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Short Review

RIFM fragrance ingredient safety assessment, 2-methyl-4-(2,6,6-trimethyl-cyclohex-1-en-1-yl)-2-butenal, CAS registry number 3155-71-3

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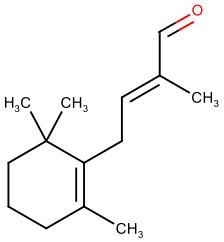

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Name: 2-Methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal
CAS Registry Number: 3155-71-3
Additional CAS Numbers*: 68555-62-4
2-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2-butenal (no reported use)
*Included in this assessment because the materials are isomers
Abbreviation/Definition List:

(*continued on next page*)

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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 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 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.

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2-Methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from the target material and read-across analogs 2,3-dimethyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butenal (CAS # 71850-78-7) and α-iso-methylionone (CAS # 127-51-5) show that 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal is not expected to be genotoxic. The repeated dose, reproductive, and local respiratory toxicity endpoints were evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material, and the exposure to 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). Data provided 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal a No Expected Sensitization Induction Level (NESIL) of 2900 μg/cm^2 for the skin sensitization endpoint. The photoirritation/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal is not expected to be photoirritating/photoallergenic. The environmental endpoints were evaluated; 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal 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 (VoU) in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

Human Health Safety Assessment

Genotoxicity: Not expected to be genotoxic.	(RIFM, 2016b; 2016a; 2015b)
Repeated Dose Toxicity: No NOAEL available. Exposure is below the TTC.	
Reproductive Toxicity: No NOAEL available. Exposure is below the TTC.	
Skin Sensitization: NESIL = 2900 μg/cm^2.	RIFM (2015a)
Photoirritation/Photoallergenicity: Not expected to be photoirritating/photoallergenic.	(UV/Vis Spectra; RIFM Database)
Local Respiratory Toxicity: NOAEC is not available. Exposure is below the TTC.	

Environmental Safety Assessment

Hazard Assessment:	
Persistence:	
Critical Measured Value: 78% (OECD 310) for CAS # 3155-71-3	RIFM (2011)
Bioaccumulation:	
Screening-level: 1336 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: 96-h Fish LC50: 0.147 mg/L	(ECOSAR v2.0; 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: 96-h Fish LC50: 0.147 mg/L	(ECOSAR v2.0; US EPA, 2012b)
RIFM PNEC is: 0.0147 μg/L	
•**Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe <1	

1. Identification

1. Chemical Name: 2-Methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal	**1. Chemical Name**: 2-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2-butenal
2. CAS Registry Number: 3155-71-3	**2. CAS Registry Number:** 68555-62-4
3. Synonyms: 2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-; β-Coronal; 1-(3-ホルミル-2-ブテニル)-2,6,6-トリメチルシクロヘキセン-2; 2-Methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl) but-2-enal; Boronal; 2-Methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal	**3. Synonyms:** 2-Butenal, 2-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-; 2-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2-butenal; 2-Methyl-4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-2-enal; Veltonal
4. Molecular Formula: $C_{14}H_{22}O$	**4. Molecular Formula:** $C_{14}H_{22}O$
5. Molecular Weight: 206.32	**5. Molecular Weight:** 206.32
6. RIFM Number: 5290	**6. RIFM Number:** 5922
7. Stereochemistry: Isomer not specified. One geometric center present, and 2 total stereoisomers possible.	**7. Stereochemistry:** Isomer not specified. One geometric center present, and 2 total stereoisomers possible.

2. Physical data

CAS # 3155-71-3	CAS # 68555-62-4
1. Boiling Point: 284.16 °C (EPI Suite)	**1. Boiling Point:** 280.95 °C (EPI Suite)
2. Flash Point: >93 °C (Globally Harmonized System [GHS])	**2. Flash Point:** >93 °C (GHS)
3. Log K_{OW}: 5.24 (EPI Suite)	**3. Log K_{OW}:** 5.11 (EPI Suite)
4. Melting Point: 53.34 °C (EPI Suite)	**4. Melting Point**: 44.02 °C (EPI Suite)
5. Water Solubility: 1.354 mg/L (EPI Suite)	**5. Water Solubility:** 1.747 mg/L (EPI Suite)
6. Specific Gravity: Not Available	**6. Specific Gravity:** Not Available
7. Vapor Pressure: 0.00135 mm Hg at 20 °C (EPI Suite v4.0), 0.00247 mm Hg at 25 °C (EPI Suite)	**7. Vapor Pressure:** 0.00358 mm Hg at 25 °C (EPI Suite), 0.00198 mm Hg at 20 °C (EPI Suite v4.0)
8. UV Spectra: Minor absorbance between 290 and 700 nm. Molar absorption coefficients (131, 0, 145 L mol^{-1} • cm^{-1} under neutral, acidic, and basic conditions, respectively) are below the benchmark (1000 L mol^{-1} • cm^{-1})	**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	**9. Appearance/Organoleptic:** Not Available

3. Volume of use (worldwide band)

1. 0.1–1 metric ton per year (IFRA, 2015).

4. Exposure to fragrance ingredient* (creme RIFM aggregate exposure model v3.0)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.13% (RIFM, 2020a)
2. **Inhalation Exposure**:** 0.00061 mg/kg/day or 0.048 mg/day (RIFM, 2020a)
3. **Total Systemic Exposure***:** 0.0064 mg/kg/day (RIFM, 2020a)

*When a safety assessment includes multiple materials, the highest exposure out of all included materials will be recorded here for the 95th Percentile Concentration in fine fragrance, inhalation exposure, and total exposure.

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

***95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section 5. It is derived from concentration survey data in the Creme RIFM Aggregate Exposure Model and includes exposure via dermal, oral, and inhalation routes whenever the fragrance ingredient is used in products that include these routes of exposure (Comiskey et al., 2015, 2017; Safford et al., 2015, 2017).

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

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

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

2. Analogs Selected:

a. **Genotoxicity:** 2,3-Dimethyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butenal (CAS # 71850-78-7) and α-iso-methylionone (CAS # 127-51-5)
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

7. Metabolism

Not considered for this risk assessment and therefore not reviewed except where it may pertain in specific endpoint sections as discussed below.

Additional References: None.

8. Natural occurrence

2-Methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal and 2-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2-butenal are 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.

9. REACH dossier

2-Methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal is pre-registered for 2010; no dossier available as of 12/09/21.2-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2-butenal is pre-registered for 2013; no dossier available as of 12/09/21.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for 2-methyl-4-(2,6,6- trimethylcyclohex-1-en-1-yl)-2-butenal 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)	0.22
2	Products applied to the axillae	0.066
3	Products applied to the face/body using fingertips	1.3
4	Products related to fine fragrances	1.2
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.32
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.32
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.32
5D	Baby cream, oil, talc	0.32
6	Products with oral and lip exposure	0.73
7	Products applied to the hair with some hand contact	2.5

(continued on next page)

(*continued*)

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
8	Products with significant ano-genital exposure (tampon)	0.13
9	Products with body and hand exposure, primarily rinse-off (bar soap)	2.4
10A	Household care products with mostly hand contact (hand dishwashing detergent)	8.7
10B	Aerosol air freshener	8.7
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	4.8
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 2-methyl-4-(2,6,6- trimethylcyclohex-1-en-1-yl)-2-butenal, the basis was a predicted skin absorption value of 40% and a skin sensitization NESIL of 2900 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).

[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, 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal does not present a concern for genotoxicity.

Risk Assessment:

2-Methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal was assessed in the BlueScreen assay and found positive for cytotoxicity (positive: <80% relative cell density) without metabolic activation, negative for cytotoxicity with metabolic activation, and negative for genotoxicity with and without metabolic activation (RIFM, 2013). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. Additional assays on a more reactive read-across material were considered to fully assess the potential clastogenic effects of the target material.

The mutagenic activity of 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation and preincubation methods. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal in dimethyl sulfoxide (DMSO) at concentrations up to 5000 μg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 2016b). Under the conditions of the study, 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal was not mutagenic in the Ames test.

There are no studies assessing the clastogenic activity of 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal; however, read-across can be made to 2,3-dimethyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butenal and α-iso-methylionone (CAS # 71850-78-7 and 127-51-5, respectively; see Section 6).

The clastogenic activity of 2,3-dimethyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butenal 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 2,3-dimethyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butenal in DMSO at concentrations up to 2000 μg/mL in the dose range finding (DRF) study; micronuclei analysis was conducted at concentrations up to 120 μg/mL in the presence and absence of metabolic activation. 2,3-Dimethyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butenal did not induce binucleated cells with micronuclei in either the presence or absence of an S9 activation system (RIFM, 2016a). Under the conditions of the study, 2,3-dimethyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butenal was considered to be non-clastogenic in the *in vitro* micronucleus test, and this can be extended to 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal.

The clastogenic activity of α-iso-methylionone 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 α-iso-methylionone in DMSO at concentrations up to 2063 μg/mL in the DRF study; micronuclei analysis was conducted at concentrations up to 200 μg/mL in the presence and absence of metabolic activation. α-iso-Methylionone did not induce binucleated cells with micronuclei when tested in either the presence or absence of an S9 activation system (RIFM, 2015b). Under the conditions of the study, α-iso-methylionone was considered to be non-clastogenic in the *in vitro* micronucleus test, and this can be extended to 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal.

Based on the data available, 2,3-dimethyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butenal and α-iso-methylionone does not present a concern for genotoxic potential, and this can be extended to 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/23/21.

11.1.2. Repeated dose toxicity

There are insufficient repeated dose toxicity data on 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal or any read-across materials. The total systemic exposure to 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal is below the TTC for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

Risk Assessment:

There are no repeated dose toxicity data on 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal or any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal (6.4 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: 01/29/21.

11.1.3. Reproductive toxicity

There are insufficient reproductive toxicity data on 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal or any read-across materials. The total systemic exposure to 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal is below the TTC for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

Risk Assessment:

There are no reproductive toxicity data on 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal or any read-across materials that can be used to support the developmental or reproductive toxicity endpoints. The total systemic exposure to 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal (6.4 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the developmental and reproductive toxicity endpoints of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 03/09/21.

11.1.4. Skin sensitization

Based on the available data, 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal is considered a weak skin sensitizer with a defined NESIL of 2900 μg/cm^2.

Risk Assessment:

Based on the available data, 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal is considered a weak skin sensitizer. The chemical structure of this material indicates that it would be expected to react directly with skin proteins (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). *In vitro*, 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal was predicted to be a sensitizer in a direct peptide reactivity assay (DPRA) and human cell line test (h-CLAT), whereas it was not predicted to be a sensitizer in a KeratinoSens assay (RIFM, 2016c, 2020d, 2017). The existing animal studies also indicated that the target material is a sensitizer. 2-Methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal was predicted to be a weak sensitizer in 2 guinea pig maximization tests (RIFM, 1985, 1991b). However, it was not predicted to be a sensitizer in a guinea pig Buehler test (RIFM, 1991a). In the murine local lymph node assay (LLNA), 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal was considered to be a skin sensitizer with an EC3 value of 11.9% in 1:3 ethanol:diethyl phthalate (1:3 EtOH:DEP) (RIFM, 2010). In a Confirmation of No Induction in Human (CNIH) test with more than 100 subjects, 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal did not induce sensitization reactions at 2.5% (2953 μg/cm^2) in 1:3 EtOH:DEP (RIFM, 2015a).

Based on the weight of evidence (WoE) from structural analysis as well as animal and human studies, 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal is a sensitizer with a WoE NESIL of 2900 μg/cm^2 (see Table 1). Section 10 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, 2020c).

Additional References: RIFM, 1965; RIFM, 1992.

Literature Search and Risk Assessment Completed On: 04/12/21.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal would not be expected to present a concern for phototoxicity or photoallergenicity.

Risk Assessment:

There are no phototoxicity studies available for 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal. UV/Vis absorption spectra indicate minor absorbance between 290 and 700 nm. The corresponding molar absorption coefficients are below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). Based on the lack of significant absorbance in the critical range, 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal does not present a concern for phototoxicity or photoallergenicity.

UV Spectra Analysis:

UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate minor absorbance in the range of 290–700 nm. The molar absorption coefficients (131, 0, 145 L mol^{-1} • cm^{-1} under neutral, acidic, and basic conditions, respectively) are 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: 04/14/21.

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 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal is below the Cramer Class I TTC value for inhalation exposure local effects.

Risk Assessment:

There are no inhalation data available on 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal. Based on the Creme RIFM Model, the inhalation exposure is 0.048 mg/day. This exposure is 29.2 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: 04/16/21.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal 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-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal 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).

Table 1
Data summary for 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal.

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
297 [1]	Weak	2953	NA	NA	2900

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.

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) did not identify 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal as possibly persistent and 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, 2017a). 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.

Risk Assessment:

Based on the current Volume of Use (2015), 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal presents a risk to the aquatic compartment in the screening-level assessment.

Key Studies:

Biodegradation:

For CAS # 3155-71-3.

RIFM, 2011: A biodegradation study was conducted using activated sludge in a CO_2 in sealed vessels (headspace test) according to the OECD 310 method. Biodegradation of 78% was reported after 28 days.

Ecotoxicity:

No data available.

Other available data:

2-Methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal has been pre-registered for REACH with no additional data at this time.

11.2.2. 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)**	0.422			1000000	0.000422	
ECOSAR Acute Endpoints **(Tier 2)** v2.0	0.147	0.268	0.449	10000	0.0147	Vinyl/Allyl aldehydes
ECOSAR Acute Endpoints **(Tier 2)** v2.0	0.208	0.159	0.408			Neutral Organics SAR

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

Exposure	Europe (EU)	North America (NA)
Log K_{OW} Used	5.24	5.24
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional Volume of Use Tonnage Band*	<1	<1
Risk Characterization: PEC/PNEC	**<1**	**<1**

*Regional Volume of Use combined for both CAS #s.

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

The RIFM PNEC is 0.0147 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: 03/24/21.

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
- **PubChem:** https://pubchem.ncbi.nlm.nih.gov/
- **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
- **National Library of Medicine Technical Bulletin:** 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 ChemView:** https://chemview.epa.gov/chemview/
- **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://pubchem.ncbi.nlm.nih.gov/source/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 12/09/21.

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.

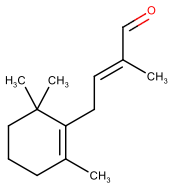
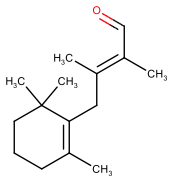
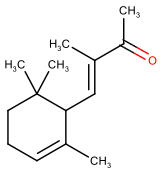
Appendix

Read-across Justification

Methods

The read-across analogs were identified using RIFM fragrance materials chemical inventory clustering and read-across search criteria (RIFM, 2020b). 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, 2017b).

- 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.

Principal Name	Target Material	Read-across Material	Read-across Material
	2-Methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal	2,3-Dimethyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butenal	α-iso-Methylionone
CAS No.	3155-71-3	71850-78-7	127-51-5
Structure			
Similarity (Tanimoto Score)		0.97	0.81
Endpoint		Genotoxicity	Genotoxicity
Molecular Formula	$C_{14}H_{22}O$	$C_{15}H_{24}O$	$C_{14}H_{22}O$
Molecular Weight	206.329	220.356	206.329
Melting Point (°C, EPI Suite)	53.34	55.24	45.26
Boiling Point (°C, EPI Suite)	284.16	295.28	271.60
Vapor Pressure (Pa @ 25°C, EPI Suite)	3.29E-01	1.76E-01	1.30E+00
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	1.35E+00	3.90E-01	2.98E+00

(*continued on next page*)

(*continued*)

Principal Name	Target Material	Read-across Material	Read-across Material
	2-Methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal	2,3-Dimethyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butenal	α-iso-Methylionone
Log K_{OW}	5.24	5.79	4.84
J_{max} (μg/cm^2/h, SAM)	0.22	0.06	0.44
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	6.16E+01	9.66E+01	2.87E+01
Genotoxicity			
DNA Binding (OASIS v1.4, QSAR Toolbox v4.2)	AN2|AN2 ≫ Nucleophilic addition to α,β-unsaturated carbonyl compounds|AN2 ≫ Nucleophilic addition to α,β-unsaturated carbonyl compounds ≫ α,β-Unsaturated Aldehydes|AN2 ≫ Schiff base formation|AN2 ≫ Schiff base formation ≫ α,β-Unsaturated Aldehydes	AN2|AN2 ≫ Nucleophilic addition to α,β-unsaturated carbonyl compounds|AN2 ≫ Nucleophilic addition to α,β-unsaturated carbonyl compounds ≫ α,β-Unsaturated Aldehydes|AN2 ≫ Schiff base formation|AN2 ≫ Schiff base formation ≫ α,β-Unsaturated Aldehydes	No alert found
DNA Binding (OECD QSAR Toolbox v4.2)	Michael addition|Michael addition ≫ Polarized Alkenes-Michael addition|Michael addition ≫ Polarized Alkenes-Michael addition ≫ α,β-unsaturated aldehydes	No alert found	Michael addition|Michael addition ≫ Polarized Alkenes-Michael addition|Michael addition ≫ Polarized Alkenes-Michael addition ≫ α,β- unsaturated ketones
Carcinogenicity (ISS)	α,β-unsaturated carbonyls (Genotox)|Structural alert for genotoxic carcinogenicity	α,β-unsaturated carbonyls (Genotox)|Structural alert for genotoxic carcinogenicity	α,β-unsaturated carbonyls (Genotox)|Structural alert for genotoxic carcinogenicity
DNA Binding (Ames, MN, CA, OASIS v1.1)	No alert found	No alert found	No alert found
***In Vitro* Mutagenicity (Ames, ISS)**	α,β-unsaturated carbonyls	α,β-unsaturated carbonyls	α,β-unsaturated carbonyls
***In Vivo* Mutagenicity (Micronucleus, ISS)**	α,β-unsaturated carbonyls	α,β-unsaturated carbonyls	α,β-unsaturated carbonyls
Oncologic Classification	Aldehyde-type Compounds	Aldehyde-type Compounds	Reactive Ketone Reactive Functional Groups
Metabolism			
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	Supplemental Data 1	Supplemental Data 2	Supplemental Data 3

Summary

There are insufficient toxicity data on the target material, 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal (CAS # 3155-71-3). Hence, *in silico* evaluation was conducted to determine a read-across material. Based on structural similarity, reactivity, metabolism data, physical–chemical properties, and expert judgment, 2,3-dimethyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butenal (CAS # 71850-78-7) and α-iso-methylionone (CAS # 127-51-5) were identified as read-across analogs with sufficient data for the genotoxicity endpoint.

Conclusions

- 2,3-Dimethyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butenal (CAS # 71850-78-7) and α-iso-methylionone (CAS # 127-51-5) were identified as read-across analogs for 2-methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)-2-butenal (CAS # 3155-71-3) for the genotoxicity endpoint.

o The materials are structurally similar and belong to the structural class of aliphatic aldehydes and ketones.
o The key structural differences between the target material and the read-across analog and WoE material are that the target material is an α,β-unsaturated aldehyde. The target material can undergo a Michael addition reaction and Schiff base formation with its free β carbon. At the same time, it can also undergo epoxidation of the endocyclic vinylene bond. The read-across analog is an α,β-unsaturated aldehyde, but its β carbon is methyl substituted. Therefore, it cannot perform a Michael addition reaction. It can form a Schiff base, and it provides endocyclic vinylene, exactly matching the target material. The weight of the evidence substance is an α,β-unsaturated ketone, and therefore can undergo Michael addition reaction. The read-across analog and the weight of the evidence substance together fulfill the reactive substructural features of the target material.
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 physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable comparison of their toxicological properties. Differences are predicted for J_{max}, which estimates skin absorption. The J_{max} values translate to 80% skin absorption for the target material and 40% absorption for the read-across analog. While percentage skin absorption estimated from J_{max} values indicate exposure to the substance, they do not represent hazard or toxicity parameters. Therefore, the J_{max} of the target material and the read-across analog material are not used directly in comparing substance hazard or toxicity. However, these parameters provide context to assess the impact of bioavailability on toxicity comparisons between the individual materials.
o According to the QSAR OECD Toolbox (v4.2), structural alerts for toxicity endpoints are consistent between the target material and the read-across analog.
o The target material, the read-across analog, and the WoE material have *in silico* alerts for Michael addition and Schiff base formation. The data on the read-across analog and the WoE material confirm that the materials do not pose a concern for genotoxicity. Therefore, based on the structural similarity between the target material, read-across analog, and the WoE material, the *in silico* alerts are superseded by the data.
o The target material and read-across analog show similar alerts for DNA binding, mutagenicity, genotoxicity, and oncologic classification.

o The target material and read-across analog are expected to be metabolized similarly. As per the OECD Toolbox, they are predicted to have similar metabolites.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2023.114047.

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