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Review

A toxicological and dermatological assessment of macrocyclic lactone and lactide derivatives when used as fragrance ingredients ☆

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ABSTRACT

The Macrocyclic Lactone and Lactide derivative (ML) group of fragrance ingredients was critically evaluated for safety following a complete literature search. For high end users, calculated maximum dermal exposures vary from 0.47% to 11.15%; systemic exposures vary from 0.0008 to 0.25 mg/kg/day. The MLs had low acute toxicity and no significant toxicity in repeat dose oral ordermal toxicity studies. Effects on blood biochemistry were reversible after 2 weeks of no treatment. No mutagenic or genotoxic activity in bacteria and mammalian cell line assays was observed. Reproductive and developmental toxicity was not observed. Human dermatological studies show MLs are generally not irritating after one application. Minor irritation was observed in a few individuals following multiple applications. At rates consistent with reported levels for current human exposure, no phototoxicity or photosensitization was observed. In animal studies, the MLs are not sensitizers at lower exposures from consumer products. Eleven ML materials were evaluated for human sensitization. Of these, only ethylene brassylate showed evidence of sensitization in 2/27 studies (sensitization frequency 4/2059 total). Based on these findings, the Panel is of the opinion that there are no safety concerns for the MLs at reported levels of use and exposure as fragrance ingredients.

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1. Introduction

In 2010 complete literature searches were conducted on the macrocyclic lactone and lactide (ML) derivative 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 ML derivatives 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 submitted for publication, 2011a–l). A complimentary environmental group summary document for the macrocyclic ketone and lactone/lactide subgroups has also been prepared (Salvito et al., 2011).

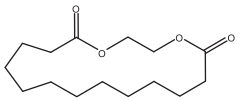
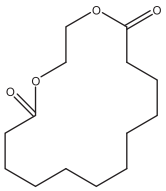
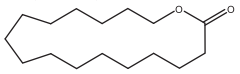
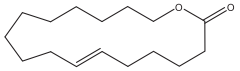
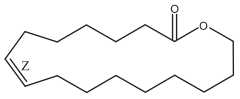
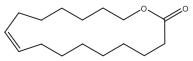
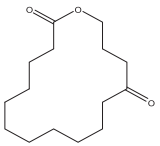
2. Chemical identity, regulatory status, and exposure

In 1998 and 2002 the International Joint FAO/WHO Expert Committee on Food Additives (JECFA) conducted and published relevant *Safety Evaluations of Certain Food Additive Safety and Contaminants* that included Aliphatic Lactones (JECFA, 1998) and Aliphatic Primary Alcohols, Aldehydes, Carboxylic Acids, Acetals and Esters Containing Additional Oxygenated Functional Groups (JECFA, 2002) that included ML flavoring agents. These publications, some of which also include the toxicology for ML fragrance ingredients described herein, were generally judged by JECFA not to present a human health safety concern at the current levels of exposures as food additives.

In the United States (US) the regulatory status of some fragrance ingredient substances (ethylene brassylate RN 105-95-3; ω-pentadecalactone RN 106-02-5; and ω-6-hexadecenlactone RN 7779-50-2) been approved by the Food and Drug Administration (FDA) as synthetic flavoring substances and food adjuvants in accordance with 21 CFR 172.515. The Flavor and Extract Manufacturers Association (FEMA) member companies have reviewed some of the ML fragrance ingredients and acknowledged them to be Generally Recognized as Safe (GRAS) for use as flavor ingredients. These include the mono ester lactones ώ-6-hexadecenlactone (CAS RN 7779-50-2); ώ-pentadecalactone (CAS RN 106-02-5); and the diester lactone ethylene brassylate (CAS RN 105-95-3). FEMA has also designated oxacycloheptadec-10-ene-2-one (CAS RN 28645-51-4) and ethylene brassylate (CAS RN 105-95-3) as Generally Recognized as Safe as a flavor ingredients – GRAS.

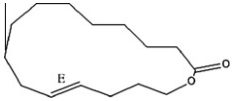
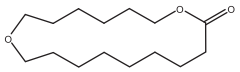
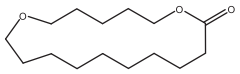
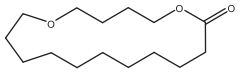
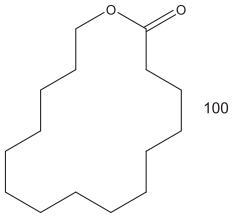
(E) and (Z)-oxacylcohexadec-(12 or 13)-en-2-one is registered in the US TSCA under four CAS numbers: 111879-79-9, 111879-80-2, 111879-81-3 and 99219-32-6. It is also registered under 0034902-57-3 in Canada, Australia, Korea and China. Four of them refer to the different isomers, while the latter refers to the non-stereo specific structure. ω-6-Hexadecenlactone (RN 7779-50-2); 16-hydroxy-7-hexadecenoic acid lactone (RN 123-69-3); and oxacycloheptadec-10-ene-2-one (RN 28645-51-4) are recognized as isomer components arising from the source and quality of the starting Aleuritic Acid. Table 1 provides a list of the ML fragrance ingredients that are evaluated in this report along with their Chemical Abstract Service registration numbers (CAS RN), synonyms, structural formulas, and some physiochemical

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] (%)
Ethylene brassylate $C_{15}H_{26}O_4$ CAS # 105-95-3 Log K_{ow} 4.7 @ 24 °CMolecular Weight: 270.37 Vapor pressure: 4.38e−007 mm Hg @ 25 °Cmm Hg @ 25 °C[e] Water solubility: 1.719 mg/l @ 25 °C[e]	• Astratone • Cyclo-1,13-ethylenedioxytridecan-1,13-dione • 1,4-Dioxacycloheptadecane-5,17-dione • Ethylene glycol brassylate,cyclic diester • Ethylene undecane dicarboxylate • Musk T • Tridecanedioic acid cyclic ethylene glycol diester		>1000	0.25	11.15%
Ethylene dodecanedioate $C_{14}H_{24}O_4$ CAS # 54982-83-1 Log K_{ow} 3.65 at 20 °C Molecular Wt: 256.35 Vapor pressure: 0.028 Pa at 25 °C Water solubility: 7.5×10^{-2} g/l at 20 °C	• Arova 16 • Cyclic ethylene dodecanedioate • 1,4-Dioxacyclohexadecane-5,16-dione • Musk 144 • Musk C-14 • Zenolide • Musk MC-4		100–1000	0.11	4.29
Hexadecanolide $C_{16}H_{30}O_2$ CAS # 109-29-5 Log K_{ow} 6.65[e] Molecular weight: 254.42 Vapor pressure: 2.48e−005 mm Hg @ 25 °C[e] Water solubility: 0.04727 mg/l @ 25 °C[e]	• Cyclohexadecanolide • Dihydro ambrettolide • Hexadecanolactone • 16-Hydroxyhexadecanoic acid lactone • Oxacycloheptadecan-2-one		10–100	0.0008	1.15
ω-6-Hexadecenlactone $C_{16}H_{28}O_2$ CAS # 7779-50-2 Log K_{ow} 5.37[e] Molecular weight: 252.4 Vapor pressure: 2.24e−005 mm Hg 25 °C[e] Water solubility: 0.5925 mg/l @ 25 °C[e]	• Ambrettolide (-2-one) • Hexadec-6-en 1,16-lactone • 6-Hexadecenolide • 16-Hydroxy-6-hexadecenoic acid,omega-lactone • Oxacycloheptadec-7-en-2-one		0.1–1	0.05	0.47
16-Hydroxy-7-hexadecenoic acid lactone $C_{16}H_{28}O_2$ CAS # 123-69-3 Log K_{ow} 5.37[e] Molecular weight: 252.39 Vapor pressure: 2.24e−005 mm Hg 25 °C[e] Water solubility: 0.5925 mg/l @ 25 °C[e]	• Ambrettolide (-16-olide) • Hexadec-7-en-16-olide • Musk ambrette (natural) • Oxacycloheptadec-8-en-2-one • Oxacycloheptadec-8-en-2-one,(8Z)- • Cyclohexadecen-7-olide		0.01–0.1	0.0005[f]	0.02[f]
Oxacycloheptadec-10-ene-2-one $C_{16}H_{28}O_2$ CAS # 28645-51-4 Log K_{ow} 6.7 at 23 °C Molecular weight: 252.39 Vapor pressure: 2.24e−005 mm Hg 25 °C[e] Water solubility: 0.5925 mg/l @ 25 °C[e]	• Ambrettolide • 9-Hexadecenoic acid,16-hydroxy-,o-lactone • D9-Isoambrettolic acid,lactone • Isoambrettolide • Oxacycloheptadec-10-en-2-one		10–100	0.024	0.83
Oxacyclohexadecane-2,13-dione$C_{15}H_{26}O_3$ CAS # 38223-29-9 Log K_{ow}: 4.1[e] Molecular weight: 254.37 Vapor pressure: $(8 \pm 1) \times 10(-2)$ Pa at 25 °C Water solubility: 80 mg/l @ 20 °C	• 12-Ketapentadecanolide • 12-Ketapentadecanolide 1 • 12-Oxopentadecanolide • 12-Oxo-15-pentadecanolide		0.1–1	0.0005[f]	0.02[f]

(*continued on next page*)

Table 1 (*continued*)

Material	Synonyms	Structure	Annual Worldwide Metric Tons[a]	Dermal systemic exposure in cosmetic products (mg/kg/day)[b]	Maximum skin level[c,d] (%)
(E) and (Z)-Oxacyclohexadec-(12 or 13)-en-2-one[g] $C_{15}H_{28}O_2$ CAS # 111879-80-2; 34902-57-3; 99219-32-6; 111879-79-9; 111879-81-3 Log K_{OW}: >3.94; >6.20 Molecular weight: 238.37 Vapor pressure: 0.16 Pa @25 °C Water solubility: 9.64 × 10(−4) g/l sol. @ 20.0 ± 0.5 °C	• Habanolide • Oxacyclohexadec-12-en-2-one, (E)- • Cyclopentadecenolide • Oxacyclohexadecen-2-one		100–1000	0.19	6.66
10-Oxahexadecanolide $C_{15}H_{28}O_3$ CAS # 1725-01-5 Log K_{ow} 4.9[e] Molecular weight: 256.39 Vapor pressure1.58e−005 mm Hg @ 25 °C[e] Water solubility: 1.433 mg/l @ 25 °C[e]	• 1,8-Dioxacycloheptadecan-9-one • 9-[(6-Hydoxyhexyl)oxy]nonanoic acid omicron-lactone • Oxalide		1–10	0.003	1.21
11-Oxahexadecanolide $C_{15}H_{28}O_3$ CAS # 3391-83-1 Log K_{ow} 4.9[e] Molecular weight: 256.39 Vapor pressure: 1.58e−005 mm Hg @ 25 °C[e] Water solubility: 1.433 mg/l @ 25 °C[e]	• 1,7-Dioxacycloheptadecan-8-one • 16-Hydroxy-11-oxahexadecanoic acid,omega lactone • Musk RI • 11-Oxahexadecan-16-olide		1–10	0.03	1.88
12-Oxahexadecanolide$C_{15}H_{28}O_3$ CAS # 6707-60-4 Log K_{ow} 4.9[e] Molecular weight: 256.39 Vapor pressure: 1.58e-005 mm Hg @ 25 °C[e] Water solubility: 1.433 mg/l @ 25 °C[e]	• Cervolide • 1,6-Dioxacycloheptadecan-7-one • Hibiscolide • 16-hydroxy-12-oxahexadecanoic acid,omega lactone • Musk 781 • 12-Oxahexadecan-16-olide		1–10	0.05	1.42
ω-Pentadecalactone $C_{15}H_{28}O_2$ CAS # 106-02-5 Log K_{ow} > 6.0 at 35 °C Molecular weight: 240.39 Vapor pressure: 5.17e−005 mm Hg @ 25 °C[e] Water solubility: 0.1484 mg/l @ 25 °C[e]	• Angelica lactone • Cyclopentadecanolid • Cyclopentadecanolide • Exaltex • Exaltolide • 15-Hydroxypentadecanoic acid,omega-lactone • Macrolide • Oxacyclohexadecan-2-one • Pentadecalactone • Pentadecanolide • Pentalide • Thibetolide • Muskalactone		100–1000	0.09	5.02

[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 by Epi Suite (EPA, 2010).
[f] A default value of 0.02% was used to calculate dermal systemic exposure.
[g] (E)- and (Z)-oxacyclohexadec-12(+13)-en-2-one is a mixture of (E)-oxacyclohexadec-12-en-2-one (CAS #111879-80-2), (E)-oxacyclohexadec-13-en-2-one (CAS # 99219-32-6), (Z)-oxacyclohexadec-12-en-2-one (CAS # 111879-79-9 b), and (Z)-oxacyclohexadec-13-en-2-one (CAS # 111879-81-3).

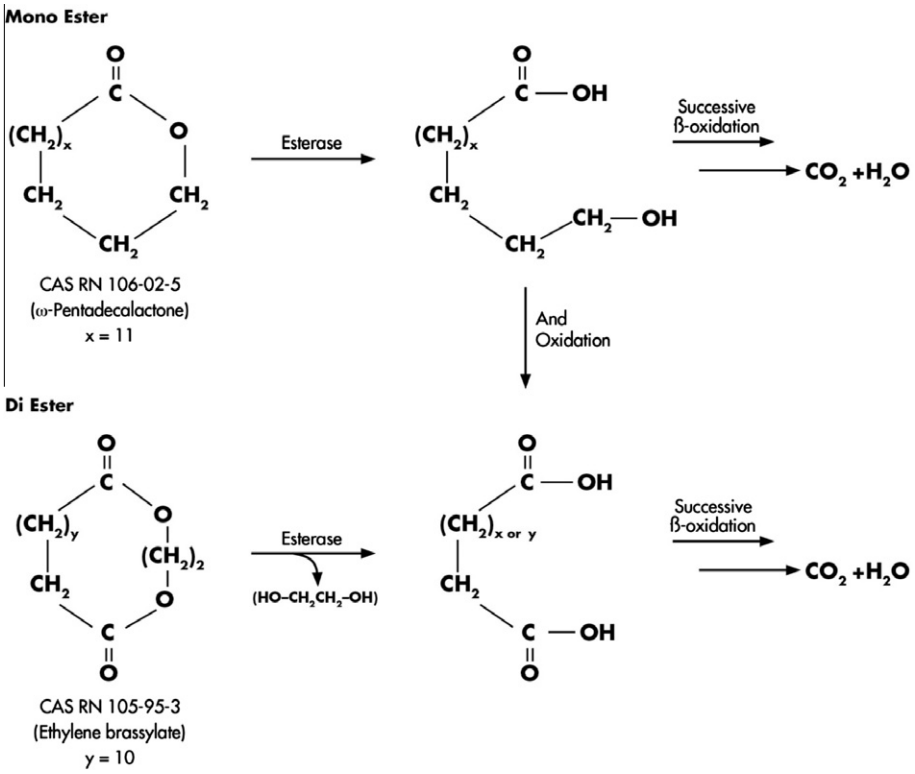


Figure 1. Proposed macrocyclic lactone metabolism and excretion (ώ-pentadecalactone and ethylene brassylate examples).

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 ML toxicology data.

2.1. Rationale for grouping macrocyclic lactones, and lactides

The ML fragrance ingredients described in Table 1 include both naturally occurring and synthetic macrocyclic lactones (cyclic mono- and di-esters).

The common structural element of the ML group of fragrance ingredients is a mono- or diester-lactone group, R–C (=O) O–R′, contained within a macrocyclic ring of C14 to C16 carbon chain length. The macrocyclic lactone fragrance ingredients described herein include 12 structurally diverse C14, C15, and C16 compounds that include (7) saturated mono-and (2) saturated di-ester lactones and (3) unsaturated lactones. For the latter, the double bond is not adjacent to (in conjugation with) the ester group. The naturally occurring macrocyclic lactones are generally derived from various plant, rather than animal, sources.

The molecular weights of the macrocyclic lactones do not vary appreciably and range from 270.37 g/mol for the C15 di-ester lactone, ethylene brassylate (CAS RN 105-95-3), to 238.37 g/mol for the C15 complex mixture of unsaturated mono lactones, (E)- and (Z)-oxacyclohexadec-(12(or 13)-en-2-one (CAS RNs 34902-57-3; 99219-32-6; 111879-79-9; 111879-81-3 and 111879-80-2). The macrocyclic lactone fragrance ingredients are generally lipophilic and log K_{ow} increases with increasing ring size. Log K_{ow} values range from 6.7 for the mono C16 saturated lactone oxacycloheptadec-10-ene-2-one (CAS RN 28645-51-4) to 3.65 for the saturated C14 diester ethylene dodecanedioate (CAS RN 54982-83-1). As a class, the macrocyclic lactone fragrance ingredients have a low volatility and are not appreciably water soluble.

JECFA (1998, 2002) reported some metabolism data for macrocyclic lactones (esters). The initial and primary metabolism would be hydrolysis of the lactone functionality to generate the corresponding long chain open carboxylic acid and alcohol which should undergo fatty acid type β-oxidation. 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

Some plants produce macrocyclic lactones that are widely used in perfumery as substitutes for the animal musks. ώ-6-Hexadecenlactone (CAS# 7779-50-2), is found in the musk seed oil extracts obtained from *Abelmoschus moschatus* (Rout et al., 2002).

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 ML fragrance ingredients and to discover new and structurally diverse synthetic musk fragrances (Sommer, 2004; Kraft et al., 2000).

As indicated in Table 1, the macrocyclic lactone, ethylene brassylate (CAS RN 105-95-3), has an annual volume of >1000 metric tons. Ethylene dodecanedioate (CAS RN 54982-83-1), ώ-pentadecalactone (CAS RN 106-02-5) and (E) and (Z)-oxacyclohexadec-(12 or 13)-en-2-one (CAS RNs 34902-57-3; 99219-32-6; 11879-79-9; 111879-81-3 and 111879-80-2) have annual volumes in the 100–1000 metric ton range.

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 ML 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 ML fragrance ingredients ranges from 0.01 to >1000 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 10 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 0.0008 to 0.25 mg/kg body weight (bw)/day for the ML 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 ML compounds that form part of the formulae of fine fragrances vary widely and have been reported to range from 0.47% to 11.15%. The maximum skin exposure for ML fragrance ingredients in fine fragrance products are listed in Table 1 (IFRA, 2007).

Exposure data for the two fragrance materials, 16-hydroxy-7-hexadecenoic acid lactone and oxacyclohexadecane-2,13-dione, were not reported. A default value of 0.02% is used to calculate the maximum daily exposure on the skin which is 0.0005 mg/kg bw for high-end users of these products.

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

3. Metabolism

The published metabolism studies for macrocyclic lactones include data on food additives as reported by JECFA (1998, 2002).

Cyclic lactones are formed *in vivo* by the intramolecular acid cyclization of linear hydroxycarboxylic acids and the elimination of one molecule of water. Ring formation is a pH-dependent action by esterases in which equilibrium is established between a linear non-cyclic hydroxycarboxylate anion and the cyclic ester (lactone ring). Basic body fluids such as blood will favor the linear form whereas acid media (e.g., stomach digestive fluids or urine) would favor the cyclic lactone (JECFA, 1998; FEMA, 1962). Both JECFA and FEMA have reported that these forms can be absorbed *in vivo* from the gastrointestinal tract. The open lactone is a long chain carboxylic acid that is similar to a fatty acid (see Fig. 1).

As noted the macrocyclic lactones comprise a structurally diverse group of macrocyclic mono- and di-esters that are saturated and non-conjugated unsaturated lactones, which may be in equilibrium and absorbed as either the lactone or acyclic hydroxycarboxylate anion. The presence of branching, unsaturation, or additional oxygen functionality (i.e., keto and or ether groups) is not expected to alter or hinder the primary metabolism route of ester hydrolysis; however, branching may inhibit the rate of β-oxidation and excretion. The hydroxycarboxylates derived from either mono- or di-ester lactones, either by the gut environment or by carboxylesterase hydrolysis, would undergo complete or partial β-oxidation and enter the tricarboxylic acid cycle to yield smaller carbon chain unit linear metabolites which are eliminated in the urine, comparable to fatty-acid metabolism (JECFA, 2002). The

Table 2-1
Acute dermal toxicity.

Material	Species	Number/dose group	LD_{50} (mg/kg)	Reference
Ethylene brassylate	Rabbit	10	>5000	RIFM (1973a)
Hexadecanolide	Rabbit	10	>5000	RIFM (1974a)
ώ-6-Hexadecenlactone	Rabbit	10	>5000	RIFM (1974b)
Oxacyclohexadecane-2,13-dione	Rabbits	6	>2000	RIFM (1982a)
Oxacyclohexadec-12(+13)en-2-one (E/Z isomer mix)	Rat	10	>2000*	RIFM (1992a)
10-Oxahexadecanolide	Rabbit	10	>5000	RIFM (1979a)
11-Oxahexadecanolide	Guinea pig	2	>5000	RIFM (1977a)
12-Oxahexadecanolide	Rat	10	>10,000	RIFM (1978a)
	Rabbit	10	>5000	RIFM (1977a)
ώ-Pentadecalactone	Rabbit	4	>5000	RIFM (1974c)

* OECD compliant study.

Table 2-2
Acute oral toxicity.

Material	Species	Number/dose group	LD_{50} (mg/kg)	Reference
Ethylene brassylate	Rat	10	>5000	RIFM (1973a)
Ethylene dodecanedioate	Rat	10	4410	RIFM (1975a)
Hexadecanolide	Rat	10	>5000	RIFM (1974a)
ώ-6-Hexadecenlactone	Rat	10	>5000	RIFM (1974b)
Oxacyclohexadecane-2,13-dione	Rat	10	>5000	RIFM (1982b)
Oxa cyclo hexa dec-12(+13)en-2-one (E/Z isomer mix)	Rat	10	>2000[a]	RIFM (1992b)
10-Oxahexadecanolide	Rat	10	>5000	RIFM (1979a)
11-Oxahexadecanolide	Rat	10	>5000	RIFM (1977a)
12-Oxahexadecanolide	Mice	10	>2000 <4000	RIFM (1977b)
	Rat	10	>5000	RIFM (1978b)
	Rat	10	>5000	RIFM (1977a)
ώ-Pentadecalactone	Rat	10	>5000	RIFM (1974c)
	Cat	7	>700 <1250	vonOettingen and Garci (1929)
	Mouse (M)	5	2820	RIFM (1971)
	Mouse (F)	5	2950	RIFM (1971)

[a] OECD compliant study.

Table 2-3
Acute intraperitoneal toxicity.

Material	Species	Number/dose group	LD_{50} (mg/kg)	Reference
12-Oxahexadecanolide	Mice	10	>500 <1000	RIFM (1977b)

proposed metabolic pathway for both mono and di-ester macrocyclic lactones is illustrated in Fig. 1; however, no *in vivo* metabolic studies of these materials are available to confirm these pathways.

4. Toxicokinetics

No toxicokinetic studies for the macrocyclic lactone and lactide derivatives were identified.

5. Toxicological studies

5.1. Acute toxicity

Nine macrocyclic lactones and lactides have been evaluated for acute dermal toxicity in rats, guinea pigs and rabbits (Table 2-1). Dermal LD_{50} values exceeded 2000 mg/kg body weight for all for these compounds, seven exceeded 5000 mg/kg, and one exceeded 10,000 mg/kg body weight.

Ten lactones used in fragrances have been evaluated for acute oral toxicity (Table 2-2). In rats, all 10 lactones exceeded an oral LD_{50} value of at least 2000 mg/kg and eight of these of exceeded an LD_{50} of 5000 mg/kg. The only material that had an LD_{50} value for rats below the highest dose tested was ethylene dodecanedioate (4410 mg/kg). In mice, 12-oxahexadecanolide had an LD_{50} greater than 2000 mg/kg but less than 4000 mg/kg. The oral LD_{50} for ώ-pentadecalactone was approximately 2800 mg/kg in the mouse. In cats, the oral LD_{50} value for ώ-pentadecalactone was between 700 mg/kg and 1250 mg/kg.

Acute intraperitoneal LD_{50} values in mice have been reported only for the lactone 12-oxahexadecanolide (LD_{50} value greater than 500 mg/kg but less than 1000 mg/kg; Tables 2 and 3).

5.2. Repeat-dose studies

There are few repeat-dose studies available for the macrocyclic lactones. These data are described below and are summarized in Tables 3-1 and 3-2.

5.2.1. Oral studies

Oral (gavage) toxicological studies have been reported for three lactones (ethylene dodecanedioate, the E/Z isomer mix of oxacyclohexadec-12-en-2-one and oxacyclohexadec-13-en-2-one, and 12-oxahexadecanolide).

After a 7-day range-finding study (RIFM, 1999a), ethylene dodecanedioate in corn oil was administered by gavage to rats (5/sex/dose and 10/sex for control and high dose groups) for 28 days at doses 0, 15, 150, 400 or 1000 mg/kg body weight/day with a concurrent high-dose group of rats maintained for 2 weeks without dosing to document recovery (RIFM, 2000a). Body weight, food consumption, clinical observations, blood and urine biochemistry, pathology, organ weights, and selected histopathological examinations were performed. No toxicological effects were associated with treatment. Mean relative kidney weights were higher for females receiving 1000 or 400 mg/kg body weight/day, but no gross or histopathological changes were observed and increases were not apparent during the recovery period. Aspartate amino transferase levels decreased in high dose males and females and at 400 mg/kg/day females with complete recovery. A slight, significant ($p < 0.01$) increase in sodium and chloride were noted in two high dose females with complete recovery. There were no adverse treatment-related findings in this study and it was concluded that 1000 mg/kg body weight/day, the highest dose tested, represented the NOAEL in the rat.

The E/Z isomer mix of oxacyclohexadec-12-en-2-one and oxacyclohexadec-13-en-2-one was administered by gavage in 0.5% carboxymethyl cellulose to rats (6/sex/dose) for 7-day range-finding study and 28-day treatment at doses of 500, 750, or 1000 mg/kg body weight/day (plus another group of 1000 mg/kg body weight/day treated rats in the 28-day study followed by a 2-week treatment free recover period) (RIFM, 1995a, 1996). The two studies measured mortality, clinical signs, body weight changes, food consumption, ocular effects, blood biochemistry, urinary parameters, organ weight changes, and gross alterations and histopathological findings. Each study (i.e., 7-day, 28-day) found no treatment-related effects and produced a NOEL of 1000 mg/kg

Table 3-1
Repeat dose toxicity by oral exposure.

Material	Route and duration	Dose (mg/kg/day)	Species (number/dose)	Results	Reference
Ethylene dodecanedioate	28-day gavage[a]	15, 150, 400, or 1000 in corn oil	Rat (5/sex, 10/sex for control and high dose)	NOAEL 1000 mg/kg Aspartate amino transferase levels decreased in high dose males and females and at 400 mg/kg/day females with complete recovery; slight, significant ($p < 0.01$) increases in sodium and chloride noted in two high dose females with complete recovery; mean relative kidney weights higher for females receiving 1000 or 400 mg/kg body weight/day (not apparent during recovery period), no gross or histopathological changes were observed	RIFM (2000a)
	7-day gavage[b]	250, 500, or 1000 in corn oil	Rat (6, 3/sex)	NOAEL 1000 mg/kg No adverse effect	RIFM (1999a)
Oxacyclohexadec-12(+13)en-2-one E/Z isomer mix)	90-day gavage[b]	50, 250, or 1000 in 0.5% carboxymethyl celluose	Rat (15/sex)	NOEL: 1000 mg/kg/day No effects	RIFM (1998a)
	28-day gavage	500, 750, or 1000 in 0.5% carboxymethyl cellulose	Rat (6/sex)	NOEL: 1000 mg/kg/day No effects	RIFM (1996)
	7-day gavage	500, 750, or 1000 in 5% caboxymethyl cellulose	Rat (6/sex)	NOEL: 1000 mg/kg/day No effects	RIFM (1995a)
12-Oxahexadecanolide	5-day gavage	240 in corn oil	Rat (6)	NOEL: 240 mg/kg/day No significant behavior or physiologic effects; four had swollen thymus glands, and one had a dark coloration of the liver; brain, spinal cord, and sciatic nerve of most of the rats contained an occasional single vacuole or scattered single vacuoles; no light microscopic changes in the nervous tissues compared to control	RIFM (1978c)

[a] Study performed under joint directive of Japanese Environmental Protection Agency and the Ministries of Health and Welfare.
[b] OECD compliant study.

Table 3-2
Repeat dose toxicity by other routes.

Material	Route and duration	Dose (mg/kg/day)	Species (number/dose)	Results	Reference
Ethylene brassylate	20-day dermal	700, 70, or 30 in mineral oil	Rabbit (6/sex)	One death at 700 mg/kg/day on day 21; Dose dependent dermal irritation at all dose levels and enlargement of regional lymph nodes at 700 mg/kg/day (no other significant gross pathology or histopathology observed)	RIFM (1974d)
ω-Pentadecalactone	10 or 11-day intraperitoneal	36	Rat (5)	40% mortality (2/5); bloated, bleeding from the nose, and diarrhea	RIFM (1976)

body weight/day. Similarly, treated rats in a 90-day study (15/sex/dose) at doses of 50, 250, or 1000 mg/kg body weight/day (plus another group of 1000 mg/kg body weight/day treated rats followed by a 28-day recovery period (10/sex/dose)) (RIFM, 1998a) also produced no treatment-related changes in the same measured parameters. The NOEL was considered to be 1000 mg/kg body weight/day.

12-Oxahexadecanolide was administered by gavage in corn oil to female rats (6) for 5 days at a dose of 240 mg/kg body weight/day. The study measured mortality, behavior, physiologic state of the animals, body weight changes, necropsy and histopathology. The authors of the study concluded that there were no significant behavior or physiologic effects in animals receiving 12-oxahexadecanolide at 240 mg/kg body weight/day for 5 days. Four (of six) rats had swollen thymus glands, and one had a dark coloration of the liver. The brain, spinal cord, and sciatic nerve of most of the rats contained an occasional single vacuole or scattered single vacuoles. It was concluded that this dose level did not produce any significant light microscopic changes in the nervous tissues of these rats when compared to rats in the control (corn oil) group (RIFM, 1978c). The Panel has concluded that 240 mg/kg body weight/day represents the NOEL.

5.2.2. Dermal studies

The only repeat dose dermal study identified was with the macrocyclic lactone, ethylene brassylate. Ethylene brassylate was applied to New Zealand White rabbits (6/sex/dose) for 20 days at doses of 0, 30, 70, or 700 mg/kg body weight/day (RIFM, 1974d). The rabbits were observed daily for changes in behavior, appearance, irritation of the skin, body weight, blood and urine biochemistry, and organ weights were recorded at necropsy. Dose-dependent dermal irritation (erythema, edema, desquamation, coriaceousness, fissuring, intradermal hemorrhaging, blanching, and necrosis) was observed at all dose levels.

Table 4-1
Genotoxicity in bacteria.

Material	Test	Bacterial strain	Concentration	Results	Reference
Ethylene brassylate	Ames reverse mutation	*Salmonella typhimurium* TA97, TA98, or TA100±S9	Up to 2000 (+S9) μg/plate Up to 100 (−S9) μg/plate	Negative	Abramsson-Zetterberg and Slanina (2002)
	Ames reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, or TA1538±S9	Up to 3600 μg/plate	Negative	Wild et al. (1983)
Ethylene dodecanedioate	Ames reverse mutation	*Salmonella typhimurium* TA97, TA98, or TA100±S9	Up to 2000 (+S9) μg/plateUp to 150 (−S9) μg/plate	Negative	Abramsson-Zetterberg and Slanina (2002)
	Ames reverse mutation[a]	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, or TA1538±S9	Up to 5000 μg/plate	Negative	RIFM (1999b)
	DNA damage activity[a]	*Escherichia coli* WP2uvrA±S9	Up to 1250 μg/plate	Negative	RIFM (1999b)
Hexadecanolide	Ames reverse mutation[a]	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, or TA1538±S9	Up to 2500 μg/plate	Negative	RIFM (1999c)
	Ames reverse mutation[a]	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, or TA1538±S9	Up to 5000 μg/plate	Negative	RIFM (1999d)
	DNA damage activity[a]	*Escherichia coli* WP2uvrA±S9	Up to 2500 μg/plate	Negative	RIFM (1999c)
	DNA damage activity[a]	*Escherichia coli* WP2uvrA±S9	Up to 5000 μg/plate	Negative	RIFM (1999d)
Oxacycloheptadec-10-ene-2-one	Ames reverse mutation[a]	*Salmonella typhimurium* TA98, TA100, TA102, TA1535, or TA1537±S9	Up to 5000 μg/plate	Negative	RIFM (2003a)
Oxacyclohexadecane-2,13-dione	Ames reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 or TA1538±S9	Up to 5000 μg/plate	Negative	RIFM (1985)
Oxacyclohexadec-12(+13)en-2-one (E/Z isomer mix)	Ames reverse mutation[b]	*Salmonella typhimurium* TA98, TA100, TA1535, or TA1537±S9	Up to 5000 μg/plate	Negative	RIFM (2005a)
	Ames reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, or TA1537±S9	Up to 2500 μg/plate	Negative	RIFM (1992c)
	DNA damage activity[b]	*Escherichia coli* WP2uvrA±S9	Up to 5000 μg/plate	Negative	RIFM (2005a)
11-Oxahexadecanolide	Ames reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, or TA1538±S9	Up to 2500 μg/plate	Negative	RIFM (1979b)
ω-Pentadecalactone	Ames reverse mutation	*Salmonella typhimurium* TA97, TA98, or TA100±S9	Up to 1300 μg/plate	Negative	Abramsson-Zetterberg and Slanina (2002)
	Ames reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, or TA1537±S9	Up to 5000 μg/plate	Negative	RIFM (1995b)
	Ames reverse mutation[a]	*Salmonella typhimurium* TA98, TA100, TA102, TA1535, or TA1537±S9	Up to 5000 μg/plate	Negative	RIFM (2001a)
	Ames reverse mutation	*Salmonella typhimurium* TA98, TA100, or TA102±S9	Up to 1250 μg/plate	Negative	Aeschbacher et al. (1989)
	Ames reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 or TA1538±S9	Up to 3300 μg/plate	Negative	RIFM (1978d)
	DNA damage activity	*Escherichia coli* WP2uvrA±S9	Up to 5000 μg/plate	Negative	RIFM (1995b)

[a] OECD compliant study.
[b] OECD and Japanese regulatory authorities (including METI, MHLW, MAFF) compliant study.

Table 4-2
Genotoxicity in mammalian cells.

Material	Test system	Species/Test system	Concentration	Results	Reference
Ethylene dodecanedioate	Chromosome aberration[a]	Human lymphocytes ± S9	Up to 275 μg/ml (−S9); Up to 700 μg/ml (+S9)	Negative	RIFM (1999e)
Hexadecanolide	Chromosome aberration[a]	Human lymphocytes ± S9	Up to 750 μg/ml (+S9); Up to 250 μg/ml (-S9)	Negative	RIFM (1999f)
Oxacyclohexadec-12(+13)en-2-one (E/Z isomer mix)	Mouse lymphoma assay[b]	Mouse L5178Y TK ± lymphoma cells ± S9	Up to 80 μg/ml (+S9); Up to 50 μg/ml (−S9)	Negative	RIFM (2001b)
	Chromosome aberration[a]	Human lymphocytes ± S9	Up to 2380 μg/ml	Negative	RIFM (1995c)

[a] OECD compliant study.
[b] Study performed under OECD and United Kingdom Environmental Mutagen Society guidelines.

Enlargement of regional lymph nodes, which the authors attributed to an immune response to irritation, was present at 700 mg/kg body weight/day. No other significant gross or histopathological changes were observed.

5.2.3. Inhalation studies

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

6. Genotoxicity studies

6.1. Bacteria

Eight of the lactones have been studied in the reverse mutation assays with *Salmonella typhimurium* (Ames test), or *Escherichia coli WP2uvrA* strains. These compounds, ethylene brassylate, ethylene dodecanedioate, hexadecanolide, ω-pentadecalactone, (E)- and

Table 4-3
Genotoxicity in mice.

Material	Test system	Mouse strain	Dosemg/kg	Results	Reference
Ethylene brassylate	Micronucleus test (erythrocytes)[a]	NMRI female	350, 700, or 1400 (IP)	Negative	Abramsson-Zetterberg and Slanina (2002)
	Micronucleus test (erythrocytes)[a]	CD-1 male	100, 1000, or 1600 (IP)	Negative	Abramsson-Zetterberg and Slanina (2002)
Ethylene dodecanedioate	Micronucleus test (erythrocytes)[a]	NMRI female	350, 700, or 1400 (IP)	Negative	Abramsson-Zetterberg and Slanina (2002)
	Micronucleus test (erythrocytes)[a]	CD-1 male	100, 1000, or 1600 (IP)	Negative	Abramsson-Zetterberg and Slanina (2002)
ώ-Pentadecalactone	Micronucleus test (erythrocytes)[a]	NMRI female	350, 700, or 1400 (IP)	Negative	Abramsson-Zetterberg and Slanina (2002)
	Micronucleus test (erythrocytes)[a]	CD-1 male	100, 1000, or 1600 (IP)	Negative	Abramsson-Zetterberg and Slanina (2002)

[a] OECD recommendations for dosing were followed.

Table 5
Reproductive and developmental toxicity.

Material	Method	Dose (mg/kg/day)	Species (number/dose)	Results	Reference
Oxacyclohexadec-12(+13)en-2-one (E/Z isomer mix) Oxacyclohexadec-12(+13)en-2-one (E/Z isomer mix)	One generation Reproductive Study[a] 12 weeks gavage through maturation, mating, gestation and lactation (adult males dosed for 72 days, adult females dosed for 16 days)	50, 250, or 1000, in 0.5% carboxymethyl cellulose	Rat (28/sex)	Reproduction: NOEL 1000 mg/kg body weight/day Offspring Development: NOEL 1000 mg/kg body weight/day Test material administered to adult male and female rats throughout the reproductive cycle for one generation resulted in no evidence of significant toxicity	RIFM (2003b)
	Developmental Study[a] Gavage from gestational day 5–19	50, 250, or 1000, in 0.5% carboxymethyl cellulose	Rat (24 pregnant females)	Adult toxicity: NOEL 1000 mg/kg body weight/day Developmental toxicity: NOEL 1000 mg/kg body weight/day No significant systemic effects on adults, on any uterine parameter examined, or on viability, growth or development of offspring	RIFM (2003c)

[a] OECD compliant study.

(Z)-oxacyclohexadec-12(+13)en-2-one (isomer mix), oxacycloheptadec-10-ene-2-one, oxacyclohexadecane-2,13-dione, and 11-oxahexadecanolide, 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. Ethylene dodecanedioate, hexadecanolide, (E)- and (Z)-oxacyclohexadec-12(+13)en-2-one and ώ-pentadecalactone did not produce mutations in *Escherichia coli WP2uvrA* strains with or without metabolic activation.

6.2. Mammalian cell lines

Four lactones have been studied *in vitro* by analyzing chromosomal aberrations in activated or non-activated human lymphocytes, human leukocytes or mouse L5178Y TK± lymphoma cells. No induction of chromosomal aberrations has been reported with the macrocyclic lactones (ethylene dodecanedioate, hexadecanolide, or oxacyclohexadec-12(+13)en-2-one (E/Z isomer mix) (RIFM, 1999e, 1999f; or RIFM, 2001b,1995c).

6.3. Mice

In a micronucleus assay, groups of female NMRI mice received a single intraperitoneal injection of ethylene brassylate, ethylene dodecandioate, or ώ-pentadecalactone at dose levels of 350, 700, or 1400 mg/kg. Groups of male CD-1 mice received an injection of 100, 1000, or 1600 mg/kg. OECD recommendations for dosing were followed. At 30 h, the mice were euthanized, the bone marrow extracted, and polychromatic and normochromatic erythrocytes were scored for the presence of micronuclei. No evidence of genotoxicity was produced (Abramsson-Zetterberg and Slanina, 2002).

7. Carcinogenicity

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

8. Reproductive and developmental toxicity

One macrocyclic lactone has been tested for reproductive and developmental toxicity (Table 5).

In the OECD compliant study (test guidelines 415), the E/Z isomer mix of (E) and (Z) oxacyclohexadec-12-en-2-one and (E) and (Z) oxacyclohexadec-13-en-2-one in 0.5% carboxymethyl cellulose was administered by gavage to groups of rats (28/sex/dose) throughout maturation, mating, gestation, and lactation at doses of 0, 50, 250 or 1000 mg/kg body weight/day (RIFM, 2003b). The males were dosed for 72 days and female animals were dosed for 16 days. 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. The NOEL for both reproduction and offspring development was 1000 mg/kg body weight/day.

In an OECD compliant developmental study (test guidelines 414), the E/Z isomer mix of (E) and (Z) oxacyclohexadec-12-en-2-one and (E) and (Z) oxacyclohexadec-13-en-2-one in 0.5% carboxy-

Table 6-1
Skin irritation in humans.

Material	Method[a]	Concentration	Subjects	Results	Reference
Ethylene brassylate	Irritation (HRIPT)	20% in 3:1 EtOH:DEP	50	2/50	RIFM (1990a)
	Irritation (HRIPT)	20% in 3:1 EtOH:DEP	50	0/50	RIFM (1990b)
	Irritation (HRIPT)	20% in 3:1 EtOH:DEP	64	1/64	RIFM (1990c)
	Irritation (HRIPT)	20% in 3:1 EtOH:DEP	67	1/67	RIFM (1990d)
	Irritation (HRIPT)	20% in 3:1 EtOH:DEP	71	0/71	RIFM (1990e)
	Irritation (HRIPT)	20% in 3:1 EtOH:DEP	94	1/94	RIFM (1990f)
	Irritation (HRIPT)	20% in 3:1 EtOH:DEP	36	0/36	RIFM (1989a)
	Irritation (HRIPT)	20% in 3:1 EtOH:DEP	103	0/103	RIFM (1989b)
	Irritation (HRIPT)	20% in 3:1 EtOH:DEP	112	1/112	RIFM (1988a)
	Irritation (HRIPT)	20% in 3:1 EtOH:DEP	38	0/38	RIFM (1988b)
	Irritation (HRIPT)	20% in 3:1 EtOH:DEP	27	0/27	RIFM (1988c)
	Irritation (HRIPT)	20% in 3:1 EtOH:DEP	34	0/34	RIFM (1988d)
	Irritation (HRIPT)	20% in 3:1 EtOH:DEP	108	0/108	RIFM (1988e)
	Irritation (HRIPT)	20% in 3:1 EtOH:DEP	106	0/106	RIFM (1988f)
	Irritation (HRIPT)	20% in 3:1 EtOH:DEP	202	0/202	RIFM (1987)
	Irritation (HRIPT)	20% in DEP	110	2/110	RIFM (1989e)
	Irritation (HRIPT)	10% in DEP	65	0/65	RIFM (1995d)
	Irritation (HRIPT)	10% in DEP	125	0/125	RIFM (1995e)
	Irritation (HRIPT)	10% in DEP	109	0/109	RIFM (1995f)
	Irritation (HRIPT)	10% in DEP	91	0/91	RIFM (1994a)
	Irritation (HRIPT)	10% in DEP	95	0/95	RIFM (1994b)
	Irritation (HRIPT)	10% in DEP	22	0/22	RIFM (1994c)
	Irritation (HRIPT)	10% in DEP	99	0/99	RIFM (1993)
	Irritation (HRIPT)	10% in DEP	107	1/107	RIFM (1991)
	Irritation (HRIPT)	10% in 3:1 EtOH:DEP (2 different samples)	69	19/69	RIFM (1990g)
	Irritation (HRIPT)	10% in 3:1 EtOH:DEP	37	1/37	RIFM (1989c)
	Irritation (HRIPT)	10% in 3:1 EtOH:DEP	36	10/36 (irritation probably due to vehicle)	RIFM (1989d)
	Maximization pre-test	30% in petrolatum	5	0/5	RIFM (1973b)
Ethylene dodecanedioate	Irritation (HRIPT)	25% in 3:1 EtOH:DEP	44	0/44	RIFM (2000b)
	Maximization pre-test	20% in petrolatum	25	2/25	RIFM (1978e)
	Closed patch test	5% in petrolatum	25	0/25	RIFM (1997b)
	Epicutaneous patch test	2% in isopropyl myristate	40	0/40	RIFM (1975b)
Hexadecanolide	Irritation (HRIPT)	2% in DMP	54	0/54	RIFM (1972c)
	Irritation (HRIPT)	0.75% in EtOH	40	0/40	RIFM (1964b)
	Maximization pre-test	4% in petrolatum	5	0/5	RIFM (1974e)
ώ-6-Hexadecenlactone	Maximization pre-test	1% in petrolatum	26	0/26	RIFM (1974f)
Oxacycloheptadec-10-ene-2-one	Irritation (HRIPT)	0.5% in EtOH	38	0/38	RIFM (1964a)

(*continued on next page*)

Table 6-1 (*continued*)

Material	Method[a]	Concentration	Subjects	Results	Reference
Oxacyclohexadecane-2,13-dione	Irritation (HRIPT)	20% in petrolatum	50	0/50	RIFM (1982c)
Oxacyclohexadec-12(+13)en-2-one (E/Z isomer mix)	Irritation (HRIPT)	15% in DEP	104	0/104	RIFM (1997c)
10-Oxahexadecanolide	Maximization pretest	10% in petrolatum	29	0/29	RIFM (1978f)
11-Oxahexadecanolide	Maximization pretest	10% in petrolatum	24	0/24	RIFM (1977c)
12-Oxahexadecanolide	Irritation (HRIPT)	5% in DMP	51	0/51	RIFM (1978h)
	Maximization pretest	10% in petrolatum	25	0/25	RIFM (1977d)
ώ-Pentadecalactone	Irritation (HRIPT)	2% in DMP	54	0/54	RIFM (1972d)
	Irritation (HRIPT)	10% in 3:1 DEP:EtOH	105	0/105	RIFM (2006)
	Maximization pretest	10% in petrolatum	5	0/5	RIFM (1974e)

[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 pretests are 48 h in duration.

methyl cellulose was administered by gavage to pregnant rats (24/dose) from gestational days 5 through 19 at doses of 0, 50, 250 or 1000 mg/kg body weight/day (RIFM, 2003c). Clinical signs, bodyweight and food consumption were recorded during the study. The females were sacrificed on Day 20 of gestation, examined macroscopically and the uterine contents examined. The number of corpora lutea, implantation number, position and type, fetal and placental weights, fetal sex, and external appearance were recorded. All live fetuses were preserved, processed and subsequently examined for skeletal or visceral anomalies. Administration of the test material resulted in no significant systemic effects on the adults, on any of the uterine parameters examined, nor upon offspring viability, growth or development. The No Observed Effect Level (NOEL) for adult toxicity and developmental toxicity was 1000 mg/kg bodyweight.

In an effort to determine if the ML fragrance ingredients have any estrogenic activity, the lactones ethylene brassylate, ethylene dodecanedioate, and ώ-pentadecalactone, were evaluated at 10 μM in the E screen assay. This assay uses estrogenic receptor-positive human mammary carcinoma cells (cell line MCF-7) and measures the proliferative effect of the test material compared to that of 17b-estradiol. Based on their inability to significantly increase proliferation, these lactones were not considered estrogenically active (Bitsch et al., 2002).

9. Irritation

9.1. Human studies

A considerable amount of data has been collected regarding human irritation from the macrocyclic lactones. Eleven macrocylic lactones were evaluated for skin irritation in approximately 2875 male and female volunteers at concentrations ranging from 0.5% to 30% (see individual studies listed in Table 6-1).

More than half of the irritation data generated were from repeat insult patch test studies with ethylene brassylate at 10% or 20% in DEP or EtOH:DEP (3:1) on approximately 2100 volunteers. During the induction phase of these studies, in which volunteers had patches reapplied three times per week for three weeks, ethylene brassylate appeared to cause a weak to moderate irritation that dissipated with time. Studies with DEP as the vehicle appear to be less irritating subjects than those with EtOH:DEP as the vehicle. Eighteen HRIPTs were performed where ethylene brassylate was used as the negative control. Of these tests 38/1338 irritation reactions were observed.

Ethylene dodecanedioate, and the remaining lactones (hexadecanolide, ώ-6-hexadecenlactone, ώ-pentadecalactone, (E)- and (Z)-oxacyclohexadec-12(+13)en-2-one (isomer mix), oxacycloheptadec-10-ene-2-one, oxacyclohexadecane-2,13-dione, 10-, 11-, and 12-oxahexadecanolide) did not appear to cause irritation among volunteers.

9.2. Animal studies

9.2.1. Skin irritation

Irritation reactions were identified for 10 macrocyclic lactones with a range of reactions from strong 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 range finding prior to or during maximization tests.

In general this group of macrocyclic lactones caused no irritation or slight temporary irritation which usually dissipated. Members of this category include ethylene brassylate (except for 100% applications), ethylene dodecanedioate, ώ-pentadecalactone, and 10-, 11-, or 12-oxahexadecanolide (except for 100% applications). Less irritation occurred as the topical dose decreased.

Hexadecanolide and oxacyclohexadecane-2,13-dione had data that showed a range from no irritation to moderate or severe irritation at concentrations of 50% or higher.

9.2.2. Mucous membrane (eye) irritation in rabbits

The potential for eight macrocyclic lactone and lactide derivatives 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 0.5% to 100% in various vehicles (Table 7). Transient slight conjunctival erythema after application was recorded for ethylene dodecanedioate and 12-oxahexadecanolide, most of which was cleared by day 2 after application. At 0.75%, mild erythema was reported for hexadecanolide and moderate conjunctival irritation was reported for oxacycloheptadec-10-ene-2-one at 0.5%, but these erythemous responses cleared by day 4 and 3, respectively. Under criteria described in OECD, EEC, CFR or FDA directives, oxacyclohexadec-12(+13)en-2-one (E/Z isomer mix), oxacyclohexadecane-2,13-dione and 11-oxahexadecanolide, were considered not-irritating to the eyes.

Table 6-2
Skin irritation in animals.

Material	Method	Concentration	Species (number)	Results	Reference
Ethylene brassylate	Irritation[a] (4h semi-occluded)	100%, 20%, 10%, 5%, or 1% in EtOH:DEP	Rabbit (4)	0/4 at all doses	RIFM (1994d)
	Irritation (phototoxicity control)	30%, 10%, or 5% in acetone	Guinea pig (5)	4/5 at 30%, 0/5 at 10% and 5%	RIFM (1997e)
	Irritation (phototoxicity control)	50% in DEP or EtOH	Guinea pig (3)	Weak irritation (DEP); Mild to weak irritation (EtOH)	RIFM (1978g)
	Irritation (phototoxicity control)	50% in EtOH	Rabbit (3)	No irritation	RIFM (1978g)
	Open epicutaneous test[b]	10% in EtOH with DMSO	Guinea pig (10)	0/10	RIFM (1983a)
	Irritation (LD_{50})	100%	Rabbit (10)	10-Mar	RIFM (1973a)
	Irritation[a] (maximization)	100%	Guinea pig (20)	20-Feb	RIFM (1995g)
Ethylene dodecanedioate	Irritation (phototoxicity control)	30,% 10%, or 5% in acetone	Guinea pig (5)	0/5 at all doses	RIFM (1997f)
Hexadecanolide	Irritation (phototoxicity control)	50% in DEP, 5% in EtOH	Guinea pig (3)	Strong to moderate irritation (DEP); no irritation (EtOH)	RIFM (1978g)
	Irritation (phototoxicity control)	5% or 1% in EtOH	Rabbit (3)	Moderate to weak irritation at 5%, no irritation at 1%	RIFM (1978g)
	Irritation (LD_{50})	100%	Rabbit (10)	10-Apr	RIFM (1974a)
Oxacyclohexadecane-2,13-dione	Irritation (24h occluded)	20% in petrolatum	Rabbit (6)	0/6	RIFM (1982d)
	Irritation (LD_{50})	100%	Rabbit (6)	6-Jun	RIFM (1982a)
Oxacyclohexadec-12(+13)en-2-one (E/Z isomer mix)	Irritation (24h occluded)	100%, 50%, 25% and 12.5% in EtOH	Guinea pig (4)	0/4	RIFM (1992f)
	Irritation[c] (4h occluded)	100%	Rabbit (4)	4-Apr	RIFM (1992d)
10-Oxahexadecanolide	Irritation (LD_{50})	100%	Rabbit (10)	10-Jul	RIFM (1979a)
	Irritation (phototoxicity control)	30%, 10%, or 5% in acetone	Guinea pig (5)	0/5 at all doses	RIFM (1997g)
	Irritation (phototoxicity control)	50% in DEP	Guinea pig (3)	No irritation	RIFM (1978g)
11-Oxahexadecanolide	Irritation (24h occluded)	100%	Rabbit (6)	0/6	RIFM (1980a)
	Irritation (phototoxicity control)	10% in EtOH	Rabbit (6)	0/6	RIFM, 1980a
	Irritation (phototoxicity control)	50% in DEP	Guinea pig (3)	No irritation	RIFM (1978g)
	Irritation (photosensitization control)	10% in EtOH then 5% in EtOH	Guinea pig (15)	0/15 at both doses	RIFM (1980b)
	Irritation (maximization)	10% in petrolatum	Guinea pig (10)	0/10	RIFM (1980c)
	Irritation (LD_{50})	100%	Guinea pig (2)	2-Feb	RIFM (1977a)
12-Oxahexadecanolide	Open epicutaneous test	100%, 30%, 10%, 3%, or 1% in EtOH (induction)	Guinea pig (6)	Strong to moderate irritation at 100%, slight to moderate at 30%, very slight at 10% and 3%	RIFM (1977e)
	Irritation	10% in EtOH with 2% DMSO	Guinea pig (10)	0/10	RIFM (1983b)
	Irritation (LD_{50})	100%	Rabbit (10)	10-Oct	RIFM (1977a)
	Irritation (phototoxicity control)	50% in DEP	Guinea pig (3)	0/3	RIFM (1978g)
ω-Pentadecalactone	Irritation (phototoxicity control)	100%	Miniature Swine (2)	0/2	Forbes et al. (1977)
	Irritation (LD_{50})	100%	Rabbit (4)	0/4 by 48h	RIFM (1974c)
	Irritation[a] (4h semi-occluded)	100%, 20%, 10%, 5%, 1% in EtOH:DEP	Rabbit (4)	0/4 at all doses by day 14 of observations	RIFM (1995h)
	Irritation (phototoxicity control)	5% or 10% in EtOH	Rabbit (3)	0/3 by 72h	RIFM (1978g)
	Irritation[a] (maximization prescreen of topical doses)	100%, 75%, 50% or 25% in FCA	Guinea pig (2)	Slight to moderate erythema at all doses	RIFM (1995i)
	Irritation (maximization pre-test)	100	Guinea pig (20)	0/20 at all doses	RIFM (1995i)
	Irritation[a] (maximization prescreen of topical doses)	100%, 50%, 25%, or 12.5% in EtOH:DEP	Guinea pig (2)	Slight erythema at both doses	RIFM (1997i)
	Irritation[a] (maximization pre-test)	25% or 12.5% in EtOH:DEP	Guinea pig (20)	Slight erythema at both doses	RIFM (1997i)

(*continued on next page*)

Table 6-2 (*continued*)

Material	Method	Concentration	Species (number)	Results	Reference
	Irritation (phototoxicity control)	50% in DEP, 10% in EtOH	Guinea pig (3)	No irritation at 10% Slight irritation at 72h with 50%	RIFM (1978g)
	Irritation (phototoxicity control)	100%	Hairless mice (6)	0/6	Forbes et al. (1977)

[a] OECD compliant study.
[b] Study performed under CTFA guidelines.
[c] Study met EEC requirements.

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

Material	Dose (No. animals)	Results	Reference
Ethylene dodecanedioate	2.5% in saline ($n = 6$)	Not irritating; slight conjunctival irritation at 8 h clear by 24 h	RIFM (1975c)
	2.5% in peanut oil ($n = 6$)	Not irritating; slight conjunctival irritation at 24 h clear by day 2	RIFM (1975d)
Hexadecanolide	0.5% in propylene glycol ($n = 3$)	Not irritating	RIFM (1972a)
	0.75% in propylene glycol ($n = 3$)	Not irritating; mild conjuncitival erythema clear by day 4	RIFM (1963a)
Oxacycloheptadec-10-ene-2-one	0.5% *vehicle not reported ($n = 3$)	Not irritating; moderate conjunctival irritation clear by day 4	RIFM (1963b)
Oxacyclohexadecane-2,13-dione	100% ($n = 6$)	Not irritating under US CFR	RIFM (1982e)
Oxacyclohexa dec-12(+13)en-2-one (E/Z isomer mix)	100% ($n = 4$)	Not irritating under EEC guidelines	RIFM (1992e)
11-Oxahexadecanolide	100% ($n = 6$)	Not irritating under FDA guidelines	RIFM (1980a)
12-Oxahexadecanolide	10% *vehicle not reported ($n = 3$)	Not irritating; slight conjuntival erythema (1/3) clear by day 2	RIFM (1977f)
	30% *vehicle not reported ($n = 3$)	Not irritating; slight conjuntival erythema (3/3) clear by day 2 (1/3)	RIFM (1977f)
	100% ($n = 3$)	Not irritating; slight conjunctival erythema (3/3) clear by day 3	RIFM (1977f)
ώ-Pentadecalactone	0.5% in propylene glycol ($n = 3$)	Not irritating	RIFM (1972b)

10. Skin sensitization

This group of macrocyclic lactones and lactide derivatives 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 approximately 2800 male and female volunteers for 11 of the macrocyclic lactone and lactide derivatives (Table 8-1a). Of these materials, only ethylene brassylate had results indicating sensitization. All studies had control volunteers.

Multiple HRIPT sensitization studies performed with ethylene brassylate at 10% or 20% in DEP or EtOH:DEP (3:1) have been identified. Of these 27 HRIPT studies, 25 indicated no evidence of sensitization; however, slight to mild irritation was reported (see Section 9.1) during the induction phase, and occasionally during the challenge phase. Of the remaining two HRIPT studies, one reported 2 of 107 sensitized (1.9%) and one reported 2 of 67 sensitized (3%). Therefore, among all the HRIPT studies performed with ethylene brassylate, there were 4 of 2059 positive reactions (0.19%).

10.1.2. Elicitation studies

One elicitation study was conducted using the macrocyclic lactone, ethylene brassylate as a negative control (Api and Letizia, 2001). This use test involved the 6 month long use of a bar of soap containing 0.05% ethylene brassylate, followed by the three month use of a moisturizing lotion containing 0.03% ethylene brassylate by 75 subjects. The final phase of the use test, which lasted for 4 months, involved the use, by 61 subjects, of four different cologne type products containing the material in question (at concentrations of 0.05%, 0.1%, 0.3% and 1%). No positive reactions due to ethylene brassylate were noted.

10.1.3. Diagnostic patch-test studies

Diagnostic patch-test studies have been reported for eight macrocyclic lactones (Table 8-1b).

One hundred seventy eight fragrance-sensitive patients were patch tested in eight centers worldwide with 5% of the following macrocyclic lactones in petrolatum: 10-oxahexadecanolide (0 reactions), 12-oxahexadecanolide (0 reactions), hexadecanolide (1 positive reaction, 0.6%) and ώ-6-hexadecenlactone (6 positive reactions, 3.4%). Of these, it was suggested that ώ-6-hexadecenlactone should be further evaluated to corroborate its allergenicity (Larsen et al., 2001).

Of 218 fragrance sensitive, male and female volunteers from seven centers worldwide, 0.9% had positive reactions with 5% ethylene dodecanedioate in petrolatum (2 positive reactions) (Larsen et al., 2002).

Table 8-1a
Skin sensitization in humans.

Material	Method	Concentration	Subjects	Results	Reference
Ethylene brassylate	HRIPT	20% in 3:1 EtOH:DEP (23,620 μg/cm^2)	106	0/106	RIFM (1988e)
	HRIPT	20% in 3:1 EtOH:DEP (23,620 μg/cm^2)	50	0/50	RIFM (1990a)
	HRIPT	20% in 3:1 EtOH:DEP (23,620 μg/cm^2)	48	0/48	RIFM (1990b)
	HRIPT	20% in 3:1 EtOH:DEP (23,620 μg/cm^2)	64	0/64	RIFM (1990c)
	HRIPT	20% in 3:1 EtOH:DEP (23,620 μg/cm^2)	67	2/67	RIFM (1990d)
	HRIPT	20% in 3:1 EtOH:DEP (23,620 μg/cm^2)	71	0/71	RIFM (1990e)
	HRIPT (2 groups)	20% in 3:1 EtOH:DEP (23,620 μg/cm^2)	47, 46	0/46, 0/47	RIFM (1990f)
	HRIPT	20% in 3:1 EtOH:DEP (23,620 μg/cm^2)	36	0/36	RIFM (1989a)
	HRIPT	20% in 3:1 EtOH:DEP (23,620 μg/cm^2)	103	0/103	RIFM (1989b)
	HRIPT	20% in 3:1 EtOH:DEP (23,620 μg/cm^2)	109	0/109	RIFM (1988a)
	HRIPT	20% in 3:1 EtOH:DEP (23,620 μg/cm^2)	38	0/38	RIFM (1988b)
	HRIPT	20% in 3:1 EtOH:DEP (23,620 μg/cm^2)	34	0/34	RIFM (1988d)
	HRIPT	20% in 3:1 EtOH:DEP (23,620 μg/cm^2)	58	0/58	RIFM (1988f)
	HRIPT	20% in 3:1 EtOH:DEP (23,620 μg/cm^2)	28	0/28	RIFM (1988c)
	HRIPT	20% in 3:1 EtOH:DEP (23,620 μg/cm^2)	197	0/197	RIFM (1987)
	HRIPT	20% in DEP (23,620 μg/ cm^2)	108	0/108	RIFM (1989e)
	HRIPT	10% in DEP (11,810 μg/cm^2)	65	0/65	RIFM (1995d)
	HRIPT	10% in DEP (11,810 μg/cm^2)	109	0/109	RIFM (1995f)
	HRIPT	10% in DEP (11,810 μg/cm^2)	91	0/91	RIFM (1994a)
	HRIPT	10% in DEP (11,810 μg/cm^2)	125	0/125	RIFM (1995e)
	HRIPT	10% in DEP (11,810 μg/cm^2)	95	0/95	RIFM (1994b)
	HRIPT	10% in DEP (11,810 μg/cm2)	22	0/22	RIFM (1994c)
	HRIPT	10% in DEP (11,810 μg/cm^2)	99	0/99	RIFM (1993)
	HRIPT	10% in DEP (11,810 μg/cm^2)	107	2/107	RIFM (1991)
	HRIPT	10% in 3:1 EtOH:DEP (11,810 μg/cm^2)	63	0/63	RIFM (1990g)
	HRIPT	10% in 3:1 EtOH:DEP (11,810 μg/cm^2)	36	0/36	RIFM (1989d)
	HRIPT	10% in 3:1 EtOH:DEP (11,810 μg/cm^2)	37	0/37	RIFM (1989c)
	Maximization	30% in petrolatum (20,700 μg/ cm^2)	25	0/25	RIFM (1973b)
Ethylene dodecanedioate	HRIPT	25% in 3:1 EtOH:DEP (13,800 μg/ cm^2)	44	0/44	RIFM (2000b)
	Maximization	20% in petrolatum (13,800 μg/ cm^2)	25	0/25	RIFM (1978e)
Hexadecanolide	HRIPT	2% in DMP (3937 μg/cm^2)[1]	54	0/54	RIFM (1972c)
	HRIPT	0.75% in EtOH (581 μg/cm^2)	40	0/40	RIFM (1964b)
	Maximization	4% in petrolatum (2760 μg/cm^2)	25	0/25	RIFM (1974e)
ώ-6-Hexadecenlactone	Maximization	1% in petrolatum (690 μg/cm^2)	26	0/26	RIFM (1974f)
Oxacycloheptadec-10-ene-2-one	HRIPT	0.5% in EtOH (382 μg/cm^2)	38	0/38	RIFM (1964a)
Oxacyclohexa decane-2,13-dione	HRIPT	20% in petrolatum (50,000 μg/cm^2)	50	0/50	RIFM (1982c)
Oxacyclohexadec-12(+13)en-2-one (E/Z isomer mix)	HRIPT	15% in DEP (7500 μg/cm^2)	104	0/104	RIFM (1997c)
10-Oxahexadecanolide	Maximization	10% in petrolatum (6900 μg/cm^2)	29	0/29	RIFM (1978f)
11-Oxahexadecanolide	Maximization	10% in petrolatum (6900 μg/cm^2)	24	0/24	RIFM (1977c)
12-Oxahexadecanolide	HRIPT	5% in DMP (3800 μg/cm^2)	51	0/51	RIFM (1978h)
	Maximization	10% in petrolatum (6900 μg/cm^2)	25	0/25	RIFM (1977d)
ώ-Pentadecalactone	HRIPT	~10% in 1:3 EtOH:DEP (13,770 μg/ cm^2)	105	0/105	RIFM (2006)
	HRIPT	2% in DMP (3940 μg/cm^2)	54	0/54	RIFM (1972d)
	48–72 h occluded				
	Maximization	10% in petrolatum (6900 μg/cm^2)	25	0/25	RIFM (1974e)

In a patch test study of 422 Korean contact dermatitis patients, there were no positive reactions with 5% oxacycloheptadec-10-ene-2-one in petrolatum. Six positive reactions occurred with 5% hexadecanolide (1.4%) (An et al., 2005).

In a patch test study of 50 patients that included 29 atopics with sensitive skin, 10% ethylene brassylate in EtOH:DEP did not cause any positive reactions (RIFM, 1997a). Likewise, when 8 mycolog cream-sensitive patients were patch tested with 5% ethylene brassylate in petrolatum there were no positive reactions (Larsen, 1979).

Of 25 asthmatic patients, none had positive reactions to 1% or 5% ethylene dodecanedioate in petrolatum (RIFM, 1977g).

In multiple patch tests of ώ-pentadecalactone in patients that included those with sensitive skin, cosmetic dermatitis, facial melanosis, eczema and dermatitis, there was no evidence of positive reactions (see Table 8-1b).

10.2. Animal studies

Nine macrocyclic lactones 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 6 macrocyclic lactones and lactide derivatives tested in the Maximization test, none reported sensitization in animals receiving the highest dose during challenge.

Sensitization was evaluated using local lymph node assays conducted with ethylene brassylate (Table 8-2b). The concentrations were not able to give rise to a 3-fold increase in lymphocyte proliferation and an EC_3 could not be established. Studies at higher concentrations were not conducted. This indicates that ethylene brassylate is unlikely to be a moderate or strong sensitizer (RIFM, 1997j; Kimber et al., 1994).

11. Phototoxicity and photosensitization

UV spectra have been obtained on 11 of the macrocyclic lactones and lactide derivatives. All 11 had maximum absorbance between approximately 190 and 210 nm, with the majority showing absorbance between 200 and 250 nm and returning to baseline by 300 nm (Table 11). Phototoxicity was assessed for one material in humans (Table 9-1), while nine of the macrocyclic lactones were assessed in guinea pigs or rabbits (Table 9-2). Two ML materials were tested for photosensitization; one in humans (Table 10-1) and another in guinea pigs (Table 10-2).

Table 8-1b
Diagnostic patch tests.

Material	Concentration	Subjects	Results (frequency)	Reference
Ethylene brassylate	10% in EtOH:DEP	50 (29 identified as atopics with sensitive skin)	0/50	RIFM (1997a)
	5% in petrolatum	8 Mycolog cream sensitive patients	0/8	Larsen (1979)
Ethylene dodecanedioate	1 or 5% in petrolatum	25 asthmatics	0/25	RIFM (1977g)
	5% in petrolatum	218 fragrance sensitive patients	2/218 (0.9%)	Larsen et al. (2002)
Hexadecanolide	5% in petrolatum	178 fragrance sensitive patients	1/178 (0.6%)	Larsen et al., 2001
	5% in petrolatum	422 Korean contact dermatitis patients	6/422 (1.4%)	An et al. (2005)
ώ-6-Hexadecenlactone	5% in petrolatum	178 fragrance sensitive patients	6/178 (3.4%)	Larsen et al. (2001)
Oxacycloheptadec-10-ene-2-one	5% in petrolatum	422 Korean contact dermatitis patients	0/422	An et al.(2005)
10-Oxahexadecanolide	5% in petrolatum	178 fragrance sensitive patients	0/178	Larsen et al. (2001)
12-Oxahexadecanolide	5% in petrolatum	178 fragrance sensitive patients	0/178	Larsen et al. (2001)
ώ-Pentadecalactone	100%	50 (11 identified as atopics with sensitive skin)	1/50 (2%) (non standard method; 1 person may have been sensitized, but results not confirmed	RIFM (1998b)
	10% in EtOH:DEP	50 (29 identified as atopics with sensitive skin)	0/50	RIFM (1997d)
	5% *vehicle not reported	85 patients with and without cosmetic dermatitis and in patients with facial melanosis	0/85	Ishihara et al. (1981)
	5% *vehicle not reported	101 eczema and dermatitis patients	0/101	Nishimura et al. (1984); Itoh et al. (1986); Itoh et al. (1988)

11.1. Phototoxicity

11.1.1. Human studies

One macrocyclic lactone was assessed for phototoxicity in humans. Twenty percent oxacyclohexadecane-2,13-dione in petrolatum was applied to the skin of 20 male and female volunteers followed by 365 nm UVA irradiation for 15 min at a distance of 38 cm (1680 μW/cm^2). None of the subjects exhibited any evidence of phototoxicity (RIFM, 1982c).

11.1.2. Animal studies

Eight of the macrocyclic lactones and lactide derivatives were tested with 20 or 1% in petrolatum or EtOH for phototoxicity on guinea pigs (Ogoshi et al., 1980; Ohkoshi et al., 1981). After a 2-h 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 the lactones ethylene brassylate, ethylene dodecanedioate, ώ-6-hexadecenlactone, oxacycloheptadec-10-ene-2-one, 10-, 11-,and 12-oxadecanolide, and ώ-pentadecalactone. In a similar study, 8 of the macrocyclic lactone and lactide derivatives were tested for phototoxicity in guinea pigs or rabbits (3/dose) at 5%, 10%, or 50% in EtOH or DEP at an energy level of 1.14×10^8 ergs/cm^2 (RIFM, 1978g). None of these materials were considered phototoxic by the authors. 10-Oxahexadecanolide at 30%, 10% or 5% in acetone with UVA irradiation of 13 J/cm^2 for 60 min was not phototoxic in guinea pigs (RIFM, 1997g). 11-Oxahexadecanolide at 10% in EtOH was also not considered phototoxic in rabbits and guinea pigs (RIFM, 1980a,b). Ten percent ethylene brassylate or 12-oxahexadecanolide in EtOH with 2% DMSO with an irradiation dose of 20 J/cm^2 UVA did not elicit a phototoxic response in guinea pigs (RIFM, 1983a,b). Undiluted ώ-pentadecalactone was tested for phototoxicity in hairless mice and miniature swine (Forbes et al., 1977). No phototoxicity was reported.

Studies with 30% ethylene brassylate and ethylene dodecanedioate in acetone with guinea pigs reported slight to moderate erythema in some of the animals, with a return to normal by 48 h, but no similar effect at 10% and 5%. The irradiation dose for these studies was 13 J/cm^2 UVA for 60 min (RIFM, 1997e,f).

11.2. Photosensitization

11.2.1. Human studies

No sensitization in humans was recorded during HRIPT with 20% oxacyclohexadecane-2,13-dione in petrolatum followed by 365 nm UVA irradiation for 15 min at a distance of 38 cm (1680 μW/cm^2) (Table 10-1; RIFM, 1982c).

11.2.2. Animal studies

A photosensitization study was performed on guinea pigs with 10% 11-oxahexadecanolide in EtOH (Table 10-2; RIFM, 1980b), and did not indicate photosensitization.

12. Conclusions

The macrocyclic lactone and lactide derivatives fragrance ingredients constitute a class of chemicals with distinct reactivity and metabolism and thus distinct toxicology. JECFA and FEMA have reported that macrocyclic lactones are acted upon by carboxylesterases and exhibit pH-dependent equilibrium between a linear hydroxycarboxylate anion and cyclic ester. No *in vivo* mammalian metabolite studies, utilizing radiolabeled materials are currently available for the macrocyclic lactones. These types of studies would be necessary to fully substantiate the proposed metabolic pathway illustrated in Fig. 1 and to conclusively identify metabolites.

Table 8-2a
Skin sensitization in animals.

Material	Method	Induction	Challenge	Species (No./group)	Results	Reference
Ethylene brassylate	Maximization[a]	5% in arachis oil BP or FCA (intradermal); 100% (topical)	50% or 25% in EtOH:DEP	Guinea pig (20)	0/10 at both doses	RIFM (1995g)
	Maximization[a]	5% in liquid parafin or FCA (intradermal); 10% in petrolatum (topical)	100%, 50%, 5%, 3%, 1% or 0.1% in EtOH	Guinea pig (5)	Moderate erythema at 50 and 100%, however it was judged to be stimulative	RIFM (2005b)
Ethylene dodecanedioate	Buehler delayed hypersensitivity	100% (topical)	100%	Guinea pig (20)	0/20	RIFM (1975e)
	Maximization	10% in FCA (intradermal); 10% in petrolatum (topical)	20%, 10%, or 5% in acetone	Guinea pig (10)	0/10 at all doses	RIFM (1997h)
Hexadecanolide	Open epicutaneous test	100%, 30%, 10%, 3%, 1%, or 0.3%∗vehicle not specified	4%∗vehicle not specified	Guinea pig (6–8)	0/6–8	Klecak (1985)
ώ-6-Hexa decenlactone	Open epicutaneous test	100, 30, 10, 3, 1, or 0.3%∗vehicle not specified	1%∗vehicle not specified	Guinea pig (6–8)	0/6–8	Klecak (1979)
Oxacyclohexadec-12(+13)en-2-one(E/Z isomer mix)	Maximization	50% in paraffin or 25% in FCA (intradermal); 100% (topical)	100% or 50% in EtOH	Guinea pig (20)	Not a sensitizer 2/20 reactions observed	RIFM (1992f)
10-Oxahexadecanolide	Maximization	10% in FCA (intradermal); 10% in petrolatum (topical)	20%, 10% or 5% in acetone	Guinea pig (10)	0/10 at all doses	RIFM (1982f)
11-Oxahexadecanolide	Maximization	2% in propylene glycol or FCA (intradermal); 10% in petrolatum (topical)	10% in petrolatum (topical)	Guinea pig (10)	0/10	RIFM (1980c)
12-Oxahexadecanolide	Freund's Complete Adjuvant Test	5% in FCA (intradermal)	1% in EtOH	Guinea Pig (8)	0/8	RIFM (1977e)
	Open epicutaneous test	100, 30, 10 or 3% in EtOH	Up to 3% EtOH	Guinea pig (6)	0/6 at all doses	RIFM (1977e)
	Open epicutaneous test	30, 10, 3, 1, 0.3% in an unspecified vehicle	10%	Guinea pig (6–8)	0/6–8	Klecak (1985)
ώ-Pentadecalactone	Maximization[a]	5% in arachis oil BP or FCA (intradermal); 100% in EtOH:DEP (topical)	75% or 50% in EtOH:DEP	Guinea pig (20)	0/2 at both doses	RIFM, 1995i
	Maximization[a]	10% in sesame oil or FCA (intradermal); 50% in 1:1 EtOH:DEP (topical)	25% in 1:1 EtOH:DEP challenge; 12.5% in 1:1 EtOH:DEP rechallenge	Guinea pig (20)	0/20	RIFM (1997i)

[a] OECD compliant study.

Table 8-2b
Local lymph node assay (LLNA).

Material	Method	Dose	Species (No./group)	Results	Reference
Ethylene brassylate	LLNA[b]	30, 10 or 1% in acetone	Mouse (4)	Non-sensitizer[a]	RIFM (1997j)

[a] Did not result in a 3-fold isotope incorporation.
[b] OECD compliant study.

Table 9-1
Phototoxicity in humans.

Material	Method	Concentration	Subjects	Results	Reference
Oxacyclohexadecane-2,13-dione	Photosensitization (induction)	20% in petrolatum, 1680 μW/cm^2 UVA	Human (20)	0/20	RIFM (1982c)

The macrocyclic lactone and lactide derivatives 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 reevaluation by the Panel. It is the opinion of the Expert Panel that safety concerns regarding the use of the macrocyclic lactone and lactide derivatives are not indicated under the reported 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 ML group of fragrance ingredients including their metabolites.

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

Table 9-2
Phototoxicity in animals.

Material	Concentration	Species (number/dose)	Results	Reference
Ethylene brassylate	10% in EtOH with 2% DMSO, 20 J/cm^2 UVA	Guinea pig (10)	0/10[a]	RIFM (1983a)
	30%, 10%, or 5% in acetone, 13 J/cm^2 UVA	Guinea pig (5)	5/5 at 30%, 0/5 at 10% and 5%	RIFM (1997e)
	20% or 1% in petrolatum or EtOH, 1.6–7.6 J/ cm^2 UVA	Guinea pig (5)	0/5	Ohkoshi et al. (1981); Ogoshi et al. (1980)
	50% in EtOH or DEP 1.14 × 10^8 ergs/cm^2	Guinea pig (3)	Not phototoxic	RIFM (1978g)
	50% in EtOH 1.14 × 10^8 ergs/cm^2	Rabbit (3)	Not phototoxic	RIFM (1978g)
Ethylene dodecanedioate	20% or 1% in petrolatum or EtOH, 1.6–7.6 J/cm^2 UVA	Guinea pig (5)	0/5	Ohkoshi et al. (1981); Ogoshi et al. (1980)
	30%, 10%, or 5% in acetone 13 J/cm^2 UVA	Guinea pig (5)	3/5 at 30%, 0/5 at 10% and 5%	RIFM, 1997f
Hexadecanolide	50% in DEP or 5% in EtOH 1.14 × 10^8 ergs/cm^2	Guinea pig (3)	Not phototoxic at either dose at 24, 48 or 72 h *no information on individual test subject reactions	RIFM (1978g)
	5 or 1% in EtOH 1.14 × 10^8 ergs/cm^2	Rabbit (3)	Not phototoxic at either dose at 24, 48 or 72 h *no information on individual test subject reactions	RIFM (1978g)
ω-6-Hexadecenlactone	20% or 1% in petrolatum or EtOH, 1.6–7.6 J/cm^2 UVA	Guinea pig (5)	0/5	Ohkoshi et al. (1981), Ogoshi et al. (1980)
	50% in DEP or 10% in EtOH 1.14 × 10^8 ergs/cm^2	Guinea pig (3)	Not phototoxic at either dose at 24, 48 or 72 h *no information on individual test subject reactions	RIFM (1978g)
	10% in EtOH 1.14 × 10^8 ergs/cm^2	Rabbit (3)	Not phototoxic at 24, 48 or 72 h *no information on individual test subject reactions	RIFM (1978g)
Oxacycloheptadec-10-ene-2-one	20% or 1% in petrolatum or EtOH, 1.6–7.6 J/ cm^2 UVA	Guinea pig (5)	0/5	Ohkoshi et al. (1981), Ogoshi et al. (1980)
10-Oxahexadecanolide	50% in DEP 1.14 × 10^8 ergs/cm^2	Guinea pig (3)	Not phototoxic at 24, 48 or 72 h *no information on individual test subject reactions	RIFM (1978g)
	20% or 1% in petrolatum or EtOH, 1.6–7.6 J/ cm^2 UVA	Guinea pig (5)	0/5	Ohkoshi et al. (1981), Ogoshi et al. (1980)
	30%, 10%, or 5% in acetone, 13 J/cm^2 UVA	Guinea pig (5)	0/5 at all doses	RIFM (1997g)
11-Oxahexadecanolide	10% in EtOH	Rabbit (6)	0/6	RIFM (1980a)
	50% in DEP 1.14 × 10^8 ergs/cm^2	Guinea pig (3)	Not phototoxic at 24, 48 or 72 h *no information on individual test subject reactions	RIFM (1978g)
	20% or 1% in petrolatum or EtOH, 1.6–7.6 J/ cm^2 UVA	Guinea pig (5)	0/5	Ohkoshi et al. (1981), Ogoshi et al. (1980)
	10% in EtOH	Guinea Pig (15)	0/15	RIFM (1980b)
12-Oxahexadecanolide	50% in DEP 1.14 × 10^8 ergs/cm^2	Guinea pig (3)	Not phototoxic at 24, 48 or 72 h *no information on individual test subject reactions	RIFM (1978g)
	20% or 1% in petrolatum or EtOH, 1.6–7.6 J/ cm^2 UVA	Guinea pig (5)	0/5	Ohkoshi et al. (1981), Ogoshi et al. (1980)
	10% in EtOH with 2% DMSO, 20 J/cm^2 UVA	Guinea pig (10)	0/10	RIFM (1983b)
ω-Pentadecalactone	20% or 1% in petrolatum or EtOH, 1.6–7.6 J/ cm^2 UVA	Guinea pig (5)	0/5	Ohkoshi et al. (1981), Ogoshi et al. (1980)
	50% in DEP and 10% in EtOH 1.14 × 10^8 ergs/cm^2	Guinea pig (3)	Not phototoxic at either dose at 24, 48 or 72 h *no information on individual test subject reactions	RIFM (1978g)
	10% or 5% in EtOH 1.14 × 10^8 ergs/cm^2	Rabbit (3)	Not phototoxic at either dose at 24, 48 or 72 h *no information on individual test subject reactions	RIFM (1978g)
	100%	Hairless mice (6)	0/6	Forbes et al. (1977)
	100%	Miniature swine (2)	0/2	Forbes et al. (1977)

[a] Study performed under CTFA guidelines.

Table 10-1
Photosensitization in humans.

Material	Method	Concentration	Subjects	Results	Reference
Oxacyclohexadecane-2,13-dione	Photosensitization	20% in petrolatum during induction, 1680 μW/cm^2 UVA	Human (20)	0/20	RIFM (1982c)

Table 10-2
Photosensitization in animals.

Material	Method	Concentration	Species (number/dose)	Results	Reference
11-Oxahexadecanolide	Photosensitization	10% in EtOH (induction); 5% in EtOH (challenge)	Guinea pig (15)	0/15	RIFM (1980b)

Table 11
Summary of UV spectra data.

Material	UV spectra range of absorption (nm)
Ethylene brassylate	Maximum at 201. Some absorbance from 200 to 280. Baseline by 300
Ethylene dodecanedioate	Maximum at 210. Some absorbance from 210 to 240. Baseline by 250
Hexadecanolide	Maximum at 203. Some absorbance from 200 to 240. Return to baseline by 250
ω-6-Hexadecenlactone	Maximum at 205–210. Some absorbance from 210 to 240. Return to baseline by 250
Oxacycloheptadec-10-ene-2-one	Maximum at 206. Absorbance between 210 and 250. Return to baseline by 270
Oxacyclohexadecane-2,13-dione	Maximum at 208. Absorbance between 208 and 330 with distinct peak at 280. Return to baseline at 340
(E) and (Z)-Oxacyclohexadec-(12 or 13))-en-2-one	Maximum at 201. Some absorbance between 225 and 260. Return to baseline by 270
10-Oxahexadecanolide	Maximum at 190. Absorbance at 200–250. Return to baseline by 270
11-Oxahexadecanolide	Maximum at 210. Absorbance between 190 and 230. Near baseline by 290.
12-Oxahexadecanolide	Maximum at 190. Second, smaller peak at 210. Return to baseline at 250
ω-Pentadecalactone	Maximum at 210. Some absorbance from 210 to 250. Return to baseline by 290

- Low acute toxicity.
- No significant toxicity 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 blood biochemistry were reversible after two weeks of no treatment.
- No genotoxic activity was observed in bacteria, mammalian cell lines or mice assays. Therefore, although carcinogenicity studies are lacking, this evidence is not indicative of carcinogenicity by this mechanism.
- No reproductive or developmental toxicity was reported for the macrocyclic lactone oxacyclohexadec-12(+13)en-2-one (E/Z isomer mix). The NOEL for this material was 1000 mg/kg body weight/day, and there was no observed treatment effect on offspring, on fertility or on reproductive performance (NOEL 1000 mg/kg body weight/day).
- Human dermatological studies show that these fragrances ingredients, with the possible exception of ethylene brassylate, are not irritating after one application. During the induction phase of HRIPTs, in which volunteers had patches reapplied three times per week, 10% or 20% ethylene brassylate appeared to cause weak to moderate irritation that dissipated with time. Animal studies indicate that irritation occurs only at high concentrations (30%, 50% or 100%) which are not consistent with the reported maximum skin levels of fragrance ingredients in consumer products and fine fragrances (see Table 1).
- The potential for eye irritation at the present maximum use level is considered minimal.
- No phototoxicity or photosensitization were observed at rates that are consistent with estimated levels for current human exposures.
- Animal studies have demonstrated that these fragrance ingredients are not sensitizers at exposures from 0.1% to 100%. In a murine local lymph node assay, up to 30% ethylene brassylate was considered non-sensitizing. Eleven ML materials were evaluated for human sensitization. Of these, only ethylene brassylate showed evidence of sensitization. Of the 27 sensitization studies with 10% or 20% ethylene brassylate, 25 had no evidence of sensitization. The remaining 2 studies showed sensitization at frequencies of 1.9% (2/101) and 3% (2/67). In total, out of 2059 HRIPTs performed with ethylene brassylate there were 4 positive reactions (0.19%). Thus, in general, the potential for human skin sensitization with ML fragrance ingredients is low. There is, however, evidence of positive patch test reactions in patients with fragrance sensitivity or contact dermatitis (highest frequency 3.4% (6/178) to a concentration of 5% ω-6-hexadecanolide).
- To calculate margin of safety, the lowest NOAEL of 1000 mg/kg body weight/day (this value was the same for all the repeat dose toxicity by oral exposure reported for the materials presented in Table 3-1) is used as a representative worst case scenario for the group (assuming 100% oral absorption). Using the highest systemic exposure for the group (0.25 mg/kg body weight/day for ethylene brassylate) again, as a representative worst case scenario, and assuming 100% dermal absorption, the margin of safety is calculated to be 4000. If a margin of safety of 100 were used, the maximum allowable exposure would be 10 mg/kg body weight/day.

Conflict of Interest

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. The authors are all members of the Expert Panel for Fragrance Materials, an independent group of experts who evaluate the safety of fragrance materials.

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