Food and Chemical Toxicology 118 (2018) S147–S155

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

RIFM FRAGRANCE INGREDIENT SAFETY ASSESSMENT, 2,6-Dimethylocta-2,4,6-triene, CAS Registry Number 673-84-7

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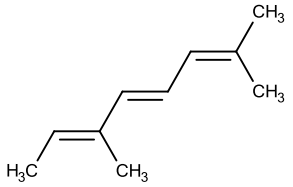
Version: 031618. This version replaces any previous versions.
Name: 2,6-Dimethylocta-2,4,6-triene
CAS Registry Number: 673-84-7
Additional CAS Numbers*:
3016-19-1 2,6-Dimethyl-2,4,6-octatriene
*This material was included in this assessment because it is a mixture of isomers.



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
EU - Europe/European Union

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https://doi.org/10.1016/j.fct.2018.06.048
Received 17 April 2018; Accepted 19 June 2018
Available online 22 June 2018
0278-6915/

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
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 under the limits 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 use of this material under current conditions is supported by existing information.
2,6-Dimethylocta-2,4,6-triene was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analog myrcene (CAS# 123-35-3) show that 2,6-dimethylocta-2,4,6-triene is not expected to be genotoxic. The skin sensitization endpoint was completed using the DST for non-reactive materials (900 μg/cm^2/day). The repeated dose, reproductive, and local respiratory toxicity endpoints were completed using the TTC for a Cramer Class I material (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). The phototoxicity/photoallergenicity endpoint was completed based on UV spectra. The environmental endpoints were evaluated; 2,6-dimethylocta-2,4,6-triene was found not to be PBT as per the IFRA Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., PEC/PNEC), are < 1.

Human Health Safety Assessment
Genotoxicity: Not expected to be genotoxic. (NTP, 2010)
Repeated Dose Toxicity: No NOAEL available. Exposure is below the TTC.
Developmental and 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. (UV Spectra, RIFM DB)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment
Hazard Assessment:
Persistence: Screening-level: 2.89 (BIOWIN 3) (EPI Suite v4.1; US EPA, 2012a)
Bioaccumulation: Screening-level: 604.1 L/kg (EPI Suite v4.1; US EPA, 2012a)
Ecotoxicity: Screening-level: 48-hour *Daphnia magna* LC50: 0.295 mg/L (ECOSAR; US EPA, 2012b)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards
Risk Assessment:
Screening-Level: PEC/PNEC (North America and Europe) > 1 (RIFM Framework; Salvito et al., 2002)

Critical Ecotoxicity Endpoint: 48-hour *Daphnia magna* LC50: 0.295 mg/L (ECOSAR; US EPA, 2012b)
RIFM PNEC is: 0.0295 µg/L
- Revised PEC/PNECs (2015 IFRA VoU): North America and Europe < 1

1. Identification

Chemical Name: 2,6-Dimethylocta-2,4,6-triene	**Chemical Name:** 2,6-Dimethyl-2,4,6-octatriene
CAS Registry Number: 673-84-7	**CAS Registry Number:** 3016-19-1
Synonyms: Allo-Ocimene (isomer unspecified); 2,4,6-Octatriene, 2,6-dimethyl-; 2,6-Dimethylocta-2,4,6-triene	**Synonyms:** (E,E)-2,6-Dimethylocta-2,4,6-triene; trans,*trans*-2,6-Dimethyl-2,4,6-octatriene; trans,*trans*-Alloocimene; 2,4,6-Octatriene, 2,6-dimethyl-, (E,E)-; 2,6-Dimethyl-2,4,6-octatriene; 2,6-Dimethylocta-2,4,6-triene; Allo-ocimene (E,E)
Molecular Formula: $C_{10}H_{16}$	**Molecular Formula:** $C_{10}H_{16}$
Molecular Weight: 136.38	**Molecular Weight:** 150.27
RIFM Number: 5208	**RIFM Number:** 1155
Stereochemistry: Isomer not specified. Two stereocenters and 4 total stereoisomers possible.	

2. Physical data

CAS# 673-84-7	CAS# 3016-19-1
Boiling Point: 172.65 °C (US EPA, 2012a)	**Boiling Point:** 172.65 °C (US EPA, 2012a)
Flash Point: 163.00 °F TCC (72.78 °C)*	**Flash Point:** 174 °F; CC (FMA), 79 °C (GHS)
Log K_{ow}: 4.72 (EPI Suite)	**Log K_{ow}:** 4.72 (US EPA, 2012a)
Melting Point: −63.36 °C (US EPA, 2012a)	**Melting Point:** −63.36 °C (US EPA, 2012a)
Water Solubility: 2.348 mg/L (US EPA, 2012a)	**Water Solubility:** Not Available
Specific Gravity: 0.80900 to 0.81500 @ 25.00 °C*	**Specific Gravity:** 0.809 (FMA)
Vapor Pressure: 0.638 mm Hg @ 20 °C (US EPA, 2012a), 0.911 mm Hg @ 25 °C (US EPA, 2012a)	**Vapor Pressure:** 0.911 mm Hg @ 25 °C (US EPA, 2012a), 0.638 mm Hg @ 20 °C (US EPA, 2012a)
UV Spectra: No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark ($1000\ L\ mol^{-1} \cdot cm^{-1}$)	
Appearance/Organoleptic: A colorless to pale, yellow, clear, liquid with a dry, woody, chrysanthemum, phenolic, powdery, and orrisl odor.*	**Appearance/Organoleptic:** A colorless to greenish yellow clear liquid with a sweet, fresh, and floral.**

*http://www.thegoodscentscompany.com/data/rw1043231.html, retrieved 09/13/17.

**http://www.thegoodscentscompany.com/data/rw1020231.html, retrieved 09/13/17.

3. Exposure***

1. **Volume of Use (worldwide band):** < 1 metric tons per year (IFRA, 2015)
2. **95th Percentile Concentration in Hydroalcoholics:** 0.014% (RIFM, 2016)
3. **Inhalation Exposure*:** 0.000022 mg/kg/day or 0.0016 mg/day (RIFM, 2016)
4. **Total Systemic Exposure**:** 0.00036 mg/kg/day (RIFM, 2016)

*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).

***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 hydroalcoholics, inhalation exposure, and total exposure.

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
I	I	I

2. **Analogs Selected:**
   a. **Genotoxicity:** Myrcene (CAS # 123-35-3)
   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: See Appendix below

6. Metabolism

No relevant data available for inclusion in this safety assessment.

7. NATURAL OCCURRENCE (discrete chemical) or COMPOSITION (NCS)

2,6-Dimethylocta-2,4,6-triene is reported to occur in the following foods* and in some natural complex substances (NCS):

Black currants (*Ribes nigrum* L.)
Calamus (sweet flag) (*Acorus calamus* L.)
Celery (*Apium graveolens* L.)
Cherimoya (*Annona cherimolia Mill.*)
Citrus fruits.
Ginger (Zingiber species)
Guava and feyoa.
Mangifera species.
Mastic (*Pistacia lentiscus*)
Oats (*Avena sativa* L.)
Ocimum species.
Olive (*Olea europaea*)
Passion fruit (*Passiflora species*)
Piper betle L. cultivars.
Salvia species.
Starfruit (*Averrhoa carambola* L.)
Wormwood oil (*Artemisia absinthium* L.)

2,6-Dimethyl-2,4,6-octatriene is reported to occur in the following foods* and in some natural complex substances (NCS):

Guava and feyoa	Ocimum species
Litchi (*Litchi chinensis sonn.*)	Olive (*Olea europaea*)
Lovage (*Levisticum officinale koch*)	Salvia species
Mangifera species	Tarragon (*Artemisia dracunculus 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. IFRA standard

None.

9. REACH dossier

Pre-registered for 2010; No dossier available as of 03/12/18.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data, 2,6-dimethylocta-2,4,6-triene does not present a concern for genetic toxicity.

10.1.1.1. Risk assessment. 2,6-Dimethylocta-2,4,6-triene was tested in the BlueScreen assay and was found negative for genotoxicity in the presence and absence of metabolic activation (RIFM, 2013). There are no studies assessing the mutagenic activity of 2,6-dimethylocta-2,4,6-triene; however, read-across can be made to myrcene (CAS # 123-35-3; see Section V). The mutagenic activity of myrcene has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and OECD TG 471 using the preincubation method. *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and *Escherichia coli* strain WP2uvrA were treated with myrcene at concentrations up to 10,000 μg/plate. No increases in the mean number of revertant colonies were observed at any tested dose in the presence or absence of S9 (NTP, 2010). Under the conditions of the study, myrcene was not mutagenic in the Ames test, and this can be extended to 2,6-dimethylocta-2,4,6-triene.

There are no studies assessing the clastogenic activity of 2,6-dimethylocta-2,4,6-triene; however, read-across can be made to myrcene (CAS # 123-35-3; see Section V). The clastogenic activity of myrcene was evaluated in an *in vivo* micronucleus test conducted in compliance with GLP regulations and with NTP guidelines. The test material was administered in doses of 250, 500, 1000, or 2000 mg/kg/bw to groups of male and female B6C3F1 mice in corn oil via gavage. Mice from each dose level were euthanized at the end of the 14-week study 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 (NTP, 2010). Under the conditions of the study, myrcene was considered to be not clastogenic in the *in vivo* micronucleus test.

Based on the available data, myrcene does not present a concern for genotoxic potential and this can be extended to 2,6-dimethylocta-2,4,6-triene.

Additional References: Kauderer et al., 1991; Roscheisen et al., 1991; Zamith et al., 1993; Gomes-Carneiro et al., 2005; Mitic-Culafic et al., 2009.

Literature Search and Risk Assessment Completed On: 08/31/2017.

10.1.2. Repeated dose toxicity

There are insufficient repeated dose toxicity data on 2,6-dimethylocta-2,4,6-triene or any read-across materials. The total systemic exposure to 2,6-dimethylocta-2,4,6-triene 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 no repeated dose toxicity data on 2,6-dimethylocta-2,4,6-triene or any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to 2,6-dimethylocta-2,4,6-triene (0.36 μg/kg bw/day) is below the TTC (30 μg/kg bw/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: 09/10/17.

10.1.3. Developmental and reproductive toxicity

There are insufficient developmental and reproductive toxicity data on 2,6-dimethylocta-2,4,6-triene or any read-across materials. The total systemic exposure to 2,6-dimethylocta-2,4,6-triene 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-dimethylocta-2,4,6-triene or any read-across materials that can be used to support the developmental or reproductive toxicity endpoints. The total systemic exposure to 2,6-dimethylocta-2,4,6-triene (0.36 μg/kg bw/day) is below the TTC

Table 1
Acceptable concentrations limits for 2,6-dimethylocta-2,4,6-triene based on non-reactive DST.

IFRA Category[a]	Description of Product Type	Acceptable Concentrations in Finished Products	Reported 95th Percentile Use Concentrations in Finished Products
1	Products applied to the lips	0.069%	0.00%[b]
2	Products applied to the axillae	0.021%	0.00%[b]
3	Products applied to the face using fingertips	0.41%	0.00%[b]
4	Fine fragrance products	0.39%	0.01%
5	Products applied to the face and body using the hands (palms), primarily leave-on	0.10%	0.00%[b]
6	Products with oral and lip exposure	0.23%	0.00%[b]
7	Products applied to the hair with some hand contact	0.79%	0.00%[b]
8	Products with significant ano-genital exposure	0.04%	0.00%[b]
9	Products with body and hand exposure, primarily rinse-off	0.75%	No Data
10	Household care products with mostly hand contact	2.70%	0.00%[b]
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate	1.50%	No Data
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted	0.14%

Note.
[a] For a description of the categories, refer to the IFRA/RIFM Information Booklet.
[b] Negligible exposure (< 0.01%).

(30 μg/kg bw/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: 09/10/17.

10.1.4. Skin sensitization

Based on the application of DST, 2,6-dimethylocta-2,4,6-triene does not present a safety concern for skin sensitization under the current, declared levels of use.

10.1.4.1. Risk assessment. The chemical structure of this material indicates that it would not be expected to react with skin proteins (Roberts et al., 2007; Toxtree 2.6.13; OECD toolbox v3.4). No predictive skin sensitization studies are available for 2,6-dimethylocta-2,4,6-triene or read-across materials. However, in a human maximization test, no skin sensitization reactions were observed (RIFM, 1982). Acting conservatively, due to the insufficient data, the reported exposure was benchmarked utilizing the non-reactive Dermal Sensitization Threshold (DST) of 900 μg/cm^2. The current exposure from the 95th percentile concentration is below the DST for non-reactive materials when evaluated in all QRA categories. Table 1 provides the acceptable concentration for 2,6-dimethylocta-2,4,6-triene, which presents no appreciable risk for skin sensitization based on the non-reactive DST.

Additional References: None.

Literature Search and Risk Assessment Completed On: 09/13/17.

10.1.5. Phototoxicity/photoallergenicity

Based on UV/Vis absorption spectra, 2,6-dimethylocta-2,4,6-triene does not present a concern for phototoxicity or photoallergenicity.

10.1.5.1. Risk assessment. There are no phototoxicity data available for 2,6-dimethylocta-2,4,6-triene. UV/Vis spectra were obtained for 2,6-dimethylocta-2,4,6-triene and indicate minor absorbance in the critical range of 290–700 nm. Molar absorption coefficient is below the benchmark of concern for phototoxicity (Henry et al., 2009). Based on the lack of absorbance in the critical range, 2,6-dimethylocta-2,4,6-triene does not present a concern for phototoxicity or photoallergenicity.

10.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained for 2,6-dimethylocta-2,4,6-triene. The spectra demonstrate minor absorbance between 290 and 700 nm. Molar absorption coefficient for λ max within that range is below the benchmark of concern for phototoxic effect, 1000 L · mol-^{1} · cm^{-1} (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 08/17/17.

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-dimethylocta-2,4,6-triene 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-dimethylocta-2,4,6-triene. Based on the Creme RIFM Model, the inhalation exposure is 0.0016 mg/day. This exposure is 875 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: 09/11/2017.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of 2,6-dimethylocta-2,4,6-triene 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, 2,6-dimethylocta-2,4,6-triene was identified as a fragrance material with 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 v4.1 did not identify 2,6-dimethylocta-2,4,6-triene as possibly being either 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 1.

10.2.2. Risk assessment

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

10.2.2.1. Key studies. ***Biodegradation:*** No data available.

Ecotoxicity: No data available.

10.2.2.2. Other available data. 2,6-Dimethylocta-2,4,6-triene has been pre-registered for REACH with no additional data at this time.

10.2.3. 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.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} used	4.72	4.72
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	< 1	< 1
Risk Characterization: PEC/PNEC	**< 1**	**< 1**

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

The RIFM PNEC is 0.0295 µg/L. The revised PEC/PNECs for EU and NA < 1, therefore does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 9/11/17.

11. Literature Search*

- **RIFM Database:** Target, Fragrance Structure Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** http://echa.europa.eu/
- **NTP:** https://ntp.niehs.nih.gov/
- OECD Toolbox
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf

- **PubMed:** http://www.ncbi.nlm.nih.gov/pubmed
- **TOXNET:** http://toxnet.nlm.nih.gov/
- **IARC:** http://monographs.iarc.fr
- **OECD SIDS:** http://webnet.oecd.org/hpv/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:** http://www.safe.nite.go.jp/english/db.html
- **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.

		(mg/L)	(mg/L)			
RIFM Framework Screening-Level **(Tier 1)**	0.791			1,000,000	0.000791	
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	0.404	0.295	0.620	10,000	0.0295	Neutral Organics

Conflicts of interest

The authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fct.2018.06.048.

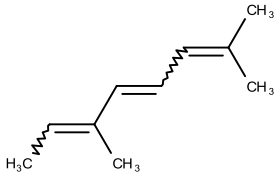
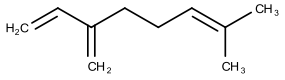
Appendix

Read-across Justification

Methods

The read-across analogs were identified following the strategy for structuring and reporting a read-across prediction of toxicity described in Schultz et al. (2015). The strategy is also 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, 2016).

- First, materials were clustered based on their structural similarity. Second, data availability and data quality on the selected cluster was 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 substance 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, and oncologic classification predictions were generated using OECD QSAR Toolbox v3.4 (OECD, 2012).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v3.4 (OECD, 2012).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010), and skin sensitization was predicted using Toxtree 2.6.13.
- Protein binding was predicted using OECD QSAR Toolbox v3.4 (OECD, 2012).
- The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox v3.4 (OECD, 2012).

	Target Material	Read-across Material
Principal Name	2,6-Dimethylocta-2,4,6-triene	Myrcene
CAS No.	**673–84–7** and 3016-19-1 (mixture)	123-35-3
Structure		
Similarity (Tanimoto Score)		0.78
Read-across Endpoint		• Genotoxicity
Molecular Formula	$C_{10}H_{16}$	$C_{10}H_{16}$
Molecular Weight	136.24	136.24
Melting Point (°C, EPI Suite)	−63.36	−64.83
Boiling Point (°C, EPI Suite)	172.65	156.22
Vapor Pressure (Pa @ 25 °C, EPI Suite)	122	320
Log Kow (KOWWIN v1.68 in EPI Suite)	4.72	4.17
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPI Suite)	2.348	5.6
J_{max} (mg/cm^2/h, SAM)	20.672	1.094
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	6.01E+004	5.30E+004
Genotoxicity		
DNA Binding (OASIS v1.4, QSAR Toolbox v3.4)	• No alert found	• No alert found
DNA Binding (OECD QSAR Toolbox v3.4)	• No alert found	• No alert found
Carcinogenicity (ISS)	• Non-carcinogen (low reliability)	• Non-carcinogen (low reliability)
DNA Binding (Ames, MN, CA, OASIS v1.1)	• No alert found	• No alert found
In Vitro Mutagenicity (Ames, ISS)	• No alert found	• No alert found

In Vivo Mutagenicity (Micronucleus, ISS)	• No alert found	• No alert found
Oncologic Classification	• Not classified	• Not classified
Metabolism		
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v3.4)	See Supplemental Data 1	See Supplemental Data 2

Summary

There are insufficient toxicity data on 2,6-dimethylocta-2,4,6-triene (CAS # 673-84-7). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, metabolism, physical–chemical properties, and expert judgment, myrcene (CAS # 123-35-3) was identified as a read-across material with sufficient data for toxicological evaluation.

Conclusions

- Myrcene (CAS # 123-35-3) was used as a read-across analog for the target material 2,6-dimethylocta-2,4,6-triene (CAS # 673-84-7) for the genotoxicity endpoint.
  - o The target substance and the read-across analog are structurally similar and belong to the class of aliphatic hydrocarbons.
  - o The target substance and the read-across analog share a triene structure.
  - o The key difference between the target substance and the read-across analog is that the target substance has 3 vinylene groups, while the read-across analog has 2 vinyl and 1 vinylene groups. This structural difference is toxicologically insignificant.
  - o Similarity between the target substance and the read-across analog is indicated by the Tanimoto score. The Tanimoto score is mainly driven by the triene structure. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The physical–chemical properties of the target substance and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - o Differences are predicted for J_{max}, which estimates skin absorption. $J_{max} \leq 80\%$ for the target substance and $\leq 40\%$ for the read-across analog. While the percentage of skin absorption estimated from J_{max} indicates exposure to the substance, it does not represent hazard or toxicity. This parameter provides context to assess the impact of bioavailability on toxicity comparisons between the materials evaluated.
  - o According to the OECD QSAR Toolbox v3.4, structural alerts for toxicological endpoints are consistent between the target substance and the read-across analog.
  - o The target substance and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - o The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

References

Api, A.M., Belsito, D., Bruze, M., Cadby, P., Calow, P., Dagli, M.L., Dekant, W., Ellis, G., Fryer, A.D., Fukayama, M., Griem, P., Hickey, C., Kromidas, L., Lalko, J.F., Liebler, D.C., Miyachi, Y., Politano, V.T., Renskers, K., Ritacco, G., Salvito, D., Schultz, T.W., Sipes, I.G., Smith, B., Vitale, D., Wilcox, D.K., 2015. Criteria for the Research Institute for fragrance materials, Inc. (RIFM) safety evaluation process for fragrance ingredients. Food Chem. Toxicol. 82, S1–S19.

Carthew, P., Clapp, C., Gutsell, S., 2009. Exposure based waiving: the application of the toxicological threshold of concern (TTC) to inhalation exposure for aerosol ingredients in consumer products. Food Chem. Toxicol. 47 (6), 1287–1295.

Cassano, A., Manganaro, A., Martin, T., Young, D., Piclin, N., Pintore, M., Bigoni, D., Benfenati, E., 2010. CAESAR models for developmental toxicity. Chem. Cent. J. 4 (Suppl. 1), S4.

Comiskey, D., Api, A.M., Barratt, C., Daly, E.J., Ellis, G., McNamara, C., O'Mahony, C., Robison, S.H., Safford, B., Smith, B., Tozer, S., 2015. Novel database for exposure to fragrance ingredients in cosmetics and personal care products. Regul. Toxicol. Pharmacol. 72 (3), 660–672.

Comiskey, D., Api, A.M., Barrett, C., Ellis, G., McNamara, C., O'Mahony, C., Robison, S.H., Rose, J., Safford, B., Smith, B., Tozer, S., 2017. Integrating habits and practices data for soaps, cosmetics and air care products into an existing aggregate exposure model. Regul. Toxicol. Pharmacol. 88, 144–156.

ECHA, 2012. Guidance on information requirements and chemical safety assessment Chapter R.11: PBT Assessment, November 2012 v1.1. http://echa.europa.eu/.

ECHA, 2016. Read-across assessment framework (RAAF). Retrieved from. www.echa.europa.eu/documents/10162/13628/raaf_en.pdf.

Gomes-Carneiro, M.R., Viana, M.E.S., Felzenszwalb, I., Paumgartten, F.J.R., 2005. Evaluation of beta-myrcene, alpha-terpinene and (+)- and (-)-alpha-pinene in the Salmonella/microsome assay. Food Chem. Toxicol. 43 (2), 247–252.

Henry, B., Foti, C., Alsante, K., 2009. Can light absorption and photostability data be used to assess the photosafety risks in patients for a new drug molecule? J. Photochem. Photobiol. B Biol. 96 (1), 57–62.

IFRA (International Fragrance Association), 2015. Volume of Use Survey, February 2015.

Kauderer, B., Zamith, H., Paumgartten, F.J.R., Speit, G., 1991. Evaluation of the mutagenicity of beta-myrcene in mammalian cells in vitro. Environ. Mol. Mutagen. 18 (1), 28–34.

Kroes, R., Renwick, A.G., Feron, V., Galli, C.L., Gibney, M., Greim, H., Guy, R.H., Lhuguenot, J.C., van de Sandt, J.J.M., 2007. Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients. Food Chem. Toxicol. 45 (12), 2533–2562.

Laufersweiler, M.C., Gadagbui, B., Baskerville-Abraham, I.M., Maier, A., Willis, A., et al., 2012. Correlation of chemical structure with reproductive and developmental toxicity as it relates to the use of the threshold of toxicological concern. Regul. Toxicol. Pharmacol. 62 (1), 160–182.

Mitic-Culafic, D., Zegura, B., Nikolic, B., Vukovic-Gacic, B., Knezevic-Vukcevic, J., Filipic, M., 2009. Protective effect of linalool, myrcene and eucalyptol against t-butyl hydroperoxide induced genotoxicity in bacteria and cultured human cells. Food Chem. Toxicol. 47 (1), 260–266.

National Toxicology Program, 2010. Toxicology and Carcinogenesis Studies of Beta-myrcene (CAS No. 123-35-3) in F344/N Rats and B6C3F1 Mice (Gavage Studies). NTP TR 557. Unpublished.

OECD, 2012. The OECD QSAR Toolbox, v3.4. Retrieved from. http://www.qsartoolbox.org/.

OECD, 2015. Guidance document on the reporting of integrated Approaches to testing and assessment (IATA). ENV/JM/HA(2015)7. Retrieved from. http://www.oecd.org/.

RIFM (Research Institute for Fragrance Materials, Inc), 1982. Report on Human Maximization Studies. Report to RIFM. RIFM report number 1643. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2013. Report on the Testing of 2,6-dimethylocta-2,4,6-triene in the BlueScreen HC Assay (-/+ S9 Metabolic Activation). RIFM report number 66150. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2016. Exposure Survey 10, March 2016.

Roberts, D.W., Patlewicz, G., Kern, P.S., Gerberick, F., Kimber, I., Dearman, R.J., Ryan, C.A., Basketter, D.A., Aptula, A.O., 2007. Mechanistic applicability domain classification of a local lymph node assay dataset for skin sensitization. Chem. Res. Toxicol. 20 (7), 1019–1030.

Rogers, D., Hahn, M., 2010. Extended-connectivity fingerprints. J. Chem. Inf. Model. 50 (5), 742–754.

Roscheisen, C., Zamith, H., Paumgartten, F.J.R., Speit, G., 1991. Influence of B-myrcene on sister-chromatid exchanges induced by mutagens in V79 and HTC cells. Mutat. Res. Lett. 264 (1), 43–49.

Safford, B., Api, A.M., Barratt, C., Comiskey, D., Daly, E.J., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Smith, B., Thomas, R., Tozer, S., 2015. Use of an aggregate exposure model to estimate consumer exposure to fragrance ingredients in personal care and cosmetic products. Regul. Toxicol. Pharmacol. 72, 673–682.

Safford, B., Api, A.M., Barratt, C., Comiskey, D., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Rose, J., Smith, B., Tozer, S., 2017. Application of the expanded Creme RIFM consumer exposure model to fragrance ingredients in cosmetic, personal care and air care products. Regul. Toxicol. Pharmacol. 86, 148–156.

Salvito, D.T., Senna, R.J., Federle, T.W., 2002. A Framework for prioritizing fragrance materials for aquatic risk assessment. Environ. Toxicol. Chem. 21 (6), 1301–1308.
Schultz, T.W., Amcoff, P., Berggren, E., Gautier, F., Klaric, M., Knight, D.J., Mahony, C., Schwarz, M., White, A., Cronin, M.T.D., 2015. A strategy for structuring and reporting a read-across prediction of toxicity. Regul. Toxicol. Pharmacol. 72 (3), 586–601.
Shen, J., Kromidas, L., Schultz, T., Bhatia, S., 2014. An *in silico* skin absorption model for fragrance materials. Food Chem. Toxicol. 74 (12), 164–176.
US EPA, 2012a. Estimation Programs Interface Suite for Microsoft Windows, v4.0–v4.11. United States Environmental Protection Agency, Washington, DC, USA.
US EPA, 2012b. The ECOSAR (ECOlogical Structure Activity Relationship) Class Program for Microsoft Windows, v1.11. United States Environmental Protection Agency, Washington, DC, USA.
Zamith, H.P.S., Vidal, M.N.P., Speit, G., Paumgartten, F.J.R., 1993. Absence of genotoxic activity of beta-myrcene in the in vivo cytogenetic bone marrow assay. Braz. J. Med. Biol. Res. 26, 93–98.