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Macrocyclic fragrance materials – A screening-level environmental assessment using chemical categorization

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Abstract

A screening-level aquatic environmental risk assessment for macrocyclic fragrance materials using a “group approach” is presented using data for 30 macrocyclic fragrance ingredients. In this group approach, conservative estimates of environmental exposure and ecotoxicological effects thresholds for compounds within two subgroups (15 macrocyclic ketones and 15 macrocyclic lactones/lactides) were used to estimate the aquatic ecological risk potential for these subgroups. It is reasonable to separate these fragrance materials into the two subgroups based on the likely metabolic pathway required for biodegradation and on expected different ecotoxicological modes of action. The current volumes of use for the macrocyclic ketones in both Europe and North America ranges from <1 (low kg quantities) to no greater than 50 metric tons in either region and for macrocyclic lactones/lactides the volume of use range for both regions is <1 to no greater than 1000 metric tons in any one region. Based on these regional tonnages, biodegradability of these two subgroups of materials, and minimal in stream dilution (3:1), the conservatively predicted exposure concentrations for macrocyclic ketones would range from <0.01 to 0.05 µg/L in Europe and from <0.01 to 0.03 µg/L in North America. For macrocyclic lactones/lactides, the concentration within the mixing zone would range from <0.01 to 0.7 µg/L in Europe and from <0.01 to 1.0 µg/L in North America. The PNECs derived for the macrocyclic ketones is 0.22 µg/L and for macrocyclic lactones/lactides is 2.7 µg/L. The results of this screening-level aquatic ecological risk assessment indicate that at their current tonnage, often referred to as volumes of use, macrocyclic fragrance materials in Europe and North America, pose a negligible risk to aquatic biota; with no PEC/PNEC ratio exceeding 1 for any material in any subgroup.

Keywords: risk assessment, chemical categorization, fragrance ingredients

1. Introduction

The Research Institute for Fragrance Materials (RIFM) “Framework for prioritizing fragrance materials for aquatic risk assessment” (termed “Environmental Framework” hereafter) was developed to screen a large database of organic compounds used as fragrance ingredients to assess their potential environmental risk and set priorities for further risk assessment, as necessary (Salvito et al., 2002). RIFM has been using a group or chemical categories approach based on structure–activity relationships for the assessment of human health safety (Bickers et al., 2003). Presented here is the first application of this group approach for the aquatic risk assessment of structurally related groups of fragrance ingredients using the example of macrocyclic fragrance ingredients. Macrocyclic fra-

grance materials are important fragrance ingredients and are widely used in cosmetics, detergents, fabric softeners, cleaning products and other household products. This approach is based on the hypothesis that if chemicals are structurally related, they behave similarly in the aquatic environment. In the group approach, available environmental fate and effects values for individual compounds within the group are used to conservatively estimate the potential for aquatic ecological risks for the entire structurally related group. The fragrance materials in the macrocyclic ketone group and the macrocyclic lactone and lactide group have been reviewed in separate human health group summaries (Belsito et al., in press a, in press b). These group summaries contain references to the Fragrance Material Reviews that provide the human health data for each of the individual materials in the group.

1.1. Principles of chemical categorization

Chemical categorization, also referred to as “chemical grouping”, is a method of identifying analogs for chemicals of interest and enabling the extrapolation of a specific endpoint(s) of data-poor chemicals from data rich chemicals. Analogs can be read-across one chemical to one chemical, one to many, or many to many. The OECD has established guidance on the formation of categories and guidance has also been prepared in preparation for REACH by a REACH Implementation Project (OECD, 2005).

1.2. General guidelines

The group approach as applied here followed the guidance provided by the OECD (OECD, 2005) for the formation of chemical categories. This guidance could be applied to both human health and environmental endpoints. The principles are:

1. Identify chemical category and assign its members.
2. Gather published and unpublished data for each category member.
3. Evaluate available data for adequacy in assessing the domain hypothesis.
4. Construct a matrix of data availability.
5. Perform an internal assessment of the category.
6. Prepare a category test plan.
7. Conduct the necessary testing.
8. Perform an external assessment of the category and fill data gaps.

The key steps for establishing a hypothesis and determining the validity of the hypothesis are steps 1 and 5; supported by the data gathering and review steps (2 through 4). Step 1 identifies the potential domain of the category and establishes the hypothesis built upon said domain. In a general sense a group of organic chemicals containing similar functional groups and covering a specified physical-chemical property range, potentially linked metabolically, should have endpoints that are predictive based on the findings of other members within the category.

The purpose of Step 5 is to test the hypothesis formulated in Step 1; i.e. trying to refute its premise that the group selected is a reasonable one for read-across. This is done using the existing information and, assessing in parallel persistence (*P*), bioaccumulation (*B*) and toxicity (*T*) criteria. This part of the analysis will involve a more careful consideration of structure, QSAR and metabolic pathways than in Step 1. For example, the endpoints should support the metabolic pathway hypothesis and/or fit a well-validated QSAR. The possible outcomes of Step 5 are:

- Reject the entire category (should occur rarely).
- Reject some members based on sound scientific reasoning—poor fit with remaining members (expected outcome).
- Develop candidate subcategories (note that the outcome here may be hierarchal; e.g. a “P” group with 2 different “BT” subgroups).

Subcategory size should be the largest possible group of chemicals that shows common features (i.e., supports the domain hypothesis). However, the category itself may be the best fit for all endpoints (i.e., no need for subcategorization).

At this point there is likely to be a need for the collection of more data (Steps 6–8) for at least two purposes. To further test the hypothesis that the category is a sound one and to fill important data gaps.

The following points have to be considered during the process:

- Categorization and its associated hypothesis testing is a weight of evidence approach. There are likely to be

outliers that should fit the category but do not. To the extent possible, these outliers should be explained; e.g., α , β unsaturation with respect to carbonyl groups present is a different category that is not part of the general group of ketones.

- Categorization is an approach in which both the favorable and unfavorable aspects of available data are applied equally.
- The use of chemical categories does not preclude minimal testing strategies (i.e., minimally, physical-chemical properties have to be measured or estimated and ready biodegradation studies will need to be performed).
- Subcategories for different endpoints may not match between endpoints relevant for human health and environmental endpoints (or even within); e.g., a skin sensitization subcategory may not contain all the same members as a persistence subcategory.
- Consideration of consistency between categories is important as well. There will be chemicals that will reside in more than one category. The development of the domain parameters and the category hypothesis should be applicable for the chemical in both (or more) categories. Furthermore, the conclusions for the appropriate subcategories, in this case where a chemical would reside in two distinct groups, should be consistent (e.g., the chemical cannot be bioaccumulative in one subcategory and not bioaccumulative in another).

1.3. Specific guidelines for endpoint assessment in categorization

While the principles outlined above provide guidance for the building and assessment of chemical categories in a broad sense, below are additional guidance for evaluating specific endpoints for environmental exposure (persistence and bioaccumulation data) and aquatic effects.

1.3.1. Persistence

If no experimental data are available the following computational models can serve as assessment tools:

- a.) CATABOL—for commonality of metabolic pathways;
- b.) METEOR—for mammalian metabolism; may be useful for P assessment and for fish metabolism;
- c.) University of Minnesota Biocatalysis/Biodegradation Database—for degradation reaction pathways.

The following questions need to be addressed:

1. Structural assessment: Does the same path exist for the chemicals in the groups for initial primary degradation (same sites of attack)?
2. Are there breaks in the available dataset between readily biodegradable materials, inherently biodegradable materials, and non-biodegradable materials that are structurally defined?
3. Structural assessment: Is the pathway for biodegradation likely to go to completion (mineralization) or are there structural biophobes that are likely to inhibit this process?
4. Do potential metabolites (i.e., breakdown products), of the chemicals that do not completely metabolize have B or T properties?
5. What is the expectation of performance in a ready test (should be able to predict % biodegradation)?
6. If no ready biodegradation is predicted:
  - a.) Is the material toxic under test conditions?
  - b.) Do organisms have difficulty growing on the chemical to permit primary degradation (i.e., co-metabolism)?
  - c.) Is the material bioavailable?
  - d.) What is expected in aerobic versus anaerobic tests?

1.3.2. Bioaccumulation

In a first step, the materials are grouped in classes with known metabolic activity versus non- or poorly metabolized materials.

- For non (poorly)-metabolizable chemicals separations between non-B/B/vB is based on the hydrophobicity indicator octanol–water partition coefficient K_{OW} ;
- For categories with more than one metabolic pathway, separate by pathway.

1.3.3. Ecotoxicity

The classes are separated via mode of action (MOA) classification. For fragrance materials this separation is largely between narcosis (both Type I and II) and reactive toxicants (e.g., aldehydes). These mode-of-action classifications were originally proposed by Verhaar et al. (1992) and have had wide application in ecotoxicology. MOA I and II (nonpolar and polar narcosis) chemicals can more than likely be grouped together as they have a common underlying mechanism (Vaes et al., 1998). The difference is not very significant. ECETOC developed a more robust method for determining MOA (Thomas et al., 2008) beyond the use of the structural alerts of Verhaar et al. (1992).

This weight of evidence approach, in whole or in part, may prove useful in categorizing for ecotoxic endpoints.

2. Materials and methods

2.1. Domain definition: macrocyclic fragrance ingredients

2.1.1. Structural similarity and physical–chemical properties (identify chemical category and assign its members)

These macrocyclic fragrance materials are important fragrance ingredients and are widely used in cosmetics, detergents, fabric softeners, cleaning products and other household products. The groups of macrocyclic fragrance ingredients identified for this study consist of two structural classes (i.e., subcategories), macrocyclic ketones and macrocyclic lactones/lactides. The materials specifically identified in this study are a complete list of macrocyclic fragrance materials, as of this publication, used in commerce and found in the RIFM Database. The process de-

scribed above for defining and assessing chemical categories was adhered to. Both of these classes are represented by a large carbon ring (typically 14–17 carbons, Figure 1). Unsaturation may also be present on the carbon ring. From the domain assessment that follows and a review of the available data, the presence or absence of unsaturation in the ring does not appear to necessitate further differentiation of these subgroups (i.e., there is no apparent effect on biodegradation or toxicity for these chemicals). The macrocyclic ketones contain only a single carbonyl functional group on the ring. The macrocyclic lactones/lactides contains one or two cyclic ester functional groups and, in the case of the lactones may also contain an additional ring oxygen or carbonyl group. Thirty different macrocyclic compounds were included in this group assessment, 15 lactones/lactides and 15 ketones. Their physical–chemical properties are presented in Table 1. Measured data are provided where available. When measured values were not available, physical–chemical property estimates were calculated using the USEPA's EPI Suite models (version 4.0).

2.1.2. Microbial metabolism

2.1.2.1. Gather published and unpublished data for each category member; evaluate available data for adequacy; construct a matrix of data availability. In addition to the structural differences between the two chemical classes, the macrocyclic ketones would have to undergo oxidation to a lactone during biodegradation and then further degrade via ester hydrolysis. The materials in the lactone/lactide subgroup would, therefore, degrade directly via ester hydrolysis. This microbial pathway was identified using the University of Minnesota Biocatalysis/Biodegradation Database (Gao et al., 2010) and shows, for cyclohexanone, Baeyer–Villiger oxidation of a cyclic ketone to a cyclic lactone (neutral under aerobic conditions) followed by the lactone forming the hydroxy carboxylate (likely under aerobic conditions). A representative reaction pathway is shown in Figure 2.

2.1.2.2. Perform an internal assessment of the category. The two principally different pathways are supported by the difference in biodegradation predicted by BIOWIN for the two classes (i.e., predict “not readily biodegradable” for the ketones and “readily biodegradable” for the lactones/lactides). This difference in prediction by the BIOWIN model is likely due to the necessity of an initial oxidation step for the ketones as noted above. Standardized experimental studies of biodegradation may not necessarily provide the data needed to demonstrate this difference. Thus, based on a weight of evidence approach, the biodegradation data for both groups of materials indicates that these compounds are readily biodegradable. However, the known difference in metabolism does support the distinction between the two subclasses (Table 2).

Subgroup	Ketones	Lactones/Lactides
Representative Structure		
Number of materials	15	15
Verhaar Mode of Action	Non-Polar Narcosis	Polar Narcosis
Biodegradation Class	Inherent to Readily	Readily
Ring Size	C14 to C17	C14 to C16
Notes	May contain unsaturation in ring and methyl side chain	May contain unsaturation in ring and methyl side chain. Lactides contain additional carbonyl group and ring oxygen (i.e, diester)

Figure 1. Domain definition.

Table 1. Physical chemical properties.

CAS#	Name	2008 Vol Global	2008 Vol EU	2008 Vol NA	MW	Formula	K _{OW}	Solubility (aq) (mg/L)	Vapor pressure (mm Hg 25 °C)
Ketones									
542-46-1	9-Cycloheptadecan-1-one	<1	<1	<1	250.43	C ₁₇ H ₃₀ O	6.31	0.096	0.00034
2550-52-9	Cyclohexadecanone	1-10	1-10	1-10	238.41	C ₁₆ H ₃₀ O	6.04	0.19	0.00026
2550-59-6	7-Cyclohexadecan-1-one	<1	NR	<1	236.4	C ₁₆ H ₂₈ O	5.82	0.30	0.00024
37609-25-9	5-Cyclohexadecan-1-one	10-100	10-100	1-10	236.99	C ₁₆ H ₂₈ O	5.82	0.30	0.00024
3100-36-5	Cyclohexadec-8-en-1-one mixture of <i>cis</i> and <i>trans</i> isomer	10-100	10-100	1-10	236.99				
502-72-7	Cyclopentadecanone	10-100	10-100	10-100	224.39	C ₁₅ H ₂₈ O	5.55	0.60	0.00042
63314-79-4	5-Cyclopentadecan-1-one, 3-methyl-	1-10	NR	1-10	236.4	C ₁₆ H ₂₈ O	6.57 (m)	0.100 (m)	0.0003 (m)
35720-57-1	4-Cyclopentadecan-1-one	1-10	1-10	NR	222.37	C ₁₅ H ₂₆ O	5.33	0.94	0.00057
14595-54-1	4-Cyclopentadecan-1-one, (Z)-	1-10	<1	1-10					
3603-99-4	Cyclotetradecan-1-one	0	0	0	210.61	C ₁₄ H ₂₆ O	5.05	1.87	0.0016
259854-70-1	5-Cyclotetradecan-1-one, 3-methyl-, (5E)-	1-10	<1	<1	222.37	C ₁₅ H ₂₆ O	5.6 (m)	1.08	0.0011
259854-71-2	5-Cyclotetradecan-1-one, 3-methyl-, (5Z)-	NR	NR	NR					
541-91-3	3-Methyl-1-cyclopentadecanone	1-10	1-10	<1	238.42	C ₁₆ H ₃₀ O	5.96	0.22	0.00047
82356-51-2	3-Methylcyclopentadecanone	10-100	10-100	10-100		C ₁₆ H ₂₈ O	>4.88 (m)	0.899 (m) ^a	0.0003 (m)
61415-11-0	3-Methylcyclotridecan-1-one	NR	NR	NR	210.61	C ₁₄ H ₂₆ O	4.98	2.16	0.0021
Lactones/lactides									
105-95-3	Ethylene brassylate	>1000	100-1000	100-1000	270.37	C ₁₅ H ₂₆ O ₄	4.7 (m)	1.72	4.4e-007
54982-83-1	Ethylene dodecanedioate	100-1000	100-1000	100-1000	256.35	C ₁₄ H ₂₄ O ₄	3.65 (m)	75 (m)	0.00021 (m)
109-29-5	Hexadecanolid	10-100	10-100	10-100	254.42	C ₁₆ H ₃₀ O ₂	6.65	0.047	2.5e-005
7779-50-2	ω-6-Hexadecenlactone	<1	<1	<1	252.4	C ₁₆ H ₂₈ O ₂	5.51 (m); 6.7 (m) —out of method range		2.2e-005
123-69-3	16-Hydroxy-7-hexadecenoic acid lactone	<1	NR	<1	252.39	C ₁₆ H ₂₈ O ₂			
28645-51-4	Oxacycloheptadec-10-ene-2-one	10-100	10-100	10-100	252.39	C ₁₆ H ₂₈ O ₂			
36508-31-3	Oxacycloheptadec-11-en-2-one	<1	<1	<1	252.39	C ₁₆ H ₂₈ O ₂			
38223-29-9	Oxacyclohexadecane-2,13-dione	<1	<1	<1	254.37	C ₁₅ H ₂₆ O ₃	4.1	80 (m)	0.0006 (m)
34902-57-3	ω-Oxacyclohexadecan-2-one	100-1000	100-1000	100-1000	238.37	C ₁₅ H ₂₆ O ₂	>6.2 (m); >3.94 (m); 4.88 calc	0.964 (m)	0.0012 (m)
111879-80-2	E- and Z-Oxacyclohexadec-12(+13)-en-2-one	10-100	10-100	10-100					
329925-33-9	Oxacycloheptadec-10-en-2-one, 13-methyl-	1-10	1-10	1-10	238.37	C ₁₅ H ₂₆ O ₂	2 isomers 5.8; 6.0 (m)	2.15	7.0 e-5
1725-01-5	10-Oxahexadecanolid	1-10	<1	1-10	256.39	C ₁₅ H ₂₈ O ₃	4.9	1.43	1.6e-005
3391-83-1	11-Oxahexadecanolid	1-10	1-10	<1	256.39	C ₁₅ H ₂₈ O ₃	4.9	1.43	1.6e-005
6707-60-4	12-Oxahexadecanolid	1-10	1-10	<1	256.39	C ₁₅ H ₂₈ O ₃	4.9	1.43	1.6e-005
106-02-5	ω-Pentadecalactone	100-1000	100-1000	100-1000	240.39	C ₁₅ H ₂₈ O ₂	6.15	0.15	5.17e-005

Properties calculated using EPI/SUITE 4.0 unless noted as measured: m.

Volume range is in metric tones.

As several of these materials are structural isomers, the physical-chemical properties for isomers are grouped and only one value is reported.

NR: None reported.

a. pH of 6.3 and 20 °C; another study at pH of 9.15 and 20 °C reports a solubility of <0.07 mg/L.

Figure 2. Representative reaction pathway for microbial metabolism of macrocyclic ketones and lactones.

2.1.3. Ecotoxicological mode of action

2.1.3.1. Gather published and unpublished data for each category member, evaluate available data for adequacy, construct a matrix of data availability. The aquatic toxicity data is summarized in Table 3.

Note: References noted in Table 2 and Table 3 represent a significant number of unpublished studies from the RIFM Database. The complete citations for these studies can be found in the Supplemental Information provided for this paper.

2.1.3.2. Perform an internal assessment of the category. According to Verhaar et al.'s (1992) rules for identifying ecotoxicological mode of action for organic compounds, the ketones would likely act as non-polar narcotics (Type I) and the lactones/lactides as polar narcotics (Type II). As noted earlier, as there is little to differentiate between these two modes of action, the measured aquatic toxicity varies little between the two subcategories. It can be noted, in support of this distinction, that the USEPA's QSAR model ECOSAR differentiates between the two classes of compounds as well. ECOSAR applies the neutral organic QSAR to the ketones and the ester QSAR to the lactones/lactides. Furthermore, comparison between the output of this QSAR and measured toxicity values is not necessarily confirmatory of correct classification of a chemical category. These QSARs both under and over predict toxicity compared with experimental results.

2.1.4. Conclusion on chemical categories.

As noted in Section 2.1.2, the measured experimental data for these two subgroups, are likely not sensitive enough to differentiate between the additional oxidation step needed for biodegradation or for differences between Type I and Type II narcosis in aquatic toxicity. However, following the Guidelines for categorization, the structural differences, microbial pathway for degradation, and ecotoxicological modes of action support the hypothesis that the macrocyclic fragrance materials are separable into two subcategories: ketones and lactones/lactides (Figure 3). As this is an exercise in hypothesis testing, the data, in and of themselves, do not refute this hypothesis.

2.2. Screening level risk assessment for the aquatic compartment

2.2.1. Problem formulation

A screening-level risk assessment was performed using the RIFM Environmental Framework (Salvito et al., 2002). This framework includes problem formulation, evaluation of exposure (via a conservative predicted environmental concentration based on each individual material's continental tonnage) and ecotoxicological effect (via a predicted no effect concentration based on the lowest aquatic toxicity endpoint measured for any one material within a subgroup, but applied to the whole subgroup) and risk characterization. The assessment was performed for each group using data from all compounds in the group.

2.2.2. Analysis – characterization of exposure: PEC

The primary pathway by which fragrance materials can enter the environment during and after consumer use is as wastewater washed down drains (Salvito et al., 2002). A portion of the fragrance materials may volatilize to the atmosphere. The amount depends upon the type of consumer product the fragrance material is used in and the volatility of the fragrance compound. Another fraction will leave the household in the wastewater. Most wastewater is discharged to sewers that connect to wastewater treatment plants (WWTPs), and thus considering the total volume of use of the fragrance materials discharged to the wastewater is an appropriately conservative approach for a screening level risk assessment as described in the Framework (Salvito et al., 2002) and is also consistent with the ERA guidelines for pharmaceuticals (EMEA, 2006).

A typical WWTP includes primary treatment to remove solids and secondary treatment to remove dissolved and suspended organic matter. The most common type of secondary treatment is activated sludge. Removal of fragrance materials can involve sorption to sewage solids in primary and secondary treatment, biodegradation or biotransformation mainly during secondary treatment, and removal by clarification in the final clarifier before discharge to the aquatic environment. During conveyance in the sewer and treatment, some material can volatilize

to the atmosphere. In addition, materials can be biodegraded, biotransformed, and sorbed to the sludge within the sewer. Materials removed by sorption to the sludge in the WWTP may be incinerated, landfilled, or amended to soil. Material not removed in the WWTP is discharged to surface waters, where biotransformation and biodegradation can continue. In the surface waters, materials may sorb to suspended solids and be transferred to the sediments.

Exposure is expressed as a PEC in the water column of the mixing zone, where WWTP effluent is diluted with surface water (e.g., stream or river). This concentration is determined by fragrance usage volume, removal during WWTP treatment, and dilution in the mixing zone (Salvito et al., 2002). This approach makes the following assumptions: the entire fragrance usage volume in a region is discharged via sewers to WWTPs; usage is evenly distributed across the population of a region; no losses occur during consumer use or conveyance in the sewer as a result of volatilization or other processes; no losses occur as a result of biodegradation, biotransformation, or abiotic chemical processes; and effluents are minimally diluted (3:1) in the mixing zone. As noted in Salvito et al. (2002) a 3:1 dilution is a conservative dilution estimate especially relative to the 10:1 dilution recommended in the technical guidance in Europe for local-scale exposure calculations (ECHA, 2008a). The assumptions used in estimating operational aspects of wastewater treatment are that wastewater undergoes primary and secondary (activated sludge) treatment, removal during sewage treatment occurs only as a result of sorption, removal of solids during primary treatment is only 50% (many plants exhibit better efficiency), the level of suspended solids in the aeration vessel of the activated sludge system is 2500 mg/L (for many plants this value is closer to 3500 mg/L), and the level of solids in final effluent is 20 mg/L (many plants have much lower solids levels in their effluents). This assessment depends on three input variables: regional usage volume (metric tons/year), octanol–water partitioning coefficient (K_{OW}), and molecular weight. The model parameters and the conservative nature of this approach have been discussed elsewhere (Salvito et al., 2002). Macrocyclic fragrance materials have estimated $\log K_{OW}$ values ranging from about 4.2 to about 6.7. Molecular weights generally are around 250 g/mol (Table 1). Population differences, per capita water usage, and influent solid levels are the variants between scenarios for European and North American screening level exposure assessments (Salvito et al., 2002).

2.2.2.1. Calculation of the PEC. The PECs for all materials were estimated individually using the model described in the RIFM Environmental Framework (Salvito et al., 2002) for the European and North American scenarios. For macrocyclic fragrance materials, the PEC was estimated based on the reported volume of use (IFRA, 2008) in Europe and North America, the individual material's $\log K_{OW}$ value, and a 3:1 dilution ratio to estimate exposure in the receiving stream. As the screening level assessment outlined in Salvito et al. (2002) is able to incorporate measured biodegradation data when available, as is the case for both macrocyclic fragrance ingredient subgroups, this rate (1/h) was included in the analysis (Salvito et al., 2002).

2.2.3. Analysis – characterization of ecological effects: PNEC

The potential toxic effects of a fragrance material in the aquatic environment were expressed in terms of a PNEC. All measured acute and chronic toxicity data available for each subgroup of compounds were evaluated and the lowest chronic no observed effect concentration (NOEC) for the subgroup was identified, based on measured concentrations. As data are not available for each compound in the particular subgroup, this NOEC was applied to all compounds within the subgroup to calculate risk quotients. This is consistent with using a "group approach". All of the toxicity tests were performed using Organization for Economic Cooperation and Development (OECD) methods (OECD, 2006a) or similar methodologies and are noted in Table 3. The PNEC was estimated by dividing the lowest chronic NOEC by an assessment factor selected using European Union guidelines (ECHA, 2008b) and the RIFM Environmental Framework (Salvito et al., 2002).

2.2.3.1. Risk characterization. Potential risk to the aquatic environment for each subgroup was estimated from the ratio of the PEC to PNEC (PEC/

Table 2. Biodegradation summary.

Material	CAS#	Method	Test duration	Results	References
(a) Ketones					
5-cyclohexadecan-1-one	37609-25-9	OECD 301B OECD 301C OECD 302C OECD 301F	28 days 26 days 28 days 28 days	81% and 83% 52%, 60% and 68% 60% 86%	RIFM (1996a) RIFM (1997a) RIFM (1997b) RIFM (1997c)
Cyclopentadecanone	502-72-7	OECD 302A	28 days	66.7%	RIFM (1997d)
4-cyclopentadecan-1-one, (Z)-	14595-54-1	OECD 301B	28 days	83.9%	RIFM (1999a)
3-Methylcyclopentadecanone	82356-51-2	OECD 302A OECD 301F OECD 301B	28 days 28 days 28 days	99.8% 61% 96.4% (10 mg/L) 93.4% (20 mg/L)	RIFM (1999b) RIFM, 1996b RIFM (1996c)
		OECD 301D SCAS	28 days 7 days	43% 99.9% removal	RIFM (1995a) RIFM (1995b)
		OECD 301B	28 days	78.8%	RIFM (2000a)
		OECD 302A	28 days	96.1%	RIFM (2000b)
		OECD 302C	28 days	85% BOD; 100% GC	RIFM (2000c)
Cyclohexadecanone	2550-52-9	OECD 301B	28 days	43%	RIFM, 2001a
5-Cyclotetradecan-1-one, 3-methyl-, (5E)	259854-70-1	OECD 302C	28 days	75%	RIFM (2004a)
5-Cyclotetradecan-1-one, 3-methyl-, (5Z)	259854-71-2	OECD 301F OECD 302C	28 days 28 days	70% [73% after 34 days] 75%	RIFM (2004b)
3-Methyl-1-cyclopentadecanone	5411-91-3	OECD 301F	28 days	70% [73% after 34 days]	RIFM, 2004a
5-Cyclopentadecan-1-one, 3-methyl-	63314-79-4	OECD 301F	28 days	80%	RIFM (2009a)
		OECD 301F	28 days	80% (100 mg/L); 81% (20 mg/L)	RIFM (2009b)
(b) Lactones/lactides					
Ethylene brassylate	105-95-3	"Closed Bottle" OECD 301F OECD 301F OECD 301F	28 days 28 days 28 days 28 days	40% 73% 77% 89%	RIFM (1996d) RIFM (1996e) RIFM (1996f) RIFM (1998a)
		OECD 301B	28 days	78.1%	RIFM (1994a)
		OECD 301F	28 days	83%	RIFM (1999c)
		OECD 301C	28 days	85% BOD; 100% GC	RIFM (1997e)
		OECD 301B	28 days	>100%	RIFM (1997f)
		OECD 301B	28 days	80%; 78%	RIFM (1997g)
		OECD 301C	28 days	73% BOD; 100% GC	RIFM (1999d)
		OECD 301B	28 days	>100%	RIFM (2000d)
		OECD 301F	28 days	84%	RIFM (1994b)
		OECD 301B	28 days	82%	RIFM (1997h)
		OECD 301B	28 days	100%	RIFM (1997i)
		OECD 301B	28 days	79.6%	RIFM (1993)
		OECD 301B	28 days	96.5%	RIFM (1996g)
		OECD 301B	28 days	93%	RIFM (1996h)
		OECD 301F	28 days	71%	RIFM (2005)
		OECD 301B	28 days	82%	RIFM (1998b)
		M.R Test C.4-D	28 days	90%	RIFM (1996)
		OECD 301B	28 days	83% (10 mg/L), 79% (20 mg/L)	RIFM (1990a)
		OECD 301F	28 days	95%	RIFM (1996)
		OECD 301B	28 days	86.1% (10 mg/L); 87.1% (20 mg/L)	RIFM (1995c)
		SCAS	7 days	Ave. removal: 95%	RIFM (1995d)
		(SDA Method)			
		OECD 301C	28 days	92% BOD; 100% GC	RIFM, 1998c
		OECD 111	120 h abiotic deg. (hydrolysis)	At pH values of 4 and 7 degradation is less than 10% after 5 days (equivalent to a half-life time higher than 1 year at 25 °C) and no more testing is required. For pH 9, about 50% degradation was observed after 120 h. In this case, a second degradation test was recommended at a lower temperature (35 °C)	RIFM (2000e)
			0.4 mg/L		
		OECD 111	120 h abiotic deg. (hydrolysis)	The low concentration measured at 120 h in the pH 7 solution is not in agreement with the results of the second preliminary test. An accidental loss during analysis may have occurred. The slow hydrolysis of the test material at pH 4 and 7, as observed at 50 °C is confirmed (half-life time higher than one year). Hydrolysis at pH 9 is slower than in the experiment at 50 °C, it also fits roughly a pseudo-first order law (straight line). From the two tests at 50 °C and 35 °C, a rate constant and half-life time at 25 °C can be calculated. The rate constant at 25 °C = 21.4 days	RIFM (2000e)
			0.4 mg/L		
		OECD 301F	28 days	87%, 89% after 39 days	RIFM (2000f)

Table 3. Aquatic ecotoxicity data.

Material	CAS#	Method	Test duration	Species	Results	References
(a) Ketones						
3-Methylcyclopentadecanone	82356-51-2	OECD 202	48 h	<i>Daphnia magna</i>	EC50: 0.39 mg/L NOEC: 0.18 mg/L	RIFM (1995e)
		OECD 211	21 days	<i>Daphnia magna</i>	EC50 (repro): 0.022 mg/L LOEC: 0.045 mg/L; NOEC: 0.011 mg/L	RIFM (2003a)
		OECD 203	96 h	<i>Oncorhynchus mykiss</i>	LC50: 0.45 mg/L NOEC: 0.18 mg/L	RIFM (1995f)
		OECD 201 OECD 210: No dose response relationship —not used for RA. See Supplemental Information	72 h 33 days	<i>Scenedesmus subspicatus</i> <i>Pimephales promelas</i>	EBC50: >30 mg/L; ERC50: >30 mg/L; NOEC: ≥30 mg/L NOEC: 0.0020 mg/L (nominal)	RIFM (1995g) RIFM (2003b)
Cyclohexadecanone	2550-52-9	OECD 202 OECD 203: Conc based on initial conc; no material detected at study termination	48 h 96 h	<i>Daphnia magna</i> <i>Brachydanio rerio</i>	0.00098 mg/L (mean measured) EC50:>0.19 mg/L LC50>0.1 mg/L	RIFM (2001b) RIFM (2001c)
		OECD 201	72 h	<i>Scenedesmus subspicatus</i>	LC50: >0.22 mg/L EBC50: >1.8 mg/L; ERC50: >1.8 mg/L; NOEC: >1.8 mg/L	RIFM (2001d)
		OECD 202	48 h	<i>Daphnia magna</i>	EC50: 0.58 mg/L NOEC: 0.32 mg/L	RIFM (2004c)
		OECD 202	48 h	<i>Daphnia magna</i>	EC50: 0.58 mg/L NOEC: 0.32 mg/L EBC50: >0.715 mg/L;	RIFM (2004c) RIFM (2009c)
5-Cyclotetradecen-1-one, 3-methyl-, (5E)	259854-70-1	OECD 202	48 h	<i>Daphnia magna</i>	EC50: 0.58 mg/L NOEC: 0.32 mg/L	RIFM (2004c)
5-Cyclotetradecen-1-one, 3-methyl-, (5Z)	259854-71-2	OECD 202	48 h	<i>Daphnia magna</i>	EC50: 0.58 mg/L NOEC: 0.32 mg/L	RIFM (2004c)
5-Cyclopentadecen-1-one, 3-methyl-	63314-79-4	OECD 201 (ERC50 was extrapolated to 754 µg/L; highest test conc. was 715 µg/L)	72 h	<i>Pseudokirchneriella subcapitata</i>	EBC50: >0.715 mg/L; NOEC: 0.371 mg/L EC50 (repro): 0.2534 mg/L	RIFM (2009c)
(b) Lactones/lactides Ethylene brassylate	105-95-3	OECD 211 (time weighted means)	21 days	<i>Daphnia magna</i>	ERC50: >0.715 mg/L; NOEC: 0.371 mg/L EC50 (repro): 0.2534 mg/L LOEC: 0.1550 mg/L; NOEC: 0.0899 mg/L	RIFM (2009)
		OECD 202 OECD 203 OECD 202	48 h 96 h 21 day	<i>Daphnia magna</i> <i>Brachydanio rerio</i> <i>Daphnia magna</i>	Geom. Mean EC0/EC100: 6.4 mg/L Geom. Mean LC0/LC100: 1.23 mg/L LOEC: 0.165 mg/L NOEC: 0.088 mg/L	RIFM (1996k) RIFM (1996l) RIFM (1996m)
		67/548/EWT (Algae Inhibition Test)	72 h	<i>Scenedesmus subspicatus</i>	EBC50: 5.2 mg/L; ERC50:>6.9 mg/L;	RIFM (1996n)
		OECD 202 OECD 203 OECD 201	48 h 96 h 72 h	<i>Daphnia magna</i> <i>Oncorhynchus mykiss</i> <i>Scenedesmus capricornutum</i>	NOEC: 3.48 mg/L EC50: >14 mg/L; NOEC: 14 mg/L LC50: 0.88 mg/L; NOEC: 0.10 mg/L EBC50: 1.9 mg/L; ERC50: 17 mg/L; NOEC: 0.61 mg/L	RIFM (1997j) RIFM (1997k) RIFM (1997l)

Table 3. Aquatic ecotoxicity data.

Material	CAS#	Method	Test duration	Species	Results	References
Oxacyclohepta dec-11-en-2-one	36508-31-3	OECD 202 OECD 203 OECD 201	48 h 96 h 72 h	<i>Daphnia magna</i> <i>Brachydanio rerio</i> <i>Scenedesmus capricornutum</i>	EC50: >66 µg/l; LC50: 42.5 µg/l; NOEC: 11.0 µg/l EBC50 and EC50>113.5 µg/l; EC0: >1.27 mg/L	RIFM (2000g) RIFM (2000h) RIFM (1997m) RIFM (1995h)
ω-Pentadeca lactone	106-02-5	(C.2), Directive 67/548/EEC (C.1) Directive 67/548/EEC OECD 202	48 h 96 h 21 days	<i>Daphnia magna</i> <i>Brachydanio rerio</i> <i>Daphnia magna</i>	LC0:>0.11 mg/L EC0 / EC 100 (Immob; Geo Mean) 0.093 mg/L (0.28 mg/L (Nominal)) EC 50 (Repro):>0.068 mg/L<0.127 mg/L [> 0.2 mg/L<0.4 mg/L (Nominal)] NOEC (Repro) 0.068 mg/l [0.2 mg/l (Nominal)] LOEC (Repro): 0.127 mg/l [0.4 mg/L (Nominal)] NOEC: 0.26 mg/L; EBC50: 0.4 mg/L; ERC50: >0.47 mg/L NOEC: 10 mg/L EC50: 21.2 mg/L NOEC>1.0 mg/L EC50 >1.0 mg/L EBC50: 11 mg/L; EC50: 16 mg/L; NOEC: 6.9 mg/L LC50: between 3.2 and 5.6 mg/L NOEC: ≤1.8 mg/L NOEC: 0.027 mg/L (mean measured); 0.16 mg/L (nominal) LOEC: 0.11 mg/L (mean measured); 0.50 mg/L (nominal) Nominal EC50: 0.88 mg/L NOEC: 0.32 mg/L; Measured EC50: 0.48 mg/L NOEC: 0.24 mg/L Nominal LC50: 2.4 mg/L NOEC: 1.0 mg/L; Measured LC50: 2.0 mg/L NOEC: 0.52 mg/L EC50 (immob): 0.27 mg/L, nominal; 0.079 mg/L, time-weighted mean measured EC50 (repro): 0.27 mg/L, nominal; 0.079 mg/L, time-weighted mean measured LOEC: 0.48 mg/L, nominal; 0.16 mg/L, time-weighted mean measured NOEC: 0.15 mg/L, nominal; 0.039 mg/L, time-weighted mean measured EBC50: 2.4 mg/L; EC50: 5.0 mg/L; NOEC: 0.625mg/L EC50:>0.686 mg/L LC50: >0.884 mg/L The NOEC corresponded with a WAF prepared at 10 mg/L for both cell growth inhibition and growth rate reduction. The NOEC exceeded the maximum solubility of 1.6 mg/L at 20 °C in distilled water and 0.6 mg/L at 20 °C and a pH of 7.0 in re-constituted medium	RIFM (1994c) RIFM,1996o
Oxacyclohexa-decane-2,13-dione	38223-29-9	(C.3), Directive 67/548/EEC OECD 202 OECD 202: OECD 201 OECD 203	72 h 48 h 21 days 72 h 96 h	<i>Scenedesmus subspicatus</i> <i>Daphnia magna</i> <i>Daphnia magna</i> <i>Scenedesmus subspicatus</i> <i>Cyprinus carpio</i>		RIFM (1996p) RIFM (1990b) RIFM (1996q) RIFM (1994d) RIFM (1990e)
E- and Z-Oxacyclohexadec-12(+13)-en-2-one	111879-80-2	OECD 210	33 days	<i>Pimephales promelas</i>		RIFM (2003c)
E- and Z-Oxacyclohexa dec-12(+13)-en-2-one	111879-80-2	OECD 202	48 h	<i>Daphnia magna</i>		RIFM (1995i)
		OECD 203	96 h	<i>Oncorhynchus mykiss</i>		RIFM (1995j)
		OECD 202	21 days	<i>Daphnia magna</i>		RIFM (2002)
Oxacyclopenta dec-10-en-2-one, 13-methyl	329925-33-9	OECD 201 OECD 202 OECD 203 OECD 201	72 h 48 h 96 h 72 h	<i>Scenedesmus subspicatus</i> <i>Daphnia magna</i> <i>Cyprinus carpio</i> <i>Selenastrum capricornutum</i>		RIFM (1995k) RIFM (2001e) RIFM (2001f) RIFM (2001g)

Figure 3. Decision process following categorization guidelines.

PNEC) which defines the risk quotient. When the PEC/PNEC ratio is below one, the risk from the macrocyclic group is considered to be negligible at current volumes of use and thus no adverse aquatic impacts are expected. The screening-level risk assessment answers the question: "Is there a potential for ecological risk?". When the risk quotient exceeds one, a more robust assessment of the environmental risk is warranted to attempt to quantify the magnitude and probability of those risks.

3. Results and discussion

Read-across plays an important role in the risk assessment that follows. Following the guidance established in Section 1 of this paper, and having supported the hypothesis that these materials can be assessed as two groups of macrocyclic compounds, it is appropriate to read across from the data presented for both biodegradation and aquatic toxicity.

3.1. Estimation of PECs – exposure assessment

The annual volume of use range for the macrocyclic ketones in both Europe and North America is from <1 (low kg quantities) to no greater than 50 metric tons in either region (resulting in the 10–100 tonne tonnage band) and for macrocyclic lactones/lactides the volume of use range for both regions is <1 to no greater than 1000 metric tons in any one region; here, resulting in a >1000 metric tons volume of use band (IFRA, 2008).

Within the RIFM/FEMA Database there are 22 biodegradation studies available for 9 macrocyclic ketones and 28 studies for 11 macrocyclic lactones/lactides (Table 2). Following OECD Guidelines (OECD, 2006b) for determining ready biodegradation (e.g., considering the "10-day window"), macrocyclic ketones range from inherent to readily biodegradable. However, the range of biodegradation (mineralization) from these studies ranges from a low of 43% to a high of 100%. In

performing this assessment the quality of the data reported in the RIFM Database was evaluated. All studies followed OECD Guidelines. While data outliers are present, there was nothing present in the data reports to warrant their exclusion. Therefore, all available biodegradation were used. The low values observed are not supported by the weight of evidence from the studies (see Table 2) evaluated for any individual compound but are included in this assessment for completeness and to provide for a conservative evaluation of exposure. In applying the RIFM Framework, a conservative value of 1 h^{-1} is used as the biodegradation rate for these compounds. Macrocyclic lactones/lactides were largely found to be readily biodegradable with biodegradation ranging from 71% to >100% in the available studies with a single outlier (40%) not supported by other studies for the same compound (ethylene brassylate). In applying the RIFM Framework, while a rate of 3 h^{-1} is acceptable, a conservative value of 1 h^{-1} was applied for biodegradation in the WWTP in the exposure assessment of these compounds. Based on these data, neither the macrocyclic ketones nor the macrocyclic lactones/lactides should be considered persistent in the aquatic environment.

Based on these input parameters, and a 3:1 dilution ratio, the Framework estimates that the predicted concentration of macrocyclic ketones in river water within the mixing zone of the WWTP discharge would be range from <0.01 to 0.05 $\mu\text{g/L}$ in Europe and from <0.01 to 0.03 $\mu\text{g/L}$ in North America. For macrocyclic lactones/lactides, the concentration within the mixing zone would range from in Europe from <0.01 to 0.7 $\mu\text{g/L}$ and from <0.01 to 1.0 $\mu\text{g/L}$ in North America. These differences between regions occur because there are different input parameters for the model for Europe and North America (e.g., per capita water use) and tonnage varies between the two regions.

Figure 4. Risk quotients for macrocyclic ketones categorized by volume bands (metric tons). Nine out of 15 materials are presented based on their EU tonnage and 9 out of 15 based on their US tonnage. Additional materials are not included as their risk quotients were too low to be presented (0.01).

3.2. Estimation of PNECs

For 5 macrocyclic ketones, 3 algae inhibition studies, 4 daphnia immobilization studies, 2 fish acute lethality studies and 2 daphnia reproduction studies are available within the RIFM database. For 7 macrocyclic lactones/lactides, 7 algae inhibition studies, 7 daphnia immobilization studies, 7 fish lethality studies, 4 daphnia reproduction studies and 1 fish early life stage study are available within the RIFM Database. For the ketones, and the lactones/lactides as well, ecotoxicity studies are often difficult to perform due to the poor solubility of the materials, their volatility, and their ability to degrade. Concerns about material solubility and the usefulness of the data do not negate the conservative nature of this assessment. In performing this assessment the quality of the data reported in the RIFM Database was evaluated. All studies followed OECD Guidelines. The chronic toxicity studies, run at lower concentrations below the water solubility, provide for reliable data to be used in this group effects assessment. The acute data, while more prone to concerns about material solubility, appear to provide relatively consistent experimental values continuing to support the group effects assessment. In only one case, a Fish, Early Life Stage study of 3-methylcyclopentadecanone, was the data determined to be of sufficiently poor quality that it could not be used in this assessment (see Supplemental Information). These data are summarized in Table 3.

For the macrocyclic ketones, the lowest acute EC_{50}/LC_{50} reported (algae, daphnia or fish) was 0.39 mg/L (*D. magna* immobilization study for 3-Methylcyclopentadecanone) and the lowest NOEC was 0.011 mg/L (*D. magna* reproduction study for 3-Methylcyclopentadecanone). Two chronic endpoints are available (algae and daphnia), therefore an assessment factor of 50, as described in Salvito et al. (2002) is applied to this NOEC. The PNEC derived for the macrocyclic ketones is 0.22 µg/L.

For the macrocyclic lactones/lactides, the lowest acute EC_{50}/LC_{50} reported (algae, daphnia or fish) was 0.0425 mg/L (*Danio rerio* lethality study for Oxacycloheptadec-11-en-2-one). This material is reported in other studies to be very poorly soluble (mean limit of solubility reported in its *D. magna* im-

Figure 5. Risk quotients for macrocyclic lactones/lactides categorized by volume bands (metric tons). Seven out of 15 materials are presented based on their EU tonnage and 7 out of 15 based on their US tonnage. Additional materials are not included as their risk quotients were too low to be presented (≤ 0.01).

bilization study is 66 µg/L). This may explain the difference observed in acute toxicity between this material and the other lactones/lactides where the next lowest acute endpoint reported is an order of magnitude higher (E_bC_{50} in an algae biomass based inhibition study for ω -pentadecalactone). The lowest NOEC from a chronic toxicity study was 0.027 mg/L (fish early life stage study for Oxacyclohexadecan-2-one). Three chronic endpoints are available (algae, daphnia, and fish), therefore an assessment factor of 10 is applied to this NOEC. The PNEC derived for the macrocyclic lactones/lactides is 2.7 µg/L.

As with biodegradation, one rate applied to all chemicals within the subgroup, the PNEC is applied to all materials within each subgroup. Risk quotients are then calculated based on individual material PECs.

3.3. Risk characterization

The screening level risk quotient, PEC/PNEC ratio, for macrocyclic lactones/lactides ranges from <0.05 to 0.27 for the North American scenario and <0.05 to 0.35 for the European scenario identified in the RIFM Framework. The screening level PEC/PNEC ratio for macrocyclic ketones ranges from <0.05 to 0.14 for the North American scenario and <0.05 to 0.23 for the European scenario identified in the RIFM Framework (Figures 4 & 5).

Since the risk quotients for all materials are less than 1.0, the potential risks of macrocyclic fragrance ingredients to aquatic biota are negligible at current use levels. The RIFM Framework, as applied, has been designed to conservatively overpredict PEC/PNEC values. This screening-level assessment only evaluated the aqueous exposure pathway. It did not consider effects to sediment organisms or exposure via bioaccumulation in food.

4. Conclusions

The results of a screening-level aquatic ecological risk assessment using a group approach indicates that at the current volume of use, of macrocyclic fragrance materials do not pose a

significant risk to aquatic biota. This is primarily a function of the high degree of degradation expected in waste water treatment of macrocyclic fragrance materials resulting in efficient removal in wastewater treatment plants. Based on current volume of use, no PEC/PNEC ratio exceeds 1 for any material in any subgroup under any set of conditions identified in the RIFM Framework. The IFRA volume of use survey is revised every 4 years allowing RIFM to reassess the aquatic risk of this class of fragrance materials.

Acknowledgments — The authors gratefully acknowledge the contributions Dr. Benjamin Parkhurst made to the development of this manuscript. The authors also acknowledge the Research Institute for Fragrance Materials for their support of this project.

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Appendix A: Supplemental Information follows.

Supplemental Information

Provided in this section is a complete set of references for the unpublished data presented in Tables 2 and 3 of the manuscript.

Other unpublished data (Table SI1) were available for sediment and terrestrial organisms, but they were not included in the main paper as this is an aquatic risk assessment for the macrocyclic fragrance ingredients. Additionally, one unpublished bioaccumulation study was also available. For completeness they are presented here.

Also of note, while one data point would not be amenable to this weight of evidence approach for chemical grouping, it should be noted that in one OECD 305 BCF study (RIFM, 1997) a macrocyclic lactide (oxacyclohexadecane-2,13-dione), the measured BCF for whole fish (*Onchorynchus mykiss*) based on total radioactivity was 1389 L/kg in the low dose and 2389 L/kg in the high dose; however, significant metabolism was observed. In edible and non-edible parts of the fish analyzed, the unchanged parent compound was not observed.

Lastly provided here are figures illustrating the rationale for eliminating a Fish, Early Life Stage study from the PNEC derivation for macrocyclic ketones are also provided. In the main paper it is noted that a Fish Early Life Stage study for 3-methylcyclopentadecenone was not useful for the risk assessment of the macrocyclic ketones in developing the PNEC because there was no evident concentration response relationship. Figures SI1 and SI2 graphically present the relationships derived from this study to support this conclusion.

Table SI1: Additional ecotoxicological data

Material (subgroup)	CAS#	Method	Test Duration	Species	Results	References
3-Methylcyclopentadecanone (Macrocyclic Ketone)	82356-51-2	OECD 216	28 days	Soil microorganisms	NOEC 1000 mg/Kg	RIFM, 2002 a
		OECD 208	280 days	Oat (A. sativa) Soybean (G. max) Tomato (L. esculentum)	For soybean and tomato for % emergence and shoot weight: EC25 and EC50: >1000 mg/Kg; NOEC 1000 mg/Kg For Oat: EC25 (shoot weight): 180 mg/Kg; EC50 >1000mg/Kg NOEC: 110 mg/Kg (shoot weight)	RIFM, 2002 b
		OECD 207	14 days	Earthworm	LC50: 250 mg/kg NOEC: 180 mg/kg	RIFM, 2001a
E- and Z-Oxacyclohexadec-12(+13)-en-2-one (Macrocyclic Lactone/Lactide)	111879-80-2	OECD 207	14 days	E. foetida (earthworm)	NOEC: 1000 mg/Kg LC50: >1000 mg/Kg	RIFM,2001b
		OECD 218	28 days	L. variegatus	Nominal EC50 (repro.): 960 mg/kg NOEC: 320 mg/kg; Mean Measured EC50 (repro.): 630 mg/kg NOEC: 210 mg/kg	RIFM,2003
		OECD 208	30 days	Oat (A. sativa) Soybean (G. max) Tomato (L. esculentum)	For all species for % emergence and shoot weight: EC25 and EC50: >1000 mg/Kg; NOEC 1000 mg/Kg	RIFM,2002 c

Figure SI1 **Figure SI2**

References (Table SI1)

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