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RIFM fragrance ingredient safety assessment, β,4-dimethylcyclohex-3-ene-1-propan-1-al, CAS Registry Number 6784-13-0

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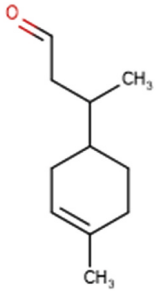

Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
CNIH – Confirmation of No Induction in Humans test. A human repeat insult patch test that is performed to confirm an already determined safe use level for fragrance ingredients (Na et al., 2021)
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., 2015a, 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 Observed 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
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

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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.

β,4-Dimethylcyclohex-3-ene-1-propan-1-al was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that β,4-dimethylcyclohex-3-ene-1-propan-1-al is not genotoxic and provided a No Expected Sensitization Induction Level (NESIL) of 5500 μg/cm^2 for the skin sensitization endpoint. Data from read-across analog 6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde (CAS # 33885-51-7) provide a calculated Margin of Exposure (MOE) for the repeated dose and reproductive toxicity endpoints. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; β,4-dimethylcyclohex-3-ene-1-propan-1-al 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 β,4-dimethylcyclohex-3-ene-1-propan-1-al is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; β,4-dimethylcyclohex-3-ene-1-propan-1-al 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.	(Symrise, 2009; RIFM, 2014)
Repeated Dose Toxicity: NOAEL = 40 mg/kg/day.	RIFM (2020c)
Reproductive Toxicity: Developmental NOAEL = 60 mg/kg/day. Fertility NOAEL = 60 mg/kg/day.	RIFM (2020c)
Skin Sensitization: NESIL = 5500 μg/cm^2.	RIFM (2006)
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic.	(UV/Vis Spectra; RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below TTC.	

Environmental Safety Assessment

Hazard Assessment:	
Persistence:	
Critical Measured Value: 80% (OECD 301 F)	RIFM (2011)
Bioaccumulation:	
Screening-level: 157.7 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: 48-h *Daphnia magna* LC50: 0.671 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	(RIFM Framework; Salvito, 2002)
Critical Ecotoxicity Endpoint: 48-h *Daphnia magna* LC50: 0.671 mg/L	(ECOSAR; US EPA, 2012b)
RIFM PNEC is: 0.0671 μg/L	
• **Revised PEC/PNECs (2015 IFRA VoU)**: North America and Europe <1	

1. Identification

1. **Chemical Name:** β,4-Dimethylcyclohex-3-ene-1-propan-1-al
2. **CAS Registry Number:** 6784-13-0
3. **Synonyms:** 3-Cyclohexene-1-propanal,.β.,4-dimethyl-; p-Menth-1-ene-9-carboxaldehyde; 3-(4-Methyl-3-cyclohexen-1-yl)butanal; 3-(4-Methylcyclohex-3-en-1-yl)butanal; Limonenal; Liminal; Reaction mass of (3 R)-3-[(1 R)-4-methyl-3-cyclohexen-1-yl]butanal and (3 S)-3-[(1 R)-4-methyl-3-cyclohexen-1-yl]butanal;.β.,4-Dimethylcyclohex-3-ene-1-propan-1-al
4. **Molecular Formula:** $C_{11}H_{18}O$
5. **Molecular Weight:** 166.26

6. **RIFM Number:** 5350
7. **Stereochemistry:** Stereoisomer not specified. Two chiral centers present, and a total of 4 enantiomers possible.

2. Physical data

1. **Boiling Point:** 196 °C (469 K) at 101.5 kPa (RIFM, 2013a), 232.75 °C (EPI Suite)
2. **Flash Point:** >93 °C (Globally Harmonized System)
3. **Log K_{OW}:** 4.1 (RIFM, 2013d), 3.84 (EPI Suite)
4. **Melting Point**: 5.24 °C (EPI Suite)
5. **Water Solubility:** 20 mg/L at 20 °C (RIFM, 2013c), 33.93 mg/L (EPI Suite)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.0439 mm Hg at 20 °C (EPI Suite v4.0), 0.28 Pa at 20 °C, 0.48 Pa at 25 °C, 5.87 Pa at 50 °C (RIFM, 2013b), 0.0677 mm Hg at 25 °C (EPI Suite)
8. **UV Spectra:** No absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** Not Available

3. Volume of use (Worldwide band)

1. 1–10 metric tons per year (IFRA, 2015)

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

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.016% (RIFM, 2017b)
2. **Inhalation Exposure*:** 0.000099 mg/kg/day or 0.0072 mg/day (RIFM, 2017b)
3. **Total Systemic Exposure**:** 0.00081 mg/kg/day (RIFM, 2017b)

*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

6.1. *Cramer Classification*

Class I, Low.

Expert Judgment	Toxtree v3.1	OECD QSAR Toolbox v4.2
I	I	I

6.2. *Analogs selected*

a. Genotoxicity: None
b. Repeated Dose Toxicity: 6,6-Dimethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde (CAS # 33885-51-7)
c. Reproductive Toxicity: 6,6-Dimethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde (CAS # 33885-51-7)
d. Skin Sensitization: None
e. Phototoxicity/Photoallergenicity: None
f. Local Respiratory Toxicity: None
g. Environmental Toxicity: None

6.3. *Read-across justification*

See Appendix below.

7. Metabolism

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

8. Natural occurrence

β,4-Dimethylcyclohex-3-ene-1-propan-1-al 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

Available; accessed on 12/10/21.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for β,4-dimethylcyclohex-3-ene-1-propan-1-al are detailed below.

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
1	Products applied to the lips (lipstick)	0.028
2	Products applied to the axillae	0.13
3	Products applied to the face/body using fingertips	0.028
4	Products related to fine fragrances	2.4
5 A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.60
5 B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.31
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.20
5D	Baby cream, oil, talc	0.066
6	Products with oral and lip exposure	0.028
7	Products applied to the hair with some hand contact	0.23
8	Products with significant ano-genital exposure (tampon)	0.066
9	Products with body and hand exposure, primarily rinse-off (bar soap)	2.0
10 A	Household care products with mostly hand contact (hand dishwashing detergent)	4.4
10 B	Aerosol air freshener	1.6
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.066

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IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
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 β,4-dimethylcyclohex-3-ene-1-propan-1-al, the basis was the subchronic reference dose of 0.40 mg/kg/day, a predicted skin absorption value of 40%, and a skin sensitization NESIL of 5500 μg/cm^2.

[b]For a description of the categories, refer to the IFRA RIFM Information Booklet (https://www.rifm.org/downloads/RIFM-IFRA%20Guidance-for-the-use-of-IFRA-Standards.pdf; December 2019).

[c]Calculations by Creme RIFM Aggregate Exposure Model v3.1.4.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, β,4-dimethylcyclohex-3-ene-1-propan-1-al does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. β,4-Dimethylcyclohex-3-ene-1-propan-1-al was assessed in the BlueScreen assay and found positive for cytotoxicity (positive: <80% relative cell density) and negative for genotoxicity, with and without metabolic activation (RIFM, 2013e). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. Additional assays were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

The mutagenic activity of β,4-dimethylcyclohex-3-ene-1-propan-1-al 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, and TA102 were treated with β,4-dimethylcyclohex-3-ene-1-propan-1-al 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 (Symrise, 2009). Under the conditions of the study, β,4-dimethylcyclohex-3-ene-1-propan-1-al was not mutagenic in the Ames test.

The clastogenic activity of β,4-dimethylcyclohex-3-ene-1-propan-1-al 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 β,4-dimethylcyclohex-3-ene-1-propan-1-al in DMSO at concentrations up to 1666.4 μg/mL in the dose range finding (DRF) study; micronuclei analysis was conducted at concentrations up to 200 μg/mL in the presence and absence of metabolic activation. β,4-dimethylcyclohex-3-ene-1-propan-1-al did not induce binucleated cells with micronuclei when tested in either the presence or absence of an S9 activation system (RIFM, 2014). Under the conditions of the study, β,4-dimethylcyclohex-3-ene-1-propan-1-al was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the data available, β,4-dimethylcyclohex-3-ene-1-propan-1-al does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 06/04/21.

11.1.2. Repeated dose toxicity

The MOE for β,4-dimethylcyclohex-3-ene-1-propan-1-al is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are no repeated dose toxicity data β,4-dimethylcyclohex-3-ene-1-propan-1-al. Read-across material, 6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde (CAS # 33885-51-7; see Section VI) has sufficient repeated dose toxicity data. In a GLP and OECD 422-compliant study, 10 Wistar Han rats/sex/dose were administered 6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde via gavage at doses of 0, 30, 60, and 120 mg/kg/day for a minimum of 28 days. Doses were selected based on a DRF study in which mortality was observed at 450 mg/kg/day, but no severe effects were observed at 90 mg/kg/day. No treatment-related mortality was seen up to the highest dose. No treatment-related effects were observed in clinical appearance, functional observations, body weight, food consumption, hematology, clotting parameters, or hormone levels. Total serum protein concentrations were reduced in males at the mid and high doses, but this effect was not considered adverse due to a lack of correlated gross or histopathological findings. Liver weight and periportal hepatocellular hypertrophy incidence were significantly increased in females at the mid dose and in both sexes at the high dose; however, these effects were slight and were not accompanied by any histopathological findings. Thus, liver effects were not considered adverse. Based on no treatment-related adverse effects seen up to the highest dose, the NOAEL for this study was considered to be 120 mg/kg/day (RIFM, 2020c).

A default safety factor of 3 was used when deriving a NOAEL from an 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 120/3 or 40 mg/kg/day.

Therefore, the β,4-dimethylcyclohex-3-ene-1-propan-1-al MOE for the repeated dose toxicity endpoint can be calculated by dividing the 6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde NOAEL in mg/kg/day by the total systemic exposure to β,4-dimethylcyclohex-3-ene-1-propan-1-al, 40/0.00081, or 49383.

In addition, the total systemic exposure to β,4-dimethylcyclohex-3-ene-1-propan-1-al (0.81 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.

Section X 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, 2020b) and a subchronic reference dose (RfD) of 0.40 mg/kg/day.

11.1.2.2. Derivation of subchronic RfD. The RIFM Criteria Document (Api, 2015) calls for a default MOE of 100 (10 × 10), based on uncertainty factors applied for interspecies (10 ×) and intraspecies (10 ×) differences. The subchronic RfD for β,4-dimethylcyclohex-3--ene-1-propan-1-al was calculated by dividing the lowest NOAEL (from the Repeated Dose and Reproductive Toxicity sections) of 40 mg/kg/day by the uncertainty factor, 100 = 0.40 mg/kg/day.

Additional References: None.

Literature Search and Risk Assessment Completed On: 06/02/21.

11.1.3. Reproductive toxicity

The MOE for β,4-dimethylcyclohex-3-ene-1-propan-1-al is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are no reproductive toxicity data on β,4-dimethylcyclohex-3-ene-1-propan-1-al. Read-across material, 6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde (CAS # 33885-51-7; see Section VI) has sufficient reproductive toxicity data. In a GLP and OECD 422-compliant study, 10 Wistar Han rats/sex/dose were administered 6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde

via gavage at doses of 0, 30, 60, and 120 mg/kg/day for a minimum of 28 days. Doses were selected based on a DRF study in which significant treatment-related changes in sperm parameters (reduced mean motile sperm, mean progressive motility, the mean number of cells with normal morphology, the mean number of cells with coiled tail, increased mean number of cells with detached head, and mean number of cells with abnormal neck) were observed at 450 mg/kg/day, but none of these effects were observed at 90 mg/kg/day. Sperm analyses were not as extensive in the main study, so it is possible that the previously mentioned sperm effects seen in the DRF study also occurred at the high dose (120 mg/kg/day) of the main study. In the main study, the fertility index was adversely affected (reduced to 50%) at the high dose, which may be related to undetected sperm effects. No treatment-related effects were observed in the mating index, precoital time, number of implantations, estrous cycle, spermatogenic profiling, or histopathological examination of reproductive organs. Based on decreased fertility index at 120 mg/kg/day, the fertility NOAEL for this study was considered to be 60 mg/kg/day (RIFM, 2020c).

No treatment-related effects at the low and mid doses were observed in gestation, viability and lactation indices, duration of gestation, parturition, sex ratio, maternal care, litter size, and early postnatal pup development consisting of mortality, clinical signs, anogenital distance, areola/nipple retention, T4 thyroid hormone levels, or macroscopic examination. However, the number of litters (N = 5) at the high dose was considered too low for toxicological evaluation. Thus, based on insufficient data at 120 mg/kg/day, the NOAEL for this study was considered to be 60 mg/kg/day (RIFM, 2020c).

Therefore, the β,4-dimethylcyclohex-3-ene-1-propan-1-al MOE for the reproductive toxicity endpoint can be calculated by dividing the 6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde NOAEL in mg/kg/day by the total systemic exposure to β,4-dimethylcyclohex-3-ene-1-propan-1-al, 60/0.00081, or 74074.

In addition, the total systemic exposure to β,4-dimethylcyclohex-3-ene-1-propan-1-al (0.81 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: 06/02/21.

11.1.4. Skin sensitization

Based on the existing data, β,4-dimethylcyclohex-3-ene-1-propan-1-al is considered a skin sensitizer with a defined NESIL of 5500 μg/cm^2.

11.1.4.1. Risk assessment. Based on the existing data, β,4-dimethylcyclohex-3-ene-1-propan-1-al is considered a skin sensitizer. The chemical structure of this material indicates that it would be expected to react with skin proteins directly (Roberts, 2007; Toxtree v3.1.0; OECD Toolbox v4.2). β,4-Dimethylcyclohex-3-ene-1-propan-1-al, was found to be negative in an *in vitro* direct peptide reactivity assay (DPRA) but positive in KeratinoSens and the human cell line activation test (h-CLAT) test (RIFM, 2016a; RIFM, 2016b; RIFM, 2017a). In 2 murine local lymph node assays (LLNAs), β,4-dimethylcyclohex-3-ene-1-propan-1-al was found to be sensitizing with EC3 values of 8.1% (2025 μg/cm^2) and 37.3% (9325 μg/cm^2) (RIFM, 2005a; RIFM, 2005b). However, in another LLNA study, β,4-dimethylcyclohex-3-ene-1-propan-1-al was not found to be sensitizing up to 50% (RIFM, 2012). In a guinea pig Buehler test, β,4-dimethylcyclohex-3-ene-1-propan-1-al presented reactions indicative of sensitization when 25% was used for induction (RIFM, 1991). Data on 3 Confirmation of No Induction in Humans tests (CNIHs) conducted with the β,4-dimethylcyclohex-3-ene-1-propan-1-al were available. In a CNIH with 10%, or 5510 μg/cm^2 of β,4-dimethylcyclohex-3-ene-1-propan-1-al in 3:1 diethyl phthalate:ethanol, stabilized with 0.1% BHT, no skin sensitization reactions were observed in 105 volunteers (RIFM, 2006). However, in another CNIH with 10% or 5510 μg/cm^2 of β,4-dimethylcyclohex-3-ene-1-propan-1-al, stabilized with 0.1% tocopherol (vehicle was not reported), reactions indicative of sensitization were observed in 3/102 volunteers (RIFM, 2008). Another CNIH with β,4-dimethylcyclohex-3-ene-1-propan-1-al stabilized with 0.1% tocopherol at a lower concentration of 2.2% or 2598 μg/cm^2 did not lead to induction of skin sensitization in any of the 110 volunteers (RIFM, 2018).

Based on the weight of evidence (WoE) from structural analysis and animal and human studies, β,4-dimethylcyclohex-3-ene-1-propan-1-al, 1-cyclohexene-1-propanal is a sensitizer with a WoE NESIL of 5500 μg/cm^2 (see Table 1). Section X 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, 2020b).

Additional References: None.

Literature Search and Risk Assessment Completed On: 06/04/21.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis absorption spectra, β,4-dimethylcyclohex-3-ene-1-propan-1-al 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 β,4-dimethylcyclohex-3-ene-1-propan-1-al in experimental models. UV/Vis absorption spectra indicate no absorption between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry, 2009). Based on the lack of absorbance, β,4-dimethylcyclohex-3--ene-1-propan-1-al 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 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: 06/02/21.

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for β,4-dimethylcyclohex-3-ene-1-propan-1-al 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 β,4-dimethylcyclohex-3-ene-1-propan-1-al. Based on the Creme RIFM Model, the inhalation exposure is 0.0072 mg/day. This exposure is

Table 1
Data summary for β,4-dimethylcyclohex-3-ene-1-propan-1-al.

LLNA Weighted Mean EC3 Value [No. Studies]	Potency Classification Based on Animal Data[a]	Human Data			
		NOEL-CNIH (induction) μg/cm^2	NOEL-HMT (induction) μg/cm^2	LOEL[b] (induction) μg/cm^2	WoE NESIL[c]
5675 [2]	Weak	5510[e]	NA	5510[d]	5500[e]

NOEL = No observed effect level; CNIH = Confirmation of No Induction in Humans test; HMT = Human Maximization Test; LOEL = lowest observed effect level; NA = Not Available.

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

[b] Data derived from CNIH or HMT.

[c] WoE NESIL limited to 2 significant figures.

[d] Material was stabilized with 0.1%Tocopherol.

[e] Material stabilized with 0.1%BHT.

194.4 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: 06/03/21.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of β,4-dimethylcyclohex-3-ene-1-propan-1-al 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, β,4-Dimethylcyclohex-3-ene-1-propan-1-al 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 β,4-dimethylcyclohex-3-ene-1-propan-1-al as possibly persistent or bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

11.2.1.1. Risk assessment. Based on the current Volume of Use (2015), β,4-dimethylcyclohex-3-ene-1-propan-1-al presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2. Key studies

11.2.2.1. Biodegradation. RIFM, 2011: The ready biodegradability of the test material was conducted using the manometric respirometry test according to the OECD 301 F guideline. Biodegradation of 80% was observed after 28 days.

11.2.2.2. Ecotoxicity. No data available.

11.2.2.3. Other available data. β,4-Dimethylcyclohex-3-ene-1-propan-1-al has been registered for REACH with no additional data available at this time.

11.2.2.4. Risk assessment refinement. Since β,4-Dimethylcyclohex-3-ene-1-propan-1-al has passed the screening criteria, measured data is included for completeness only and has not been used in PNEC derivation.

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)**	3.34			1000000	0.00334	
ECOSAR Acute Endpoints **(Tier 2)** v1.11	1.039	0.671	1.552	10000	0.0671	Aldehydes (Mono)
ECOSAR Acute Endpoints **(Tier 2)** v1.11	3.067	2.063	3.097			Neutral Organics SAR (baseline toxicity)

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	4.1	4.1
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	1–10	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 0.0671 μg/L. The revised PEC/PNECs for EU and NA are <1; therefore, the material does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 06/01/21.

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:** https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm
- **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 12/10/21.

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.2022.113174.

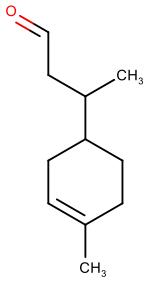
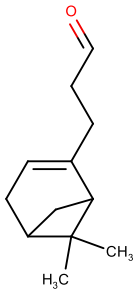
Appendix

Read-across Justification

Methods

The read-across analogs were identified using RIFM fragrance materials chemical inventory clustering and read-across search criteria (RIFM, 2020a). These criteria follow the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015) and are consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemical 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 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). The parameters were calculated using the consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts, oncologic classification, ER binding, and repeat dose categorization predictions were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010).
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018), and skin sensitization was predicted using Toxtree.
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- To keep continuity and compatibility with *in silico* alerts, OECD QSAR Toolbox v4.2 was selected as the alert system.

	Target Material	Read-across Material
Principal Name	β,4-Dimethylcyclohex-3-ene-1-propan-1-al	6,6-Dimethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde
CAS No.	6784-13-0	33885-51-7
Structure		
Similarity (Tanimoto Score)		0.80
SMILES	CC(CC═O)C1CCC(C)═CC1	CC1(C)C2CC═C(CCC═O)C1C2
Endpoint		Repeated dose toxicity Reproductive toxicity
Molecular Formula	$C_{11}H_{18}O$	$C_{12}H_{18}O$
Molecular Weight	166.264	178.275
Melting Point (°C, EPI Suite)	5.24	44.75
Boiling Point (°C, EPI Suite)	232.75	246.66
Vapor Pressure (Pa @ 25°C, EPI Suite)	9.03 E+00	2.79 E+00
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	3.39 E+01	3.44 E+01
Log K_{OW}	3.84	3.76
J_{max} (μg/cm^2/h, SAM)	4.53	3.76
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	4.03 E+01	2.36 E+01
Repeated Dose Toxicity		
Repeated Dose (HESS)	Not categorized	Not categorized
Reproductive Toxicity		
ER Binding (OECD QSAR Toolbox v4.2)	Non-binder, without OH or NH2 group	Non-binder, without OH or NH2 group
Developmental Toxicity (CAESAR v2.1.6)	Toxicant (moderate reliability)	Toxicant (moderate reliability)
Metabolism		
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	See Supplemental Data 1	See Supplemental Data 2

aSummary

There are insufficient toxicity data on β,4-dimethylcyclohex-3-ene-1-propan-1-al (CAS # 6784-13-0). 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, 6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde (CAS # 33885-51-7) was identified as a read-across analog with sufficient data for toxicological evaluation.

Conclusions

- 6,6-Dimethylbicyclo[3.1.1]hept-2-ene-2-propionaldehyde (CAS # 33885-51-7) was used as a read-across analog for the target material β,4-dimethylcyclohex-3-ene-1-propan-1-al (CAS # 6784-13-0) for the repeated dose toxicity and reproductive toxicity endpoints.
  - o The target material and the read-across analog are structurally similar and belong to the structural class of aliphatic aldehydes.
  - o The target material and the read-across analog share an aldehyde functional group and methyl-substituted cyclohexene ring.
  - o The key difference between the target material and the read-across analog is that the target has a shorter aliphatic chain between the aldehyde group and the cyclohexene ring compared to the read-across analog. The read-across analog contains the structural features of the target material that are relevant to this endpoint and is expected to have equal or greater potential for toxicity as compared to the target.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score in the above table. Differences between the structures that affect the Tanimoto score do not affect consideration of the toxicity endpoint.
  - o The 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 a 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 The CAESAR model for developmental toxicity has alerted the target material and the read-across analog to be toxicant with moderate reliability. The data for the read-across analog confirms that the material has adequate MOE under current levels of use. Therefore, based on the structural similarity between the target material and the read-across analog and data on the read-across analog, the alerts will be superseded by the availability of the data.
  - 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.

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