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Short Review

RIFM fragrance ingredient safety assessment, 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one, CAS registry number 13215-88-8

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Phototoxicity/photoallergenicity
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Environmental safety

ABSTRACT

The existing information supports the use of this material as described in this safety assessment.

4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one is not genotoxic. Data on 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one provide a calculated margin of exposure (MOE) > 100 for the repeated dose toxicity endpoint. The 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-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one is below the TTC (0.03 mg/kg/day and 1.4 mg/day, respectively). The skin sensitization endpoint was completed using the dermal sensitization threshold (DST) for reactive materials (64 μg/cm^2); exposure is below the DST. The phototoxicity/photoallergenicity endpoints were evaluated based on data; 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one 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.

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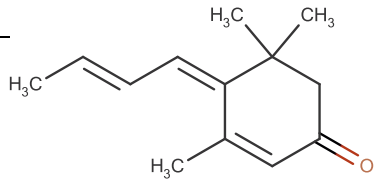
Name: 4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one
CAS Registry Number: 13215-88-8
Additional CAS*: 163440-97-9 (no reported use)
*Included because the materials are an isomeric mixture



Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 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 Observable Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
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.

4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one is not genotoxic. Data on 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one provide a calculated margin of exposure (MOE) > 100 for the repeated dose toxicity endpoint. The 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-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one is below the TTC (0.03 mg/kg/day and 1.4 mg/day, respectively). The skin sensitization endpoint was completed using the dermal sensitization threshold (DST) for reactive materials (64 μg/cm^2); exposure is below the DST. The phototoxicity/photoallergenicity endpoints were evaluated based on data; 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one 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, 2000a; RIFM, 2000b)
Repeated Dose Toxicity: Derived NOAEL = 5.33 mg/kg/day. RIFM (2003)

Reproductive Toxicity: No NOAEL available. Exposure is below the TTC.
Skin Sensitization: No safety concerns at current, declared use levels; Exposure is below the DST.
Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic. RIFM (1984a)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment
Hazard Assessment:
Persistence:
Critical Measured Value: 3.1% (OECD 301 D) RIFM (1992)
Bioaccumulation:
Screening-level: 277.8 L/kg (EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:
Screening-level: Fish LC50: 3.07 mg/L (RIFM Framework; Salvito, 2002)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:
Screening-level: PEC/PNEC (North America and Europe) < 1 (RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: Fish LC50: 3.07 mg/L (RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.00307 μg/L
- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe are not applicable; cleared at screening-level

1. Identification

Chemical Name: 4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one	**Chemical Name**: 2-Cyclohexen-1-one, 4-[3-(acetyloxy)-1-buten-1-yl]-3,5,5-trimethyl-, pyrolyzed
CAS Registry Number: 13215-88-8	**CAS Registry Number:** 163440-97-9
Synonyms: 4-(2-Butenylidene)-3,5,5-trimethyl-2-cyclohexen-1-one; 2-Cyclohexen-1-one, 4-(2-butenylidene)-3,5,5-trimethyl-; 4,6,8-Megastigmatrien-3-one; Megastigmatrienone; 4-But-2-en-1-ylidene-3,5,5-trimethylcyclohex-2-en-1-one; 4-(2-Buten-1-ylidene)-3,5,5-trimethyl-2-cyclohexen-1-one; (4E)-4-[(2E/Z)-Butenylidene]-3,5,5-trimethylcyclohex-2-en-1-one; Tabanon; 4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one	**Synonyms:** Tabanon; 2-Cyclohexen-1-one, 4-[3-(acetyloxy)-1-buten-1-yl]-3,5,5-trimethyl-, pyrolyzed
Molecular Formula: $C_{13}H_{18}O$	**Molecular Formula:** $C_{13}H_{18}O$
Molecular Weight: 190.28	**Molecular Weight:** 190.28
RIFM Number: 5398	**RIFM Number:** 5398
Stereochemistry: 4E, 2E/Z isomer specified. Two stereocenters and 4 total stereoisomers possible.	**Stereochemistry:** No isomer specified. Two stereocenters and 4 total stereoisomers possible.

2. Physical data

CAS # 13215-88-8	CAS # 163440-97-9
Boiling Point: 280.67 °C (EPI Suite)	**Boiling Point:** Not Available
Flash Point: Not Available	**Flash Point:** Not Available
Log K_{OW}: 4.21 (EPI Suite)	**Log K_{OW}:** Not Available
Melting Point: 65.7 °C (EPI Suite)	**Melting Point:** Not Available
Water Solubility: 12.48 mg/L (EPI Suite)	**Water Solubility:** 361 mg/L (20 ± 0.5 °C, pH 5.0) RIFM, 2017e
Specific Gravity: Not Available	**Specific Gravity:** Not Available
Vapor Pressure: 0.00219 mm Hg @ 20 °C (EPI Suite v4.0), 0.00393 mm Hg @ 25 °C (EPI Suite)	**Vapor Pressure:** Not Available
UV Spectra: Significant absorbance between 290 and 700 nm, with peak at 330 nm and returning to baseline by 390 nm. Molar absorption coefficient is above the benchmark (1000 L $mol^{-1} \cdot cm^{-1}$)	**UV Spectra:** Not Available
Appearance/Organoleptic: Not Available	**Appearance/Organoleptic:** Not available

3. Volume of use (worldwide band)

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

4. Exposure to fragrance ingredient (Creme RIFM Aggregate Exposure Model v1.0)

1. **95th Percentile Concentration in Hydroalcoholics:** 0.025% (RIFM, 2017a)
2. **Inhalation Exposure*:** 0.000012 mg/kg/day or 0.00090 mg/day (RIFM, 2017a)
3. **Total Systemic Exposure**:** 0.00020 mg/kg/day (RIFM, 2017a)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM Aggregate Exposure Model (Comiskey et al., 2015; Safford, 2015a, 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 (Comiskey et al., 2015; Safford, 2015a, 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; see Table 1)

Table 1
Cramer class.

Expert Judgment	Toxtree v 2.6	OECD QSAR Toolbox v 3.2
I	II	I

*Due to potential discrepancies with the current *in silico* tools (Bhatia et al., 2015), the Cramer Class of the target material was determined using expert judgment based on the Cramer decision tree (Cramer et al., 1978). See the Appendix below for further details.

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 (discrete chemical) or composition (NCS)

4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one is reported to occur in the following foods by the VCF*:
Citrus fruits.
Starfruit (*Averrhoa carambola* L.)
2-Cyclohexen-1-one, 4-[3-(acetyloxy)-1-buten-1-yl]-3,5,5-trimethyl-, pyrolyzed 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 for 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one (ECHA, 2018; accessed 09/06/19); no dossier available for 2-Cyclohexen-1-one, 4-[3-(acetyloxy)-1-buten-1-yl]-3,5,5-trimethyl-, pyrolyzed.

10. Conclusion

The existing information supports the use of this material as described in this safety assessment.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. The mutagenic activity of 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the preincubation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one in dimethyl sulfoxide (DMSO) at concentrations up to 600 μg/plate. No increase in the mean number of revertant colonies was observed at any tested concentration in the presence or absence of S9 (RIFM, 2000a). Under the conditions of the study, 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one was not mutagenic in the Ames test.

The clastogenic activity of 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one was evaluated in an *in vivo* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 474. The test material was administered in olive oil via intraperitoneal to groups of male and female CD-1 mice. Doses of 125, 250, or 500 mg/kg body weight were administered. Mice from each dose level were euthanized at 24 and 48 h after dosing, and the bone marrow was extracted and examined for polychromatic erythrocytes. The test material did not induce a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow (RIFM, 2000b). Under the conditions of the study, 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one was considered to be not clastogenic in the *in vivo* micronucleus test.

Based on the data available, 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/15/19.

Table 2
Maximum acceptable concentrations for 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one that present no appreciable risk for skin sensitization based on reactive DST.

IFRA Category[a]	Description of Product Type	Maximum Acceptable Concentrations in Finished Products Based on Reactive DST	Reported 95th Percentile Use Concentrations in Finished Products
1	Products applied to the lips	0.0049%	NRU[b]
2	Products applied to the axillae	0.0015%	8.0×10^{-4}%
3	Products applied to the face using fingertips	0.029%	1.2×10^{-5}%
4	Fine fragrance products	0.027%	0.025%
5	Products applied to the face and body using the hands (palms), primarily leave-on	0.0070%	0.0018%
6	Products with oral and lip exposure	0.016%	NRU[b]
7	Products applied to the hair with some hand contact	0.056%	2.2×10^{-4}%
8	Products with significant ano-genital exposure	0.0029%	No Data[c]
9	Products with body and hand exposure, primarily rinse-off	0.054%	0.0025%
10	Household care products with mostly hand contact	0.19%	NRU[b]
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate	0.11%	No Data[c]
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not restricted	0.20%

Note:
[a] For a description of the categories, refer to the IFRA/RIFM Information Booklet.
[b] No reported use.
[c] Fragrance exposure from these products is very low. These products are not currently in the Creme RIFM Aggregate Exposure Model.

11.1.2. Repeated Dose Toxicity

The MOE for 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data for the target material. In a 28-day repeated dose toxicity study, 10 Crj:CD (SD) IGS rats/sex/dose were administered 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one orally at doses of 0, 16, 80, and 400 mg/kg/day. An additional 6 rats/sex/dose were used as a recovery group at 0 and 400 mg/kg/day. No treatment-related mortalities occurred throughout the study. Additionally, no treatment-related effects were observed in body condition, body weights, or hematology. There were dose-dependent increases in kidney weights, with statistical significance at the mid-dose (80 mg/kg/day) in males and the high-dose (400 mg/kg/day) in both sexes. There were also dose-dependent increases in liver weights, with statistical significance at mid-dose in females and high-dose in both sexes. Females also exhibited increased absolute liver and kidney weight at 400 mg/kg/day. Mid and high-dose males exhibited a dose-dependent increase in hyaline droplets. There were slight decreases in sodium (not dose-dependent), potassium (dose-dependent), and potassium excretion in high-dose males, as well as in chlorine in both sexes at high-dose. Based on increased liver weight in females and increased kidney weight and hyaline droplets in males at 80 mg/kg/day, the study authors determined that the NOAEL of 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one is 16 mg/kg/day.

A default safety factor of 3 was used when deriving a NOAEL from the 28-day 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 16/3 or 5.33 mg/kg/day.

Therefore, the MOE can be calculated by dividing the NOAEL (in mg/kg/day) for 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one by the total systemic exposure (in mg/kg/day) of -(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one, 5.33/0.0002 or 26650.

In addition, the total systemic exposure to 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one (0.2 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: 10/21/19.

11.1.3. Reproductive Toxicity

There are insufficient reproductive toxicity data on 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one or any read-across materials. The total systemic exposure to 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one 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-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one or on any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one (0.2 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: 10/09/19.

11.1.4. Skin Sensitization

Based on the existing data, 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one is a sensitizer. However, based on the application of DST, 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one does not present a concern for skin sensitization.

11.1.4.1. Risk assessment. The chemical structure of this material indicates that it would be expected to react with skin proteins (Roberts et al., 2007; Toxtree 3.1.0; OECD Toolbox v4.2). In a murine local lymph node assay, 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one was found to be sensitizing with an EC1.6 value of 17.3% (RIFM, 2017b). In a guinea pig maximization test, 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one was classified as a mild sensitizer (RIFM, 1984b). 4-(2-Butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one is a sensitizer. However, limited data exist to derive a NESIL. Acting conservatively due to the limited data, the reported exposure was benchmarked utilizing the reactive DST of 64 μg/cm^2 (Safford, 2008, 2011, 2015b; Roberts et al., 2015). The current exposure from the 95th percentile concentration is below the DST for reactive materials when evaluated in all QRA categories. Table 2 provides the maximum acceptable concentrations for 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one that present no appreciable risk for skin sensitization based on the reactive DST. These levels represent maximum acceptable concentrations based on the DST approach. However, additional studies may show it could be used at higher levels.

Additional References:

None.

Literature Search and Risk Assessment Completed On: 10/21/19.

11.1.5. Phototoxicity/Photoallergenicity

Based on the available *in vivo* study data, 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one would not be expected to present a concern for phototoxicity or photoallergenicity.

11.1.5.1. Risk assessment. UV/Vis absorption spectra indicate significant absorption between 290 and 700 nm, with a peak absorbance at 330 nm and returning to baseline by 390 nm. The corresponding molar absorption coefficient is above the benchmark of

Table 3
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)	Assessment Factor (AF)	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	<u>3.07</u>			1000000	0.00307	

concern for phototoxicity and photoallergenicity (Henry et al., 2009). In studies conducted in guinea pigs, 10% 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one did not result in phototoxicity or photoallergy when applied to the skin and exposed to UVA irradiation (RIFM, 1984a). Based on *in vivo* study data, 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one does not present a concern for phototoxicity or photoallergenicity.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were generated for 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one. The spectra demonstrate that the material absorbs in the range of 290–700 nm, with a peak at 330 nm and returning to baseline by 390 nm. The molar absorption coefficient for λ max within this range is above 1000 L $mol^{-1} \cdot cm^{-1}$, the benchmark of concern for phototoxic effects (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/09/19.

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to the lack of appropriate data. The exposure level for 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one 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-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one. Based on the Creme RIFM Model, the inhalation exposure is 0.00090 mg/day. This exposure is 1556 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: 10/10/19.

11.2. Environmental endpoint summary

11.2.1. Screening-level Assessment

A screening-level risk assessment of 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one 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, 2012), 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 tables below (see Table 3 and Table 4). 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-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one was identified as a fragrance material with no potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC < 1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) identified 4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one as possibly persistent but not 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-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one presents no risk to the aquatic compartment in the screening-level assessment.

11.2.3. Key Studies

11.2.3.1. Biodegradation. For CAS # 13215-88-8.

RIFM, 1992: The ready biodegradability of the test material was evaluated using the closed bottle test according to the OECD 301D guideline. Biodegradation of 28% was observed after 28 days.

11.2.3.2. Ecotoxicity. For CAS # 13215-88-8.

RIFM, 2017d: An acute immobilization test to *Daphnia magna* was conducted according to the OECD 202 method under static conditions in a closed system without headspace. Due to the low water solubility of the test material, the test concentrations were individually prepared as Water Accommodated Fraction/Water Soluble Fraction (WAF) at loading levels in the range of 6.25–100 mg/L in a geometric series with a separation factor of 2. All the loading levels of the test material and the control were analytically verified via GC-MS analysis of the 4 main components in fresh media at the start of the exposure (0 h) and at the end of the test (48 h). Under the conditions of the study and based on the nominal loadings of the test material, the 48 h-EL50 was 15.7 mg/L (95% CI: 14.2–18.0 mg/L).

RIFM, 2017c: An algae acute growth inhibition test was conducted according to the OECD 201 method under static conditions. Due to the low water solubility of the test material, the test concentrations were prepared as Water Accommodated Fraction/Water Soluble Fraction (WAF) with the nominal test materials loadings at 1.00, 3.16, 10.0, 31.6, and 100 mg/L. The concentrations of the test material in all the loadings were analytically verified by GC-MS at the start (0 h) and at the end of exposure (72 h). Based on nominal loadings of the test material, the 72-EL50 values for growth rate and yield were reported to be 20.2 mg/L) (95% CI: 18.8–21.6 mg/L) and 10.3 mg/L (95% CI: 8.63–13.2 mg/L), respectively.

Table 4
Exposure information and PEC calculation (following RIFM Framework: Salvito et al., 2002).

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	4.21	4.21
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band[a]	< 1	< 1
Risk Characterization: PEC/PNEC	**< 1**	**< 1**

[a] Combined regional Volumes of Use.

11.2.4. Other available data

4-(2-butenylidene)-3,5,5-trimethylcyclohex-2-en-1-one has been registered for REACH with no additional information available at this time.

11.3. Risk assessment refinement

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

The RIFM PNEC is 0.00307 μg/L. The revised PEC/PNECs for EU and NA are not applicable. The material was cleared at the screening-level; therefore, it does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 10/10/19.

12. Literature Search*

- **RIFM Database:** Target, Fragrance Structure-Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** https://echa.europa.eu/
- **NTP:** https://ntp.niehs.nih.gov/
- **OECD Toolbox**
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
- **TOXNET:** https://toxnet.nlm.nih.gov/
- **IARC:** https://monographs.iarc.fr
- **OECD SIDS:** https://hpvchemicals.oecd.org/ui/Default.aspx
- **EPA ACToR:** https://actor.epa.gov/actor/home.xhtml
- **US EPA HPVIS:** https://ofmpub.epa.gov/oppthpv/public_search.publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&EndPointRpt=Y#submission
- **Japanese NITE:** https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com
- **ChemIDplus:** https://chem.nlm.nih.gov/chemidplus/

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 01/31/20.

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

Explanation of Cramer Classification

Due to potential discrepancies between the current *in silico* tools (Bhatia et al., 2015), the Cramer Class of the target material was determined using expert judgment, based on the Cramer decision tree.

1N,2N,3N,5N,6N,7N,16N,17N, 19N,23N,24N,25N,26Y.

Q1. A normal constituent of the body? No
Q2. Contains functional groups associated with enhanced toxicity? No
Q3. Contains elements other than C, H, O, N, and divalent S? No
Q5. Simply branched aliphatic hydrocarbon or a common carbohydrate? No
Q6. Benzene derivative with certain substituents? No
Q7. Heterocyclic? No
Q16. Common terpene? (see Cramer et al., 1978 for detailed explanation)? No
Q17. Readily hydrolyzed to a common terpene? No
Q19. Open chain? No
Q23. Aromatic? No
Q24. Monocarbocyclic with simple substituents? No
Q25. Cyclopropane (see explanation in Cramer et al., 1978)? No
Q26. Monocycloalkanone or a bicyclo compound? Yes, Intermediate (Class II)

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