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Short review

RIFM fragrance ingredient safety assessment, ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate, CAS Registry Number 94333-50-3

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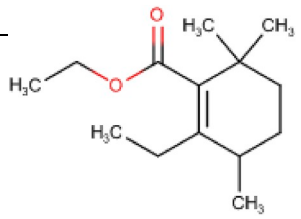


Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. Proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
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., 2015, 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observable 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
QRA - Quantitative Risk Assessment
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 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.

Ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate and read-across analog ethyl 2,3,6,6-tetramethylcyclohex-2-ene-1-carboxylate (CAS # 77851-07-1) show that ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate is not expected to be genotoxic. The repeated dose, reproductive, and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class I material, and the exposure to ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). Data from read-across analog 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R,2S)-rel- (CAS # 540734-22-3) show that there are no safety concerns for ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate for skin sensitization under the current declared levels of use. The phototoxicity/photoallergenicity endpoints were evaluated based on data and UV spectra; ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate was found not to be PBT as per the IFRA Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., PEC/PNEC), are < 1.

Human Health Safety Assessment

Genotoxicity: Not expected to be genotoxic. (RIFM, 2002; RIFM, 2015)
Repeated Dose Toxicity: No data available. Exposure is below the TTC.
Reproductive Toxicity: No NOAEL available. Exposure is below the TTC.
Skin Sensitization: Not a sensitization concern under the current, declared levels of use. RIFM (2004)

Phototoxicity/Photoallergenicity: Not phototoxic/not expected to be photoallergenic. (UV Spectra, RIFM, 1977a)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.
Environmental Safety Assessment
Hazard Assessment:
Persistence: Screening-level: 2.6 (BIOWIN 3) (EPI Suite v4.1; US EPA, 2012a)
Bioaccumulation: Screening-level: 1529 L/kg (EPI Suite v4.1; US EPA, 2012a)
Ecotoxicity: Screening-level: 96 h Algae EC50: 0.125 mg/L (ECOSAR v1.11; 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: 0.125 mg/L (ECOSAR v1.11; US EPA, 2012b)
RIFM PNEC is: 0.0125 μg/L
- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: < 1

1. Identification

1. **Chemical Name:** Ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate
2. **CAS Registry Number:** 94333-50-3
3. **Synonyms:** 1-Cyclohexene-1-carboxylic acid, 2-ethyl-3,6,6-trimethyl-, ethyl ester; Myrascone; Ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate
4. **Molecular Formula:** $C_{14}H_{24}O_2$
5. **Molecular Weight:** 224.34
6. **RIFM Number:** 5516
7. **Stereochemistry:** Isomer not specified. One chiral center and 2 total enantiomers possible.

2. Physical data

1. **Boiling Point:** 265.69 °C (EPI Suite)
2. **Flash Point:** > 93 °C (GHS)
3. **Log K_{OW}:** 5.33 (EPI Suite)
4. **Melting Point:** 38.37 °C (EPI Suite)
5. **Water Solubility:** 0.8913 mg/L (EPI Suite)
6. **Specific Gravity:** Not available
7. **Vapor Pressure:** 0.00896 mm Hg @ 25 °C (EPI Suite), 0.00505 mm Hg @ 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 $mol^{-1} \cdot cm^{-1}$)
9. **Appearance/Organoleptic:** Not available

3. Exposure to fragrance ingredient

1. **Volume of Use (Worldwide Band):** 0.1–1 metric tons per year (IFRA (International Fragrance Association), 2015)
2. **95th Percentile Concentration in Hydroalcoholics:** 0.0048% (RIFM, 2017)
3. **Inhalation Exposure*:** 0.000016 mg/kg/day or 0.0011 mg/day (RIFM, 2017)
4. **Total Systemic Exposure**:** 0.00014 mg/kg/day (RIFM, 2017)

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

**95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section 4. 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, 2017; Safford et al., 2015, 2017).

4. Derivation of systemic absorption

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

5. Computational toxicology evaluation

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

Expert Judgment	Toxtree v2.6	OECD QSAR Toolbox v3.2
I	I	I

2. Analogs Selected:
   a. **Genotoxicity:** Ethyl 2,3,6,6-tetramethylcyclohex-2-ene-1-carboxylate (CAS # 77851-07-1)
   b. **Repeated Dose Toxicity:** None
   c. **Reproductive Toxicity:** None
   d. **Skin Sensitization:** 3-Cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R, 2S)-rel- (CAS # 540734-22-3)
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. Read-across Justification: See Appendix below

6. Metabolism

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

7. Natural occurrence (discrete chemical) or composition (NCS)

Ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate 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.

8. IFRA STANDARD

None

9. REACH Dossier

Pre-registered; no dossier available as of 12/12/18.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data, ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate does not present a concern for genotoxicity.

10.1.1.1. Risk assessment. Ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate (CAS # 94333-50-3) 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 method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102 were treated with ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate 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 concentration in the presence or absence of S9 (RIFM, 2002). Under the conditions of the study, ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate was not mutagenic in the Ames test.

There are no data assessing the clastogenic activity of ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate; however, read-across can be made to ethyl 2,3,6,6-tetramethylcyclohex-2-ene-1-carboxylate (CAS # 77851-07-1; see Section 5). The clastogenic activity of ethyl 2,3,6,6-tetramethylcyclohex-2-ene-1-carboxylate (CAS # 77851-07-1) has been evaluated in an *in vitro* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes were treated with ethyl 2,3,6,6-tetramethylcyclohex-2-ene-1-carboxylate in DMSO at concentrations up to 2100 μg/mL in dose range finding study (DRF) study. Micronuclei analysis in the main study was conducted up to 80 μg/mL in the presence and absence of metabolic activation (S9) for 4 h and in the absence of metabolic activation for 24 h. Ethyl 2,3,6,6-tetramethylcyclohex-2-ene-1-carboxylate did not induce binucleated cells with micronuclei when tested up to cytotoxic levels in either the presence or absence of an S9 activation system (RIFM, 2015). Under the conditions of the study, ethyl 2,3,6,6-tetramethylcyclohex-2-ene-1-carboxylate was considered to be non-clastogenic in the *in vitro* micronucleus test.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/15/18.

10.1.2. Repeated dose toxicity

There are insufficient repeated dose toxicity data on ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate nor any read-across materials. The total systemic exposure to ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate is below the TTC for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

10.1.2.1. Risk assessment. There are no repeat dose toxicity data on ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate or any read-across materials that can be used to support the repeat dose toxicity endpoint. The total systemic exposure to ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate (0.14 μg/kg bw/day) is below the TTC (1800 μg/kg bw/day; Kroes et al., 2007) 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: 11/16/18.

10.1.3. Reproductive toxicity

There are no reproductive toxicity data on ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate or on any read-across materials. The total systemic exposure to ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate is below the TTC for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

10.1.3.1. Risk assessment. There are no reproductive toxicity data on ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate or on any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate (0.14 μg/kg bw/day) is below the TTC (30 μg/kg bw/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: 11/15/18.

10.1.4. Skin sensitization

Based on the existing data and read-across material 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R, 2S)-rel- (CAS # 540734-22-3), ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate does not present a concern for skin sensitization under the current, declared levels of use.

10.1.4.1. Risk assessment. Limited skin sensitization studies are available for ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate. Based on the read-across material 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R, 2S)-rel- (CAS # 540734-22-3; see Section 5), ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate is not considered a skin sensitizer. The chemical structure of these materials indicate that they would not be expected to react with skin proteins (Roberts et al., 2007; Toxtree 3.1.0; OECD Toolbox v4.2). In a guinea pig maximization test, no reactions indicative of sensitization were observed with ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate (RIFM, 1976a). In a murine local lymph node assay (LLNA), read-across material 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R, 2S)-rel- was not found to be sensitizing up to 40% (RIFM, 2004). Additionally, in an HRIPT with 5906 μg/cm^2 of read-across material 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R, 2S)-rel-, no reactions indicative of sensitization were observed in any of the 106 volunteers (RIFM, 2009).

Based on weight of evidence (WoE) from structural analysis and read-across material 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R, 2S)-rel-, ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate does not present a concern for skin sensitization under the current, declared levels of use.

Additional References: RIFM, 1977b; RIFM, 1976b.

Literature Search and Risk Assessment Completed On: 11/21/18.

10.1.5. Phototoxicity/photoallergenicity

Based on the available *in vivo* study data and UV/Vis spectra, ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate would not be expected to present a concern for phototoxicity or photoallergenicity.

10.1.5.1. Risk assessment. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. The corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). In a phototoxicity study conducted in guinea pigs, there were no phototoxic reactions (RIFM, 1977a). Based on the lack of absorbance and the available *in vivo* study data, ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate does not present a concern for phototoxicity or photoallergenicity.

10.1.5.2. 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 et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/16/18.

10.1.6. Local Respiratory Toxicity

The margin of exposure could not be calculated due to a lack of appropriate data. The exposure level for ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate is below the Cramer Class I TTC value for inhalation exposure local effects.

10.1.6.1. Risk assessment. There are no inhalation data available on ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate. Based on the Creme RIFM Model, the inhalation exposure is 0.0011 mg/day. This exposure is 1272.7 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: 11/01/18.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate 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, ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate 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 ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate as possibly 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 ≥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).

10.2.1.1. Risk assessment. Based on the current Volume of Use (2015), ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate presents a risk to the aquatic compartment in the screening-level assessment.

10.2.1.2. Key studies

10.2.1.2.1. Biodegradation. No data available.

10.2.1.2.2. Ecotoxicity. No data available.

10.2.1.2.3. Other available data. Ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate has been pre-registered for REACH with no additional data at this time.

10.2.2. Risk assessment refinement

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)**	<u>0.4066</u>			1000000	0.0004066	
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	0.646	0.499	<u>0.125</u>	10000	0.0125	Esters
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	0.190	0.146	0.389	10000		Neutral Organics

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

Exposure	Europe	North America
Log K_{ow} Used	5.3	5.3
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	< 1	< 1
Risk Characterization: PEC/PNEC	**< 1**	**< 1**

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

The RIFM PNEC is 0.0125 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: 11/18/18.

11. 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**
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
- **TOXNET:** 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 05/31/19.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.110880.

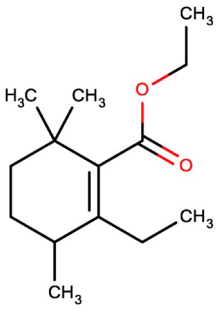
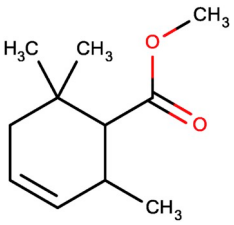
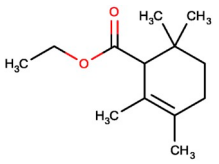
Appendix

Read-across Justification

Methods

The read-across analogs were identified following the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015). The strategy is also consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemicals Agency read-across assessment framework (ECHA, 2016).

- 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 material 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).
- DNA binding, mutagenicity, genotoxicity alerts, and oncologic classification predictions were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010).
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018), 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, 2018).

	Target Material	Read-across Material	Read-across Material
Principal Name	Ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate	3-Cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R,2S)-rel-	Ethyl 2,3,6,6-tetramethylcyclohex-2-ene-1-carboxylate
CAS No.	94333-50-3	540734-22-3	77851-07-1
Structure			
Similarity (Tanimoto Score)		0.47	0.88
Read-across Endpoint		• Skin sensitization	• Genotoxicity
Molecular Formula	$C_{14}H_{24}O_2$	$C_{11}H_{18}O_2$	$C_{13}H_{22}O_2$
Molecular Weight	224.34	182.26	210.32
Melting Point (°C, EPI Suite)	58.10	10.54	47.61
Boiling Point (°C, EPI Suite)	274.55	219.71	257.92
Vapor Pressure (Pa @ 25 °C, EPI Suite)	0.49	17.70	1.47
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	5.38	3.72	4.88
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPI Suite)	0.8374	35.98	2.609
J_{max} (μg/cm^2/h, SAM)	11.49	761.33	47.786
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.16E+002	6.65E+001	1.63E+002
Genotoxicity			
DNA Binding (OASIS v1.4, QSAR Toolbox v4.2)	• No alert found		• No alert found
DNA Binding (OECD QSAR Toolbox v4.2)	• No alert found		• No alert found
Carcinogenicity (ISS)	• Non-carcinogen		• Non-carcinogen
DNA Binding (Ames, MN, CA, OASIS v1.1)	• No alert found		• No alert found
In Vitro Mutagenicity (Ames, ISS)	• No alert found		• No alert found
In Vivo Mutagenicity (Micronucleus, ISS)	• No alert found		• No alert found
Oncologic Classification	• Acrylate reactive function		• Not classified function
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)	• Alert for Schiff base formation	• No alert found	
Metabolism			
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	• See Supplemental Data 1	• See Supplemental Data 2	• See Supplemental Data 3

Summary

There are insufficient toxicity data on ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate (CAS # 94333-50-3). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, physical–chemical properties, and expert judgment, 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R, 2S)-rel- (CAS # 540734-22-3) and ethyl 2,3,6,6-tetramethylcyclohex-2-ene-1-carboxylate (CAS # 77851-07-1) were identified as read-across analogs with sufficient data for toxicological evaluation.

Conclusions

- 3-Cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R, 2S)-rel- (CAS # 540734-22-3) was used as a read-across analog for the target material ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate (CAS # 94333-50-3) for the skin sensitization endpoint.
  - ○ The target material and the read-across analog are structurally similar and belong to a class of monocyclic unsaturated esters.
  - ○ The target material and the read-across analog share a 6,6-dimethylcyclohexene-1- carboxylate structure.
  - ○ The key differences between the target material and the read-across analog is that the target material has an ethyl carboxylate group in the 1 position, whereas the read-across analog has a methyl carboxylate group in the same position; the target material has an ethyl moiety in the 2 position, whereas the read-across analog has a methyl group in the same position, and the target material has a methyl in the 3 position. Additionally, the cyclohexene ring in the target material has a double bond in the 1 position, whereas the read-across analog has a double bond in the 3 position. These structural differences are toxicologically insignificant.
  - ○ Similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - ○ The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - ○ According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.

- The target has an alert for forming a Schiff base, while the read-across analog lacks such an alert. This is due to the fact that the target has a,β-unsaturated carbonyl group, which is not present in the read-across analog. Since the α,β unsaturation in the target has an alkyl substitution on both the α- and β-carbons, rendering the unsaturation inactive, the alert can be ignored.
- The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
- The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

- Ethyl 2,3,6,6-tetramethylcyclohex-2-ene-1-carboxylate (CAS # 77851-07-1) was used as a read-across analog for the target material ethyl 2-ethyl-3,6,6-trimethylcyclohexenecarboxylate (CAS # 94333-50-3) for the genotoxicity endpoint clastogenesis.
  - The target material and the read-across analog are structurally similar and belong to a class of monocyclic unsaturated esters.
  - The target material and the read-across analog share cyclohexene-1-carboxylate structures.
  - The key structural differences between the target material and the read-across analog are that the target material has an ethyl carboxylate in the 1 position, whereas the read-across analog has a methyl carboxylate in the same position; the target material also has a methyl in the 3 position and lastly, the cyclohexene ring in the target material has a double bond in the 1 position, whereas the read-across analog has a double bond in the 2-position. These structural differences are toxicologically insignificant.
  - Similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - The target is classified as acrylate reactive according to oncologic classification, while the read-across analog lacks this classification. This difference in reactivity is due to the fact that the target has a β-unsaturated carbonyl group, which is not present in the read-across analog. The α,β unsaturation in the target has an alkyl substitution on both α-and β-carbons rendering it inactive. Therefore, the alert can be ignored.
  - The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

References

Api, A.M., Belsito, D., Bruze, M., Cadby, P., Calow, P., Dagli, M.L., Dekant, W., Ellis, G., Fryer, A.D., Fukayama, M., Griem, P., Hickey, C., Kromidas, L., Lalko, J.F., Liebler, D.C., Miyachi, Y., Politano, V.T., Renskers, K., Ritacco, G., Salvito, D., Schultz, T.W., Sipes, I.G., Smith, B., Vitale, D., Wilcox, D.K., 2015. Criteria for the Research Institute for fragrance materials, Inc. (RIFM) safety evaluation process for fragrance ingredients. Food Chem. Toxicol. 82, S1–S19.

Carthew, P., Clapp, C., Gutsell, S., 2009. Exposure based waiving: the application of the toxicological threshold of concern (TTC) to inhalation exposure for aerosol ingredients in consumer products. Food Chem. Toxicol. 47 (6), 1287–1295.

Cassano, A., Manganaro, A., Martin, T., Young, D., Piclin, N., Pintore, M., Bigoni, D., Benfenati, E., 2010. CAESAR models for developmental toxicity. Chem. Cent. J. (4 Suppl. 1), S4.

Comiskey, D., Api, A.M., Barratt, C., Daly, E.J., Ellis, G., McNamara, C., O'Mahony, C., Robison, S.H., Safford, B., Smith, B., Tozer, S., 2015. Novel database for exposure to fragrance ingredients in cosmetics and personal care products. Regul. Toxicol. Pharmacol. 72 (3), 660–672.

Comiskey, D., Api, A.M., Barrett, C., Ellis, G., McNamara, C., O'Mahony, C., Robison, S.H., Rose, J., Safford, B., Smith, B., Tozer, S., 2017. Integrating habits and practices data for soaps, cosmetics and air care products into an existing aggregate exposure model. Regul. Toxicol. Pharmacol. 88, 144–156.

ECHA, 2012. Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT Assessment, November 2012 v1.1. http://echa.europa.eu/.

ECHA, 2016. Read-across Assessment Framework (RAAF). Retrieved from. www.echa.europa.eu/documents/10162/13628/raaf_en.pdf.

Henry, B., Foti, C., Alsante, K., 2009. Can light absorption and photostability data be used to assess the photosafety risks in patients for a new drug molecule? J. Photochem. Photobiol. B Biol. 96 (1), 57–62.

IFRA (International Fragrance Association), 2015. Volume of Use Survey. February 2015.

Kroes, R., Renwick, A.G., Feron, V., Galli, C.L., Gibney, M., Greim, H., Guy, R.H., Lhuguenot, J.C., van de Sandt, J.J.M., 2007. Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients. Food Chem. Toxicol. 45 (12), 2533–2562.

Laufersweiler, M.C., Gadagbui, B., Baskerville-Abraham, I.M., Maier, A., Willis, A., et al., 2012. Correlation of chemical structure with reproductive and developmental toxicity as it relates to the use of the threshold of toxicological concern. Regul. Toxicol. Pharmacol. 62 (1), 160–182.

OECD, 2015. Guidance Document On The Reporting Of Integrated Approaches To Testing And Assessment (IATA). ENV/JM/HA 2015. pp. 7. Retrieved from. http://www.oecd.org/.

OECD, 2018. The OECD QSAR Toolbox, v3.2–4.2. Retrieved from. http://www.qsartoolbox.org/.

RIFM (Research Institute for Fragrance Materials, Inc), 1976. Capacity for Allergic Sensitization Determined by the Maximization Test on guinea Pigs with Ethyl 2-Ethyl-3,6,6-Trimethylcyclohexenecarboxylate (Myrascone). Unpublished report from Givaudan. RIFM report number 57269. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1976. Skin Irritation and Capacity for Allergic Sensitization Determined by the Open Epicutaneous Test (OET) on guinea Pigs with Ethyl 2-Ethyl-3,6,6-Trimethylcyclohexenecarboxylate (Myrascone). Unpublished report from Givaudan. RIFM report number 57271. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1977. Determination of Phototoxicity in guinea Pigs of Ethyl 2-Ethyl-3,6,6-Trimethylcyclohexenecarboxylate (Myrascone). Unpublished report from Givaudan. RIFM report number 57268. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1977. Human Repeat Insult Patch Test with Ethyl 2-Ethyl-3,6,6-Trimethylcyclohexenecarboxylate (Myrascone). Unpublished report from Givaudan. RIFM report number 57270. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2002. Salmonella typhimurium Reverse Mutation Assay with Ethyl 2-Ethyl-3,6,6-Trimethylcyclohexenecarboxylate (Myrascone). Unpublished report from Givaudan. RIFM report number 41967. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2004. 3-Cyclohexene-1-carboxylic Acid, 2,6,6-trimethyl-,methyl Ester: Murine Local Lymph Node Assay. Unpublished report from Firmenich SA. RIFM report number 47326. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2009. Repeated Insult Patch Study of 3-Cyclohexene-1-Carboxylic Acid, 2,6,6-trimethyl-, Methyl Ester, (1R,2S)-Rel- at 5% in 75% Diethyl Phthalate (DEP)/25% Ethanol. Unpublished report from Firmenich SA. RIFM report number 58916. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2015. Ethyl 2,3,6,6-Tetramethylcyclohex-2-Ene-1-Carboxylate: in Vitro Mammalian Cell Micronucleus Assay in Human Peripheral Blood Lymphocytes (HPBL). RIFM report number 68317. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2017. Expo. Surv. 14 January 2017.

Roberts, D.W., Patlewicz, G., Kern, P.S., Gerberick, F., Kimber, I., Dearman, R.J., Ryan, C.A., Basketter, D.A., Aptula, A.O., 2007. Mechanistic applicability domain classification of a local lymph node assay dataset for skin sensitization. Chem. Res. Toxicol. 20 (7), 1019–1030.

Rogers, D., Hahn, M., 2010. Extended-connectivity fingerprints. J. Chem. Inf. Model. 50 (5), 742–754.

Safford, B., Api, A.M., Barratt, C., Comiskey, D., Daly, E.J., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Smith, B., Thomas, R., Tozer, S., 2015. Use of an aggregate exposure model to estimate consumer exposure to fragrance ingredients in personal

care and cosmetic products. Regul. Toxicol. Pharmacol. 72, 673–682.
Safford, B., Api, A.M., Barratt, C., Comiskey, D., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Rose, J., Smith, B., Tozer, S., 2017. Application of the expanded Creme RIFM consumer exposure model to fragrance ingredients in cosmetic, personal care and air care products. Regul. Toxicol. Pharmacol. 86, 148–156.
Salvito, D.T., Senna, R.J., Federle, T.W., 2002. A Framework for prioritizing fragrance materials for aquatic risk assessment. Environ. Toxicol. Chem. 21 (6), 1301–1308.
Schultz, T.W., Amcoff, P., Berggren, E., Gautier, F., Klaric, M., Knight, D.J., Mahony, C., Schwarz, M., White, A., Cronin, M.T., 2015. A strategy for structuring and reporting a read-across prediction of toxicity. Regul. Toxicol. Pharmacol. 72 (3), 586–601.
US EPA, 2012a. Estimation Programs Interface Suite for Microsoft Windows, v4.0–v4.11. United States Environmental Protection Agency, Washington, DC, USA.
US EPA, 2012b. The ECOSAR (ECOlogical Structure Activity Relationship) Class Program for Microsoft Windows, v1.11. United States Environmental Protection Agency, Washington, DC, USA.