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Short Review

RIFM fragrance ingredient safety assessment, 2,4,6-trimethyl-3-cyclohexene-1-methanol, CAS Registry Number 68527-77-5

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Name: 2,4,6-Trimethyl-3-cyclohexene-1-methanol
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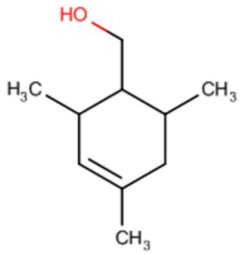

Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015; Safford et al., 2015a; Safford et al., 2017; Comiskey et al., 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
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
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines

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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 et al., 2015a), 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.

2,4,6-Trimethyl-3-cyclohexene-1-methanol was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from 2,4,6-trimethyl-3-cyclohexene-1-methanol and read-across analog 3,5-dimethylcyclohexene-1-methanol (CAS # 67634-16-6) show that 2,4,6-trimethyl-3-cyclohexene-1-methanol is not expected to be genotoxic. Data provided 2,4,6-trimethyl-3-cyclohexene-1-methanol a NESIL of 3800 μg/cm^2 for the skin sensitization endpoint. The repeated dose, reproductive, and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class I material, and the exposure to 2,4,6-trimethyl-3-cyclohexene-1-methanol is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; 2,4,6-trimethyl-3-cyclohexene-1-methanol is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; 2,4,6-trimethyl-3-cyclohexene-1-methanol 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, 2014a; RIFM, 2017a)

Repeated Dose Toxicity: No NOAEL available. Exposure is below the TTC.

Reproductive Toxicity: No NOAEL available. Exposure is below the TTC.

Skin Sensitization: NESIL = 3800 μg/cm^2 (RIFM, 2005c)

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: 10% (OECD 301D) (ECHA REACH Dossier: 2,4,6-Trimethylcyclohex-3-ene-1-methanol; ECHA, 2017)

Bioaccumulation: Screening-level: 69-.4 L/kg (EPI Suite v4.11; US EPA, 2012a)

Ecotoxicity: Screening-level: *Daphnia magna* 48-h LC50: 5.561 mg/L (ECOSAR; US EPA, 2012b)

Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:

Screening-level: PEC/PNEC (North America and Europe) > 1 (Salvito et al., 2002)

Critical Ecotoxicity Endpoint: 48-h *Daphnia magna* LC50: 5.561 mg/L (ECOSAR; US EPA, 2012b)

RIFM PNEC is: 0.5561 μg/L.

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

1. Identification

1. **Chemical name:** 2,4,6-trimethyl-3-cyclohexene-1-methanol.
2. **CAS Registry Number:** 68527-77-5.
3. **Synonyms:** 3-Cyclohexene-1-methanol, 2,4,6-trimethyl-; Isocyclogeraniol; (2,4,6-Trimethylcyclohex-3-en-1-yl)methanol; 2,4,6-Trimethyl-3-cyclohexene-1-methanol.
4. **Molecular Formula:** $C_{10}H_{18}O$.
5. **Molecular Weight:** 154.25.
6. **RIFM Number:** 1330.

2. Physical data

1. **Boiling Point:** 237 °C (EPI Suite).
2. **Flash Point:** > 93 °C (GHS).
3. **Log K_{OW}:** 3.3 (EPI Suite).
4. **Melting Point**: 13.51 °C (EPI Suite).
5. **Water Solubility:** 360.2 mg/L (EPI Suite).
6. **Specific Gravity:** 0.92600 to 0.93400 @ 20 °C*.
7. **Vapor Pressure:** 0.00747 mm Hg @ 25 °C (EPI Suite), 0.00439 mm Hg @ 20 °C (EPI Suite v4.0).
8. **UV Spectra:** No significant absorbance between 290 and 700 nm; the molar absorption coefficient is below the benchmark (1000 L mol^{-1} · cm^{-1}).
9. **Appearance/Organoleptic:** The material is estimated to be a pale to colorless clear liquid. It has an odor described as spicy, floral, carnation, herbal, green, and woody with a camphoraceous and bitter flavor.*.

*http://www.thegoodscentscompany.com/data/rw1006901.html, retrieved 05/05/2017.

3. Exposure

1. **Volume of Use (worldwide band):** 10–100 metric tons per year (IFRA, 2015)
2. **95**th **Percentile Concentration in Hydroalcoholics*****:** 0.12% (RIFM, 2017c)
3. **Inhalation Exposure*:** 0.0011 mg/kg/day or 0.074 mg/day (RIFM, 2017c)
4. **Total Systemic Exposure**:** 0.0029 mg/kg/day (RIFM, 2017c)

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

4. Derivation of systemic absorption

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

3. **Inhalation:** Assumed 100%

5. Computational toxicology evaluation

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

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

2. Analogs Selected:
   a. **Genotoxicity:** 3,5-dimethylcyclohexene-1-methanol (CAS # 67634-16-6)
   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

Not considered for this risk assessment.

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

2,4,6-Trimethyl-3-cyclohexene-1-methanol is not reported to occur in food 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. Reach dossier

Available; accessed 02/18/19 (ECHA, 2017).

9. Conclusion

The maximum acceptable concentrations[a] in the finished products for 2,4,6-trimethyl-3-cyclohexene-1-methanol are detailed below.

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)
1	Products applied to the lips (lipstick)	0.29
2	Products applied to the axillae	0.087
3	Products applied to the face/body using fingertips	1.8
4	Products related to fine fragrances	1.6
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.41
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.41
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.41
5D	Baby cream, oil, talc	0.41
6	Products with oral and lip exposure	0.96
7	Products applied to the hair with some hand contact	3.3
8	Products with significant ano-genital exposure (tampon)	0.17
9	Products with body and hand exposure, primarily rinse-off (bar soap)	3.2
10A	Household care products with mostly hand contact (hand dishwashing detergent)	11
10B	Aerosol air freshener	11
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	6.3
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted

Note: [a]Maximum acceptable concentrations for each product category are based on the lowest maximum acceptable concentrations (based on systemic toxicity, skin sensitization, or any other endpoint evaluated in this safety assessment). For 2,4,6-trimethyl-3-cyclohexene-1-methanol the basis was the skin sensitization NESIL of 3800 μg/cm^2.
[b]For a description of the categories refer to the IFRA RIFM Information Booklet. (http://www.rifm.org/doc).

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the existing data, 2,4,6-trimethyl-3-cyclohexene-1-methanol does not present a concern for genotoxicity.

10.1.1.1. Risk assessment. 2,4,6-Trimethyl-3-cyclohexene-1-methanol was assessed in the BlueScreen assay and found negative for genotoxicity, with and without metabolic activation (RIFM, 2013a). The mutagenic activity of 2,4,6-trimethyl-3-cyclohexene-1-methanol has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and according to guidelines similar to OECD TG 471 using the preincubation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with 2,4,6-trimethyl-3-cyclohexene-1-methanol 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 dose in the presence or absence of S9 (RIFM, 2014a). Under the conditions of the study, 2,4,6-trimethyl-3-cyclohexene-1-methanol was not mutagenic in the Ames test.

There are no studies assessing the clastogenic activity of 2,4,6-trimethyl-3-cyclohexene-1-methanol. However, read-across can be made to 3,5-dimethylcyclohexene-1-methanol (CAS # 67634-16-6; see Section V). The clastogenic activity of 3,5-dimethylcyclohexene-1-methanol 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 3,5-dimethylcyclohexene-1-methanol in DMSO at concentrations up to 1400 μg/mL in the presence and absence of metabolic activation (S9) for 4 and 24 h 3,5-Dimethylcyclohexene-1-methanol did not induce binucleated cells with micronuclei when tested up to the maximum dose either with or without S9 (RIFM, 2017a). Under the conditions of the study, 3,5-dimethylcyclohexene-1-methanol was considered to be non-clastogenic in the *in vitro* micronucleus test, and this can be extended to 2,4,6-trimethyl-3-cyclohexene-1-methanol.

Based on the data available, 2,4,6-trimethyl-3-cyclohexene-1-methanol does not present a concern for genotoxicity.

Additional References: RIFM, 2013b; RIFM, 2017b.

Literature Search and Risk Assessment Completed On: 04/27/17.

10.1.2. Repeated dose toxicity

There are insufficient repeated dose toxicity data on 2,4,6-trimethyl-3-cyclohexene-1-methanol or any read-across materials evaluated. The total systemic exposure to 2,4,6-trimethyl-3-cyclohexene-1-

methanol 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 2,4,6-trimethyl-3-cyclohexene-1-methanol or any read-across materials evaluated that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to 2,4,6-trimethyl-3-cyclohexene-1-methanol (2.9 μg/kg/day) is below the TTC (30 μg/kg/day or 0.03 mg/kg/day, the RfD for a Cramer Class I material; Kroes et al., 2007; Laufersweiler et al., 2012) for the repeated dose toxicity endpoint at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/28/17.

10.1.3. Reproductive toxicity

There are insufficient reproductive toxicity data on 2,4,6-trimethyl-3-cyclohexene-1-methanol or on any read-across materials. The total systemic exposure to 2,4,6-trimethyl-3-cyclohexene-1-methanol 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 2,4,6-trimethyl-3-cyclohexene-1-methanol or on any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to 2,4,6-trimethyl-3-cyclohexene-1-methanol (2.9 μg/kg/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: 04/28/17.

10.1.4. Skin sensitization

Based on the existing data, 2,4,6-trimethyl-3-cyclohexene-1-methanol is considered a weak skin sensitizer with a defined NESIL of 3800 μg/cm^2.

10.1.4.1. Risk assessment. Based on the existing data, 2,4,6-trimethyl-3-cyclohexene-1-methanol is considered a weak skin sensitizer. The chemical structure of this material indicates that it would not be expected to react with skin proteins (Roberts et al., 2007; Toxtree 2.6.13; OECD Toolbox v3.4). 2,4,6-Trimethyl-3-cyclohexene-1-methanol was found to be negative in an *in vitro* direct peptide reactivity assay (DPRA), KeratinoSens, and U937-CD86 test but positive in the human cell line activation test (h-CLAT) (RIFM, 2014b; RIFM, 2015; unpublished RIFM results). In a murine local lymph node assay (LLNA), 2,4,6-trimethyl-3-cyclohexene-1-methanol was found to be non-sensitizing up to 25% (6250 μg/cm^2) (RIFM, 2005b). In a Buehler study, 60% 2,4,6-trimethyl-3-cyclohexene-1-methanol presented 7/10 reactions indicative of sensitization (RIFM, 1981). In a confirmatory human repeated insult patch test (HRIPT) with 3897 μg/cm^2 of 2,4,6-trimethyl-3-cyclohexene-1-methanol in 1:3 ethanol:diethyl phthalate (EtOH:DEP) no reactions indicative of sensitization were observed in any of the 103 volunteers (RIFM, 2005c). In additional HRIPTs, each with less than 100 subjects, 2,4,6-trimethyl-3-cyclohexene-1-methanol did induce sensitization reactions at 10% (5000 μg/cm^2) (RIFM, 1982) or 5% (2500 μg/cm^2) (RIFM, 1983). Based on the weight of evidence from structural analysis and animal and human studies, 2,4,6-trimethyl-3-cyclohexene-1-methanol is a weak sensitizer with a Weight of Evidence No Expected Sensitization Induction Level (WoE NESIL) of 3800 μg/cm^2 (Table 1). Section IX provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2008; IDEA [International Dialogue for the Evaluation of Allergens] project Final Report on the QRA2: Skin Sensitization Quantitative Risk Assessment for Fragrance Ingredients, September 30, 2016, http://www.ideaproject.info/uploads/Modules/Documents/qra2-dossier-final–september-2016.pdf

Table 1
Data summary for 2,4,6-trimethyl-3-cyclohexene-1-methanol.

LLNA Weighted Mean EC3 Value μg/cm^2 (No. Studies)	Skin Sensitization Potency Classification Based on Animal Data[a]	Human Data			
		NOEL-HRIPT (Induction) μg/cm^2	NOEL-HMT (Induction) μg/cm^2	LOEL[b] (Induction) μg/cm^2	WoE NESIL[c] μg/cm^2
> 6250 [1]	Weak sensitizer	3897	NA	5000	3800

NOEL = No observed effect level; LOEL = lowest observable effect level; HRIPT = Human Repeat Insult Patch Test; HMT = Human Maximization Test; NA = Not Available.

[a] Based on animal data using classification defined in ECETOC, Technical Report No. 87, 2003.

[b] Data derived from HRIPT or HMT.

[c] WoE NESIL limited to 3 significant figures.

Additional References: RIFM, 2005a; ICCVAM, 2011; RIFM, 2009; Api et al., 2015b; Safford et al., 2015b.

Literature Search and Risk Assessment Completed On: 02/25/19.

10.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 2,4,6-trimethyl-3-cyclohexene-1-methanol 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 2,4,6-trimethyl-3-cyclohexene-1-methanol 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, 2,4,6-trimethyl-3-cyclohexene-1-methanol 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: 02/22/19.

10.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 2,4,6-trimethyl-3-cyclohexene-1-methanol 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 2,4,6-trimethyl-3-cyclohexene-1-methanol. Based on the Creme RIFM Model, the inhalation exposure is 0.074 mg/day. This exposure is 18.92 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: 02/26/19.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of 2,4,6-trimethyl-3-cyclohexene-1-methanol 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, 2,4,6-trimethyl-3-cyclohexene-1-methanol was identified as a fragrance material with the potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC > 1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) did not identify 2,4,6-trimethyl-3-cyclohexene-1-methanol as possibly being 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., 2015a). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11).

10.2.1.1. Risk assessment. Based on current VoU (2015), 2,4,6-trimethyl-3-cyclohexene-1-methanol presents a risk to the aquatic compartment in the screening-level assessment.

10.2.2. Key studies

10.2.2.1. Biodegradation. No data available.

10.2.2.2. Ecotoxicity. No data available.

10.2.2.3. Other available data. 2,4,6-Trimethyl-3-cyclohexene-1-methanol has been registered under REACH with the following data available:

The ready biodegradability of the test material was evaluated in a closed bottle test according to the OECD 301D method. Biodegradation of 10% was observed after 28 days (ECHA, 2017).

10.3. 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>15.38</u>			1000000	0.01538	
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	8.689	<u>5.561</u>	6.794	10000	0.5561	Neutral Organics

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	3.3	3.3
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	1–10	< 1
Risk Characterization: PEC/PNEC	**< 1**	**< 1**

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

The RIFM PNEC is 0.5561 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: 04/01/19.

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 05/03/19.

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.

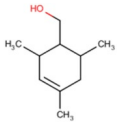
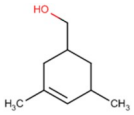
Appendix

Read-across Justification

Methods

The read-across analog was identified following the strategy for structuring and reporting a read-across prediction of toxicity 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 (ECHA) read-across assessment framework or RAAF (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 (US EPA, 2012a).
- J_{max} values were calculated using RIFM's skin absorption model (SAM). The parameters were calculated using the consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts, and oncologic classification were generated using OECD QSAR Toolbox (v3.4) (OECD, 2018).
- ER binding and repeat dose categorization were estimated using OECD QSAR Toolbox (v3.4) (OECD, 2018).
- Developmental toxicity and skin sensitization were estimated using CAESAR v.2.1.7 and 2.1.6, respectively (Cassano et al., 2010).
- Protein binding was estimated using OECD QSAR Toolbox (v3.4) (OECD, 2018).
- The major metabolites for the target material and the read-across analog were determined and evaluated using OECD QSAR Toolbox (v3.4) (OECD, 2018).

	Target Material	Read-across Material
Principal Name	2,4,6-Trimethyl-3-cyclohexene-1-methanol	3,5-Dimethylcyclohexene-1-methanol
CAS No.	68527-77-5	67634-16-6
Structure		
Similarity (Tanimoto Score)		0.84
Read-across Endpoint		• Genotoxicity
Molecular Formula	$C_{10}H_{18}O$	$C_9H_{16}O$
Molecular Weight	154.25	140.23
Melting Point (°C, EPI SUITE)	13.51	6.17
Boiling Point (°C, EPI SUITE)	237	223.88
Vapor Pressure (Pa @ 25°C, EPI Suite)	0.996	2.27
Log K_{ow} (KOWWIN v1.68 in EPI Suite)	3.3	2.88
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	360.2	941.7
J_{max} (μg/cm^2/h, SAM)	234.963	393.224
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.60E+000	1.21E+000
Genotoxicity		
DNA Binding (OASIS v1.4 QSAR Toolbox v3.4)	• No alert found	• No alert found
DNA Binding by OECD QSAR Toolbox (3.4)	• No alert found	• No alert found
Carcinogenicity (Genotoxicity and Non-genotoxicity) Alerts (ISS)	• Non-carcinogen (low reliability)	• Non-carcinogen (low reliability)
DNA Alerts: Ames, MN, CA by OASIS v 1.1	• No alert found	• No alert found
***In Vitro* Mutagenicity (Ames test) Alerts by ISS**	• No alert found	• No alert found
***In Vivo* Mutagenicity (Micronucleus) ISS Alerts**	• No alert found	• No alert found
Oncologic Classification	• Not classified	• Not classified

Metabolism		
OECD QSAR Toolbox (3.4)	See Supplemental Data 1	See Supplemental Data 2
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites		

Summary

There are insufficient toxicity data on the 2,4,6-trimethyl-3-cyclohexene-1-methanol (CAS # 68527-77-5). Hence, *in silico* evaluation was conducted to determine a read-across analog for this material. Based on structural similarity, reactivity, metabolism data, physical–chemical properties and expert judgment, analog 3,5-dimethylcyclohexene-1-methanol (CAS # 67634-16-6) was identified as a read-across material with data for the genotoxicity endpoint.

Conclusions

- 3,5-dimethylcyclohexene-1-methanol (CAS # 67634-16-6) was used as a read-across analog for the target material 2,4,6-trimethyl-3-cyclohexene-1-methanol (CAS # 68527-77-5) for the genotoxicity endpoint.
  - o The target material and the read-across analog are structurally similar and belong to the structural class of alkyl cyclic alcohols.
  - o The target material and the read-across analog share a common cyclohexene-1-methanol fragment.
  - o The key difference between the target material and the read-across analog is that the target material has 3 methyl substitutions on the cyclohexene ring while the read-across analog has 2 methyl substitutions. This structural difference between the target material and the read-across analog are not toxicologically significant.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score in the above table. The Tanimoto score is mainly driven by the cyclohexene-1-methanol fragment. Differences between the structures that affect the Tanimoto score are not toxicologically significant.
  - 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 QSAR OECD Toolbox (v3.4), structural alerts for the genotoxicity endpoint are consistent between the target material and the read-across analog.
  - 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 genotoxicity endpoint are consistent between the metabolites of the read-across analog and the target material.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.110951.

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