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RIFM fragrance ingredient safety assessment, cyclohexaneacetic acid, α-methyl-, ethyl ester, CAS Registry Number 2511-00-4

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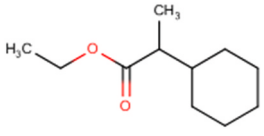

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Name: Cyclohexaneacetic acid, α-methyl-, ethyl ester
CAS Registry Number: 2511-00-4

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

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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., 2020)
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, 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, 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 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.

Cyclohexaneacetic acid, α-methyl-, ethyl ester was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that cyclohexaneacetic acid, α-methyl-, ethyl ester is not genotoxic. The repeated dose, reproductive, and local respiratory toxicity endpoints were completed using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material; exposure is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). Data from read-across material methyl cyclopentylideneacetate (CAS # 40203-73-4) show that there are no safety concerns for cyclohexaneacetic acid, α-methyl-, ethyl ester for skin sensitization under the current declared levels of use. The phototoxicity/photoallergenicity endpoint was completed based on ultraviolet/visible (UV/Vis) spectra; cyclohexaneacetic acid, α-methyl-, ethyl ester is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; cyclohexaneacetic acid, α-methyl-, ethyl ester 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.

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Human Health Safety Assessment

Genotoxicity: Not genotoxic.	(ECHA REACH Dossier: Ethyl 2-cyclohexylpropionate; ECHA, 2013)
Repeated Dose Toxicity: No data available. Exposure is below the TTC.	
Reproductive Toxicity: No data available. Exposure is below the TTC.	
Skin Sensitization: Not a sensitization concern under the current, declared levels of use.	RIFM (2014)
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic.	(UV/Vis Spectra; RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.	

Environmental Safety Assessment

Hazard Assessment:

Persistence: Critical Measured Value: Screening-level: 13% (OECD 301D)	(ECHA REACH Dossier: Ethyl 2-cyclohexylpropionate; ECHA, 2013)
Bioaccumulation: Screening-level: 215 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Screening-level: 96-h Algae EC50: 0.813 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, 2002)
Critical Ecotoxicity Endpoint: 96-h Algae EC50: 0.813 mg/L	(ECOSAR; US EPA, 2012b)

RIFM PNEC is: 0.0813 μg/L

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

1. Identification

1. **Chemical Name:** Cyclohexaneacetic acid, α-methyl-, ethyl ester
2. **CAS Registry Number:** 2511-00-4
3. **Synonyms:** Ethyl 2-cyclohexylpropionate; Ethyl-2-cyclohexyl propionate; Poirenate; Poirenate (ethyl 2-cyclohexylpropionate); Poirenate (ethyl 2 cyclohexyl propionate); Poirenate; ethyl 2-cyclohexylpropionate; Cyclohexaneacetic acid,.α.-methyl-, ethyl ester
4. **Molecular Formula:** $C_{11}H_{20}O_2$
5. **Molecular Weight:** 184.27
6. **RIFM Number:** 6952
7. **Stereochemistry:** One stereocenter and 2 possible stereoisomers.

2. Physical data

1. **Boiling Point:** Not Available
2. **Flash Point:** 92 °C (Globally Harmonized System)
3. **Log K_{OW}:** 4.04
4. **Melting Point**: −3.00 °C (EPI Suite)
5. **Water Solubility:** 18.52 mg/L at 25 °C (EPI Suite)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.0605 mm Hg at 20 °C (EPI Suite v4.0)
8. **UV Spectra:** No significant 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 v1.0)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.11% (RIFM, 2017)
2. **Inhalation Exposure*:** 0.00041 mg/kg/day or 0.032 mg/day (RIFM, 2017)
3. **Total Systemic Exposure**:** 0.0013 mg/kg/day (RIFM, 2017)

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

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

6.1. Cramer classification

Class I, Low

Expert Judgment	Toxtree v3.1	OECD QSAR Toolbox v4.2
I	I	I

6.2. Analogs selected

a. **Genotoxicity:** None
b. **Repeated Dose Toxicity:** None
c. **Reproductive Toxicity:** None
d. **Skin Sensitization:** Methyl cyclopentylideneacetate (CAS # 40203-73-4)
e. **Phototoxicity/Photoallergenicity:** None
f. **Local Respiratory Toxicity:** None
g. **Environmental Toxicity:** None

6.3. Read-across justification

See Appendix below

7. Metabolism

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

8. Natural occurrence

Cyclohexaneacetic acid, α-methyl-, ethyl ester 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 09/17/21 (ECHA, 2013).

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, cyclohexaneacetic acid, α-methyl-, ethyl ester does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. Cyclohexaneacetic acid, α-methyl-, ethyl ester was assessed in the BlueScreen assay and found negative for both cytotoxicity (positive: <80% relative cell density) and 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 cyclohexaneacetic acid, α-methyl-, ethyl ester 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 method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 were treated with cyclohexaneacetic acid, α-methyl-, ethyl ester in ethanol at concentrations up to 5000 μg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (ECHA, 2013). Under the conditions of the study, cyclohexaneacetic acid, α-methyl-, ethyl ester was not mutagenic in the Ames test.

The clastogenicity of cyclohexaneacetic acid, α-methyl-, ethyl ester was assessed in an *in vitro* chromosome aberration study conducted in compliance with GLP regulations and in accordance with OECD TG 473. Human peripheral blood lymphocytes were treated with cyclohexaneacetic acid, α-methyl-, ethyl ester in ethanol at concentrations up to 400 μg/mL in the presence and absence of metabolic activation. No statistically significant increases in the frequency of cells with structural chromosomal aberrations or polyploid cells were observed with any concentration of the test item, either with or without S9 metabolic activation (ECHA, 2013). Under the conditions of the study, cyclohexaneacetic acid, α-methyl-, ethyl ester was considered to be non-clastogenic in the *in vitro* chromosome aberration assay.

Based on the data available, cyclohexaneacetic acid, α-methyl-, ethyl ester does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/02/20.

11.1.2. Repeated dose toxicity

There are insufficient repeated dose toxicity data on cyclohexaneacetic acid, α-methyl-, ethyl ester, or any read-across materials. The total systemic exposure to cyclohexaneacetic acid, α-methyl-, ethyl ester is below the TTC for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

11.1.2.1. Risk assessment. There are no repeated dose toxicity data on cyclohexaneacetic acid, α-methyl-, ethyl ester, or any of the read-across materials. The total systemic exposure to cyclohexaneacetic acid, α-methyl-, ethyl ester (1.3 μg/kg bw/day) is below the TTC (30 μg/kg bw/day; Kroes, 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 09/08/20.

11.1.3. Reproductive toxicity

There are insufficient reproductive toxicity data on cyclohexaneacetic acid, α-methyl-, ethyl ester, or any read-across materials. The total systemic exposure to cyclohexaneacetic acid, α-methyl-, ethyl ester is below the TTC for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

11.1.3.1. Risk assessment. There are no reproductive toxicity data on cyclohexaneacetic acid, α-methyl-, ethyl ester, or any of the read-across materials. The total systemic exposure to cyclohexaneacetic acid, α-methyl-, ethyl ester (1.3 μg/kg bw/day) is below the TTC (30 μg/kg bw/day; Kroes, 2007; Laufersweiler, 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: 09/29/20.

11.1.4. Skin sensitization

Based on existing data and read-across to methyl cyclopentylideneacetate (CAS # 40203-73-4), cyclohexaneacetic acid, α-methyl-, ethyl ester presents no concern for skin sensitization under the current, declared levels of use.

11.1.4.1. Risk assessment. Limited skin sensitization studies are available for cyclohexaneacetic acid, α-methyl-, ethyl ester. Based on the existing data and read-across material methyl cyclopentylideneacetate (CAS # 40203-73-4; see Section VI), cyclohexaneacetic acid, α-methyl-, ethyl ester is not considered a skin sensitizer. The chemical structure of these materials indicates that they would not be expected to react with skin proteins directly (Roberts, 2007; Toxtree v3.1.0; OECD Toolbox v4.2). In a murine local lymph node assay (LLNA), read-across material methyl cyclopentylideneacetate was not found to be sensitizing when tested up to 100% (ECHA, 2015; RIFM, 2014). In a guinea pig maximization test, cyclohexaneacetic acid, α-methyl-, ethyl ester did not present reactions indicative of sensitization (ECHA, 2013). Additionally, in a Confirmation of No Induction in Humans test (CNIH) with 10% or 5000 μg/cm^2 of read-across material methyl cyclopentylideneacetate in petrolatum, no reactions indicative of sensitization was observed in any of the 51 volunteers (RIFM, 1977).

Based on weight of evidence (WoE) from structural analysis, animal studies, and read-across material methyl cyclopentylideneacetate, cyclohexaneacetic acid, α-methyl-, ethyl ester 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: 09/03/20.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, cyclohexaneacetic acid, α-methyl-, ethyl ester would not be expected to present a concern for phototoxicity or photoallergenicity.

11.1.5.1. Risk assessment. There are no phototoxicity studies available for cyclohexaneacetic acid, α-methyl-, ethyl ester in experimental models. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry, 2009). Based on the lack of absorbance, cyclohexaneacetic acid, α-methyl-, ethyl ester does not present a concern for phototoxicity or photoallergenicity.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate no significant 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, 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 09/01/20.

11.1.6. Local Respiratory Toxicity

The margin of exposure could not be calculated due to a lack of appropriate data. The exposure level for cyclohexaneacetic acid, α-methyl-, ethyl ester 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 cyclohexaneacetic acid, α-methyl-, ethyl ester. Based on the Creme RIFM Model, the inhalation exposure is 0.032 mg/day. This exposure is 43.8 times lower than the Cramer Class I TTC value of 1.4 mg/day (based on human lung weight of 650 g; Carthew, 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: None.

Literature Search and Risk Assessment Completed On: 09/30/20.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of cyclohexaneacetic acid, α-methyl-, ethyl ester was performed following the RIFM Environmental Framework (Salvito, 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, cyclohexaneacetic acid, α-methyl-, ethyl ester 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) identified cyclohexaneacetic acid, α-methyl-, ethyl ester as possibly persistent but not 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, 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 ≥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).

11.2.2. Risk assessment

Based on the current Volume of Use (2015), cyclohexaneacetic acid, α-methyl-, ethyl ester presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Key studies. ***Biodegradation:*** No data available.

Ecotoxicity: No data available.

Other available data: Cyclohexaneacetic acid, α-methyl-, ethyl ester has been registered under REACH, and the following data is available (ECHA, 2013):

A ready biodegradation test was conducted according to the OECD 301D method, and biodegradation of 13% was observed after 28 days.

A fish (*Oncorhynchus mykiss*) acute toxicity study was conducted according to the OECD 203 method under semi-static conditions. The 96-h LC50 value was reported to be 8.6 mg/L based on measured concentrations.

A *Daphnia magna* immobilization test was conducted according to the OECD 202 method. The 48-h EC50 value based on nominal test concentration was reported to be 1.1 mg/L.

An algae growth inhibition assay was conducted according to the OECD 201 method, under static conditions. The 72-h EC50 value based on nominal test concentrations for growth rate was reported to be 94.8 mg/L.

11.2.3. Risk assessment refinement

Since cyclohexaneacetic acid, α-methyl-, ethyl ester 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.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	4.04	4.04
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	1–10	<1
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 0.0813 μg/L. The revised PEC/PNECs for EU and NA are <1, and therefore, the material does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 10/04/20.

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

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	4.175			1,000,000	0.004175	
ECOSAR Acute Endpoints **(Tier 2)** v1.11	1.58	2.64	<u>0.813</u>	10,000	0.0813	Esters
ECOSAR Acute Endpoints **(Tier 2)** v1.11	2.217	1.52	2.469			Neutral Organic SAR (Baseline toxicity)

- **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 09/15/21.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2021.112726.

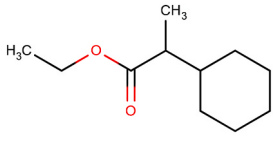
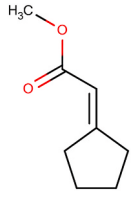
Appendix

Read-across Justification

Methods

The read-across analogs were identified using RIFM fragrance chemicals inventory clustering and read-across search criteria (RIFM, 2020). These criteria are in compliance with the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015) and are consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemical Agency read-across assessment framework (ECHA, 2017).

- First, materials were clustered based on their structural similarity. Second, data availability and data quality on the selected cluster were examined. Third, appropriate read-across analogs from the cluster were confirmed by expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physical–chemical properties of the target substance and the read-across analogs were calculated using EPI Suite v4.11 (US EPA, 2012a).
- J_{max} values were calculated using RIFM's Skin Absorption Model (SAM). The parameters were calculated using the consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts, oncologic classification, ER binding, and repeat dose categorization predictions were generated using OECD QSAR Toolbox v4.2 (OECD, 2020).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010).
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2020), and skin sensitization was predicted using Toxtree.
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2020).
- To keep continuity and compatibility with *in silico* alerts, OECD QSAR Toolbox v4.2 was selected as the alert system.

	Target Material	Read-across Material
Principal Name	Cyclohexaneacetic acid, α-methyl-, ethyl ester	Methyl cyclopentylideneacetate
CAS No.	2511-00-4	40203-73-4
Structure		
Similarity (Tanimoto Score)		0.18
Endpoint		• Skin Sensitization
Molecular Formula	$C_{11}H_{20}O_2$	$C_8H_{12}O_2$
Molecular Weight	184.28	140.18
Melting Point (°C, EPI Suite)	−3.00	−15.15
Boiling Point (°C, EPI Suite)	226.74	186.28
Vapor Pressure (Pa @ 25°C, EPI Suite)	12.35	94.79
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	18.52	547.20
Log K_{OW}	4.04	2.56
J_{max} (μg/cm^2/h, SAM)	2.28	33.73
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	75.59	17.83

(*continued on next page*)

(*continued*)

	Target Material	Read-across Material
Skin Sensitization		
Protein Binding (OASIS v1.1)	No alert found	No alert found
Protein Binding (OECD)	No alert found	No alert found
Protein Binding Potency	Not possible to classify according to these rules (GSH)	Not possible to classify according to these rules (GSH)
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	No alert found	No alert found
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	No skin sensitization reactivity domains alerts identified.	Alert for Schiff base formation identified.
Metabolism		
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	See Supplemental Data 1	See Supplemental Data 2

Summary

There are insufficient toxicity data on cyclohexane acetic acid, α-methyl-, ethyl ester (CAS 2511-00-4). Therefore, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, metabolism data, physical–chemical properties, and expert judgment, methyl cyclopentylideneacetate (CAS # 40203-73-4) was identified as a read-across material with sufficient data for toxicological evaluation.

Conclusions

- Methyl cyclopentylideneacetate (CAS # 40203-73-4) was used as a read-across analog for the target material cyclohexaneacetic acid, α-methyl-, ethyl ester (CAS # 2511-00-4) for the skin sensitization endpoint.
  - o The target material and the read-across analog belong to the structural class of esters.
  - o The key difference between the target material and the read-across analog is that the target has methyl and cyclohexane substituents on the second position whereas the read-across analog has a cyclopentadiene substituent on the second position. Moreover, the target has an ethyl group on the alcohol side whereas the read-across analog has a methyl group on the alcohol side. These structural differences are toxicologically insignificant.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score presented in the above table. The Tanimoto score is mainly driven by the ester fragment. The differences in the structure that are responsible for a Tanimoto score <1 are not relevant from a toxicological endpoint perspective.
  - o The target material and the read-across analog have similar physical–chemical properties. Any differences in some of the physical–chemical properties of the target material and the read-across analog are estimated to be toxicologically insignificant for the skin sensitization endpoint.
  - o According to the QSAR OECD Toolbox (v3.4), structural alerts for the skin sensitization endpoint are consistent between the target material and the read-across analog as seen in the table above.
  - o The target material and the read-across analog are expected to be metabolized similarly as shown by the metabolism simulator.
  - o The structural alerts for the skin sensitization endpoint are consistent between the metabolites of the read-across analog and the target material.

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