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Short Review

RIFM fragrance ingredient safety assessment, 2,6,6-trimethylcyclohexa-1,3-dienyl methanal, CAS Registry Number 116-26-7

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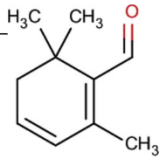
Version: 032619. This version replaces any previous versions.

Name: 2,6,6-trimethylcyclohexa-1,3-dienyl methanal
CAS Registry Number: 116-26-7



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
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., 2015, 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union

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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
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.

2,6,6-Trimethylcyclohexa-1,3-dienyl methanal was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 2,6,6-trimethylcyclohexa-1,3-dienyl methanal is not genotoxic. Data show that 2,6,6-trimethylcyclohexa-1,3-dienyl methanal is a strong skin sensitizer and provided a No Expected Sensitization Induction Level (NESIL) of 29 μg/cm^2 for the skin sensitization endpoint. The repeated dose toxicity, developmental and reproductive toxicity, and local respiratory toxicity endpoints were evaluated using the threshold of toxicological concern (TTC) for a Cramer Class I material, and the exposure to 2,6,6-trimethylcyclohexa-1,3-dienyl methanal is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). The phototoxicity/photoallergenicity endpoints were evaluated based on data and ultraviolet (UV) spectra; 2,6,6-trimethylcyclohexa-1,3-dienyl methanal is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; 2,6,6-trimethylcyclohexa-1,3-dienyl methanal 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, 2010b; RIFM, 2010c)
Repeated Dose Toxicity: No NOAEL available. Exposure is below TTC.
Developmental and Reproductive Toxicity: No NOAEL available. Exposure is below the TTC.
Skin Sensitization: NESIL = 29 μg/cm^2. (RIFM, 2010a; RIFM, 2015a)
Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic. (UV Spectra, RIFM Database; RIFM, 1983; RIFM, 1984b)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:

Persistence: Critical Measured Value: 61% (Day 70) RIFM, (2014a)
Bioaccumulation: Screening-level: 62 L/kg (EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Screening-level: LC50: 49.85 mg/L (RIFM Framework; Salvito et al., 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: LC50: 49.85 mg/L (RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.04985 μg/L

- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: Not Applicable; Cleared atScreening-level

1. Identification

1. **Chemical Name:** 2,6,6-trimethylcyclohexa-1,3-dienyl methanal
2. **CAS Registry Number:** 116-26-7
3. **Synonyms:** 1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-; Dehydro-β-cyclocitral; 2,3-Dihydro-2,2,6-trimethylbenzaldehyde; Safranal; 2,6,6-Trimethyl-1,3-cyclohexadien-1-carboxaldehyde; 1,1,3-Trimethyl-2-formylcyclohexa-2,4-diene; 2,6,6-Trimethyl-1,3-cyclohexadienal; 2,6,6-Trimethylcyclohexa-1,3-diene-1-carbaldehyde; Safranal P; 2,6,6-Trimethylcyclohexa-1,3-dienyl methanal
4. **Molecular Formula:** $C_{10}H_{14}O$
5. **Molecular Weight:** 150.22
6. **RIFM Number:** 6247
7. **Stereochemistry:** No stereocenters present. No stereoisomers possible.

2. Physical data

1. **Boiling Point:** 216.25 °C (EPI Suite)
2. **Flash Point:** 186.00 °F. TCC (85.56 °C)
3. **Log K_{OW}:** Log Pow = 2.7 (RIFM, 2014a), 3.22 (EPI Suite)
4. **Melting Point:** 20.87 °C (EPI Suite)
5. **Water Solubility:** 134.2 mg/L (EPI Suite)
6. **Specific Gravity:** 0.96800 to 0.98000 @ 20.00 °C
7. **Vapor Pressure:** 0.105 mm Hg @ 20 °C (EPI Suite v4.0), 0.07 mm Hg 20 °C (FMA Database), 0.159 mm Hg @ 25 °C (EPI Suite)
8. **UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} cm^{-1})
9. **Appearance/Organoleptic:** A pale yellowish oily liquid with a very powerful, sweet, green-floral and somewhat tobacco-herbaceous odor (Arctander, 1969)

3. Exposure

1. **Volume of Use (worldwide band):** 0.1–1 metric tons per year (IFRA, 2015)
2. **95**th **Percentile Concentration in Hydroalcoholics:** 0.00011% (RIFM, 2015b)
2. **Inhalation Exposure*:** 0.000022 mg/kg/day or 0.0017 mg/day (RIFM, 2015b)
3. **Total Systemic Exposure** **: 0.00023 mg/kg/day (RIFM, 2015b)

*95th percentile calculated exposure derived from concentration survey data in the Creme 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 IV. It is derived from concentration survey data in the Creme RIFM Aggregate Exposure Model and includes exposure via dermal, oral, and inhalation routes whenever the fragrance ingredient is used in products that include these routes of exposure (Comiskey et al., 2015, 2017; Safford et al., 2015, 2017)).

4. Derivation of systemic absorption

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

5. Computational toxicology evaluation

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

Expert Judgment	Toxtree v 2.6	OECD QSAR Toolbox v 3.2
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2. **Analogs** Selected:
   a. **Genotoxicity:** None
   b. **Repeated Dose Toxicity:** None
   c. **Developmental and 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

6. Metabolism

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

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

2,6,6-Trimethylcyclohexa-1,3-dienyl methanal is reported to occur in the following foods by the VCF*:

Capsicum species.
Honey.
Mate (*Ilex paraguayensis*).
Tea.
Citrus fruits.
Rooibos tea (*Aspalathus linearis*).
Sweet grass oil (*Hierochloe odorata*).
Vanilla.
Saffron (Crocus sativus L.)
Lemon balm (*Melissa officinalis* L.)

*VCF Volatile Compounds in Food: Database/Nijssen, L.M.; Ingen-Visscher, C.A. van; Donders, J.J.H. (eds). – Version 15.1 – Zeist (The Netherlands): TNO Triskelion, 1963–2014. A continually updated database containing information on published volatile compounds that have been found in natural (processed) food products. Includes FEMA GRAS and EU-Flavis data.

8. REACH dossier

Dossier available; accessed 02/21/17.

9. Conclusion

The maximum acceptable concentrations[a] in finished products for 2,6,6-trimethylcyclohexa-1,3-dienyl methanal are detailed below.

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)
1	Products applied to the lips (lipstick)	0.0022
2	Products applied to the axillae	0.00066
3	Products applied to the face/body using fingertips	0.013
4	Products related to fine fragrances	0.012
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.0032
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.0032
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.0032
5D	Baby cream, oil, talc	0.0032
6	Products with oral and lip exposure	0.0073
7	Products applied to the hair with some hand contact	0.025
8	Products with significant ano-genital exposure (tampon)	0.0013
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.024
10A	Household care products with mostly hand contact (hand dishwashing detergent)	0.087
10B	Aerosol air freshener	0.087
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.048
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 2,6,6-trimethylcyclohexa-1,3-dienyl methanal, the basis was a predicted skin absorption value of 80%, and a skin sensitization NESIL of 29 μg/cm^2. [b]For a description of the categories, refer to the IFRA RIFM Information Booklet. (www.rifm.org/doc).

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data, 2,6,6-trimethylcyclohexa-1,3-dienyl methanal does not present a concern for genetic toxicity.

10.1.1.1. Risk assessment. 2,6,6-Trimethylcyclohexa-1,3-dienyl methanal was assessed in the BlueScreen assay and found negative for genotoxicity, with and without metabolic activation (RIFM, 2016). The mutagenic activity of 2,6,6-trimethylcyclohexa-1,3-dienyl methanal 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 TA102 were treated with 2,6,6-trimethylcyclohexa-1,3-dienyl methanal in dimethyl sulfoxide (DMSO) at concentrations up to 5000 μg/plate. Statistically significant increases in revertant numbers were observed in strain TA102 (1.3-fold increase maximum) in the absence and presence of S9; however, they were not concentration-related and were of insufficient magnitude to be considered as clear evidence of mutagenic activity. Two additional experiments were performed over the same range of concentrations using both the plate incorporation and preincubation methodologies and did not induce statistically significant increases in revertants. Therefore, the initial increases were not reproducible and were considered to have been due to normal biological variability and not evidence of mutagenic activity. No increases in the mean number of revertant colonies were observed in any other strains in the presence or absence of S9 (RIFM, 2010b). Under the conditions of the study, 2,6,6-trimethylcyclohexa-1,3-dienyl methanal was not mutagenic in the Ames test.

The clastogenic activity of 2,6,6-trimethylcyclohexa-1,3-dienyl methanal 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 for 3 and 24 h with 2,6,6-trimethylcyclohexa-1,3-dienyl methanal in DMSO at concentrations up to 200 μg/mL (limited by toxicity) in the presence and absence of S9 metabolic activation. A single concentration in the 3-h exposure group with metabolic activation treatment induced a statistically significant increase in binucleated cells with micronuclei. However, this was at a concentration that induced 62% cytotoxicity and was the maximum concentration analyzed in this group. Additionally, this increase was small and set against a low mean vehicle control and fell within historical control ranges. 2,6,6-Trimethylcyclohexa-1,3-dienyl methanal did not induce binucleated cells with micronuclei when tested up to cytotoxic levels in any other treatment group in the non-activated or S9-activated test systems (RIFM, 2010c). Under the conditions of the study, 2,6,6-trimethylcyclohexa-1,3-dienyl methanal was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the data available, 2,6,6-trimethylcyclohexa-1,3-dienyl methanal does not present a concern for genotoxic potential.

Additional References: Florin et al., (1980); Abdullaev et al., (2003); Hasseinzadeh and Sadeghnia, (2007).

Literature Search and Risk Assessment Completed On: 2/11/17.

10.1.2. Repeated dose toxicity

There are insufficient repeated dose toxicity data on 2,6,6-trimethylcyclohexa-1,3-dienyl methanal or any read-across materials. The total systemic exposure to 2,6,6-trimethylcyclohexa-1,3-dienyl methanal is below the TTC for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

10.1.2.1. Risk assessment. There are insufficient repeated dose toxicity data on 2,6,6-trimethylcyclohexa-1,3-dienyl methanal or any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to 2,6,6-trimethylcyclohexa-1,3-dienyl methanal (0.23 μg/kg/day) is below the TTC (30 μg/kg/day) 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: 01/06/17.

10.1.3. Developmental and reproductive toxicity

There are insufficient developmental and reproductive toxicity data on 2,6,6-trimethylcyclohexa-1,3-dienyl methanal or any read-across materials. The total systemic exposure to 2,6,6-trimethylcyclohexa-1,3-dienyl methanal is below the TTC for the developmental and reproductive toxicity endpoints of a Cramer Class I material at the current level of use.

10.1.3.1. Risk assessment. There are no developmental or reproductive toxicity data on 2,6,6-trimethylcyclohexa-1,3-dienyl methanal or any read-across materials that can be used to support the developmental or reproductive toxicity endpoints. The total systemic exposure to 2,6,6-trimethylcyclohexa-1,3-dienyl methanal (0.23 μg/kg/day) is below the TTC (30 μg/kg/day) 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: 01/06/17.

10.1.4. Skin sensitization

2,6,6-Trimethylcyclohexa-1,3-dienyl methanal is considered to be a strong skin sensitizer with a defined NESIL of 29 μg/cm^2.

10.1.4.1. Risk assessment. Based on the existing data, 2,6,6-

Table 1
Data Summary for 2,6,6-trimethylcyclohexa-1,3-dienyl methanal.

LLNA weighted mean EC3 value μg/cm^2 [No. Studies]	Potency Classification Based on Animal Data[1]	Human Data			
		NOEL-HRIPT (induction) μg/cm^2	NOEL-HMT (induction) μg/cm^2	LOEL[2] (induction) μg/cm^2	WoE NESIL[3] μg/cm^2
< 250 [1]	Strong	29.5	NA	59	29

NOEL = No observed effect level; HRIPT = Human Repeat Insult Patch Test; HMT = Human Maximization Test; LOEL = lowest observed effect level; NA = Not Available.
[1] Based on animal data using classification defined in ECETOC, Technical Report No. 87, 2003.
[2] Data derived from HRIPT or HMT.
[3] WoE NESIL limited to 2 significant figures.

trimethylcyclohexa-1,3-dienyl methanal presents a concern for skin sensitization. The chemical structure of this material indicates that it would be expected to react with skin proteins (Roberts et al., 2007; Toxtree 2.6.6; OECD toolbox v3.3). The material 2,6,6-trimethylcyclohexa-1,3-dienyl methanal was found to be positive in the *in vitro* Direct Peptide Reactivity Assay (DPRA), KeratinoSens, and U937-CD86 test (Natsch et al., 2013; Piroird et al., 2015). In 2 murine local lymph node assays (LLNAs), 2,6,6-trimethylcyclohexa-1,3-dienyl methanal was found to be sensitizing with an EC3 value of less than 1% (< 250 μg/cm^2; RIFM, 2012) and an EC3 value of 7.5% (1875 μg/cm^2; Gerberick et al., 2005). In a guinea pig maximization test and a Buehler test, the material did present reactions indicative of sensitization in 20/20 animals (RIFM, 1984a). In human repeated insult patch tests (HRIPTs), 2,6,6-trimethylcyclohexa-1,3-dienyl methanal was found to be sensitizing at 59 μg/cm^2 in 3:1 DEP:EtOH in 1/99 subjects (RIFM, 2010a) and at 250 μg/cm^2 in dimethyl phthalate in 5/53 subjects (RIFM, 1996). However, no reactions indicative of sensitization were observed with 29.5 μg/cm^2 in 3:1 EtOH:DEP in 105 subjects (RIFM, 2015a) or at 25 μg/cm^2 in dimethyl phthalate in 54 subjects (RIFM, 1998).

Based on weight of evidence (WoE) from structural analysis and animal and human studies, 2,6,6-trimethylcyclohexa-1,3-dienyl methanal is a strong sensitizer with a WoE NESIL of 29 μg/cm^2 (Table 1). Section IX provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2008; IDEA [International Dialogue for the Evaluation of Allergens] project Final Report on the QRA2: Skin Sensitization Quantitative Risk Assessment for Fragrance Ingredients, September 30, 2016, http://www.ideaproject.info/uploads/Modules/Documents/qra2-dossier-final–september-2016.pdf).

Additional References: McKim et al., (2010); Natsch and Gfeller, (2008).

Literature Search and Risk Assessment Completed On: 02/16/17.

10.1.5. Phototoxicity/photoallergenicity

Based on UV/Vis absorption spectra and available *in vivo* study data, 2,6,6-trimethylcyclohexa-1,3-dienyl methanal would not be expected to present a concern for phototoxicity or photoallergenicity.

10.1.5.1. Risk assessment. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. The corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). Phototoxicity and photoallergenicity of 2,6,6-trimethylcyclohexa-1,3-dienyl methanal were evaluated *in vivo* in guinea pigs, and there were no reactions observed (RIFM, 1983; RIFM, 1984b). Based on UV/Vis absorption spectra and the *in vivo* studies, 2,6,6-trimethylcyclohexa-1,3-dienyl methanal would not be expected to present a concern for phototoxicity or photoallergenicity.

10.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate no significant absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark of concern for phototoxic effects, 1000 L mol^{-1} cm^{-1} (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 02/06/17.

10.1.6. Local Respiratory Toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The exposure level for 2,6,6-trimethylcyclohexa-1,3-dienyl methanal is below the Cramer Class I TTC value for inhalation exposure local effects.

10.1.6.1. Risk assessment. There are no inhalation data available on 2,6,6-trimethylcyclohexa-1,3-dienyl methanal. Based on the Creme RIFM Model, the inhalation exposure is 0.0017 mg/day. This exposure is 824 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: 02/16/17.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of 2,6,6-trimethylcyclohexa-1,3-dienyl methanal was performed following the RIFM Environmental Framework (Salvito et al., 2002) which provides for 3 levels of screening for aquatic risk. In Tier 1, only the material's volume of use in a region, its log K_{ow} and molecular weight are needed to estimate a conservative risk quotient (RQ; Predicted Environmental Concentration/Predicted No Effect Concentration or PEC/PNEC). In Tier 1, a general QSAR for fish toxicity is used with a high uncertainty factor as discussed in Salvito et al. (2002). At Tier 2, the model ECOSAR (US EPA, 2012b) (providing chemical class specific ecotoxicity estimates) is used and a lower uncertainty factor is applied. Finally, if needed, at Tier 3, measured biodegradation and ecotoxicity data are used to refine the RQ (again, with lower uncertainty factors applied to calculate the PNEC). Provided in the table below are the data necessary to calculate both the PEC and the PNEC determined within this Safety Assessment. For the PEC, while the actual regional tonnage is not provided, the range from the most recent IFRA Volume of Use Survey is reported. The PEC is calculated based on the actual tonnage and not the extremes noted for the range. Following the RIFM Environmental Framework, 2,6,6-Trimethylcyclohexa-1,3-dienyl methanal 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 did not identify 2,6,6-Trimethylcyclohexa-1,3-dienyl methanal 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 v4.1). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 11.

10.2.1.1. Risk assessment. Based on current VoU (IFRA, 2015), 2,6,6-trimethylcyclohexa-1,3-dienyl methanal does not present a risk to the aquatic compartment in the screening-level assessment.

10.2.1.2. Key studies

10.2.1.2.1. Biodegradation. RIFM, 2014b: Ready biodegradability of the test material was evaluated according to the OECD 301F method. No biodegradation was observed after 28 days, but biodegradation of

61% was observed when the study was extended to day 70.

10.2.1.2.2. Ecotoxicity. No data available.

10.2.1.3. Other available data. 2,6,6-Trimethylcyclohexa-1,3-dienyl methanal has been registered under REACH with no additional data at this time.

10.2.2. 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)**	49.85			1,000,000	0.04958	

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} used	2.7	2.7
Biodegradation Factor Used	0	0
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 0.04985 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 environmental at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 03/05/19.

11. Literature Search*

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

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 01/22/19.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. RIFM staff are employees of the Research Institute for Fragrance Materials, Inc. (RIFM). The Expert Panel receives a small honorarium for time spent reviewing the subject work.

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