Food and Chemical Toxicology 49 (2011) S126–S141

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Review

A toxicological and dermatological assessment of macrocyclic ketones when used as fragrance ingredients ☆
The RIFM Expert Panel

D. Belsito [a], D. Bickers [b], M. Bruze [c], P. Calow [d], M.L. Dagli [e], A.D. Fryer [f], H. Greim [g], Y. Miyachi [h], J.H. Saurat [i], I.G. Sipes [j]

[a] Columbia University Medical Center, Department of Dermatology, 161 Fort Washington Avenue, New York, NY 10032, USA
[b] Columbia University Medical Center, Department of Dermatology, 161 Fort Washington Ave., New York, NY 10032, USA
[c] Malmo University Hospital, Department of Occupational & Environmental Dermatology, Sodra Forstadsgatan 101, Entrance 47, Malmo SE-20502, Sweden
[d] Science and Public Policy, Office of Research and Economic Development, 230 Whittier Research Center, Lincoln NE 68583-0857, USA
[e] University of Sao Paulo, School of Veterinary Medicine and Animal Science, Department of Pathology, Av. Prof. dr. Orlando Marques de Paiva, 87, Sao Paulo CEP 05508-900, Brazil
[f] Oregon Health Science University, 3181 SW Sam Jackson Park Rd., Portland, OR 97239, USA
[g] Technical University of Munich, Institute for Toxicology & Environmental Hygiene, Hohenbachernstrasse 15-17, Freising-Weihenstephan D-85354, Germany
[h] Department of Dermatology, Kyoto University Graduate School of Medicine, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan
[i] Swiss Centre for Human Applied Toxicology, University Medical Center, University of Geneva, Rue Michel Servat, 1211 Geneve 4 CH, Switzerland
[j] Department of Pharmacology, University of Arizona, College of Medicine, 1501 North Campbell Avenue, P.O. Box 245050, Tucson, AZ 85724-5050, USA

ARTICLE INFO

Article history:
Available online 23 July 2011

Keywords:
Safety
Review
Fragrance
Macrocyclic ketone

ABSTRACT

The macrocyclic ketone (MK) group of fragrance ingredients was evaluated for safety following a complete literature search. For high end users, calculated maximum dermal exposures vary from 0.13% to 1.10%; systemic exposures vary from 0.0005 to 0.0441 mg/kg/day. The MKs had low acute toxicity and no significant repeat dose toxicity. Liver weight and blood biochemistry effects were reversible after 2 weeks. No genotoxicity in bacteria and mammalian cell lines was observed. Reproductive toxicity was not observed for 3-methylcyclopentadecenone in an OECD compliant study. In humans, MKs are generally not irritating after one application. Animal studies showed irritation for some materials at concentrations higher than current consumer exposure. At rates consistent with current human exposure, phototoxicity and photosensitization were not observed. In animals, some MKs are sensitizers only at concentrations of 20%, 30%, or 100%, which are higher than current consumer exposure. No evidence of sensitization was observed in human tests. In patients with fragrance allergy, reactions were seen with cyclopentadecanone (3/178). Based on these findings, the Panel is of the opinion that there are no safety concerns for the MKs at reported levels of use and exposure as fragrance ingredients.

Contents

☆ All correspondence should be addressed to A.M. Api, Research Institute for Fragrance Materials Inc., 50 Tice Boulevard, Woodcliff Lake, NJ 07677, USA. E-mail address: AApi@RIFM.org (A.M. Api).

0278-6915/$ - see front matter © 2011 Published by Elsevier Ltd.
doi:10.1016/j.fct.2011.07.040

1. Introduction

In 2010 complete literature searches were conducted on the macrocyclic ketones (MK) group of fragrance ingredients. This document provides a risk assessment of these materials as fragrance ingredients. These fragrance ingredients are blended with other fragrance ingredients that may or may not be MK for use in decorative cosmetics, fine perfumes, personal care products such as shampoos, soaps, and in household products such as cleaners, air fresheners and detergents. 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 includes a group summary evaluation paper and individual Fragrance Material Reviews on discrete chemicals. The group summary is an evaluation of relevant data selected from the large bibliography of studies and reports on the individual chemicals. These studies and reports are both primary data from RIFM and RIFM member companies, and peer reviewed publications. The selected data were deemed to be relevant based on the currency of protocols, quality of the data, statistical significance and appropriate exposure. These are identified in tabular form in the group summary (Tables 2–11). Details that are provided in the tables are not always discussed in the text of the group summary. The separate Fragrance Material Reviews, which cover individual fragrance materials, contain a comprehensive summary of all unpublished and published reports including complete bibliographies (McGinty et al., in press-a–h). A complimentary environmental group summary document for the macrocyclic ketone and lactone/lactide subgroups has also been prepared (Salvito et al., 2001).

2. Chemical identity, regulatory status, and exposure

In the United States (US) some fragrance ingredient substances have been approved as synthetic flavoring substances and food adjuvants. The Joint Expert Committee on Food Additives have reviewed 3-methyl-1-cylopentadecanone (CAS RN 541-91-3) and cycloheptadeca-9-en-1-one (CAS RN 542-46-1) as they are used for flavoring and concluded that they do not present a safety concern at current levels of intake when used as a flavoring agent. The Flavor and Extract Manufacturers Association (FEMA) companies have designated 3-methyl-1-cylopentadecanone (CAS RN 541-91-3) and cycloheptadeca-9-en-1-one (CAS RN 542-46-1) as Generally Recognized as Safe (GRAS) for use as flavor ingredients.

Table 1 provides a list of the MK fragrance ingredients that are evaluated in this report along with their Chemical Abstract Service registration numbers (CAS RN), synonyms, structural formulas, and some physiochemical properties (e.g., calculated log K_{ow}, vapor pressure, and water solubility), annual worldwide production, and estimated dermal systemic exposure data for these compounds. Tables 2–10 summarize the available MK toxicology data. Two structurally related compounds, 3-methylcyclotridecan-1-one (CAS RN 61415-11-0) and cyclotetradecan-1-one (CAS RN 3603-99-4) are listed in the RIFM database but not used as fragrance ingredients. No toxicity data on these compounds were identified.

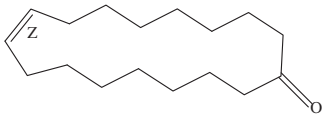
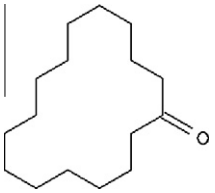
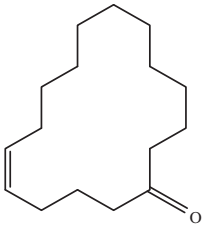
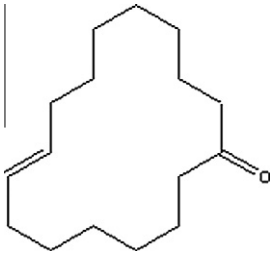
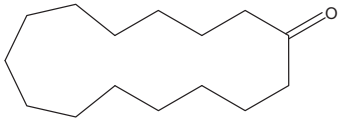
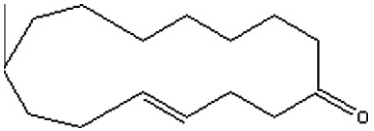
2.1. Rationale for grouping macrocyclic ketones

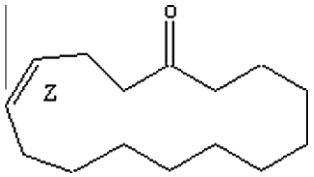
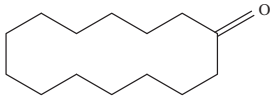
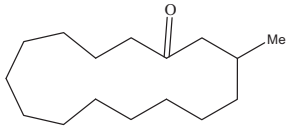
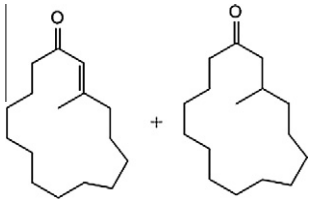
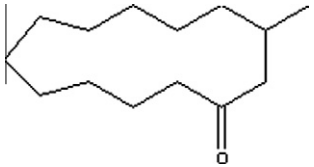
The MK fragrance ingredients described in Table 1 include both naturally occurring and synthetic macrocyclic ketones The common structural element of the MK group of fragrance ingredients is a keto group, R–C (=O)-R′, contained within a macrocyclic ring of C15 to C17 carbon chain length.

The macrocyclic ketone fragrance ingredients described herein include 11 structurally diverse C15, C16 and C17 compounds that include three saturated and eight unsaturated ketones. For the latter, the double bond is not adjacent (in conjugation with) to the ketone group. The naturally occurring macrocyclic ketones are derived from various animal rather than plant sources.

The molecular weights of the macrocyclic ketones do not vary appreciably and range from a high of 250.4 g/mol for the C17 congener cycloheptadeca-9-en-1-one (CAS RN 542-46-1) to a low of 222.4 g/mol for the C15 congener (Z)-4-cyclopentadecen-1-one (CAS RN 14595-54-1). The macrocyclic ketone fragrance ingredients are generally lipophilic and log K_{ow} increases with increasing with ring size. Log K_{ow} values range from 6.31 for the C17, cycloheptadeca-9-en-1-one (CAS RN 542-46-1), to 5.33 for the C15,

Table 1
Material identification, volume of use, and dermal exposure.

Material	Synonyms	Structure	Annual worldwide metric tons[a]	Dermal systemic exposure in cosmetic products (mg/kg/day)[b]	Maximum skin level[c,d] (%)
Cycloheptadeca-9-en-1-one $C_{17}H_{30}O$ CAS # 542-46-1 Log K_{ow}: 6.31[e] Molecular weight: 250.43 Vapor pressure: 0.000339 mm Hg 25 °C[e] Water solubility: 0.09556 mg/L at 25 °C[e]	• Civetone • Civettone • alpha-*trans*-Civettone • 9-Cycloheptadecen-1-one • Cycloheptadec-9-en-1-one		0.01–0.1	0.0005	0.13
Cyclohexadecanone $C_{16}H_{30}O$ CAS # 2550-52-9 Log K_{ow}: 6.04[e] Molecular weight: 238.41 Vapor pressure: 0.000262 mm Hg 25 °C[e] Water solubility: 0.1915 mg/L[e]	• Homoexaltone • Isomuscone		1–10	0.01402	0.52
5-Cyclohexadecen-1-one $C_{16}H_{28}O$ CAS # 37609-25-9 Log K_{ow}: >6.0[e] Molecular weight: 236.99 Vapor pressure: 0.000238 mm Hg 25 °C[e] Water solubility: 0.2997 mg/L at 25 °C[e]	• Cyclohexadec-5-en-1-one • Toray-musk • Velvione • Musk amberol • Ambrettone • 5-Cyclohexadecanone • Musk TM-II		10–100	0.0405	1.10
Cyclohexadec-8-en-1-one (mix of *cis* and *trans* isomer) $C_{16}H_{28}O$ CAS # 3100-36-5 Log K_{ow}: 5.82[e] Molecular weight: 236.99 Vapor pressure: 0.000238 mm Hg 25 °C[e] Water solubility: 0.2997 mg/L	• 8-Cyclohexadecen-1-one • *cis*,*trans*-Cyclohexadec-8-en-1-one • Globanone		10–100	0.0382	0.86
Cyclopentadecanone $C_{15}H_{28}O$ CAS # 502-72-7 Log K_{ow}: 5.55[e] Molecular weight: 224.39 Vapor pressure: 0.000418 mm Hg 25 °C[e] Water solubility: 0.5989 mg/L at 25 °C[e]	• Exaltone • Normuscone		10–100	0.0201	0.77
4-Cyclopentadecen-1-one $C_{15}H_{26}O$ CAS # 35720-57-1 Log K_{ow}: 5.33[e] Molecular weight: 222.37 Vapor pressure: 0.000565 mm Hg 25 °C[e] Water solubility: 0.9369 mg/L[e]	• Cyclopentadec-4-en-1-one		1–10	0.0217	0.78

4-Cyclopentadecen-1-one, (Z)- $C_{15}H_{26}O$ CAS # 14595-54-1 Log K_{ow}: 5.33[e] Molecular weight: 222.37 Vapor pressure: 0.00126 mm Hg 25 °C[e] Water solubility: 0.2435 mg/L	• 4-Cyclopentadecen-1-one, (4Z)- • *cis*-4-Cyclopentadecenone • *cis*-4-Cyclopentadecen-1-one • Exaltenone • Musk pentane		1–10	0.0184	0.31
Cyclotetradecan-1-one $C_{14}H_{26}O$ CAS # 3603–99-4[g] Log K_{ow}: 5.05[e] Molecular weight: 210.61 Vapor pressure: 0.00158 mm Hg 25 °C Water solubility: 1.866 mg/L at 25 °C	• Cyclotetradecanone		0	0.0005[f]	0.02[f]
3-Methyl-1-cyclopentadecanone $C_{16}H_{30}O$ CAS # 541-91-3 Log K_{ow}: 5.96[e] Molecular weight: 238.42 Vapor pressure: 0.000469 mm Hg 20 °C[e] Water solubility: 0.2213 mg/L at 25 °C[e]	• Muscone • Cyclopentadecanone, 3-methyl- • 3-Methylcyclopentadecanone • Methylexaltone • D,L-Muscone		1–10	0.0296	0.45
3-Methylcyclopentadecenone (mixed isomers) $C_{16}H_{28}O$ CAS # 82356-51-2 Log K_{ow}: >4.88 Molecular weight: 236.39 Vapor pressure: 4.0×10^{-2} Pa at 25 °C Water solubility: 8.99 × 10e−4 g/L	• Muscenone δ • Cetolide		10–100	0.0441	1.01
3-Methylcyclotridecan-1-one $C_{14}H_{26}O$ CAS # 61415-11-0[g] Log K_{ow}: 4.98[e] Molecular weight: 210.61 Vapor pressure: 0.00213 mm Hg 25 °C[e] Water solubility: 2.156 mg/L[e]	• Cyclotridecanone, 3-methyl- • 3-Methylcyclotridecanone		0	0.0005[f]	0.02[f]

[a] 2008 volume of use survey (IFRA, 2008).
[b] Based on a 60 kg adult.
[c] Upper 97.5 percentile levels of the fragrance ingredient in the fragrance mixture used in these products.
[d] 2007 use level survey (IFRA, 2007).
[e] Physical properties have been calculated Epi Suite (EPA, 2010).
[f] A default value of 0.02% was used to calculate dermal systemic exposure.
[g] Cyclotetradecan-1-one (CAS # 3603-99-4) and 3-methylcyclotridecan-1-one (CAS #61415-11-0) are not used as fragrance materials but are considered structurally related.

4-cyclopentadecen-1-one (CAS RN 35720-57-1) macrocyclic ketone.

The availability of macrocyclic ketone metabolism studies is limited. Enzyme induction studies have been published for 3-methyl-1-cyclopentadecanone but the metabolites were not identified. By analogy, and for lack of other available information, cyclohexanone may serve as an example of how higher order macrocyclic ketones may be metabolized. As with cyclohexanone, it is proposed that the macrocyclic ketone may also be acted upon by reductases to generate a macrocyclic alcohol metabolite, which may also be either converted back to the macrocyclic ketone or conjugated with glucuronic acid and excreted. It is believed that all the materials in this group have similar metabolism and are detoxified in the same manner. Their toxicological profiles would, then, be similar.

2.2. Occurrence and use

Naturally occurring animal and plant musks have been used as fragrances, foods, flavors, and for medicinal purposes for hundreds of years (Sommer, 2004; Groom, 1997). Historically, the most economically important musks have included those derived from animal sources. These include 3-methyl-1-cyclopentadecanone (CAS RN 541-91-3), and its demethylated congener cyclopentadecanone (CAS RN 502-72-7), and 3-methyl-1-cyclopentadecanone (CAS RN 542-46-1). 3-Methyl-1-cyclopentadecanone and its related compounds were originally isolated from musk gland secretions of the musk deer, *Moschus moschiferus* (Moschidae), which are found throughout Asia in Pakistan, India, Tibet, China, Siberia and Mongolia (Kraft, 2005). Musk-like materials have also been identified in various other animals, including the muskrat (*Ondatra zibethicus*) of North America, the musk duck (*Biziura lobata*) of southern Australia, a musk shrew, the musk beetle (*Aromia moschata*) (Groom, 1997).

The limited availability and cost of obtaining compounds from their naturally occurring sources has provided great economic incentive to develop manufacturing processes to both supplement and replace the naturally occurring MK fragrance ingredients and to discover new and structurally diverse synthetic musk fragrances (Sommer, 2004; Kraft et al., 2000). The popularity of the alternative synthetic musks, sometimes referred to as "white musks", has fostered preservation of the deer musk and civet cat, both of which had become endangered animals.

As indicated in Table 1 the yearly worldwide production of the macrocyclic ketone fragrances ranges from very low tonnage, 0.01–0.1 metric tons for cycloheptadeca-9-en-1-one (CAS RN 542-46-1), to moderate tonnage (10–100 metric tons for 5-cyclohexadecen-1-one (CAS RN 37609-25-9) and cyclopentadecanone (CAS RN 502-72-7)).

2.3. Estimated consumer exposure

Exposure data have been provided by the fragrance industry. Potential consumer exposure to fragrance materials occurs through the dermal and inhalation routes. Published human inhalation exposure studies are not available for the MK fragrance ingredients. Worst-case scenario calculations indicate that the depositions on the surface of the skin following use of cosmetics represents the major route of exposure to fragrance ingredients when conservative estimates for evaporation, rinsing and other forms of product removal are employed (Cadby et al., 2002). Therefore, the dermal route was the major route examined in assessing the safety of these compounds.

The fragrance industry has developed three types of approaches to estimate potential exposure for consumers to fragrance materials. All three types of exposure are summarized in Table 1. The first is volume of use. The total worldwide volume of use for fragrance materials in the MK fragrance ingredients ranges from 0.01 to 100 metric tons per year (IFRA, 2008). The reported volume is for the fragrance ingredient as used in fragrance compounds (mixtures) in all finished consumer product categories. The volume of use is determined by IFRA approximately every four years through a comprehensive survey of IFRA and RIFM member companies. As such, the volume of use data from this survey provides volume of use of fragrance ingredients for the majority of the fragrance industry.

The second method estimates potential percutaneous (total human skin exposure) absorption from the entire body based on the use of multiple consumer personal care products containing the same fragrance ingredient. The dermal systemic exposure in cosmetic products is based on the concentrations in ten types of the most frequently used personal care and cosmetic products (anti-perspirant, bath products, body lotion, eau de toilette, face cream, fragrance cream, hair spray, shampoo, shower gel, and toilet soap). The concentration of the fragrance ingredient in fine fragrances is obtained from examination of several thousand commercial formulations. The upper 97.5 percentile concentration is calculated from the data obtained. This upper 97.5 percentile concentration is then used for all ten consumer products. These concentrations are multiplied by the amount of product applied, the number of applications per day for each product type, and a "retention factor" (ranging from 0.001 to 1.0) to account for the length of time a product may remain on the skin and/or the likelihood of the fragrance ingredient being removed by washing. The resultant calculation represents the total consumer exposure (mg/kg/day) (Cadby et al., 2002; Ford et al., 2000). In view of all the above assumptions, the total calculated consumer exposure is conservative; it is unlikely that a consumer will consistently use a number of different consumer products which are all perfumed with the upper 97.5 percentile level of the fragrance ingredient from a fine fragrance type product (Cadby et al., 2002; Ford et al., 2000). The total consumer exposure to fragrance ingredients range from 0.0005 to 0.0441 mg/kg body weight (bw)/day for the MK fragrance ingredients in high-end user of cosmetic products containing these materials (see Table 1) (IFRA, 2007).

The third method provides maximum skin levels. For consideration of potential sensitization, the exposure is calculated as the percent concentration of the fragrance ingredient applied to the skin based on the use of 20% of the fragrance mixture in fine fragrance consumer product (IFRA, 2007). The maximum skin exposure levels of the MK compounds that form part of the formulae of fine fragrances vary widely and have been reported to range from 0.13% to 1.10%. The maximum skin exposures for the MK fragrance ingredients in fine fragrance products are listed in Table 1 (IFRA, 2007).

In assessing safety, the calculated dermal systemic exposure in cosmetic products can then be compared to the indices of systemic toxicity such as NOAEL and LOAEL that are obtained from the repeat dose sub-chronic, chronic and reproductive toxicity studies to derive a margin of exposure (MOE). Systemic exposures (i.e., the dose absorbed through the skin and available to the systemic circulation) were estimated based on dermal absorption rates. Where such data were lacking as a conservative measure, dermal absorption was considered to be 100% (i.e., the maximum skin exposure value was considered as the estimate of systemic exposure).

All exposure data were provided by the fragrance industry. Further explanation of how the data were obtained and of how

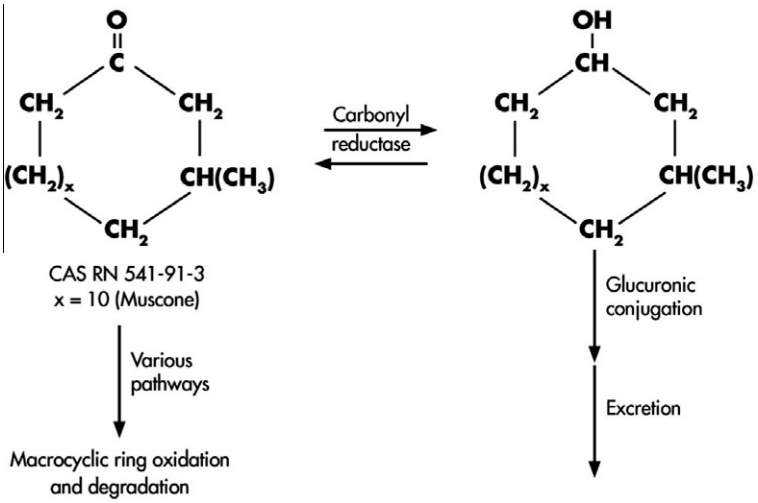


Fig. 1. Proposed macrocyclic ketone metabolism and excretion (3-methyl-1-cyclopentadecanone example).

exposures were determined has been previously reported by Cadby et al. (2002) and Ford et al. (2000).

3. Metabolism

The availability of macrocyclic ketone metabolism studies is limited primarily to two enzyme induction studies for 3-methyl-1-cyclopentadecanone (CAS RN 541-91-3).

An initial metabolism study in rats exposed to non-radiolabeled 3-methyl-1-cyclopentadecanone administered by intraperitoneal injection showed an increased level of cytochrome P450 and increased activity of aminopyridine demethylase, aniline hydroxylase and δ-aminolevulinic acid (ALA) synthetase (Peng et al., 1986). It was also reported that this type of induction was comparable to hepatic enzyme induction patterns generated by the structurally and biologically unrelated drug phenobarbital. A follow up enzyme induction study (Tanaka et al., 1987) also investigated changes in testosterone hydroxylation activity by microsomes in the livers of rats following intraperitoneal injection with phenobarbital (80 mg/kg) and unlabeled 3-methyl-1-cyclopentadecanone. These findings confirmed that 3-methyl-1-cyclopentadecanone causes an increase in mainly CYP2B1/2B2 and to a lesser extent the CYP3A2, 2C6 and 2B1/2B2, which are induced by phenobarbital.

By analogy, and for lack of other available information, cyclohexanone may serve as an example of how higher order macrocyclic ketones may be metabolized. The metabolism of cyclohexanone is well-studied (Eastman Chemical Company, 2007; Martis et al., 1980) and involves reduction of the ketone by carbonyl reductases to generate a secondary cyclic alcohol which may either be converted back to cyclohexanone or conjugated with glucuronic acid and excreted in the bile and urine.

Therefore, reversible reduction of the macrocyclic ketone to a macrocyclic alcohol, followed by excretion, is also a plausible route of primary metabolism for macrocyclic ketones as well and is illustrated below for 3-methyl-1-cyclopentadecanone (CAS RN 541-91-3) (see Fig. 1).

The macrocyclic ketones are larger molecules and more lipophilic than the simpler cyclohexanone (CAS RN 108-94-1) which may affect the route and rate of excretion. For example, the proposed alcohol metabolite of 3-methyl-1-cyclopentadecanone, 3-methyl-1-cyclopentadecanol, has a calculated log K_{ow} of 6.47, which is a more lipophilic than cyclohexanol which has a measured log K_{ow} of 1.23.

4. Toxicokinetics

One toxicokinetic study for the macrocyclic ketone, 3-methyl-1-cylcopentadecanone, was identified.

Following a single intravenous administration of 3-methyl-1-cyclopentadecanone at various doses, kinetics were measured in rats (12, 18, or 24 mg/kg), rabbits (24 mg/kg), and dogs (18 mg/kg) (Zhu et al., 1993). 3-Methyl-1-cyclopentadecanone appears to distribute in rats rapidly and uniformly in the central compartment, and then move to a second peripheral compartment that releases the 3-methyl-1-cyclopentadecanone more slowly. The half life of this two-compartment plasma-concentration curve in the rat was reported to be 118–131 min. In rabbits and dogs, a three-compartment model was reported and included a more rapid distribution in the central compartment, but a slower release from two subsequent peripheral compartments resulting in a half life of 332 and 366 min for rabbits and dogs, respectively (Zhu et al., 1993).

An analysis of nitro-musk compounds in 53 human breast milk samples indicated that there were no samples containing detectable levels of 3-methyl-1-cyclopentadecanone (Zehringer and Herrmann, 2001). The limit of detection for the material was 0.02 μg/kg fat. It was not possible to conclude whether this result could be attributed to a lack of exposure to this chemical in the women sampled or that this chemical does not compartmentalize in breast milk.

5. Toxicological studies

5.1. Acute toxicity

Five macrocyclic ketones have been evaluated for acute dermal toxicity with rats and rabbits (Table 2.1). Dermal LD_{50} values exceeded at least 2000 mg/kg body weight for all for these compounds, and two exceeded 5000 mg/kg.

Seven ketones used in fragrances have been evaluated for acute oral toxicity (Table 2.2). In rats, all seven macrocyclic ketones exceeded an oral LD_{50} value of at least 2000 mg/kg and four of these of exceeded an LD_{50} of 5000 mg/kg. The oral LD_{50} value for the ketone 3-methyl-1-cyclopentadecanone was greater than 2000 mg/kg in dogs. All macrocyclic ketones had LD_{50} values that exceeded the highest dose tested.

Acute intraperitoneal LD_{50} values in rats have been reported for the macrocyclic ketone 3-methyl-1-cyclopentadecanone (1920 mg/kg, see Table 2.3).

Table 2.1
Acute dermal toxicity.

Material	Species	Number per dose group	LD_{50} (mg/kg)	References
Cycloheptadeca-9-en-1-one	Rabbit	2	>2000	RIFM (1974a)
Cyclohexadecanone	Rat	10	>2000[a]	RIFM (2001a)
Cyclopentadecanone	Rabbit	10	>5000	RIFM (1975a)
3-Methyl-1-cyclopentadecanone	Rabbit	10	>5000	RIFM (1977)
3-Methylcyclopentadecenone (mixed isomers)	Rat	10	>2000[a]	RIFM (1991a)

[a] OECD compliant study.

Table 2.2
Acute oral toxicity.

Material	Species	Number per dose group	LD_{50} (mg/kg)	References
Cycloheptadeca-9-en-1-one	Rat	4	>5000	RIFM (1974a)
Cyclohexadecanone	Rat	6	>2000[a]	RIFM (2001b)
5-Cyclohexadecen-1-one	Rat	5	>2000[b]	RIFM (2000a)
Cyclopentadecanone	Mice	NA	>10,000	Caujolle and Caujolle (1965)
	Rat	10	>5000	RIFM (1975a)
4-Cyclopentadecen-1-one, (Z)-	Rat	10	>2000[b]	RIFM (2000b)
3-Methyl-1-cyclopentadecanone	Rat	10	>5000	RIFM (1977)
	Rat	5	>5000	Oh et al. (1997)
	Dog	6	>2000	You et al. (1997)
3-Methylcyclopentadecenone (mixed isomers)	Rat	10	>2000[b]	RIFM (1992a)

NA: data not available in the original report.
[a] OECD, FDA, EEC compliant study.
[b] OECD compliant study.

Table 2.3
Acute intraperitoneal toxicity.

Material	Species	Number per dose group	LD_{50} (mg/kg)	Reference
3-Methyl-1-cyclopentadecanone	Rat	5	1920	Oh et al. (1997)

5.2. Repeat-dose studies

There are few repeat-dose studies available for the macrocyclic ketones. These data are described below and are summarized in Table 3.

5.2.1. Oral studies

Oral toxicological studies have been reported for three macrocyclic ketones (cyclohexadecanone, 3-methyl-1-cyclopentadecanone, and 3-methylcyclopentadecenone).

Cyclohexadecanone was administered by gavage in corn oil to rats (5/sex/dose, 10/sex/dose for vehicle control and high dose) for 28-days at doses of 0, 300, 600, or 1000 mg/kg body weight/day followed by a 2-week treatment free recovery period (animals receiving vehicle control or high dose) (RIFM, 2001c). The animals were observed daily for visible clinical signs and body weight and consumption rates were observed weekly. Hematological, coagulation, and clinical biochemistry parameters were determined prior to the termination of the study. A detailed necropsy and organ weights of adrenals, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes, and thymus were recorded and preserved for further histological examination (high-dose only). No treatment-related differences in clinical symptoms, body weight, or food consumption were reported. The absolute and relative liver weights were increased and fat accumulation was observed in hepatocytes in all treated animals in a similar pattern to the corn oil control group. The authors concluded that these effects were probably caused by the corn oil vehicle. Prothrombin time was reversibly decreased in the female animals without dose dependence. Serum total protein and albumin level were reversibly increased in mid and high-dose female groups. No macro- or microscopic changes were reported. The authors concluded that the NOAEL was 1000 mg/kg body weight/day.

3-Methyl-1-cyclopentadecanone in 0.1% Tween 80 was administered by gavage to rats (5/sex/dose) for 28 days at doses of 0, 10, 100, or 1000 mg/kg body weight/day (Oh et al., 1997). The animals were observed for mortality, changes in body weight, food and water consumption, clinical signs; tested for urine, hematology, and serum biochemical parameters; and organs were weighed and both gross examination and histopathology (heart, liver, spleen, kidney, adrenals, prostate, testes, ovaries, brain, pituitary, and thymus) exams were completed. Liver weights were increased in both sexes in the high-dose groups; however, the authors concluded that no toxicity existed because blood chemistry data and histopathological findings did not show abnormalities. The authors suggested that the NOAEL was 1000 mg/kg body weight/day.

In another study, 3-methyl-1-cyclopentadecanone in 1% Tween 80 was administered by gavage to dogs (3/sex/dose) for 28 days at doses of 0, 0.2, 2 or 20 mg/kg body weight/day (You et al., 1997). The same parameters were observed as for the rat (Oh et al., 1997). The following organs were weighed: heart, liver, spleen, kidney, adrenals, prostate, testes, ovary, uterus, pituitary, thymus, thyroid, submandibular salivary gland, lung, and pancreas. No changes in mortality, clinical signs, body weight, food consumption, urine analyses, or organ weights were observed. Hematological and serum changes were all within normal ranges and not considered to be treatment-related. No adverse effects were observed. Based on the lack of treatment-related effects, the Panel concluded a NOAEL of 20 mg/kg body weight/day.

In a dose range-finding study, 3-methylcyclopentadecenone was administered by gavage in 0.5% aqueous carboxymethylcellulose to rats (3/sex/dose) for 7 days at doses of 0, 500, 750, or 1000 mg/kg body weight/day (RIFM, 1995a). Animals were observed daily,

Table 3
Repeat dose toxicity by oral exposure.

Material	Route and duration	Dose (mg/kg/day)	Species (number/dose)	Results (mg/kg/day)[a]	References
Cyclohexadecanone	28-day gavage	300, 600, 1000 in corn oil	Rat (5/sex, 10/sex)	NOAEL 1000 mg/kg/day No adverse effects	RIFM (2001c)
3-Methyl-1-cyclopentadecanone	28-day gavage	10, 100 or 1000 in 0.1% Tween 80	Rat (10)	NOAEL 1000 mg/kg/day Increased liver weight (relative and absolute) in high dose groups	Oh et al. (1997)
	28-day gavage	0.2, 2, 20 in 1% Tween 80	Dog (6)	NOAEL 20 mg/kg/day No adverse effects	You et al. (1997)
3-Methylcyclopentadecenone (mixed isomers)	7-day gavage	500, 750, or 1000 in 0.5% carboxy methyl cellulose	Rat (3/sex)	LOAEL 500 mg/kg/day (conclusion of Panel) Liver weight (relative and absolute) were higher in all treated male groups and mid and high-dose female groups	RIFM (1995a)
	28-day gavage	250, 500, or 1000 in 0.5% carboxymethyl cellulose	Rat (24, 12)	NOEL 250 mg/kg/day NOAEL 1000 mg/kg/day (conclusion of Panel) Activated partial prothrombin time (PTT) levels increased 21% and 34% in mid and high dose males, respectively. Fibrinogen levels increased 15% in males in high dose. Recovery occurred for both; increased cholesterol in all females (non-dose dependent)	RIFM (1996a)

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

weighed weekly, and subjected to detailed macroscopic necropsy. The following organs were weighed: adrenals, kidney, spleen, brain, liver, testes, heart, ovaries, and thymus. No deaths occurred nor were body weights affected by treatment. At 750 and 1000 mg/kg body weight/day, both males and females exhibited post-dose salivation on days six and seven. Relative and absolute liver weights were higher in all treated male groups and mid- and high-dose female groups, but not in a dose-related manner. This study was followed up with the same compound and route of administration in rats (12/sex/dose) for 28 days at doses 0, 250, 500, or 1000 mg/kg body weight/day. Concurrent high-dose groups of rats were maintained for 2 weeks after treatment to document recovery (RIFM, 1996a). Animals were observed daily, body weights and food consumption were recorded weekly, blood samples were obtained during weeks four and five, urine was collected during week four, and detailed necropsy and organ weights were recorded at sacrifice. A microscopic examination of tissues was performed on control and high dose animals and all gross lesions were examined. Additional blood samples were obtained at the end of the treatment-free period for examination of blood chemistry and coagulation parameters. No deaths,

Table 4.1
Genotoxicity in bacteria.

Material	Test	Bacterial strain	Concentration[a]	Results	References
Cyclohexadecanone	Ames reverse mutation[b]	*Salmonella typhimurium* TA98, TA100, TA1535, or TA1537 ± S9	Up to 1000 μg/plate	Negative	RIFM (2001d)
	DNA damage activity[b]	*Escherichia coli* WP2uvrA ± S9	Up to 1000 μg/plate	Negative	RIFM (2001d)
5-Cyclohexadecen-1-one	Ames reverse mutation[c]	*Salmonella typhimurium* TA98, TA100, TA1535, or TA1537 ± S9	Up to 5000 μg/plate	Negative	RIFM (1996b)
	Ames reverse mutation[b]	*Salmonella typhimurium* TA98, TA100, TA102, TA1535, or TA1537 ± S9	Up to 5000 μg/plate	Negative	RIFM (2000c)
	DNA damage activity	*Escherichia coli* WP2uvrA ± S9	Up to 5000 μg/plate	Negative	RIFM (1996b)
Cyclopentadecanone	Ames reverse mutation[b]	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, or TA1538 ± S9	Up to 5000 μg/plate	Negative	RIFM (1999a)
	Microscreen assay[b]	*Escherichia coli* WP2uvrA ± S9	Up to 5000 μg/plate	Negative	RIFM (1999a)
3-Methyl-1-cyclopentadecanone	Ames reverse mutation[d]	*Salmonella typhimurium* TA98, TA100, TA1535, or TA1537 ± S9	Up to 5000 μg/plate	Negative	RIFM (2004)
	DNA damage activity[d]	*Escherichia coli* WP2uvrA ± S9	Up to 5000 μg/plate	Negative	RIFM (2004)
3-Methylcyclopentadecenone (mixed isomers)	Ames reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, or TA1537 ± S9	Up to 5000 μg/plate	Negative	RIFM (2005)
	DNA damage activity	*Escherichia coli* WP2uvrA ± S9	Up to 5000 μg/plate	Negative	RIFM (2005)
	Ames reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, or TA1537 ± S9	Up to 2000 μg/plate	Negative	RIFM (1991b)

[a] Units may have been converted to make easier comparisons; original units are in the individual Fragrance Material Reviews.
[b] OECD compliant study.
[c] The study was designed in accordance with Japanese guideline: notification no. 1, 24 of the Pharmaceutical Affairs Bureau, Ministry of Health and Welfare.
[d] Study performed under guidelines of Japanese Regulatory Authorities and OECD compliant.

Table 4.2
Genotoxicity in mammalian cells.

Material	Test system	Species/test system	Concentration[a]	Results	References
Cyclohexadecanone	Chromosome aberration[b]	Chinese hamster lung fibroblast V79 cells ± S9	Up to 25 μg/ml (−S9) Up to 50 μg/ml (+S9)	Negative	RIFM (2001e)
Cyclopentadecanone	Chromosome aberration[b]	Human lymphocytes ± S9	Up to 250 μg/ml (±S9)	Negative	RIFM (1999b)
3-Methylcyclopentadecenone (mixed isomers)	Mammalian cell mutation with and without S9 activation[b]	Mouse lymphoma cells L5178Y TK $^{+/-}$	Up to 25 μg/ml (−S9, 3 h exposure and 24 h exposure) Up to 40 μg/ml (+S9, 3 h exposure and 24 h exposure)	Negative	RIFM (2001f)
	Chromosome aberration[b]	Human lymphocytes ± S9	Up to 213 μg/ml (+S9) Up to 53 μg/ml (−S9)	Negative	RIFM (1995b)

[a] Units may have been converted to make easier comparisons; original units are in the individual Fragrance Material Reviews.
[b] OECD compliant study.

treatment-related clinical signs, changes in body weight, food consumption, or ocular effects were recorded. No changes in urinary parameters, organ weights, or microscopic findings were noted. Activated partial prothrombin time increased in the mid- and high-dose males. Fibrinogen levels increased in high-dose males. Two weeks after the last exposure, these levels recovered to normal. Increased cholesterol levels were measured in the treated females; however, this observation was not dose-dependent and returned near normal after 2-week treatment-free period. The NOEL was 250 mg/kg body weight/day, apart from a marginal increase in female cholesterol levels, which was considered unlikely to be of any toxicological significance.

5.2.2. Dermal studies

No repeat-dose dermal toxicity studies were available for the macrocyclic ketones.

5.2.3. Inhalation studies

No repeat-dose inhalation toxicity studies were available for the macrocyclic ketones.

6. Genotoxicity studies

6.1. Bacteria

Five of the macrocyclic ketones have been studied in the reverse mutation assays with *Salmonella typhimurium* (Ames test), or *Escherichia coli WP2uvrA* strains. These compounds, cyclohexadecanone, 5-cyclohexadecen-1-one, cyclopentadecanone, 3-methyl-1-cyclopentadecanone and 3-methylcyclopentadecenone, were all inactive at producing reverse mutations in *S. typhimurium* including strains TA97, TA98, TA100, TA102, TA1535, TA1537, or TA1538. The assays were performed at concentrations ranging up to cytotoxicity, both in the presence and in the absence of metabolic activation (S9 fraction) obtained from the livers of Aroclor- or methylcholanthrene-induced rats or hamsters. The same materials did not produce mutations in *E. coli WP2uvrA* strains with or without metabolic activation.

6.2. Mammalian cell lines

Three macrocyclic ketones have been studied *in vitro* by analyzing chromosomal aberrations in activated or non-activated human lymphocytes, mouse lymphoma cells or Chinese hamster lung fibroblast V79 cells. No chromosomal aberrations have been reported with the macrocyclic ketones (cyclohexadecanone, cyclopentadecanone, 3-methylcyclopentadecenone) (RIFM, 1999b, 1995b, 2001f,e).

7. Carcinogenicity

No bioassays or long-term chronic studies for macrocyclic ketones were available.

8. Reproductive toxicity

One macrocyclic ketone, 3-methylcyclopentadecenone has been tested for reproductive toxicity (Table 5).

Table 5
Reproductive toxicity.

Material	Method	Dose (mg/kg/day)	Species (number/dose)	Results[a]	References
3-Methyl cyclopentadecenone (mixed isomers)	One generation[b] – 12 week gavage through maturation, mating, gestation and lactation	50, 250, or 1000 in 1% carboxymethyl cellulose for	Rat (28/sex)	Maternal: NOAEL 1000 mg/kg/day Fetal: NOEL 1000 mg/kg/day Maternal: increased salivation considered to be an adaptive response to oral administration of unpleasant tasting material; increased liver weights in males at 250 and 1000 mg/kg/day; increased incidence of hepatocyte enlargement for both sexes at 250 and 1000 mg/kg/day and females only at 50 mg/kg/day. Adult toxicity considered to be adaptive and non-specific responses and not an adverse effect Fetal: no treatment effects (litter size, viability, growth and physical development) on fertility or reproductive performance	RIFM (2003a)

[a] Units may have been converted to make easier comparisons; original units are in the individual Fragrance Material Reviews.
[b] OECD compliant study.

In this OECD compliant study (test guidelines 415), 3-methylcyclopentadecenone in 1% carboxy methylcellulose was administered by gavage to rats (28/sex/dose) throughout maturation, mating, gestation and lactation at doses of 0, 50, 250 or 1000 mg/kg body weight/day (RIFM, 2003a). Animals were observed daily; body weights and food consumption were recorded weekly. Offspring of these animals were observed daily for clinical signs and developmental landmarks; litter size and pup body weights were recorded on specific days. Macroscopic examinations were performed on all adults and offspring. Histopathology was performed on reproductive and target organs. In adults, there were no effects on fertility or reproductive performance observed at any dose. Response in adults (post-dose salivation, increased liver weights and hepatocyte enlargement) were considered to be adaptive and non-specific and not an adverse effect. Based on these findings the Panel has concluded a NOAEL 1000 mg/kg body weight/day and LOEL 50 mg/kg body weight/day. In offspring, there were no significant treatment related effects on litter size at birth or during lactation nor were there differences in offspring growth and physical development during lactation. The NOEL for reproduction and offspring viability was 1000 mg/kg body weight/day.

In an effort to determine if a macrocyclic ketone had any estrogenic activity, the ketone 3-methyl-1-cyclopentadecanone, was evaluated for its ability to increase proliferation of an estrogen receptor-positive human mammary carcinoma cell line (MCF-7). Based on the proliferation, 3-methyl-1-cyclopentadecanone was weakly active; however, this result was considered by the authors to be a negligible effect (Bitsch et al., 2002).

9. Irritation

9.1. Human studies

A considerable amount of data has been collected regarding human irritation from the macrocyclic ketones. Six macrocyclic ketones were evaluated for skin irritation in approximately 660 male and female volunteers at dose levels ranging from 0.5% to 30% (see individual studies listed in Table 6.1). Of the ketones tested, cyclopentadecanone; 4-cyclopentadecen-1-one, (Z)-; 5-cyclohexadecen-1-one; cycloheptadeca-9-en-1-one; 3-methyl-1-cyclopentadecanone and 3-methylcyclopentadecenone, none appeared to produce any irritation during closed patch test, repeat-insult patch test, or during the pretesting phase of a maximization test.

9.2. Animal studies

9.2.1. Skin irritation

Irritation reactions were identified for six macrocyclic ketones with a range of reactions from moderate to none (Table 6.2). Irritation studies on animals included observations from acute dermal toxicity tests, primary irritation tests on the skin of rabbits, phototoxicity control treatments, and irritation prior to or during sensitization tests. Irritations noted during the challenge phases of sensitization studies were not included; please refer to McGinty et al. (in press-a–h) for more details.

In general this group of macrocyclic ketones caused no irritation or slight temporary irritation, which usually dissipated within 24–72 h. Members of this category include cycloheptadeca-9-en-1-one; cyclohexadecanone; 5-cyclohexadecen-1-on; cyclopentadecanone; 4-cyclopentadecen-1-one, (Z)-; 3-methyl-1-cyclopentadecanone and 3-methylcyclopentadecenone (mixed isomers). Overall, less irritation occurred in fewer animals as the topical dose decreased.

9.2.2. Mucous membrane (eye) irritation in rabbits

The potential for three macrocyclic ketones to cause mucous membrane irritation in the eye has been evaluated by the Draize test or modified Draize test in rabbits at concentrations ranging from 30% to 100% in various vehicles (Table 7). Under criteria described in OECD, EEC, CFR or FDA directives, cyclopentadecanone, cyclohexadecanone and 3-methylcyclopentadecenone were considered non-irritating to the eyes.

10. Skin sensitization

This group of macrocyclic ketones has been evaluated for the potential to induce sensitization. The details of the individual studies can be found in Tables 8.1a,b and 8.2a,b, or within the individual Fragrance Material Reviews (FMRs).

10.1. Human studies

10.1.1. Induction of human sensitization

Induction of dermal sensitization was measured by standard human repeat-insult patch tests (HRIPT) and maximization tests in 545 male and female volunteers for six of the macrocyclic ketones (Table 8.1a). Of these materials none showed evidence of sensitization. All studies had control volunteers.

Table 6.1
Skin irritation in humans.

Material	Method[a]	Concentration	Subjects	Results	References
Cycloheptadeca-9-en-1-one	Maximization pre-test	4% in petrolatum	5	0/5	RIFM (1974b)
	Closed patch test	0.5–5% (animal source) in EtOH or cream base	35	0/35	Takenaka et al. (1986)
	Closed patch test	0.5–5% (synthetic) in EtOH or cream base	60	0/60	Takenaka et al. (1986)
5-Cyclohexadecen-1-one	Irritation (HRIPT)	6% in EtOH	49	0/49	RIFM (2000d)
Cyclopentadecanone	Irritation (HRIPT)	2% in DMP	54	0/54	RIFM (1972)
	Maximization pre-test	10% in petrolatum	5	0/5	RIFM (1975b)
	Closed patch test	0.5–5% (animal source) in EtOH or cream base	60	0/60	Takenaka et al.(1986)
4-Cyclopentadecen-1-one, (Z)-	Irritation (HRIPT)	10% in DEP	106	0/106	RIFM (1998a)
	Irritation (HRIPT)	2% in 3:1 EtOH:DEP	51	0/51	RIFM (1998b)
3-Methyl-1-cyclopentadecanone	Maximization pre-test	30% in petrolatum	25	0/25	RIFM (1976b)
3-Methylcyclopentadecenone (mixed isomers)	Irritation (HRIPT)	10% in DEP	102	0/102	RIFM (1995c)
	Irritation (HRIPT)	20% in DEP	108	0/108	RIFM (1999c)

[a] Irritation is observed as part of a Human Repeated Insult Patch Test (HRIPT). Induction generally consists of nine induction patches and one challenge patch. Irritation reported in this table is during the induction phase only. Patch applications are 24 h in duration unless otherwise noted. Maximization pre-tests are 48 h in duration.

Table 6.2
Skin irritation in animals.

Material	Method	Concentration	Species (number)	Results	References
Cycloheptadeca-9-en-1-one	Irritation (LD_{50})	100%	Rabbit (2)	2/2	RIFM (1974a)
Cyclohexadecanone	Irritation[a] (4 h occluded)	50%, 25%, 10% in EtOH:DEP	Rabbit (4)	0/4 at all doses	RIFM (2001g)
	Irritation[a] (LD_{50})	100%	Rabbit (10)	0/10	RIFM (2001a)
	Irritation (Maximization)	25% in EtOH:DEP	Guinea pig (20)	0/20	RIFM (2001h)
5-Cyclohexadecen-1-one	Irritation[b] (4 h semi-occluded)	100%	Rabbits (3)	0/3	RIFM (1999d)
	Irritation (phototoxicity control)	20%, 5%, or 1% in EtOH	Guinea pig (5)	0/5	RIFM (2000e)
	Irritation[a] (OET induction)	30%, 10%, or 5% in EtOH (topical induction, 5 d/wk, for 4 weeks)	Guinea pig (6)	6/6 at 30% and 10%, 1/6 at 5%	RIFM (1999e)
	Irritation (phototoxicity control)	30%, 10%, or 5% in acetone	Guinea pig (5)	5/5 at 30%, 1/5 at 10%, 0/5 at 5%	RIFM (1982a)
	Irritation (Maximization)	10% in FCA	Guinea pig (8)	0/8 at 10%	RIFM (1982b)
	Irritation (Maximization)	20% in FCA	Guinea pig (5)	0/5 at 20%	RIFM (2000f)
Cyclopentadecanone	Irritation (LD_{50})	100%	Rabbits (10)	7/10	RIFM (1975a)
	Irritation (phototoxicity control)	10% in EtOH	Rabbit (3)	0/3	RIFM (1978)
	Irritation (phototoxicity control)	50% in DEP, 10% in EtOH	Guinea pig (3)	0/3 (DEP);0/3 (EtOH)	RIFM (1978)
	Irritation (phototoxicity control)	10% in EtOH	Guinea pig (10)	1/10 slight irritation at 48 h	RIFM (1986a)
	Irritation (OET screen)	1%, 3%, 10%, 30% in EtOH (topicial)	Guinea pig (6)	0/6	RIFM (1986b)
	Irritation (FCAT pre-test)	1%, 3%, 10%, 30% in EtOH (topical)	Guinea pig (4)	0/4	RIFM (1986d)
	Irritation (FCAT induction)	5% (i.d.) in FCA	Guinea pig (20)	0/20	RIFM (1986d)
4-Cyclopentadecen-1-one, (Z)-	Irritation (Maximization)	40% (i.d.) in mineral oil 80% in acetone (topical)	Guinea pig (10)	1/10	RIFM (1998c)
3-Methyl-1-cyclopentadecanone	Irritation (LD_{50})	100%	Rabbit (10)	10/10	RIFM (1977)
	Irritation (phototoxicity control)	50% in DEP 10% in EtOH	Guinea pig (3)	Mild to weak irritation (DEP); no irritation (EtOH)	RIFM (1978)
	Irritation (phototoxicity control)	50% in EtOH	Rabbit (3)	Weak irritation	RIFM (1978)
3-Methylcyclopentadecenone (mixed isomers)	Irritation[c] (4 h semi-occluded)	100%	Rabbit (4)	4/4	RIFM (1992b)
	Irritation (Maximization topical pre-test)	100%, 50%, 25%, 12.5% in EtOH	Guinea pig (4)	0/4 at all doses	RIFM (1992c)
	Irritation (Maximization)	100% or 50% in EtOH	Guinea pig (20)	0/20	RIFM (1992c)
	Irritation (Modified Buehler pretest)	100%, 75%, 50%, 25%	Guinea pig (4)	0/4 at all doses	RIFM (1999f)
	Irritation (Modified Buehler)	100%	Guinea pig (20)	1/20	RIFM (1999f)
	Irritation[a] (Maximization)	100% (induction)	Guinea pig (20)	6/20	RIFM (2000g)

[a] OECD compliant study.
[b] OECD and EEC compliant study.
[c] EEC compliant study.

Table 7
Mucous membrane (eye) irritation studies in rabbits.

Material	Dose (No. animals)	Results	References
Cyclohexadecanone	100% ($n = 4$)	Not irritating under EEC guidelines	RIFM (2001i)
Cyclopentadecanone	100% or 30% ($n = 3$) *Vehicle not reported	Not irritating under OECD guidelines	RIFM (1986e)
3-Methylcyclopentadecenone (mixed isomers)	100% ($n = 4$)	Not irritating under EEC guidelines	RIFM (1992d)

10.1.2. Diagnostic patch-tests

Diagnostic patch-test studies have been reported for two macrocyclic ketones (Table 8.1b).

One-hundred seventy-eight fragrance-sensitive patients were patch tested in eight centers worldwide with 5% cyclopentadecanone in petrolatum. Three volunteers (1.7%) had positive reactions (Larsen et al., 2001).

Seventy-four contact dermatitis patients were patch tested with 0.2%, 0.5%, 1%, 2%, 5% or 10% 5-cyclohexadecen-1-one in petrolatum. None of the patients had positive reactions to the test material (RIFM, 1976a).

10.2. Animal studies

Five macrocyclic ketones were evaluated for sensitization in guinea pigs using various test methods including the Magnusson–Kligman Maximization test, a Modified Buehler delayed hypersensitivity test, and the Open Epicutaneous Test (Table 8.2a). Of the macrocyclic ketones tested in the Maximization test, three reported sensitization in animals receiving the highest dose during challenge. These included 100% 3-methylcyclopentadecenone, 30% cyclopentadecanone and 20% 5-cyclohexadecen-1-one.

Table 8.1a
Skin sensitization in humans.

Material	Method	Concentration	Subjects	Results	References
Cycloheptadeca-9-en-1-one	Maximization	4% in petrolatum (2760 μg/cm^2)	25	0/25	RIFM (1974b)
5-Cyclohexadecen-1-one	HRIPT	6% in EtOH (3000 μg/cm^2)	49	0/49	RIFM (2000d)
Cyclopentadecanone	HRIPT	2% in DMP (2360 μg/cm^2)	54	0/54	RIFM (1972)
	Maximization	10% in petrolatum (6900 μg/cm^2)	25	0/25	RIFM (1975b)
4-Cyclopentadecen-1-one, (Z)-	HRIPT	10% in DEP (5000 μg/cm^2)	106	0/106	RIFM (1998a)
	HRIPT	2% in EtOH:DEP (1100 μg/cm^2)	51	0/51	RIFM (1998b)
3-Methyl-1-cyclopentadecanone	Maximization	30% in petrolatum (20,700 μg/cm^2)	25	0/25	RIFM (1976b)
3-Methylcyclopentadecenone (mixed isomers)	HRIPT	10% in DEP (5000 μg/cm^2)	102	0/102	RIFM (1995c)
	HRIPT	20% in DEP (10,000 μg/cm^2)	108	0/108	RIFM (1999c)

Table 8.1b
Diagnostic patch tests in humans.

Material	Concentration	Subjects	Results (frequency)	References
Cyclopentadecanone	5% in petrolatum	178 Fragrance sensitive patients	3/178 (1.7%)	Larsen et al. (2001)
5-Cyclohexadecen-1-one	0.2%, 0.5%, 1%, 2%, 5% or 10% in petrolatum	74 Contact dermatitis patients	0/74	RIFM (1976a)

Table 8.2a
Skin sensitization in animals.

Material	Method	Induction	Challenge	Species (No./group)	Results	References
Cyclohexadecanone	Maximization	5% in arachis oil or FCA (intradermal); 25% in EtOH:DEP (topical)	25% in EtOH:DEP	Guinea pig (20)	0/20	RIFM (2001h)
5-Cyclohexadecen-1-one	Photo-sensitization (control)	10% in EtOH	3% in EtOH	Guinea pig (10)	0/10	RIFM (1986c)
	Open epicutaneous test	30%, 10%, 5% in EtOH	1%, 0.5%, 0.1%, or 0.05% in EtOH	Guinea pig (6)	0/6	RIFM (1999e)
	Maximization	10% in FCA (intradermal); 10% in petrolatum (topical)	20%, 10%, or 5% in acetone	Guinea pig (8)	5/8 at 20%, 1/8 at 10%, 0/8 at 5%	RIFM (1982b)
	Maximization	20% in FCA (intradermal); 20% in FCA (topical)	20%, 5%, 1% or 0.2% in ethanol	Guinea pig (5)	5/5 at 20%, 0/5 at 10%, 5% and 0.2%	RIFM (2000f)
Cyclopentadecanone	Open epicutaneous test	30%, 10%, 3%, 1% in EtOH	30% in EtOH	Guinea pig (6)	0/6	RIFM (1986b)
	FCAT	5% in FCA (intradermal)	30%, 10%, 3% or 1% in EtOH (topical)	Guinea pig (20)	4/20 at 30%, 0/20 at 10%, 3% and 1%	RIFM (1986d)
4-Cyclopentadecen-1-one, (Z)-	Maximization	40% in mineral oil or FCA (intradermal); 80% in acetone (topical)	5% or 2.5% in acetone	Guinea pig (10)	0/10	RIFM (1998c)
3-Methylcyclopentadecenone (mixed isomers)	Maximization	50% in FCA and 25% in parrafin or FCA (intradermal); 100% (topical)	100% or 50% in EtOH	Guinea pig (20)	12/20 at 100% (first challenge), 4/20[a] at 100% (second challenge); 0/20 at 50%	RIFM (1992c)
	Modified Buehler	100%	50% in mineral oil (topical)	Guinea pig (20)	0/20	RIFM (1999f)
	Maximization[b]	40% in mineral oil or 50% in FCA (intradermal); 100% (topical)	100% (topical)	Guinea pig (20)	0/20	RIFM (2000g)

[a] Authors of study concluded 4/20 sensitization reactions; but sponsors indicate it may also be irritation reactions.
[b] OECD compliant study.

In one local lymph node assay, 20% 3-methylcyclopentadecenone was labeled as sensitizing because of the increased percent of B220+ cells in the treated animals (test to vehicle ratio was greater than 1.25); however, it did not induce ear swelling in the mouse or increase the lymph node cell counts (RIFM, 2003b). In a local lymph node assay, 100%, 50% and 25% concentrations of the same material were labeled as sensitizing (stimulation index increased by greater than 3-fold at each concentration). The calculated EC3 for the material was 5.7% (RIFM, 2000h).

11. Phototoxicity and photosensitization

UV spectra have been obtained on nine of the macrocyclic ketones. All nine had maximum absorbance between approximately 190–200 nm, with the majority showing absorbance between 200 and 250 nm and returning to baseline by 300 nm (Table 11). Four of the macrocyclic ketones were assessed in guinea pigs or rabbits for phototoxicity and one was tested for photosensitization in guinea pigs (Tables 9 and 10).

Table 8.2b
Local lymph node assays (LLNA).

Material	Method	Dose	Species (No./group)	Results	References
3-Methylcyclopentadecenone (mixed isomers)	LLNA	20%, 10%, 3% in acetone:olive oil (topical)	Mouse (3)	Sensitizing at 20% only (test: vehicle ratio of %B220+ cells was greater than 1.25); lymph node cell counts, however were not increased	RIFM (2003b)
	LLNA	100%, 50%, 25% in acetone:olive oil (4:1)	Mouse (6)	Sensitizing (all concentrations caused a 3-fold or greater stimulation index(SI)); calculated EC_3 of the test material was 5.7%	RIFM (2000h)

Table 9
Phototoxicity.

Material	Method	Concentration	Species (number/group)	Results	References
Cycloheptadeca-9-en-1-one	Phototoxicity	20% or 1% in petrolatum or EtOH 1.6–7.6 J/ cm^2 UVA	Guinea pig (5)	0/5 phototoxic reactions	Ohkoshi et al. (1981) and Ogoshi et al. (1980)
5-Cyclohexadecen-1-one	Phototoxicity	20% or 1% in petrolatum or EtOH for 2 h 1.6–7.6 J/ cm^2 UVA	Guinea pig (5)	0/5 phototoxic reactions	Ohkoshi et al. (1981) and Ogoshi et al. (1980)
	Phototoxicity	30%, 10%, or 5% in acetone 13 J/cm^2 UVA	Guinea pig (5)	0/5 phototoxic reactions (all doses)	RIFM (1982a)
	Phototoxicity	20, 5 or 1% in EtOH 11 J/ cm^2 UVA	Guinea pig (5)	0/5 phototoxic reactions (all doses)	RIFM (2000e)
Cyclopentadecanone	Phototoxicity	20% in petrolatum or EtOH 1.6–7.6 J/ cm^2 UVA	Guinea pig (5)	0/5 phototoxic reactions	Ohkoshi et al. (1981) and Ogoshi et al. (1980)
	Phototoxicity	50% in DEP or 10% in EtOH	Guinea pig (3)	Weakly phototoxic at 50% at 24, 48 and 72 h; not phototoxic at 10% at 24, 48 or 72 h *No information on individual test subject reaction	RIFM (1978)
	Phototoxicity	10% in EtOH	Rabbit (3)	Not phototoxic at 24, 48 or 72 h *No information on individual test subject reaction	RIFM (1978)
	Phototoxicity	10% in EtOH 20 J/cm^2 UVA	Guinea pig (10)	0/10 phototoxic reactions	RIFM (1986a)
3-Methyl-1-cyclopentadecanone	Phototoxicity	20% or 1% in petrolatum or EtOH 1.6–7.6 J/ cm^2 UVA	Guinea pig (5)	0/5 phototoxic reactions	Ohkoshi et al. (1981) and Ogoshi et al. (1980)
	Phototoxicity	50% in DEP or 10% in EtOH	Guinea pig (3)	Moderately phototoxic at 50% at 24 and 72 h; not phototoxic at 10% at 24, 48 or 72 h *No information on individual test subject reaction	RIFM (1978)
	Phototoxicity	10% in EtOH	Rabbit (3)	Not phototoxic at 24, 48 or 72 h *No information on individual test subject reaction	RIFM (1978)

Table 10
Photosensitization in animals.

Material	Method	Concentration	Species (number/group)	Results	Reference
Cyclopentadecanone	Photo-sensitization (OET)	10% in EtOH induction; 3% in EtOH challenge 10 J/cm^2 UVA 1.8 J/cm^2 UVB	Guinea pig (10)	0/10	RIFM (1986c)

11.1. Phototoxicity

Cycloheptadeca-9-en-1-one, 5-cyclohexadenen-1-one, cyclopentadecanone and 3-methyl-1-cyclopentadecanone (1% or 20% in petrolatum or ethanol) were tested for phototoxicity in guinea pigs (Ogoshi et al., 1980; Ohkoshi et al., 1981). After a 2-hour application, UVA irradiation (300–430 nm) at 15–20 cm from the skin was carried out for 30, 60, or 120 min at an approximate energy level of 1.6–7.6 J/cm^2. No phototoxicity was reported for these ketones. In a similar study, cyclopentadecanone and 3-methyl-1-cylopentadecanononе were tested for phototoxicity (3/dose) in guinea pigs or rabbits at 10% or 50% in EtOH or DEP (RIFM, 1978). The authors concluded that the macrocyclic ketones tested were not phototoxic (RIFM, 1978).

11.2. Photosensitization

Photosensitization studies were performed on guinea pigs with 10% (induction) and 3% (challenge) cyclopentadecanone in EtOH followed by an irradiation dose of 10 J/cm^2 UVA (RIFM, 1986c). It has been concluded that no photosensitization occurred.

Table 11
Summary of UV spectra data.

Material	UV spectra range of absorption (nm)
Cycloheptadeca-9-en-1-one	Maximum at 200. Absorbance between 200 and 270, second peak at 233. Baseline by 300
Cyclohexadecanone	Maximum at 190. Absorbance between 190–200 and 210–310. Second distinct peak at 280. Baseline by 340
5-Cyclohexadecen-1-one	Maximum at 190. Absorbance between 190 and 210. Return to baseline by 240
Cyclohexadec-8-en-1-one (mix of *cis* and *trans* isomers)	Maximum at 190. Absorbance between 190 and 200. Return to baseline by 210
Cyclopentadecanone	Maximum at 190. Absorbance between 190 and 310. Return to baseline by 330
4-Cyclopentadecen-1-one	Maximum at 190. Absorbance between 190 and 210. Baseline by 220
4-Cyclopentadecen-1-one, (Z)-	Maximum at 208. Absorbance between 208 and 310. Return to baseline by 310
3-Methyl-1-cyclopentadecanone	Maximum at 200. Absorbance from 200 to 310, with distinct peaks at 243 and 284. Baseline by 310
3-Methylcyclopentadecenone (mixed isomers)	Maximum at 201. Some absorbance from 200 to 270. Return to baseline by 270

12. Conclusions

The macrocyclic ketones may be reversibly metabolized to a macrocyclic alcohol which may then be conjugated with glucuronic acid and excreted. No *in vivo* mammalian metabolite studies utilizing radiolabeled materials are currently available for the macrocyclic ketones. These types of studies would be necessary to fully substantiate the proposed metabolic pathways illustrated in Fig. 1 and to conclusively identify metabolites.

The macrocyclic ketones have not been evaluated at exposure levels other than those reported in this group summary. Use of these fragrance ingredients beyond the higher maximum dermal levels or higher systemic exposure levels requires revaluation by the Panel. It is the opinion of the Expert Panel that safety concerns regarding the use of the macrocyclic ketones are not indicated under the anticipated levels of exposure for their use in fine fragrance and consumer products. Since all the short term and repeated dose studies revealed a low toxicity, this conclusion applies to the MK group of fragrance ingredients including their metabolites.

The following general conclusions can be made for macrocyclic ketones based on the available and reviewed data provided by RIFM and additional literature searches. MK fragrance ingredients have:

- Low acute toxicity.
- No significant toxicity was observed in repeat dose oral or dermal toxicity studies. There were no treatment-related effects at the highest doses tested, which are substantially higher than consumer exposure. Effects on liver weight and blood biochemistry effects were reversible after two weeks of no treatment.
- No genotoxic activity in bacteria or mammalian cell lines was observed. Therefore, although carcinogenicity studies are lacking, this evidence is not indicative of potential carcinogenicity via the genotoxic mechanism.
- No reproductive toxicity was reported for the macrocyclic ketone 3-methylcyclopentadecenone in an OECD compliant study. The NOEL for this material was 1000 mg/kg body weight/day, and no effect on reproduction was observed.
- Human dermatological studies show that these fragrances ingredients are not irritating after one application. Animal studies indicate that irritation occurs for some materials (4-cyclopentadecen-1-one, (Z)-, 5-cyclohexadecen-1-one, cycloheptadeca-9-en-1-one, 3-methyl-1-cyclopentadecanone and 3-methylcyclopentadecenone) at high concentrations. Of these, all but 4-cyclopentadecen-1-one and 5-cyclopentadecen-1-one, were at concentrations of 30% or 100%, which are not consistent with the currently recommended concentrations of fragrance ingredients in consumer products and fine fragrances. Likewise, the potential for eye irritation at the present maximum use is considered minimal.
- No phototoxicity or photosensitization was observed at rates that are consistent with estimated levels for current human exposures.
- Among 178 patients with known previous positive patch tests to fragrance allergens, 3 (1.7%) reacted to cyclopentadecanone. None of 74 contact dermatitis patients reacted to a patch test of 5-cyclohexadecen-1-one. Animal studies have also demonstrated that these fragrance ingredients are sensitizers only at concentrations of 20%, 30% or 100%, which are higher than current consumer exposure. All other materials in this group showed no evidence of dermal sensitization.
- To calculate margin of safety, the lowest NOAEL of 20 mg/kg body weight/day (28-day gavage study of 3-methyl-1-cyclopentadecanone in rats) is used as a representative worst case scenario for the group (assuming 100% oral absorption). Using the highest systemic exposure for the group (0.04 mg/kg body weight/day for 5-cyclohexadecen-1-one) again, as a representative worst case scenario, and assuming 100% dermal absorption, the margin of safety is calculated to be 500. If a margin of safety of 100 were used, the maximum allowable exposure would be 0.2 mg/kg body weight/day.

Conflict of Interest

This research was supported by the Research Interest for Fragrance Materials, an independent research institute that is funded by the manufacturers of fragrances and consumer products containing fragrances. The authors are all members of the Expert Panel of the Research Institute for Fragrance Materials, an independent group of experts who evaluate the safety of fragrance materials.

Acknowledgements

The Panel wishes to express its sincere appreciation to Dr. A.E. Rogers for her help and guidance in the preparation of this manuscript.

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