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Short Review

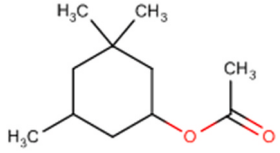
RIFM fragrance ingredient safety assessment, 3,3,5-trimethylcyclohexyl acetate, CAS Registry Number 67859-96-5

A.M. Api [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], M. Kumar [a], A. Lapczynski [a], M. Lavelle [a], I. Lee [a], D.C. Liebler [i], H. Moustakas [a], M. Na [a], T.M. Penning [j], G. Ritacco [a], J. Romine [a], N. Sadekar [a], T.W. Schultz [k], D. Selechnik [a], F. Siddiqi [a], I.G. Sipes [l], G. Sullivan [a,*], Y. Thakkar [a], Y. Tokura [m]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ, 07677, USA*
[b] *Member Expert Panel, Columbia University Medical Center, Department of Dermatology, 161 Fort Washington Ave., New York, NY, 10032, USA*
[c] *Member Expert Panel, Malmo University Hospital, Department of Occupational & Environmental Dermatology, Sodra Forstadsgatan 101, Entrance 47, Malmo, SE, 20502, Sweden*
[d] *Member Expert Panel, School of Natural Resources & Environment, University of Michigan, Dana Building G110, 440 Church St., Ann Arbor, MI, 58109, USA*
[e] *Member Expert Panel, Fraunhofer Institute for Toxicology and Experimental Medicine, Nikolai-Fuchs-Strasse 1, 30625, Hannover, Germany*
[f] *Member 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 Expert Panel, University of Wuerzburg, Department of Toxicology, Versbacher Str. 9, 97078, Würzburg, Germany*
[h] *Member Expert Panel, Oregon Health Science University, 3181 SW Sam Jackson Park Rd., Portland, OR, 97239, USA*
[i] *Member 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 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 Expert Panel, The University of Tennessee, College of Veterinary Medicine, Department of Comparative Medicine, 2407 River Dr., Knoxville, TN, 37996- 4500, USA*
[l] *Member 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 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: 070,120. This version replaces any previous versions.

Name: 3,3,5-Trimethylcyclohexyl acetate
CAS Registry Number: 67,859-96-5



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
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 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

(*continued on next page*)

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

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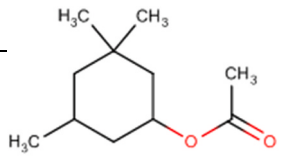


NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
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.

3,3,5-Trimethylcyclohexyl acetate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 3,3,5-trimethylcyclohexyl acetate is not genotoxic. Data on 3,3,5-trimethylcyclohexyl acetate provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and reproductive toxicity endpoints. Data from read-across material menthyl acetate (1α,2β,5α) (CAS # 89-48-5) show that there are no safety concerns for 3,3,5-trimethylcyclohexyl acetate for skin sensitization under the current declared levels of use. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet (UV) spectra; 3,3,5-trimethylcyclohexyl acetate is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material, and the exposure to 3,3,5-trimethylcyclohexyl acetate is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; 3,3,5-trimethylcyclohexyl acetate was found not to be Persistent, Bioaccumulative, and Toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

Human Health Safety Assessment

Genotoxicity: Not genotoxic.	(RIFM, 2013a; RIFM, 2015b)
Repeated Dose Toxicity: NOAEL = 150 mg/kg/day.	RIFM (2016b)
Reproductive Toxicity: Developmental toxicity: 500 mg/kg/day Fertility: 150 mg/kg/day.	RIFM (2016b)
Skin Sensitization: Not a concern for skin sensitization under the current, declared use levels.	RIFM (2012)

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:	
Critical Measured Value: 56.3% (OECD 301 C)	RIFM (2003)
Bioaccumulation:	
Screening-level: 182.4 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Critical Ecotoxicity Endpoint: 96-h Fish LC50: 8.43 mg/L	RIFM (2016c)
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 Fish LC50: 8.43 mg/L	RIFM (2016c)

RIFM PNEC is: 1.686 μg/L
- Revised PEC/PNECs (2015 IFRA VoU): North America and Europe <1

1. Identification

1. Chemical Name: 3,3,5-Trimethylcyclohexyl acetate
2. CAS Registry Number: 67,859-96-5
3. Synonyms: Homomenthol acetate; Mintonat; 3,3,5-Trimethylcyclohexyl acetate
4. Molecular Formula: $C_{11}H_{20}O_2$
5. Molecular Weight: 184.27
6. RIFM Number: 7362
7. Stereochemistry: No isomer specified. Two stereocenters and 4 total stereoisomers possible.

2. Physical data

1. Boiling Point: 210.2 °C (483.4 K) at 102.0 kPa (RIFM, 2013b), 217 °C (RIFM, 2004a), 217.21 °C (EPI Suite)
2. Flash Point: 84.5 °C (average corrected and rounded down to the nearest multiple of 0.5 °C) (RIFM, 2015a), 76 °C (RIFM, 2004a), moderate hydrolysis rate (t1/2≤30 d) at pH 9 (30 and 50 °C); slow hydrolysis (t1/2 > 30 d) at pH 4 and 7 (50 °C) and pH 9 (20 °C); no significant hydrolysis (t1/2 > 1 yr) at pH 4 and 7 (20 and 30 °C) (RIFM, 2016a)
3. Log K_{OW}: 4.4 (RIFM, 2014), 3.93 (EPI Suite), 3.93 (RIFM, 2004a)
4. Melting Point: 9 °C (RIFM, 2004a), 9.43 °C (EPI Suite)
5. Water Solubility: 23.04 mg/L (EPI Suite)
6. Specific Gravity: 0.9130–0.9230 (RIFM, 2004a)
7. Vapor Pressure: 0.151 mm Hg @ 25 °C (EPI Suite)
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. 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. 95th Percentile Concentration in Hydroalcoholics: 0.66% (RIFM, 2016d)
2. Inhalation Exposure*: 0.0028 mg/kg/day or 0.20 mg/day (RIFM, 2016d)
3. Total Systemic Exposure**: 0.016 mg/kg/day (RIFM, 2016d)

*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

1. Cramer Classification: Class I, Low

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

2. Analogs Selected:
   a. Genotoxicity: None
   b. Repeated Dose Toxicity: None
   c. Reproductive Toxicity: None
   d. Skin Sensitization: Menthyl acetate (1α,2β,5α) (CAS # 89-48-5)
   e. Phototoxicity/Photoallergenicity: None
   f. Local Respiratory Toxicity: None
   g. Environmental Toxicity: None
3. Appendix Read-across Justification: See below

7. Metabolism

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

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

3,3,5-Trimethylcyclohexyl acetate 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

3,3,5-Trimethylcyclohexyl acetate has been pre-registered as of 2010; no dossier available as of 01/02/20.

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, 3,3,5-trimethylcyclohexyl acetate does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. The mutagenic activity of 3,3,5-trimethylcyclohexyl acetate 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 pre-incubation methods. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with 3,3,5-trimethylcyclohexyl acetate 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, 2013a). Under the conditions of the study, 3,3,5-trimethylcyclohexyl acetate was not mutagenic in the Ames test.

The clastogenicity of 3,3,5-trimethylcyclohexyl acetate 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 3,3,5-trimethylcyclohexyl acetate in ethanol at concentrations up to 1850 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 (RIFM, 2015b). Under the conditions of the study, 3,3,5-trimethylcyclohexyl acetate was considered to be non-clastogenic to human/mammalian cells.

Based on the data available, 3,3,5-trimethylcyclohexyl acetate does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 01/23/20.

11.1.2. Repeated dose toxicity

The MOE for 3,3,5-trimethylcyclohexyl acetate is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on 3,3,5-trimethylcyclohexylacetate. In an OECD TG 422 and GLP-compliant combined repeated dose toxicity study with a reproduction/developmental toxicity study, 12 Crl:CD (SD) SPF rats/sex/dose were administered 3,3,5-trimethylcyclohexylacetate via gavage at doses of 0 (vehicle control: corn oil), 50, 150, and 500 mg/kg/day. An additional group of 6 rats/sex/dose (control and high-dose) were maintained as recovery groups for 2 weeks after termination of treatment. Males were treated for 6 weeks (2 weeks prior to, during, and post-mating), and females were treated for 2 weeks prior to mating, throughout gestation, and for 5 days after delivery. Also, both sexes of the recovery groups were dosed for 6 weeks during the treatment period. At 500 mg/kg/day, mortality was reported in 2 females of the main group on post-partum

day (PPD) 5 and day 3. Clinical signs such as soiled perineal region, staining around the mouth, and/or hematuria were reported in these 2 females before death as well as small thymus (2/2) and spleen (1/2), enlargement of adrenals (1/2), black focus in the forestomach (1/2), marked thymic lymphoid atrophy (2/2), mild splenic lymphoid atrophy (2/2), mild adrenal cortical hypertrophy (1/2), and mild erosion/ulceration of stomach (1/2). These effects were found to be test substance-related. No treatment-related adverse effects were reported for body weight, food consumption, sensory function, motor activity, urinalysis, hematology, clinical chemistry, organ weights, necropsy, and histopathology examination in either sex at any dose level. Based on mortality and associated clinical signs in females at the high dose, the no observed adverse effect level (NOAEL) for repeated dose toxicity was considered to be 150 mg/kg/day (RIFM, 2016b; RIFM, 2015c).

A default safety factor of 3 was used when deriving a NOAEL from the OECD 422 study (ECHA, 2012). The safety factor has been approved by the Expert Panel for Fragrance Safety*.

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

Therefore, the MOE can be calculated by dividing the NOAEL (in mg/kg/day) for 3,3,5-trimethylcyclohexylacetate by the total systemic exposure (in mg/kg/day) of 3,3,5-trimethylcyclohexylacetate, 50/0.016 or 3125.

In addition, the total systemic exposure to 3,3,5-trimethylcyclohexylacetate (16 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes, 2007) for the repeated dose endpoint of a Cramer Class I material at the current level of use.

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

Additional References: None.

Literature Search and Risk Assessment Completed On: 01/22/20.

11.1.3. Reproductive toxicity

The MOE for 3,3,5-trimethylcyclohexylacetate is adequate for the fertility and developmental toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are sufficient reproductive and developmental toxicity data on 3,3,5-trimethylcyclohexylacetate. In an OECD TG 422 and GLP-compliant combined repeated dose toxicity study with a reproduction/developmental toxicity study, 12 Crl:CD (SD) SPF rats/sex/dose were administered 3,3,5-trimethylcyclohexylacetate via gavage at doses of 0 (vehicle control: corn oil), 50, 150, and 500 mg/kg/day. An additional group of 6 animals/sex/dose (control and high-dose) were maintained as recovery groups for 2 weeks after termination of treatment. Males were treated for 6 weeks (2 weeks prior to, during, and after mating), and females were treated for 2 weeks prior to mating, throughout gestation, and for 5 days after delivery. Also, both sexes of the recovery groups were dosed for 6 weeks during the treatment period. At 500 mg/kg/day, mortality was reported in 2 females on PPDs 5 and 3. No treatment-related adverse effects were reported for clinical signs, body weight, and food consumption in either sex of parental animals at any dose level. No treatment-related histopathological findings were reported in the reproductive organs of either sex at any dose level. No treatment-related adverse effects were reported for mating period, mating index, gestation period, male and female fertility indices, gestation index, pre-and post-implantation loss rates, live birth index, mean litter size, external examination of pups, pup body weight, sex ratio of pups, and viability index of post-natal days 0 and 4 at any dose level. Therefore, the NOAEL for fertility and developmental toxicity was considered to be 500 mg/kg/day (RIFM, 2016b).

The 3,3,5-trimethylcyclohexylacetate MOE for the fertility and developmental toxicity endpoint can be calculated by dividing the 3,3,5-trimethylcyclohexylacetate NOAEL (in mg/kg/day) by the total systemic exposure to 3,3,5-trimethylcyclohexylacetate (in mg/kg/day), 500/0.016 or 31,250.

In addition, the total systemic exposure to 3,3,5-trimethylcyclohexylacetate (16 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes, 2007; Laufersweiler, 2012) for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: RIFM, 2015c.

Literature Search and Risk Assessment Completed On: 01/13/20.

11.1.4. Skin sensitization

Based on the existing data and read-across material menthyl acetate (1α,2β,5α) (CAS # 89-48-5), 3,3,5-trimethylcyclohexyl acetate has 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 3,3,5-trimethylcyclohexyl acetate. Based on the existing data and read-across material menthyl acetate (1α,2β,5α) (CAS # 89-48-5; see Section VI), 3,3,5-trimethylcyclohexyl acetate 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, 2007; Toxtree v3.1.0; OECD toolbox v4.2). In a murine local lymph node assay (LLNA), the read-across material menthyl acetate (1α,2β,5α) was not found to be sensitizing when tested up to 100% (RIFM, 2012). In a guinea pig maximization test, 3,3,5-trimethylcyclohexyl acetate did not present reactions indicative of sensitization at 100% (RIFM, 2004b).

Based on the weight of evidence (WoE) from structural analysis, animal studies, and read-across material menthyl acetate (1α,2β,5α), 3,3,5-trimethylcyclohexyl acetate 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: 12/27/19.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 3,3,5-trimethylcyclohexyl acetate 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 3,3,5-trimethylcyclohexyl acetate 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, 2009). Based on the lack of absorbance, 3,3,5-trimethylcyclohexyl acetate 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: 01/13/20.

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 3,3,5-trimethylcyclohexyl acetate 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 3,3,5-trimethylcyclohexyl acetate. Based on the Creme RIFM Model, the inhalation exposure is 0.20 mg/day. This exposure is 7 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: 01/24/20.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 3,3,5-trimethylcyclohexyl acetate 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, 3,3,5-trimethylcyclohexyl acetate 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 3,3,5-trimethylcyclohexyl acetate 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). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

11.2.2. Risk assessment

Based on the current Volume of Use (2015), 3,3,5-trimethylcyclohexyl acetate presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Biodegradation. RIFM, 2003: The ready biodegradability of the test material was evaluated according to the OECD 301C guidelines. Biodegradation of 56.3% was observed after 28 days.

11.2.2.2. Ecotoxicity. RIFM, 2016c: The acute fish (*Danio rerio*) toxicity test was conducted according to the OECD 203 guidelines under semi-static conditions. The 96-h LC50 value based on geometric mean measured concentration was reported to be 8.43 mg/L.

11.2.2.3. Other available data. 3,3,5-Trimethylcyclohexyl acetate has been pre-registered for REACH with no additional information available at this time.

11.2.3. 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>2.03</u>			1,000,000	0.00203	
ECOSAR Acute Endpoints **(Tier 2) *Ver 1.11***	1.835	3.102	<u>0.972</u>	10,000	0.0972	Esters
ECOSAR Acute Endpoints **(Tier 2) *Ver 1.11***	2.789	1.893	2.948			Neutral Organics SAR
Tier 3: Measured Data						
	LC50	EC50	NOEC	AF	PNEC	Comments
Fish	<u>8.43</u>			5000	1.686	
Daphnia						
Algae						

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	4.4	4.4
Biodegradation Factor Used	0.1	0.1
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	10–100	1–10
Risk Characterization: PEC/PNEC	<1	<1

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

The RIFM PNEC is 1.686 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 volumes of use.

Literature Search and Risk Assessment Completed On: 01/15/20.

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
- SciFinder: https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- PubMed: https://www.ncbi.nlm.nih.gov/pubmed
- National Library of Medicine's Toxicology Information Services: https://toxnet.nlm.nih.gov/
- IARC: https://monographs.iarc.fr
- OECD SIDS: https://hpvchemicals.oecd.org/ui/Default.aspx
- EPA ACToR: https://actor.epa.gov/actor/home.xhtml
- US EPA HPVIS: https://ofmpub.epa.gov/oppthpv/public_search.publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&EndPointRpt=Y#submission
- Japanese NITE: https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- Japan Existing Chemical Data Base (JECDB): http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- Google: https://www.google.com
- ChemIDplus: https://chem.nlm.nih.gov/chemidplus/

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 09/30/19.

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.2020.111852.

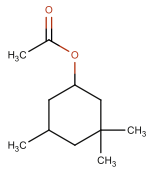
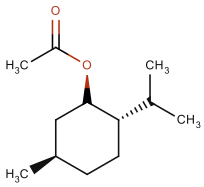
Appendix

Read-across Justification

Methods

The read-across analog was 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, 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) (Shen, 2014). The parameters were calculated using the consensus model (Shen et al., 2014).
- 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 and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).

	Target Material	Read-across Material
Principal Name	3,3,5-Trimethylcyclohexyl acetate	Menthyl acetate (1α,2β,5α)
CAS No.	67,859-96-5	89-48-5
Structure		
Similarity (Tanimoto Score)		0.90
Read-across Endpoint		• Skin Sensitization
Molecular Formula	$C_{11}H_{20}O_2$	$C_{12}H_{22}O_2$
Molecular Weight	184.27	198.30
Melting Point (°C, EPI Suite)	9.43	0.67
Boiling Point (°C, EPI Suite)	217.21	227.00
Vapor Pressure (Pa @ 25°C, EPI Suite)	20.13	13.87
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	3.93	4.00
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	23.04	17.13
J_{max} (μg/cm^2/h, SAM)	2.633	35.786
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	7.56 E+001	1.00 E+002
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 alert found	• 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

Summary

There are insufficient toxicity data on 3,3,5-trimethylcyclohexyl acetate (CAS # 67,859-96-5). 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, menthyl acetate (1α,2β,5α) (CAS # 89-48-5) was identified as a read-across analog with sufficient data for toxicological evaluation.

Conclusions

- Menthyl acetate (1α,2β,5α) (CAS # 89-48-5) was used as a read-across analog for the target material 3,3,5-trimethylcyclohexyl acetate (CAS # 67,859-96-5) for the skin sensitization endpoint.
  - o The target substance and the read-across analog are structurally similar and belong to a class of esters with a cyclohexanol moiety.
  - o The target substance and the read-across analog share an acetic acid moiety and a cyclohexanol moiety.
  - o The key difference between the target substance and the read-across analog is that the target material has a 3,3,5-trimethylcyclohexanol moiety whereas the read-across analog has a menthol moiety. This structural difference is toxicologically insignificant.
  - o The similarity between the target substance and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The physical–chemical properties of the target substance and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - o Differences are predicted for J_{max}, which estimates skin absorption. J_{max} for the target substance corresponds to skin absorption ≤40% and J_{max} for the read-across analog corresponds to skin absorption ≤80%. While percentage skin absorption estimated from J_{max} indicates exposure to the substance, it does not represent hazard or toxicity. This parameter provides context to assess the impact of bioavailability on toxicity comparisons between the materials evaluated.
  - o According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target substance and the read-across analog.
  - o The target substance and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - o The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

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