Food and Chemical Toxicology 144 (2020) 111547

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Short Review

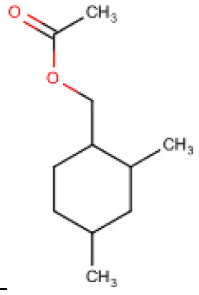
RIFM fragrance ingredient safety assessment, 2,4-dimethylcyclohexylmethyl acetate, CAS Registry Number 67634-22-4

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], 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], F. Siddiqi [a], 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 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: 050319. This version replaces any previous versions.

Name: 2,4-Dimethylcyclohexylmethyl acetate
CAS Registry Number: 67634-22-4



Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration

(*continued on next column*)

(*continued*)

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

(*continued on next page*)

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

https://doi.org/10.1016/j.fct.2020.111547
Received 17 April 2020; Received in revised form 5 June 2020; Accepted 19 June 2020
Available online 6 July 2020

(*continued*)

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
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.

2,4-Dimethylcyclohexylmethyl acetate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from the read-across analog cyclohexaneethyl acetate (CAS # 21722-83-8) show that 2,4-dimethylcyclohexylmethyl acetate is not expected to be genotoxic. The repeated dose, reproductive, and local respiratory toxicity endpoints were evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material, and the exposure to 2,4-dimethylcyclohexylmethyl acetate is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). Data and read-across to octahydro-4,7-methano-1H-indenemethyl acetate (CAS # 30772-69-1) show that there are no safety concerns for 2,4-dimethylcyclohexylmethyl acetate for skin sensitization under the current declared levels of use. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet (UV) spectra; 2,4-dimethylcyclohexylmethyl acetate is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; 2,4-dimethylcyclohexylmethyl 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 expected to be genotoxic. (RIFM, 2019; RIFM, 2020)
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 concern for skin sensitization under the current, declared levels of use. (RIFM, 1991a; RIFM, 1991b)

(*continued on next column*)

(*continued*)

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: 2.9 (BIOWIN 3) (EPI Suite v4.11; US EPA, 2012a)
Bioaccumulation: Screening-level: 193.1 L/kg (EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Screening-level: Fish LC50: 4.80 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: 4.80 mg/L (RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.00480 μg/L

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

1. Identification

1. **Chemical Name:** 2,4-Dimethylcyclohexylmethyl acetate
2. **CAS Registry Number:** 67634-22-4
3. **Synonyms:** Cyclohexanemethanol, 2,4-dimethyl-, acetate; Dihydro Agruman Acetate; (2,4-Dimethylcyclohexyl)methyl acetate; 2,4-Dimethylcyclohexylmethyl acetate
4. **Molecular Formula:** $C_{11}H_{20}O_2$
5. **Molecular Weight:** 184.27
6. **RIFM Number:** 5829
7. **Stereochemistry:** Stereoisomer not specified. Three stereocenters present and 6 total stereoisomers possible.

2. Physical data

1. **Boiling Point:** 227.17 °C (EPI Suite)
2. **Flash Point:** 89 °C (Globally Harmonized System)
3. **Log K_{OW}:** 3.97 (EPI Suite)
4. **Melting Point**: 0.39 °C (EPI Suite)
5. **Water Solubility:** 21.4 mg/L (EPI Suite)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.0591 mm Hg @ 20 °C (EPI Suite v4.0), 0.0906 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. 0.1–1 metric ton per year (IFRA, 2015).

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v1.0)

1.	**95th Percentile Concentration in Hydroalcoholics:** 0.0088%	RIFM (2016)
2.	**Inhalation Exposure*:** 0.000018 mg/kg/day or 0.0013 mg/day	RIFM (2016)
3.	**Total Systemic Exposure**:** 0.0010 mg/kg/day	RIFM (2016)

*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 5. It is derived from concentration survey data in the Creme RIFM Aggregate Exposure Model and includes exposure via dermal, oral, and inhalation routes whenever the fragrance ingredient is used in products that include these routes of exposure (Comiskey et al., 2015; Safford et al., 2015; Safford et al., 2017; and Comiskey et al., 2017).

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

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

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

2. Analogs Selected:
a. **Genotoxicity:** Cyclohexaneethyl acetate (CAS # 21722-83-8)
b. Repeated Dose Toxicity:
c. **Reproductive Toxicity:** None
d. **Skin Sensitization:** Octahydro-4,7-methano-1H-indenemethyl acetate (CAS # 30772-69-1)
e. **Phototoxicity/Photoallergenicity:** None
f. **Local Respiratory Toxicity:** None
g. **Environmental Toxicity:** None
3. Read-across Justification: See Appendix below

7. Metabolism

No relevant data available for inclusion in this safety assessment.

7.1. Additional References

None.

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

2,4-Dimethylcyclohexylmethyl 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

Pre-registered for 2010; no dossier available as of 05/03/19.

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, 2,4-dimethylcyclohexylmethyl acetate does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. 2,4-Dimethylcyclohexylmethyl acetate was assessed in the BlueScreen assay and found negative for both cytotoxicity (positive: <80% relative cell density) and genotoxicity, with and without metabolic activation (RIFM, 2013). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. Additional assays on a more reactive read-across material were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

There are no data assessing the mutagenic and clastogenic activity of 2,4-dimethylcyclohexylmethyl acetate; however, read-across can be made to cyclohexaneethyl acetate (CAS # 21722-83-8) (see Section 6).

The mutagenic activity of cyclohexaneethyl 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 method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538, and *Escherichia coli* strain WP2uvrA were treated with cyclohexaneethyl 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, 2019). Under the conditions of the study, cyclohexaneethyl acetate was not mutagenic in the Ames test, and this can be extended to 2,4-dimethylcyclohexylmethyl acetate.

The clastogenic activity of cyclohexaneethyl acetate was 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 cyclohexaneethyl acetate in DMSO at concentrations up to 1702 μg/mL in the presence and absence of metabolic activation (S9) for 3 h and in the absence of metabolic activation for 24 h. Cyclohexaneethyl acetate did not induce binucleated cells with micronuclei when tested up to the maximum concentration in either the presence or absence of an S9 activation system (RIFM, 2020). Under the conditions of the study, cyclohexaneethyl acetate was considered to be non-clastogenic in the *in vitro* micronucleus test, and this can be extended to 2,4-dimethylcyclohexylmethyl acetate.

Based on the available data, cyclohexaneethyl acetate does not present a concern for genotoxic potential, and this can be extended to 2,4-dimethylcyclohexylmethyl acetate.

Additional References: None.

Literature Search and Risk Assessment Completed On: 06/13/19.

11.1.2. Repeated dose toxicity

There are no repeated dose toxicity data on 2,4-dimethylcyclohexylmethyl acetate or any read-across materials. The total systemic exposure to 2,4-dimethylcyclohexylmethyl acetate is below the TTC for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

11.1.2.1. Risk assessment. There are no repeated dose toxicity data on 2,4-dimethylcyclohexylmethyl acetate or on any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to 2,4-dimethylcyclohexylmethyl acetate (1.0 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes, 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/14/19.

11.1.3. Reproductive toxicity

There are no reproductive toxicity data on 2,4-dimethylcyclohexylmethyl acetate or on any read-across materials. The total systemic exposure to 2,4-dimethylcyclohexylmethyl acetate is below the TTC for the reproductive toxicity endpoint of a Cramer Class I material at the

current level of use.

11.1.3.1. Risk assessment. There are no reproductive toxicity data on 2,4-dimethylcyclohexylmethyl acetate or on any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to 2,4-dimethylcyclohexylmethyl acetate (1.0 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: None.

Literature Search and Risk Assessment Completed On: 05/07/19.

11.1.4. Skin sensitization

Based on the existing data and read-across material octahydro-4,7-methano-1H-indenemethyl acetate (CAS # 30772-69-1), 2,4-dimethylcyclohexylmethyl acetate does not present a concern for skin sensitization under the current, declared levels of use.

11.1.4.1. Risk assessment. Limited skin sensitization studies are available for 2,4-dimethylcyclohexylmethyl acetate. Based on the existing data and read-across material octahydro-4,7-methano-1H-indenemethyl acetate (CAS # 30772-69-1; see Section 6), 2,4-dimethylcyclohexylmethyl acetate does not present a concern for skin sensitization under the current, declared levels of use. The chemical structures 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 guinea pigs, maximization tests with read-across material octahydro-4,7-methano-1H-indenemethyl acetate did not present reactions indicative of sensitization (RIFM, 1991a; RIFM, 1991b). In a confirmatory human repeat insult patch test (HRIPT) with 6.25% 2,4-dimethylcyclohexylmethyl acetate, no reactions indicative of sensitization were observed in any of the 41 volunteers. In another HRIPT with 2500 μg/cm^2 of read-across material octahydro-4,7-methano-1H-indenemethyl acetate in petrolatum, no reactions indicative of sensitization were observed in any of the 50 volunteers (RIFM, 1976). Additionally, in another HRIPT with 3876 μg/cm^2 of octahydro-4,7-methano-1H-indenemethyl acetate in alcohol SDA 39C, no reactions indicative of sensitization were observed in any of the 42 volunteers (RIFM, 1972).

Based on weight of evidence (WoE) from structural analysis, animal and human studies, and read-across material octahydro-4,7-methano-1H-indenemethyl acetate, 2,4-dimethylcyclohexylmethyl 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: 05/13/19.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 2,4-dimethylcyclohexylmethyl 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 2,4-dimethylcyclohexylmethyl 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, 2,4-dimethylcyclohexylmethyl 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: 05/06/19.

11.1.6. Local Respiratory Toxicity

The margin of exposure could not be calculated due to a lack of appropriate data. The exposure level for 2,4-dimethylcyclohexylmethyl 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 2,4-dimethylcyclohexylmethyl acetate. Based on the Creme RIFM Model, the inhalation exposure is 0.0013 mg/day. This exposure is 1077 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: 05/09/19.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 2,4-dimethylcyclohexylmethyl acetate 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, 2,4-dimethylcyclohexylmethyl acetate 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 2,4-dimethylcyclohexylmethyl acetate 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, 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value $<$ 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value $<$ 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF $\geq$2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or

die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11).

11.2.2. Risk assessment

Based on the current Volume of Use (2015), 2,4-dimethylcyclohexylmethyl acetate presents no risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Key studies

11.2.2.1.1. Biodegradation. No data available.

11.2.2.1.2. Ecotoxicity. No data available.

11.2.2.1.3. Other available data. 2,4-dimethylcyclohexylmethyl acetate has been pre-registered for REACH with no additional data 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.

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

Exposure	Europe (EU)	North America (NA)
Log K_{OW} Used	3.97	3.97
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 further assessment is necessary.

The RIFM PNEC is 0.00480 μg/L. The revised PEC/PNECs for EU and NA are not applicable. The material was cleared at the screening-level; therefore, it does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 05/28/19.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2020.111547.

Appendix

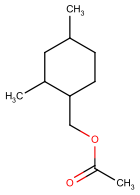
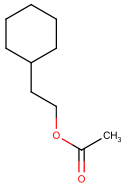
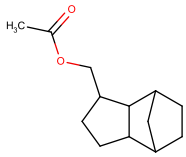
Read-across Justification

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).

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*)	EC50 (Algae)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	4.80			1,000,000	0.00480	

- 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, 2020).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v4.2 (OECD, 2020).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010).
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2020), and skin sensitization was predicted using Toxtree.
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2020).

	Target Material	Read-across Material	Read-across Material
Principal Name	2,4-Dimethylcyclohexylmethyl acetate	Cyclohexaneethyl acetate	Octahydro-4,7-methano-1H-indenemethyl acetate
CAS No.	67634-22-4	21722-83-8	30772-69-1
Structure			
Similarity (Tanimoto Score)		0.91	0.87
Read-across Endpoint		• Genotoxicity	• Skin Sensitization
Molecular Formula	$C_{11}H_{20}O_2$	$C_{10}H_{18}O_2$	$C_{13}H_{20}O_2$
Molecular Weight	184.27	170.25	208.30
Melting Point (°C, EPI Suite)	0.39	−3.31	44.24
Boiling Point (°C, EPI Suite)	227.17	219.25	265.26
Vapor Pressure (Pa @ 25 °C, EPI Suite)	1.21E+01	1.81E+01	1.08E+00
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	3.97	3.62	3.55
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPI Suite)	21.4	49.20	36.64
J_{max} (μg/cm^2/h, SAM)	35.789	38.971	12.285
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	7.56E+01	5.69E+01	2.59E+01
Genotoxicity			
DNA Binding (OASIS v1.4, QSAR Toolbox v4.2)	• AN2|AN2 ≫ Schiff base formation after aldehyde release|AN2 ≫ Schiff base formation after aldehyde release ≫ Specific Acetate Esters|SN1|SN1 ≫ Nucleophilic attack after carbenium ion formation|SN1 ≫ Nucleophilic attack after carbenium ion formation ≫ Specific Acetate Esters|SN2|SN2 ≫ Acylation|SN2 ≫ Acylation ≫ Specific Acetate Esters|SN2 ≫ Nucleophilic substitution at sp3 Carbon atom|SN2 ≫ Nucleophilic substitution at sp3 Carbon atom ≫ Specific Acetate Esters	• AN2|AN2 ≫ Schiff base formation after aldehyde release|AN2 ≫ Schiff base formation after aldehyde release ≫ Specific Acetate Esters|SN1|SN1 ≫ Nucleophilic attack after carbenium ion formation|SN1 ≫ Nucleophilic attack after carbenium ion formation ≫ Specific Acetate Esters|SN2|SN2 ≫ Acylation|SN2 ≫ Acylation ≫ Specific Acetate Esters|SN2 ≫ Nucleophilic substitution at sp3 Carbon atom|SN2 ≫ Nucleophilic substitution at sp3 Carbon atom ≫ Specific Acetate Esters	
DNA Binding (OECD QSAR Toolbox v4.2)	• No alert found	• No alert found	
Carcinogenicity (ISS)	• No alert found	• No alert found	
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	
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)
	• No alert found		• No alert found

(continued on next page)

(*continued*)

	Target Material	Read-across Material	Read-across Material
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)			
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	See Supplemental Data 3

Summary

There are insufficient toxicity data on 2,4-dimethylcyclohexylmethyl acetate (CAS # 67634-22-4). 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, cyclohexaneethyl acetate (CAS # 21722-83-8) and octahydro-4,7-methano-1H-indenemethyl acetate (CAS # 30772-69-1) were identified as read-across analogs with sufficient data for toxicological evaluation.

Conclusions

- Cyclohexaneethyl acetate (CAS # 21722-83-8) was used as a read-across analog for the target material 2,4-dimethylcyclohexylmethyl acetate (CAS # 67634-22-4) for the genotoxicity endpoint.
  - o The target substance and the read-across analog are structurally similar and belong to a class of aliphatic cyclic esters.
  - o The target material and the read-across analog are both acetates and share a primary aliphatic cyclic alcohol moiety in the ester functionality.
  - o The key difference between the target material and the read-across analog is that the read-across analog is the acetate ester of a cyclohexaneethanol alcohol, whereas the target material is the acetate ester of 2,4-dimethylcyclohexylmethanol alcohol. This structural difference is toxicologically insignificant for the skin sensitization endpoint.
  - o 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 comparison of their toxicological properties.
  - 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 Both target material and read-across analog have several DNA binding alerts by OASIS because they are acetate esters. However, a more detailed inspection of this alert shows that neither the target material nor the read-across analog have any active structural fragments belonging to the training set compounds that are mutagenic. Therefore, the predictions are superseded by data.
  - 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.
- Octahydro-4,7-methano-1H-indenemethyl acetate (CAS # 30772-69-1) was used as a read-across analog for the target material 2,4-dimethylcyclohexylmethyl acetate (CAS # 67634-22-4) for the skin sensitization endpoint.
  - o The target material and the read-across analog are structurally similar and belong to the class of aliphatic cyclic esters.
  - o The target material and the read-across analog are both acetates and share a primary aliphatic cyclic alcohol moiety in the ester functionality.
  - o The key difference between the target material and the read-across analog is that the read-across analog is the acetate ester of a fused-bridged cyclic alcohol, whereas the target material is the acetate ester of a monocyclic primary alcohol. This structural difference is toxicologically insignificant for the skin sensitization endpoint.
  - o 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.
  - o The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - o According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - o There are no toxicological alerts for the target material as well as for the read-across analog. Data are consistent with *in silico* alerts.
  - o The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - o The structural alerts for the 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 (Unpublished report).
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, 2020. The OECD QSAR Toolbox, v3.2–4.4. Retrieved from. http://www.qsartoolbox.org/.
RIFM (Research Institute for Fragrance Materials, Inc), 1972. Repeated Insult Patch Test of Octahydro-4,7-Methano-1h-Indenemethyl Acetate. Unpublished report from International Flavor and Fragrances. RIFM report number 53357. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 1976. Human Repeated Insult Patch Test with Octahydro-4,7-Methano-1h-Indenemethyl Acetate. Unpublished report from Firmenich SA. RIFM report number 37333. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 1991a. Guinea Pig Maximization Test of Skin Sensitization with Octahydro-4,7-Methano-1h-Indenemethyl Acetate. Unpublished report from Symrise. RIFM report number 57759. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 1991b. Guinea Pig Maximization Test of Skin Sensitization with Octahydro-4,7-Methano-1h-Indenemethyl Acetate (TCD-M Acetate). Unpublished report from Symrise. RIFM report number 59910. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2013. Report on the Testing of 2,4-dimethylcyclohexylmethyl Acetate in the BlueScreen HC Assay (-/+ S9 Metabolic Activation). RIFM report number 66116. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2016. Exposure Survey 10, March 2016 (Unpublished report).
RIFM (Research Institute for Fragrance Materials, Inc), 2019. Cyclohexaneethyl Acetate: Genetic Toxicity Evaluation Using a Bacterial Reverse Mutation Test in Salmonella typhimurium LT2 Strains TA1535, TA1537, TA98, and TA100 and Escherichia coli WP2 uvrA/pKM101. Unpublished report from Mee, C. RIFM report number 75960. RIFM, Woodcliff Lake, NJ, USA.
RIFM (Research Institute for Fragrance Materials, Inc), 2020. Cyclohexaneethyl Acetate: Genetic Toxicity Evaluation Using a Micronucleus Test in Human Lymphocyte Cells. RIFM report number 76244. RIFM, Woodcliff Lake, NJ, USA.
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–v.2.0. United States Environmental Protection Agency, Washington, DC, USA.