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RIFM fragrance ingredient safety assessment, cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel-, CAS Registry Number 22471-55-2

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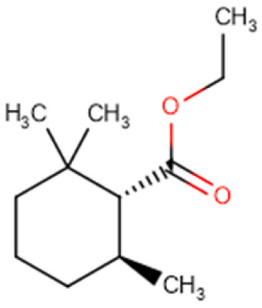

Name: Cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel-
CAS Registry Number: 22471-55-2

Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. Proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
CNIH – Confirmation of No Induction in Humans test. A human repeat insult patch test that is performed to confirm an already determined safe use level for fragrance ingredients (Na et al., 2021)
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015a; Safford et al., 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DRF - Dose Range Finding
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observed Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
Perfumery - In this safety assessment, perfumery refers to fragrances made by a perfumer used in consumer products only. The exposures reported in the safety assessment include consumer product use but do not include occupational exposures.
QRA - Quantitative Risk Assessment
QSAR - Quantitative Structure-Activity Relationship
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use
vPvB - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

The Expert Panel for Fragrance Safety* concludes that this material is safe as described in this safety assessment.

This safety assessment is based on the RIFM Criteria Document (Api et al., 2015), which should be referred to for clarifications.

Each endpoint discussed in this safety assessment includes the relevant data that were available at the time of writing (version number in the top box is indicative of the date of approval based on a 2-digit month/day/year), both in the RIFM Database (consisting of publicly available and proprietary data) and through publicly available information sources (e.g., SciFinder and PubMed). Studies selected for this

(*continued on next column*)

(*continued*)

safety assessment were based on appropriate test criteria, such as acceptable guidelines, sample size, study duration, route of exposure, relevant animal species, most relevant testing endpoints, etc. A key study for each endpoint was selected based on the most conservative endpoint value (e.g., PNEC, NOAEL, LOEL, and NESIL).

*The Expert Panel for Fragrance Safety is an independent body that selects its own members and establishes its own operating procedures. The Expert Panel is comprised of internationally known scientists that provide RIFM with guidance relevant to human health and environmental protection.

Summary: The existing information supports the use of this material as described in this safety assessment.

Cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- is not genotoxic, provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and reproductive toxicity endpoints, and show that there are no safety concerns for skin sensitization under the current declared levels of use. The phototoxicity/photoallergenicity endpoints were evaluated based on data and ultraviolet/visible (UV/Vis) spectra; cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material; exposure is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- was found not to be Persistent, Bioaccumulative, and Toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

Human Health Safety Assessment

Genotoxicity: Not genotoxic.	(RIFM, 1992; RIFM, 1996a)
Repeated Dose Toxicity: NOAEL = 50 mg/kg/day.	RIFM (1993d)
Reproductive Toxicity: NOAEL = 1000 mg/kg/day.	RIFM (2017)
Skin Sensitization: No concern for skin sensitization under the current, declared levels of use.	(RIFM, 1993c; RIFM, 1991b)
Phototoxicity/Photoallergenicity: Not phototoxic/not expected to be photoallergenic.	(UV/Vis Spectra; RIFM Database; RIFM, 1991a)

Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:

Persistence: Critical Measured Value: 5% (OECD 301D)	RIFM (1993b)
Bioaccumulation: Critical Measured Value: BCF: 159 (OECD 305C)	RIFM (1994)
Ecotoxicity: Screening-level: 96-h Algae EC50: 0.474 mg/L	(ECOSAR; US EPA, 2012b)

Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:

Screening-level: PEC/PNEC (North America and Europe) > 1	(RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: 96-h Algae EC50: 0.474 mg/L	(ECOSAR; US EPA, 2012b)

RIFM PNEC is: 0.0474 μg/L

- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe <1

1. Identification

1. **Chemical Name:** Cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel-
2. **CAS Registry Number:** 22471-55-2
3. **Synonyms:** Ethyl *trans*-2,2,6-trimethylcyclohexanecarboxylate; エチル = 2,2,6−トリメチルシクロヘキサンカルボキシラート; Thesaron; Tetrahydro ethyl safranate; Cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, *trans*-; Cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel-
4. **Molecular Formula:** $C_{12}H_{22}O_2$

5. **Molecular Weight:** 198.3 g/mol
6. **RIFM Number:** 6531
7. **Stereochemistry:** 1R,6S isomer specified. Two chiral centers and a total of 4 enantiomers are possible.

2. Physical data

1. **Boiling Point:** 501 ± 1 K corrected to a pressure of 101.325 kPa (RIFM, 1993g)
2. **Flash Point:** <10% hydrolysis after 5 days at 50 °C in pH 4, 7, or 9 buffer solutions (RIFM, 1993g), 87.8 ± 2 °C (RIFM, 1993h), 88 °C (Globally Harmonized System)
3. **Log K_{OW}:** 4.51 (RIFM, 1993g)
4. **Melting Point**: Not Available
5. **Water Solubility:** Not Available
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.000741 mm Hg at 20 °C (EPI Suite v4.0)
8. **UV Spectra:** No absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** Not Available

3. Volume of use (Worldwide band)

1. 10–100 metric tons per year (IFRA, 2015)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v3.0)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.056% (RIFM, 2020)
2. **Inhalation Exposure*:** 0.00031 mg/kg/day or 0.023 mg/day (RIFM, 2020)
3. **Total Systemic Exposure**:** 0.0069 mg/kg/day (RIFM, 2020)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM Aggregate Exposure Model (Comiskey et al., 2015; Safford et al., 2015; Safford et al., 2017; and Comiskey et al., 2017).

**95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section V. It is derived from concentration survey data in the Creme RIFM Aggregate Exposure Model and includes exposure via dermal, oral, and inhalation routes whenever the fragrance ingredient is used in products that include these routes of exposure (Comiskey et al., 2015; Safford et al., 2015; Safford et al., 2017; and Comiskey et al., 2017).

5. Derivation of systemic absorption

1. **Dermal:** Assumed 100%
2. **Oral:** Assumed 100%
3. **Inhalation:** Assumed 100%

6. Computational toxicology evaluation

1. **Cramer Classification:** Class I, Low

Expert Judgment	Toxtree v3.1	OECD QSAR Toolbox v4.2
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2. Analogs Selected:
   a. **Genotoxicity:** None
   b. **Repeated Dose Toxicity:** None
   c. **Reproductive Toxicity:** None
   d. **Skin Sensitization:** None
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. **Read-across Justification:** None

7. Metabolism

No relevant data available for inclusion in this safety assessment.
Additional References:
None.

8. Natural occurrence

Cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- is not reported to occur in foods by the VCF*.

*VCF (Volatile Compounds in Food): Database/Nijssen, L.M.; Ingen-Visscher, C.A. van; Donders, J.J.H. (eds). – Version 15.1 – Zeist (The Netherlands): TNO Triskelion, 1963–2014. A continually updated database containing information on published volatile compounds that have been found in natural (processed) food products. Includes FEMA GRAS and EU-Flavis data.

9. REACH dossier

Available; accessed on 02/25/22.

10. Conclusion

The existing information supports the use of this material as described in this safety assessment.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. Cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- was assessed in the BlueScreen assay and found positive for cytotoxicity (positive: <80% relative cell density) without metabolic activation, negative for cytotoxicity with metabolic activation, and negative for genotoxicity, with and without metabolic activation (RIFM, 2013). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. Additional assays were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

The mutagenic activity of cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation and preincubation methods. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- in dimethyl sulfoxide (DMSO) at concentrations up to 5000 μg/plate. No increases in the mean number of revertant colonies were observed at any tested dose in the presence or absence of S9 (RIFM, 1992). Under the conditions of the study, cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- was not mutagenic in the Ames test.

The clastogenic activity of cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- was evaluated in an *in vivo* micronucleus test conducted in compliance with GLP regulations and in

accordance with OECD TG 474. A single dose of the test material at 1250 mg/kg body weight was administered in Arachis oil via intraperitoneal injection to groups of male and female CD-1 mice. Mice from each dose level were euthanized at 24, 48, and 72 h, the bone marrow was extracted and examined for polychromatic erythrocytes. The test material did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow (RIFM, 1996a). Under the conditions of the study, cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- was considered to be not clastogenic in the *in vivo* micronucleus test.

Based on the data available, cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- does not present a concern for genotoxic potential.

Additional References: RIFM, 1993a.

Literature Search and Risk Assessment Completed On: 10/15/21.

11.1.2. Repeated dose toxicity

The MOE for cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel-. In a GLP-compliant study, groups of 5 SD rats/sex/dose were administered cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- via gavage at dose levels of 0, 150, or 1000 mg/kg/day for 28 days. Additional satellite groups of 5 rats/sex were administered test material at 0 and 1000 mg/kg/day for 28 days and then were observed for 14 days, without treatment. Significant increases in alanine aminotransferase and bilirubin were reported among high-dose group females. High-dose group animals showed dark and/or enlarged livers and, in males only, patchy pallor of kidneys. Mid-dose group males were also reported to have patchy pallor of kidneys. Significant increases in absolute and relative liver weights were reported among animals of the high-dose group. Mid-dose group males were also reported to have a significant increase in relative liver weights. The high-dose recovery group males also had a significant increase in relative liver weights as compared to the control. Microscopic alterations included the hepatocellular enlargement and increased cytoplasm density in hepatocytes among high-dose group animals only; no such alterations were reported among other treatment groups or recovery group animals. Absolute and relative kidney weights were significantly increased among high-dose males, and relative kidney weights were significantly increased in mid-dose males as compared to control group animals. Kidney changes reported in treated males were consistent with documented changes of α-2u-globulin nephropathy, which is species-specific to male rats in response to treatment with some hydrocarbons. This effect is not considered a hazard to human health (Lehman-McKeeman and Caudill, 1992; Lehman-McKeeman et al., 1990). Since the liver weight increases were associated with an increase in plasma aminotransferase levels among high-dose group animals, this alteration was considered to be an adverse treatment-related alteration, according to conclusions that provided guidance during the 3rd International European Society of Toxicologic Pathology (ESTP) Expert Workshop to distinguish adverse and adaptive liver effects following a repeated dose toxicity study. Thus, the NOAEL was considered to be 150 mg/kg/day (RIFM, 1993d).

A default safety factor of 3 was used when deriving a NOAEL from a 28-day study (ECHA, 2012). The safety factor has been approved by the Expert Panel for Fragrance Safety*.

Thus, the derived NOAEL for the repeated dose toxicity data is 150/3, or 50 mg/kg/day.

Therefore, the cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- MOE is equal to the cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel-NOAEL in mg/kg/day divided by the total systemic exposure to cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel-, 50/0.0069, or 7246.

In addition, the total systemic exposure to cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- (6.9 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007) for Cramer Class I material for the repeated dose toxicity endpoint at the current level of use.

*The Expert Panel for Fragrance Safety is composed of scientific and technical experts in their respective fields. This group provides advice and guidance.

Additional References: None.

Literature Search and Risk Assessment Completed On: 09/23/21.

11.1.3. Reproductive toxicity

The MOE for cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are sufficient reproductive toxicity data on cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel-. In a GLP/OECD-421-compliant study, 10 Sprague Dawley rats/sex/dose were administered cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- via gavage at doses of 0, 100, 300, or 1000 mg/kg/day for 2 weeks before mating, during mating, and (for females) throughout gestation and until day 4 post-partum. No mortality was observed throughout the study. No adverse effects were observed in food consumption, body weight, bodyweight gain, mating or fertility parameters, delivery, pup mortality, pup clinical signs, pup viability, pup sex ratio, or pup body weights and weight gain. Based on no adverse fertility or developmental toxicity effects up to the highest dose, the fertility and developmental toxicity NOAEL for this study was considered to be 1000 mg/kg/day (RIFM, 2017).

Therefore, the cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel-MOE for the fertility and developmental toxicity endpoints can be calculated by dividing the cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- NOAEL in mg/kg/day by the total systemic exposure to cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel-, 1000/0.0069, or 144927.

In addition, the total systemic exposure to cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- (6.9 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 09/23/21.

11.1.4. Skin sensitization

Based on the existing data, cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- does not present a concern for skin sensitization under the current, declared levels of use.

11.1.4.1. Risk assessment. Based on the existing data, cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- is not considered a skin sensitizer. The chemical structure of this material indicates that it would not be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). In 2 guinea pig maximization tests, cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- did not lead to skin sensitization reactions (RIFM, 1993c; RIFM, 1991b).

Based on the weight of evidence (WoE) from structural analysis and animal study, cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- does not present a concern for skin sensitization under the current, declared levels of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/08/21.

11.1.5. Phototoxicity/photoallergenicity

Based on UV/Vis absorbance spectra and available data, cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- would not be expected to present a concern for phototoxicity. Based on UV/Vis absorbance spectra, cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- would not be expected to present a concern for photoallergenicity.

11.1.5.1. Risk assessment. UV/Vis absorption spectra indicate no absorption between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). In an *in vivo* guinea pig phototoxicity test, application of 5%, 10%, 30%, and 50% cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- in acetone did not result in skin reactions (RIFM, 1991a). Based on lack of absorbance and the available *in vivo* study data, cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- does not present a concern for phototoxicity. Based on lack of absorbance, cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- does not present a concern for photoallergenicity.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate no absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark of concern for phototoxic effects, 1000 L mol^{-1} • cm^{-1} (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/13/21.

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- is below the Cramer Class I TTC value for inhalation exposure local effects.

11.1.6.1. Risk assessment. There are no inhalation data available on cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel-. Based on the Creme RIFM Model, the inhalation exposure is 0.023 mg/day. This exposure is 60.9 times lower than the Cramer Class I TTC value of 1.4 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/15/21.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- was performed following the RIFM Environmental Framework (Salvito et al., 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log K_{OW}, and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity, as discussed in Salvito et al. (2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 2012b), which provides chemical class-specific ecotoxicity estimates. Finally, if necessary, Tier 3 is conducted using measured biodegradation and ecotoxicity data to refine the RQ, thus allowing for lower PNEC uncertainty factors. The data for calculating the PEC and PNEC for this safety assessment are provided in the table below. For the PEC, the range from the most recent IFRA Volume of Use Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- was identified as a fragrance material with the potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC >1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) did not identify cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- as possibly being persistent or bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF $\geq$2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

11.2.2. Risk assessment

Based on the current Volume of Use (IFRA, 2015), cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Key studies. Biodegradation

RIFM, 1993b: The ready biodegradability of the test material was evaluated using the closed bottle test according to the OECD 301D method. After 28 days, biodegradation of 5% was observed.

RIFM, 1994: A bioaccumulation assay was conducted with carp, according to the OECD 305C method under flow-through conditions. The Bioconcentration Factors (BCFs) after 56 days were calculated to be 134 at a concentration of 0.0085 mg/L and 159 at a concentration of 0.085 mg/L.

Ecotoxicity

RIFM, 1996b: An algae growth inhibition test was conducted according to the OECD 201 method, under static conditions. Based on measured test concentrations, the EbC50 was calculated to be 0.6 mg/L (72 h), and ErC50 was calculated to be 0.8 mg/L (0–24 h).

RIFM, 1993e: A *Daphnia magna* immobilization test was conducted according to the OECD 202 method under static conditions. The 48-h EC50 value based on nominal test concentration was calculated to be 3.2 mg/L.

RIFM, 1993f: A 96-h fish (rainbow trout) acute toxicity study was conducted according to the OCD 203 method under flow-through conditions. The LC50 values based on the mean measured concentration were reported to be 6 mg/L.

RIFM, 1996c: A 21-day *Daphnia magna* reproduction test was conducted according to the OECD 202 method, under semi-static conditions. Based on the nominal concentrations, the 21-day EC50 (reproduction) was reported to be between 1 and 3.2 mg/L, the 14- and

21-day EC50 (immobilization; parent *Daphnia magna*) was 1.8 mg/L, and NOEC for both reproduction and immobilization was 1 mg/L.

Other available data

Cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- has been registered for REACH with no additional data available at his time.

11.2.3. Risk assessment refinement

Since cyclohexanecarboxylic acid, 2,2,6-trimethyl-, ethyl ester, (1R,6S)-rel- has passed the screening criteria, measured data is included for completeness only and has not been used in PNEC derivation.

Ecotoxicological data and PNEC derivation (all endpoints reported in mg/L; PNECs in μg/L).

Endpoints used to calculate PNEC are underlined.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	1.752			1000000	0.001752	
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	1.020	1.636	0.474	10000	0.0474	Esters
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	1.087	0.772	1.450			Neutral Organic SAR (Baseline toxicity)

Exposure information and PEC calculation (following RIFM Environmental Framework: Salvito et al., 2002).

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	4.51	4.51
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	<1	1–10
Risk Characterization: PEC/PNEC	**< 1**	**< 1**

Based on available data, the RQ for this material is < 1. No further assessment is necessary.

The RIFM PNEC is 0.0474 μg/L. The revised PEC/PNECs for EU and NA are <1; therefore, the material does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 09/30/21.

12. Literature Search*

- **RIFM Database:** Target, Fragrance Structure-Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** https://echa.europa.eu/
- **NTP:** https://ntp.niehs.nih.gov/
- **OECD Toolbox:** https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
- **National Library of Medicine's Toxicology Information Services:** https://toxnet.nlm.nih.gov/
- **IARC:** https://monographs.iarc.fr
- **OECD SIDS:** https://hpvchemicals.oecd.org/ui/Default.aspx
- **EPA ACToR:** https://actor.epa.gov/actor/home.xhtml
- **US EPA HPVIS:** https://ofmpub.epa.gov/oppthpv/public_search.publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&EndPointRpt=Y#submission
- **Japanese NITE:** https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com
- **ChemIDplus:** https://chem.nlm.nih.gov/chemidplus/

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 09/30/22.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. RIFM staff are employees of the Research Institute for Fragrance Materials, Inc. (RIFM). The Expert Panel receives a small honorarium for time spent reviewing the subject work.

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