactions	Klecak (1979)
Isodamascone	Maximization test	50% in peanut oil	20 Pirbright white guinea pigs	No reactions	RIFM (1991)
α-*iso*-Methylionone	LLNA	2.5%, 5%, 10%, 25%, 50% in 3:1 DEP: EtOH	24 female CBA/Ca mice	EC3 = 21.8%	RIFM (2005)
Methyl ionone	OET	100%	6–8 Outbred Himalayan white-spotted male and female guinea pigs	No reactions	Klecak et al. (1977)
Methyl ionone	Maximization test	10% (vehicle not reported)	Guinea pigs	No reactions	Ishihara et al. (1986)
Methyl ionone	Draize test	0.1% in isotonic saline	6–8 Himalayan white-spotted guinea pigs	No reactions	Klecak et al. (1977)
Methyl ionone	Maximization test	25% in petrolatum	6–8 Himalayan white-spotted guinea pigs	No reactions	Klecak et al. (1977)
Methyl ionone	FCAT	50% in Freund's Complete Adjuvant	6–8 Himalayan white-spotted guinea pigs	No reactions	Klecak et al. (1977)
Methyl ionone	OET	10% (vehicle not reported)	6–8 Outbred Himalayan white-spotted male and female guinea pigs	No reactions	Klecak (1979, 1985)
Methyl-β-ionone	Delayed hypersensitivity test	12.5% and 25% in ethanol	20 female Dunkin–Hartley Albino guinea pigs	12.5%: 4/20 reactions 25%: 8/20 reactions	RIFM (1989)

[a] This material is not one of the materials being reviewed as it is not used in fragrances, but it is included in this table because it is structurally related.

Table 13
Phototoxicity studies in humans

Material	Concentration	Subjects	Results	Reference
Damascenone	3% in triacetoin	20 male and female volunteers	No phototoxicity was observed	RIFM (1979q)
α-Damascone	10% in petrolatum	20 male and female volunteers	No phototoxicity was observed	RIFM (1992a)
cis-β-Damascone	5% in petrolatum	20 male and female volunteers	No phototoxicity was observed	RIFM (1992a)

series of experiments to determine its dissipation in the soil compartment and potential to leach from the upper 10 cm of soil. α-*iso*-Methylionone was undetected after 3 months in the soil compartment and not detected in the leachate.

Simonich et al. (2002) reported that removal of γ-methyl ionone in a variety of wastewater treatment plants in Europe and the United States exceeded 87%. Final effluent concentrations in these plants were consistently below 0.5 μg/L. This confirmed earlier work reported in Simonich et al. (2000).

The ionones present a negligible environmental risk and would not be considered persistent, bioaccumulative or toxic chemicals as indicated by applying the RIFM framework (Salvito et al., 2002) and reviewing the limited environmental data.

4. Summary

1. In the scientific literature and in studies in the RIFM database, there are no definitive data from which to quantify the *in vivo* absorption of ionones and/or rose ketones following dermal exposure. Similarly, there are no oral pharmacokinetic studies available from which the bioavailability of this class of compounds can be quantitatively determined. By analogy with

Table 14
Phototoxicity studies in animals

Material	Concentration	Species	Results	Reference
β-Ionone	5%, 10%, 30%, and 50% in acetone	5 Hartley Albino guinea pigs	No phototoxicity observed	RIFM (1999e)
Ionone	100%	10 Wistar Albino rats	No phototoxicity observed	RIFM (1981b)
Isodamascone	1.5% in petrolatum	20 Pirbright White guinea pigs	No phototoxicity observed	RIFM (1979s)
α-*iso*-Methylionone	30% in ethanol	10 Wistar rats	No phototoxicity observed	RIFM (1981c)
Methyl ionone	0.1%, 0.25%, 0.5%, 1.0%, 2.5%, 5%, 10%, and 25% in 6% acetone/saline	4 guinea pigs	Phototoxic reactions observed at 25% Questionable reactions observed at lower doses (no further details reported)	RIFM (1982b)
Methyl ionone	1.5, 5, 17, 60, 200 and 660 mg/kg in olive oil	C57 BL Colworth mice	No phototoxicity observed	RIFM (1982d)
Methyl ionone	30% in ethanol	Rats	Phototoxicity was observed (no further details reported)	RIFM (1982c)

Table 15
Photoallergy studies in humans

Material	Method	Concentration	Subjects	Results	Reference
Damascenone	HRIPT procedure with UV irritation after the 1st, 4th, 7th and 9th induction applications and again after the challenge application	3% in triacetoin	20 male and female volunteers	No reactions (0/20)	RIFM (1979q)

Table 16
Photoallergy studies in animals

Material	Method	Concentration	Subjects	Results	Reference
Isodamascone	Nine induction applications (3 times a week for 3 weeks) followed by irradiation after each application; then a 3-week rest period, followed by a challenge application and irradiation	1.5% in petrolatum	19 Pirbright white guinea pigs	No reactions (0/19)	RIFM (1979s)

Table 17
Summary of UV spectra data for ionones

Material	UV spectra range of absorption (nm)
Dihydro-α-ionone	Peaked at 235–255 nm range minor absorption in 260–300 nm region
β-Ionone	Peaked at 285–295 nm range minor absorption in 300–340 nm region
Ionone (mixed isomers)	Peaked at 290–295 nm range minor absorption in 300–320 nm region
α-*iso*-Methylionone	Does not absorb UV light at wavelengths in range of 290–400 nm
Methyl ionone (mixture of isomers)	Peaked at 230–235 nm range minor absorption in 245–320 nm region
4-(2,4,6-Trimethyl-3-cyclohexen-1-yl)-3-buten-2-one (Iritone)	Does not absorb UV light at wavelengths in the range of 290–400 nm
Damascenone	Peaked within 238–280 nm range minor absorption in 290–340 nm
cis-β-Damascone	Peaked within 220–280 nm range minor absorption in 260–300 nm
δ-Damascone	Does not absorb UV light at wavelengths in range of 290–400 nm
α-Isodamascone	Does not absorb UV light at wavelengths in range of 290–400 nm

fragrance ketones and aldehydes for which *in vivo* absorption data are available, dermal or oral absorption of ionones/rose ketones is likely to be significant and is conservatively assumed for purposes of risk assessment to be 100%. Based on metabolic studies on α-ionone and β-ionone in which ionone-specific metabolites were recovered in the urine of treated rabbits and dogs, oral absorption of these compounds does occur; it is assumed to be 100%. Bioavailability by the oral route is likely to be considerably greater than by the dermal route, based on *in vitro* rat and pig skin absorption studies.

2. The primary differences in the chemical structure of members of this class of compounds that could affect metabolism, and potentially the toxicity of metabolites, are the position of the double bond in the allylic side chain (ionones *versus* rose ketones) and the potential for epoxidation depending upon the number and position of the double bonds in the cyclohexene ring. Since the allylic side chain of the rose ketones does not appear to have strong electrophilic activity, the rose ketone metabolites are unlikely to be of greater toxicity than those of the ionones. However, based on metabolic considerations, unique epoxide metabolites could be generated for each of *trans*,*trans*-δ-damascones, δ-damascone, damascone, and methyl-δ-ionone. Thus, these compounds may have greater toxic potential than other members of this class.
3. The limited metabolic data on α- and β-ionone obtained in animals demonstrate the activity of biotransformation pathways involving combinations of hydroxylation/oxygenation of the cyclohexene ring, reduction of the butenone group to a secondary alcohol, oxidation of the angular methyl groups, reduction of the double bond in the exocyclic alkenyl side chain to form dihydro derivatives, and conjugation of the hydroxylated metabolites with glucuronic acid. Although there are no data available on the metabolic fate of the ionones and rose ketones in humans, the animal metabolic data (i.e., showing oxidative and reductive transformation followed by conjugation) and the theoretical considerations discussed above, are likely to be applicable to humans.
4. The acute oral and dermal toxicity of ionones is low to moderate. Many of the ionones have oral LD_{50} values of >2 g/kg body weight, the normal limit dose in this assay.
5. There appear to be no clear differences in the toxicity of the ionones following dermal or oral routes of exposure. This conclusion is tentative because appropriate studies comparing the two routes of exposure or to assess the subchronic dermal toxicity of any individual ionone/rose ketones or the group as a whole have not been reported. The most appropriate 90-day dermal toxicity study was conducted on α-*iso*-methyl ionone; however, its interpretation related to systemic effects is significantly compromised by severe effects of the test chemical on the skin. The available data do not indicate systemic toxicity in the absence of severe effects on the skin. Tentatively, a systemic NOAEL of 50 mg/kg body weight/day associated with dermal exposure to α-*iso*-methylionone and an oral NOAEL of 30 mg/kg body weight/day can be used for quantitative human health risk assessment of the use of the ionones as fragrance compounds. There are no dermal or subchronic oral toxicity studies available on those ionones and rose ketones that may undergo epoxidation [i.e., *trans*,*trans*-δ-damascones; δ-damascone; and 1-(2,6,6-trimethyl-3-cyclohexa-1-e-dienyl)-2-buten-1-one)], and hence have a higher potential for the generation of toxic metabolites.
6. The ionones tested are non-mutagenic in standard bacterial reverse mutation assays. In *in vitro* chromosome aberration tests, increases in structural aberrations have been reported with methyl ionone and α-ionone at high concentrations. There is one negative and one equivocal *in vivo* mouse micronucleus test with methyl ionone and a negative mouse micronucleus test with α-ionone at doses as high as 1200 mg/kg.
7. There are no long-term studies that directly evaluated the carcinogenicity of ionones. Based on the lack of significant genotoxic potential, a lack of tumor promoting activity, and the reported anti-carcinogenic effects of one ionone, it appears that ionones have no significant carcinogenicity under the recommended current conditions of use as fragrance ingredients (some uncertainty remains for those rose ketones and ionones that may be subject to metabolism by epoxidation).
8. The reproductive/developmental toxicity studies of α-*iso*-methylionone and ionone, demonstrate that these materials do not cause reproductive/developmental effects at doses that approach the maternal NOAEL.
9. At concentrations likely to be encountered by humans through the use of the ionones and rose ketones as fragrance ingredients, these chemicals are considered to be non-irritating. Rose ketones could produce some skin irritation in sensitive individuals. As neat solutions (100% concentration), the ionones and rose ketones are irritants in laboratory animals.
10. The eye irritation data indicate weak eye irritation potential of certain ionones. The rose ketones tested have shown no evidence of eye irritation potential. Under the conditions of use, fragrance ingredients at low concentrations in cosmetic products, both ionones and rose ketones evaluated in this report are expected to be non-irritating to mucous membranes (eyes).
11. The ionones are without significant skin sensitization potential. The rose ketones can be sensitizers but not when present at concentrations of 0.2% or less (based on human data). IFRA (2007) has established Standards on the methyl ionones and the rose ketones using a Quantitative Risk Assessment (QRA) for dermal sensitization (see the individual fragrance material reviews on these materials for more information).
12. The ionones included in this summary are likely to have no phototoxic or photoallergic potential.

5. Conclusion

- For evaluation of the ionones and rose ketones 100% bioavailability should be assumed for the dermal and oral routes of exposure.

- The limited metabolic data on ionones demonstrate biotransformation pathways involving combinations of hydroxylation/oxygenation, reduction, oxidation, and conjugation. Metabolism of the majority of this class of compounds is not likely to increase the toxicity of parent compounds. Those that could undergo epoxidation have not been subjected to subchronic testing and are considered to be inadequately characterized for the purposes of human health safety assessment.
- Ionones have low to moderate oral toxicity (LD_{50} values of 1.5 g to >5 g/kg body weight). In acute dermal toxicity studies, LD_{50} values are greater than 2 or 5 g/kg body weight (the limit doses commonly used in LD_{50} assays).
- No systemic toxicity was observed in uncomplicated subchronic oral or dermal 90-day toxicity studies in rats. It is concluded that these materials administered by the dermal route have a systemic NOAEL value of 50 mg/kg/day. They have an oral NOAEL value of 10 mg/kg body weight.
- Under intended conditions of use the ionones and rose ketones do not have significant genotoxic, reproductive or developmental potential.
- The ionones at concentrations likely to be encountered by humans through their use as fragrance ingredients are non-irritating, and the rose ketones have limited irritation potential in sensitive subjects.
- The ionones are considered to be without significant skin sensitization potential, while the rose ketones are sensitizers when present at concentrations in excess of 0.2% (based on human data). IFRA (2007) has established Standards on the methyl ionones and the rose ketones using a Quantitative Risk Assessment (QRA) for dermal sensitization (see the individual fragrance material reviews on these materials for more information).
- Use of the ionones and rose ketones in fragrances produces low levels of exposure relative to doses that elicit adverse dermal or systemic effects in laboratory animals exposed *via* dermal or oral routes. The estimate for maximum systemic exposure of humans using cosmetic products containing ionones or rose ketones ranges from 0.0002 to 0.331 mg/kg/day. If the estimate of 100% absorption is used and using the NOAEL of 10 mg/kg body weight/day, a margin of safety for systemic exposure of humans to the individual ionones in cosmetic products can be calculated to range from 30 to 50,000 times the maximum daily exposure.

Conflict of interest statement

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

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