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RIFM fragrance ingredient safety assessment, 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal, CAS Registry Number 65405-84-7

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

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

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ABSTRACT

The existing information supports **the use of this material as described in this safety assessment**. 4-(2,6,6-Trimethyl-2-cyclohexen)-2-methylbutanal was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal is not 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 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). Data provided 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal a No Expected Sensitization Induction Level (NESIL) of 1100 μg/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on data and ultraviolet/visible (UV/Vis) spectra; 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal 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

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

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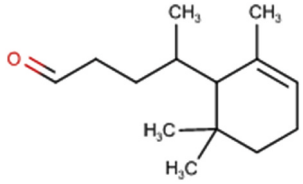
volume of use in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

Version: 111121. Initial publication. All fragrance materials are evaluated on a five-year rotating basis. Revised safety assessments are published if new relevant data become available. Open access to all RIFM Fragrance Ingredient Safety Assessments is here: fragrance materialsafetyresource.elsevier.com.



Name: 4-(2,6,6-Trimethyl-2-cyclohexen)-2-methylbutanal

CAS Registry Number: 65405-84-7

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., 2020)
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
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 et al., 2015), which should be referred to for clarifications.

Each endpoint discussed in this safety assessment includes the relevant data that were available at the time of writing (version number in the top box is indicative of the

(*continued on next column*)

(*continued*)

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.

4-(2,6,6-Trimethyl-2-cyclohexen)-2-methylbutanal was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal is not 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 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). Data provided 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal a No Expected Sensitization Induction Level (NESIL) of 1100 μg/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on data and ultraviolet/visible (UV/Vis) spectra; 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal was found not to be Persistent, Bioaccumulative, and Toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

Human Health Safety Assessment

Genotoxicity: Not genotoxic. (RIFM, 2003a; RIFM, 2015b)
Repeated Dose Toxicity: No NOAEL available. Exposure is below TTC.
Reproductive Toxicity: No NOAEL available. Exposure is below the TTC.
Skin Sensitization: NESIL = 1100 μg/cm^2. RIFM (2009)
Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic. (UV/Vis Spectra; RIFM Database; RIFM, 1981c; RIFM, 1981d)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:
Persistence:
Critical Measured Value: 70% (OECD 301F) RIFM (2003b)
Bioaccumulation:
Screening-level: 1249 L/kg (EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:
Screening-level: 48-h *Daphnia magna* LC50: 0.083 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 et al., 2002)
Critical Ecotoxicity Endpoint: 48-h *Daphnia magna* LC50: 0.083 mg/L (ECOSAR; US EPA, 2012b)
RIFM PNEC is: 0.0083 μg/L

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

1. Identification

1. **Chemical Name:** 4-(2,6,6-Trimethyl-2-cyclohexen)-2-methylbutanal
2. **CAS Registry Number:** 65405-84-7

3. **Synonyms:** Cetonal; Cyclohexenebutanal,.α.,2,2,6-tetramethyl-; a,2,2,6-Tetramethylcyclohexene-1-butyraldehyde; Amerinal; 4-(2,6,6-Trimethyl-2-cyclohexen)-2-methylbutanal
4. **Molecular Formula:** $C_{14}H_{24}O$
5. **Molecular Weight:** 208.34
6. **RIFM Number:** 1038
7. **Stereochemistry:** Isomer not specified. One chiral center is present, and 2 total enantiomers are possible.

2. Physical data

1. **Boiling Point:** 270.68 °C (EPI Suite), 267.3 °C at 1013 hPa (RIFM, 2015f)
2. **Flash Point:** 97.5 °C (corrected and rounded down to the nearest multiple of 0.5 °C) (RIFM, 2015g)
3. **Log K_{OW}:** 5.4/5.5 (RIFM, 2004), 5.2 (EPI Suite)
4. **Melting Point:** 43.04 °C (EPI Suite), −88.5 to −87.4 °C at 1013 hPa (RIFM, 2015f)
5. **Water Solubility:** 1.442 mg/L (EPI Suite)
6. **Specific Gravity:** 0.9131 at 25 °C (RIFM)
7. **Vapor Pressure:** 0.0035 mm Hg at 20 °C (EPI Suite v4.0), 0.003 mm Hg at 20 °C (Fragrance Materials Association), 0.00626 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. 0.1–1 metric tons per year (IFRA, 2015)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v3.1.4)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.13% (RIFM, 2020a)
2. **Inhalation Exposure*:** 0.00010 mg/kg/day or 0.0076 mg/day (RIFM, 2020a)
3. **Total Systemic Exposure**:** 0.0025 mg/kg/day (RIFM, 2020a)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM Aggregate Exposure Model (RIFM, 2015a; 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 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 (RIFM, 2015a; 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 v3.1	OECD QSAR Toolbox v4.2
I	I	I

2. Analogs Selected:
   a. **Genotoxicity:** None
   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:** None

7. Metabolism

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

8. Natural occurrence

4-(2,6,6-Trimethyl-2-cyclohexen)-2-methylbutanal 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

Dossier available; accessed 11/11/21 (ECHA, 2017).

10. Conclusion

The maximum acceptable concentrations[a] in finished products for 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal are detailed below.

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)
1	Products applied to the lips (lipstick)	0.085
2	Products applied to the axillae	0.025
3	Products applied to the face/body using fingertips	0.51
4	Products related to fine fragrances	0.47
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.12
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.12
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.12
5D	Baby cream, oil, talc	0.12
6	Products with oral and lip exposure	0.28
7	Products applied to the hair with some hand contact	0.96
8	Products with significant ano-genital exposure (tampon)	0.050
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.92
10A	Household care products with mostly hand contact (hand dishwashing detergent)	3.3
10B	Aerosol air freshener	3.3
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	1.8
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	No Restriction

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 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal, the basis was a skin sensitization NESIL of 1100 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).

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the available data, 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal does not present a concern for genotoxic potential.

11.1.1.1. Risk assessment. 4-(2,6,6-Trimethyl-2-cyclohexen)-2-methylbutanal 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, 2015c). 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 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal was assessed in an Ames assay conducted in compliance with GLP regulations and accordance with OECD TG 471. *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, and TA102 were treated with 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal in dimethyl sulfoxide (DMSO) at the concentration range of 1.22–5000 μg/plate in the presence and absence of metabolic activation (S9). No increase in the frequency of revertant colonies was detected in either of the strains at the concentrations tested (RIFM, 2003a). Under the conditions of the test, 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal was considered not mutagenic in bacteria.

The clastogenic activity of 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal was assessed in an *in vitro* micronucleus assay conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes from female donors were treated with 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal in DMSO at concentrations up to 86.1 μg/mL (1.10%) in the presence and absence of S9 for 3 and 24 h. A statistically significant increase in the frequency of binucleated cells with micronuclei (BNMN) was observed at the 2 lowest evaluated concentrations in the 3-h treatment without S9 and at 86.1 μg/mL with S9. However, the BNMN frequency observed for all 3 doses was within the historical control 95% reference range for this treatment condition. Moreover, no concentration-related response was observed in the test conditions. Therefore, a significant increase in the BNMN frequency was observed at 45.8 and 62.8 μg/mL in the 3-h treatment without S9, and 86.1 μg/mL in the 3-h treatment with S9 was considered biologically non-relevant. No significant increase in the BNMN frequencies was observed in the approximate 24-h treatment without S9. The BNMN frequencies of the vehicle and the positive controls fell within the acceptable range (RIFM, 2015b). The test material, 4 (2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal, was considered negative for inducing micronuclei in the binucleated cells of human peripheral blood lymphocytes from a female donor in the 3-h treatment with and without S9 and the approximate 24-h treatment without S9 when evaluated up to the limit of cytotoxicity.

Based on the available data, 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 01/27/21.

11.1.2. Repeated dose toxicity

There are insufficient repeated dose toxicity data on 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal or any read-across materials. The total systemic exposure to 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal 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 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal or any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal (2.5 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 12/15/20.

11.1.3. Reproductive toxicity

There are insufficient reproductive toxicity data on 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal or any read-across materials. The total systemic exposure to 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal 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 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal or any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal (2.5 μg/kg/day) is below the TTC (30 μg/kg/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: 02/06/21.

11.1.4. Skin sensitization

Based on the available data, 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal is considered to be a skin sensitizer with a defined NESIL of 1100 μg/cm^2.

11.1.4.1. Risk assessment. Based on the existing data, 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal is considered a skin sensitizer. The chemical structure of this material indicates that it would be expected to react with skin proteins (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). 4-(2,6,6-Trimethyl-2-cyclohexen)-2-methylbutanal was not predicted to be a sensitizer in a direct peptide reactivity assay (DPRA) and KeratinoSens assay (RIFM, 2015e; ECHA, 2017). In a murine local lymph node assay (LLNA), 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal was found to be positive with an EC3 value of 20.6% (5150 μg/cm^2) (ECHA, 2017). Similarly, in a guinea pig open epicutaneous test, 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal was found to be a weak sensitizer (RIFM, 1981a). In a human maximization test, no skin sensitization reactions were observed with 10% or 6900 μg/cm^2 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal in petrolatum (RIFM, 1977). Additionally, in a CNIH with 1%, or 1181 μg/cm^2, 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal in 3:1 diethyl phthalate:ethanol, no reactions indicative of sensitization were observed in any of the 107 volunteers (RIFM, 2009).

Based on the available data, 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal is considered to be a skin sensitizer with a defined NESIL of 1100 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

Table 1
Data summary for 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal.

LLNA Weighted Mean EC3 Value μg/cm^2 [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] μg/cm^2
5150 [[a]]	weak	1181	6897	NA	1100

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.

(QRA2) described by Api et al. (RIFM, 2020b).

Additional References: RIFM, 1978; RIFM, 1981b.

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

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra along with existing *in vivo* study data, 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal would not be expected to present a concern for phototoxicity or photoallergenicity.

11.1.5.1. Risk assessment. 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 et al., 2009). Phototoxicity and photoallergenicity of 3% and 10% 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal, respectively, were evaluated in guinea pigs; there were no effects in either study (RIFM, 1981c; RIFM, 1981d). Based on the lack of absorbance in the critical range and the *in vivo* studies 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal would not be expected to 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 et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 02/10/21.

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 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal 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 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal. Based on the Creme RIFM Model, the inhalation exposure is 0.0076 mg/day. This exposure is 184.2 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: 02/12/21.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal 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, 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal 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 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal 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.

11.2.2. Risk assessment

Based on the current Volume of Use (2015), 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Key studies

11.2.2.1.1. Biodegradation. RIFM, 2003b: A ready biodegradability of the test material was determined by the manometric respirometry test following the OECD 301F method. The average biodegradation after 28 days was 70%.

11.2.2.1.2. Ecotoxicity. RIFM, 2016: A *Daphnia magna* acute immobilization test was conducted according to the OECD 202 guideline under semi-static conditions in a closed system without headspace. The 48-h EC50 value based on geometric mean measured concentration was reported to be 0.739 mg/L (95% CI: 0.707–0.774 mg/L).

RIFM, 2016: A *Daphnia magna* acute immobilization test was conducted according to the OECD 202 guidelines under semi-static conditions in a closed system without headspace. The 48-h EC50 value based on geometric mean measured concentration was reported to be 0.739 mg/L (95% CI: 0.707–0.774 mg/L).

RIFM, 2015d: A 72-h algae growth inhibition test was conducted following the OECD 201 method under static conditions. The EC50 based on time-weighted average mean measured concentration for growth rate and yield was reported to be > 3.29 mg/L.

11.2.2.1.3. Other available data. 4-(2,6,6-Trimethyl-2-cyclohexen)-2-methylbutanal has been registered under REACH, and the following data is available (ECHA, 2017):

A *Daphnia magna* acute test was conducted according to the OECD 202 method under static conditions, and the 48-h EC50 based on mean measured concentration was reported to be 0.92 mg/L.

A 72-h algae growth inhibition test was conducted following the OECD 201 method under static conditions. The EC50 based on nominal concentration for growth rate was reported to be > 100 mg/L.

11.2.3. Risk assessment refinement

Since 4-(2,6,6-trimethyl-2-cyclohexen)-2-methylbutanal has passed the screening criteria (Tier II), 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.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	5.5	5.5
Biodegradation Factor Used	1	1
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 0.0083 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: 01/06/21.

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

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	0.25			1000000	0.00025	
ECOSAR Acute Endpoints **(Tier 2)** v1.11	0.252	0.083	0.260	10000	0.0083	Aldehydes
ECOSAR Acute Endpoints **(Tier 2)** v1.11	0.230	0.175	0.443			Neutral Organics

&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 11/11/21.

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.

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