Food and Chemical Toxicology 138 (2020) 111054

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

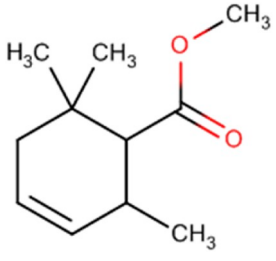
Short Review

RIFM fragrance ingredient safety assessment, 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, CAS registry number 815580-59-7

A.M. Api[a], F. Belmonte[a], D. Belsito[b], S. Biserta[a], D. Botelho[a], M. Bruze[c], G.A. Burton Jr.[d], J. Buschmann[e], M.A. Cancellieri[a], M.L. Dagli[f], M. Date[a], W. Dekant[g], C. Deodhar[a], A.D. Fryer[h], S. Gadhia[a], L. Jones[a], K. Joshi[a], A. Lapczynski[a], M. Lavelle[a], D.C. Liebler[i], M. Na[a], D. O'Brien[a], A. Patel[a], T.M. Penning[j], G. Ritacco[a], F. Rodriguez-Ropero[a], J. Romine[a], N. Sadekar[a], D. Salvito[a], T.W. Schultz[k], I.G. Sipes[l], G. Sullivan[a,*], Y. Thakkar[a], Y. Tokura[m], S. Tsang[a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ, 07677, USA*
[b] *Member RIFM Expert Panel, Columbia University Medical Center, Department of Dermatology, 161 Fort Washington Ave., New York, NY, 10032, USA*
[c] *Member RIFM Expert Panel, Malmo University Hospital, Department of Occupational & Environmental Dermatology, Sodra Forstadsgatan 101, Entrance 47, Malmo, SE, 20502, Sweden*
[d] *Member RIFM Expert Panel, School of Natural Resources & Environment, University of Michigan, Dana Building G110, 440 Church St., Ann Arbor, MI, 58109, USA*
[e] *Member RIFM Expert Panel, Fraunhofer Institute for Toxicology and Experimental Medicine, Nikolai-Fuchs-Strasse 1, 30625, Hannover, Germany*
[f] *Member RIFM Expert Panel, University of Sao Paulo, School of Veterinary Medicine and Animal Science, Department of Pathology, Av. Prof. dr. Orlando Marques de Paiva, 87, Sao Paulo, CEP, 05508-900, Brazil*
[g] *Member RIFM Expert Panel, University of Wuerzburg, Department of Toxicology, Versbacher Str. 9, 97078, Würzburg, Germany*
[h] *Member RIFM Expert Panel, Oregon Health Science University, 3181 SW Sam Jackson Park Rd., Portland, OR, 97239, USA*
[i] *Member RIFM Expert Panel, Vanderbilt University School of Medicine, Department of Biochemistry, Center in Molecular Toxicology, 638 Robinson Research Building, 2200 Pierce Avenue, Nashville, TN, 37232-0146, USA*
[j] *Member of RIFM Expert Panel, University of Pennsylvania, Perelman School of Medicine, Center of Excellence in Environmental Toxicology, 1316 Biomedical Research Building (BRB) II/III, 421 Curie Boulevard, Philadelphia, PA, 19104-3083, USA*
[k] *Member RIFM Expert Panel, The University of Tennessee, College of Veterinary Medicine, Department of Comparative Medicine, 2407 River Dr., Knoxville, TN, 37996-4500, USA*
[l] *Member RIFM Expert Panel, Department of Pharmacology, University of Arizona, College of Medicine, 1501 North Campbell Avenue, P.O. Box 245050, Tucson, AZ, 85724-5050, USA*
[m] *Member RIFM Expert Panel, The Journal of Dermatological Science (JDS), Editor-in-Chief, Professor and Chairman, Department of Dermatology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, 431-3192, Japan*

Version: 121218. This version replaces any previous versions.
Name: 3-Cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester CAS Registry Number: 815580-59-7
Additional CAS Numbers*:
540734-22-3 3-Cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R,2S)-rel-; Firascone (No Reported Use)
*Included in this assessment because the materials are isomers



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

* Corresponding author.
E-mail address: gsullivan@rifm.org (G. Sullivan).

https://doi.org/10.1016/j.fct.2019.111054
Received 10 September 2019; Accepted 10 December 2019
Available online 14 December 2019
0278-6915/

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.

3-Cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester and read-across analog ethyl 2,3,6,6-tetramethylcyclohex-2-ene-1-carboxylate (CAS # 77851-07-1) show that 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester 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 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). Data show that there are no safety concerns for 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester for skin sensitization under the current declared levels of use. The phototoxicity/photoallergenicity endpoints were evaluated based on data and UV spectra; 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester 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, 2000; RIFM, 2008c)
Repeated Dose Toxicity: No NOAEL 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 (2004a)
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic. (UV Spectra, RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:
Persistence:
Screening-level: 2.72 (BIOWIN 3) (EPI Suite v4.11; US EPA, 2012a)
Bioaccumulation:
Screening-level: 131.5 L/kg (EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:
Screening-level: Fish LC50: 7.90 mg/L (RIFM Framework; Salvito et al., 2002)
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: Fish LC50: 7.90 mg/L (RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.00790 μg/L

- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: not applicable; cleared at screening-level

1. Identification

Chemical Name: 3-Cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester	Chemical Name: 3-Cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R,2S)-rel-
CAS Registry Number: 815580-59-7 **Synonyms:** Firascone; 3-Cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester **Molecular Formula:** $C_{11}H_{18}O_2$ **Molecular Weight:** 182.26 **RIFM Number:** 1391 **Stereochemistry:** Isomer not specified. One chiral center and 2 total enantiomers possible.	**CAS Registry Number:** 540734-22-3 **Synonyms:** 3-Cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R,2S)-rel-; Firascone **Molecular Formula:** $C_{11}H_{18}O_2$ **Molecular Weight:** 182.26 **RIFM Number:** 10283 **Stereochemistry:** 1R,2S enantiomer specified. One chiral center and 2 total enantiomers possible.

2. Physical data

CAS # 815580-59-7	CAS # 540734-22-3
Boiling Point: Not available	**Boiling Point:** 207.3 °C (RIFM, 2006), 213.1 °C (RIFM, 2008b), 479 ± 2 K (206 ± 2 °C) at 96.6 kPa (RIFM, 2003)
Flash Point: Not available	**Flash Point:** 68 °C (RIFM, 2006), 69 °C (RIFM, 2008b), 74 ± 2 °C (RIFM, 2003), pH 1.2, 37 ± 0.5 °C, no signif. hydrolysis @ 24 h (RIFM, 2009b)
Log K_{OW}: Not available	**Log K_{OW}:** 8.33 x 10(3), log10 Pow 3.92 (RIFM, 2004b)
Melting Point: Not available	**Melting Point**: < -20 °C (Firmenich)
Water Solubility: Not available	**Water Solubility:** 0.119 g/L of solution at 20 ± 0.5 °C (RIFM, 2004b)
Specific Gravity: Not available	**Specific Gravity:** Not Available
Vapor Pressure: Not available	**Vapor Pressure:** 0.681513 hPa at 25 °C (RIFM, 2006), 0.550926 hPa at 25 °C (RIFM, 2008b), 151 Pa at 25 °C (RIFM, 2009c), 3.5 x 10(1) Pa at 25 °C; 2.4 x 10(1) at 20 °C (RIFM, 2009d), 0.0877 mm Hg @ 20 °C (EPI Suite v4.0)
UV Spectra: No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L $mol^{-1} \cdot cm^{-1}$)	**UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L $mol^{-1} \cdot cm^{-1}$)
Appearance/Organoleptic: Not available	**Appearance/Organoleptic:** Not Available

*3. Exposure to fragrance ingredient****

1. **Volume of Use (Worldwide Band):** 0.1–1 metric tons per year (IFRA , 2015)
2. **95**th **Percentile Concentration in Hydroalcoholics:** 0.055% (RIFM, 2017)
3. **Inhalation Exposure*:** 0.00012 mg/kg/day or 0.0087 mg/day (RIFM, 2017)
4. **Total Systemic Exposure**:** 0.0014 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; 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 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; Safford et al., 2015; Safford et al., 2017; and Comiskey et al., 2017).

***When a safety assessment includes multiple materials, the highest exposure out of all included materials will be recorded here for the 95th Percentile Concentration in hydroalcoholics, inhalation exposure, and total exposure.

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)

Expert Judgment	Toxtree v 2.6	OECD QSAR Toolbox v 3.2
I	III	I

*Due to potential discrepancies with the current *in silico* tools (Bhatia et al., 2015), the Cramer Class of the target material was determined using expert judgment based on the Cramer decision tree (Cramer et al., 1978). See Appendix below for further details.

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:** None
   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)

Neither 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester nor 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R,2S)-rel-is are 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

No dossier available for either material as of 11/21/18.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data, 3-cyclohexene-1-carboxylic acid,

2,6,6-trimethyl-, methyl ester does not present a concern for genotoxicity.

10.1.1.1. Risk assessment. There are no data assessing the mutagenic activity of 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester; however, read-across can be made to ethyl 2,3,6,6-tetramethylcyclohex-2-ene-1-carboxylate (CAS # 77851-07-1; see Section 5). Ethyl 2,3,6,6-tetramethylcyclohex-2-ene-1-carboxylate (CAS # 77851-07-1) 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 TA102 were treated with ethyl 2,3,6,6-tetramethylcyclohex-2-ene-1-carboxylate in solvent 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, 2000). Under the conditions of the study, ethyl 2,3,6,6-tetramethylcyclohex-2-ene-1-carboxylate was not mutagenic in the Ames test.

The clastogenic activity of an additional material of this assessment, 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R,2S)-rel- (CAS # 540734-22-3) has been evaluated 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 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R,2S)-rel- in DMSO at concentrations up to 1823 μg/mL in the dose range finding study; the main test was conducted at 180 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 material, either with or without S9 metabolic activation (RIFM, 2008c). Under the conditions of the study, 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R,2S)-rel- was considered to be non-clastogenic to in the *in vitro* chromosome aberration assay.

Additional References: RIFM, 2008a.

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

10.1.2. Repeated dose toxicity

There are no repeated dose toxicity data on 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester or any read-across materials. The total systemic exposure to 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester 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 repeated dose toxicity data on 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester or any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester (1.4 μg/kg bw/day) is below the TTC (1800 μg/kg bw/day; Kroes et al., 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: 11/10/18.

10.1.3. Reproductive toxicity

There are no reproductive toxicity data on 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester or on any read-across materials. The total systemic exposure to 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester 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 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester or on any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester (1.4 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: 11/10/18.

10.1.4. Skin sensitization

Based on the existing data, 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester (CAS # 815580-59-7) and 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R,2S)-rel- (CAS # 540734-22-3) do not present a concern for skin sensitization.

10.1.4.1. Risk assessment. Based on the existing data, 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester and 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R,2S)-rel- are not considered skin sensitizers. The chemical structure of these materials indicates 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 murine local lymph node assay (LLNA), 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R,2S)-rel- was found to be not sensitizing when tested up to 40% (10000 μg/cm^2) (RIFM, 2004a). Additionally, in a confirmatory human repeat insult patch test (HRIPT) with 5906 μg/cm^2 of cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester, (1R,2S)-rel- in 3:1 diethylphthalate:ethanol (DEP:EtOH), no reactions indicative of sensitization were observed in any of the 105 volunteers (RIFM, 2009a).

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

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/20/18.

10.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester would not be expected to present a concern for phototoxicity or photoallergenicity.

10.1.5.1. Risk assessment. There are no phototoxicity studies available for 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester in experimental models. 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). Based on the lack of absorbance, 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester 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: 10/17/18.

10.1.6. Local Respiratory Toxicity

The margin of exposure could not be calculated due to lack of

appropriate data. The exposure level for 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester 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 3-Cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester. Based on the Creme RIFM Model, the inhalation exposure is 0.0087 mg/day. This exposure is 161 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 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester 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, 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester was identified as a fragrance material with no 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 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester 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).

10.2.2. Risk assessment

Based on the current Volume of Use (2015), 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester presents no risk to the aquatic compartment in the screening-level assessment.

10.2.2.1. Key studies. Biodegradation

Biodegradation: No data available.

Ecotoxicity

Ecotoxicity: No data available.

Other available data

Other available data: 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester has been pre-registered for REACH with no additional data at this time.

10.2.3. 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>7.90</u>			1000000	0.00790	

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	3.7	3.7
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 these materials is < 1. No further assessment is necessary.

The RIFM PNEC is 0.00790 μg/L. The revised PEC/PNECs for EU and NA: not applicable. The material was cleared at the screening-level and therefore does not present a risk to the aquatic environment at the current reported volumes of use.

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

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. 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.2019.111054.

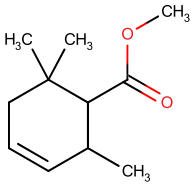
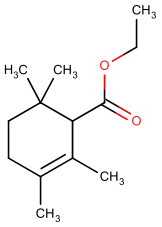
Appendix

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 ECHA, 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
Principal Name	3-Cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester	Ethyl 2,3,6,6-tetramethylcyclohex-2-ene-1-carboxylate
CAS No.	815580-59-7	77851-07-1
Structure		
Similarity (Tanimoto Score)		0.86
Read-across Endpoint		• Genotoxicity
Molecular Formula	$C_{11}H_{18}O_2$	$C_{13}H_{22}O_2$
Molecular Weight	182.26	210.31
Melting Point (°C, EPI Suite)	10.54	47.61

Boiling Point (°C, EPI Suite)	219.71	257.92
Vapor Pressure (Pa @ 25°C, EPI Suite)	17.7	1.47
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	3.72	4.88
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	35.98	2.609
J_{max} (μg/cm^2/h, SAM)	3.59	47.79
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	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 (low reliability)	• Non-carcinogen (low reliability)
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	• Not classified	• Not classified
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 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester (CAS # 815580-59-7). 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, 1-methyl-3-(4-methyl-3-pentenyl)-,methyl ester (CAS # 65652-28-0) was identified as a read-across analog with sufficient data for toxicological evaluation.

Conclusions

- 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 3-cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester (CAS # 815580-59-7) for the genotoxicity 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 cyclohex-2-ene carboxylate moiety.
  - ○ The key difference between the target material and the read-across analog is that the read-across analog has an ethyl ester while the target material has a methyl ester. The read-across analog also has an extra methyl group in position 3 in the cylohexene ring. 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 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.

Explanation of Cramer Classification

Due to potential discrepancies between the current *in silico* tools (Bhatia et al., 2015), the Cramer Class of the target material was determined using expert judgment, based on the Cramer decision tree.

1N,2N,3N,43N,5N,6N,42N,7N,16N,17N, 19N, 23N, 24Y, 18N

Q1. Normal constituent of the body? No
Q2. Contains functional groups associated with enhanced toxicity? No
Q3. Contains elements other than C, H, O, N, and divalent S? No
Q4. Elements not listed in Q3 occurs only as a Na, K, Ca, Mg, N salt, sulfamate, sulfonate, sulfate, hydrochloride? No
Q5. Simply branched aliphatic hydrocarbon or a common carbohydrate? No
Q6. Benzene derivative with certain substituents? No
Q7. Heterocyclic? No
Q16. Common terpene (see Cramer et al., 1978 for detailed explanation)? No
Q17. Readily hydrolyzed to a common terpene? No
Q19. Open chain? No
Q23. Aromatic? No
Q24. Monocarbocyclic with simple substituents? No
Q18. One of the list (see Cramer et al., 1978 for detailed explanation on list of categories)? No, Class I (Class Low)

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.

Bhatia, S., Schultz, T., Roberts, D., Shen, J., Kromidas, L., Api, A.M., 2015. Comparison of cramer classification between toxtree, the OECD QSAR Toolbox and expert judgment. Regul. Toxicol. Pharmacol. 71 (1), 52–62.

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.
Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard—a decision tree approach. Food Cosmet. Toxicol. 16 (3), 255–276.
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)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), 2000. Salmonella typhimurium Reverse Mutation Assay of Ethyl 2,3,6,6-Tetramethylcyclohex-2-Ene-1-Carboxylate (Givescone). Unpublished Report from Givaudan. RIFM Report Number 41960. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2003. 3-Cyclohexene-1-carboxylic Acid, 2,6,6-trimethyl-, Methyl Ester, (1R,2S)-Rel-: Determination of General Physico-Chemical Properties: Melting/freezing Temperature, Boiling Temperature, Relative Density and Flash Point. Unpublished Report from Firmenich SA. RIFM Report Number 62793. 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), 2004. 3-Cyclohexene-1-carboxylic Acid, 2,6,6-trimethyl-, Methyl Ester, (1R,2S)-Rel-: Determination of Water Solubility and Partition Coefficient. Unpublished Report from Firmenich SA. RIFM Report Number 62794. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2006. Vapor Pressure of 3-Cyclohexene-1-Carboxylic Acid, 2,6,6-trimethyl-, Methyl Ester, (1R,2S)-Rel-(Firascone). Unpublished Report from Firmenich SA. RIFM Report Number 62790. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2008. Salmonella typhimurium Reverse Mutation Assay with Ethyl 2,3,6,6-Tetramethylcyclohex-2-Ene-1-Carboxylate (Givescone). Unpublished Report from Givaudan. RIFM Report Number 55928. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2008. Vapor Pressure of 3-Cyclohexene-1-Carboxylic Acid, 2,6,6-trimethyl-, Methyl Ester, (1R,2S)-Rel-(Firascone). Unpublished Report from Firmenich SA. RIFM Report Number 62791. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2008. 3-Cyclohexene-1-carboxylic Acid, 2,6,6-trimethyl-, Methyl Ester, (1R,2S)-Rel-: Chromosome Aberration Test in Human Lymphocytes in Vitro. Unpublished Report from Firmenich SA. RIFM Report Number 62796. 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), 2009. 3-Cyclohexene-1-carboxylic Acid, 2,6,6-trimethyl-, Methyl Ester, (1R,2S)-Rel-: Determination of General Physico-Chemical Properties. Unpublished Report from Firmenich SA. RIFM Report Number 62789. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2009. 3-Cyclohexene-1-carboxylic Acid, 2,6,6-trimethyl-, Methyl Ester, (1R,2S)-Rel-: Determination of Hazardous Physico-Chemical Properties. Unpublished Report from Firmenich SA. RIFM Report Number 62792. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2009. 3-Cyclohexene-1-carboxylic Acid, 2,6,6-trimethyl-, Methyl Ester, (1R,2S)-Rel-: Determination of Vapor Pressure. Unpublished Report from Firmenich SA. RIFM Report Number 62795. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2017. Exposure Survey 17 August 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, 2012. Estimation Programs Interface Suite for Microsoft Windows, v4.0–v4.11. United States Environmental Protection Agency, Washington, DC, USA.
US EPA, 2012. The ECOSAR (ECOlogical Structure Activity Relationship) Class Program for Microsoft Windows, v1.11. United States Environmental Protection Agency, Washington, DC, USA.