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Short Review

RIFM fragrance ingredient safety assessment, δ-3-carene, CAS Registry Number 13466-78-9

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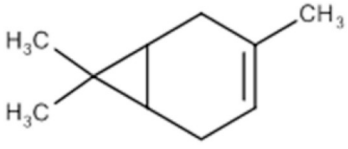
Version: 091218. This version replaces any previous versions.
Name: δ-3-Carene
CAS Registry Number: 13466-78-9



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
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observable Effect Level

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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.

δ-3-Carene was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analog α-pinene (CAS # 80-56-8) show that δ-3-carene is not expected to be genotoxic. The repeated dose and reproductive toxicity endpoints were evaluated using the TTC for a Cramer Class I material, and the exposure to δ-3-carene is below the TTC (0.03 mg/kg/day for both endpoints). The skin sensitization endpoint was completed using data from δ-3-carene and the application of DST for reactive materials (64 μg/cm^2); exposure is below the DST. The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; δ-3-carene is not expected to be phototoxic/photoallergenic. For the local respiratory endpoint, a calculated MOE > 100 was provided by the read-across analog *d*-limonene (CAS # 5989-27-5). The environmental endpoints were evaluated; δ-3-carene 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, 2016; ECHA Reach Dossier: Pin-2(3)-ene)
Repeated Dose Toxicity: No NOAEL available. Exposure is below the TTC.
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 expected to be phototoxic/photoallergenic. (UV Spectra, RIFM Database)
Local Respiratory Toxicity: NOEC = 54.3 mg/m^3. RIFM (2013)

Environmental Safety Assessment

Hazard Assessment:

Persistence: Screening-level: 2.68 (BIOWIN 3) (EPI Suite v4.11; US EPA, 2012a)
Bioaccumulation: Screening-level: 360 L/kg (EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Screening-level: 48-h *Daphnia magna* LC50: 0.366 mg/L (ECOSAR; US EPA, 2012b)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:

Screening-level: PEC/PNEC (North America and Europe) > 1 (RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: 48-h *Daphnia magna* LC50: 0.366 mg/L (ECOSAR; US EPA, 2012b)
RIFM PNEC is: 0.0366 μg/L

- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: Not applicable; cleared at screening-level

1. Identification

1. **Chemical Name:** δ-3-Carene
2. **CAS Registry Number:** 13466-78-9
3. **Synonyms:** Bicyclo[4.1.0]hept-3-ene, 3,7,7-trimethyl-; Isodiprene; 3,7,7-Trimethylbicyclo-[4.1.0]hept-3-ene; 3-Carene; Car-3-ene; 4,7,7-Trimethyl-3-norcarene; 3,7,7-Trimethylbicyclo[4.1.0]-3-heptene; 3,7,7-Trimethylbicyclo[4.1.0]heptene-3; 2-(又は3-) カレン; 3,7,7-Trimethylbicyclo[4.1.0]hept-3-ene; δ-3-Carene
4. **Molecular Formula:** $C_{10}H_{16}$
5. **Molecular Weight:** 136.24
6. **RIFM Number:** 288
7. **Stereochemistry:** No isomeric center and no isomers possible.

2. Physical data

1. **Boiling Point:** 157.25 °C (EPI Suite)
2. **Flash Point:** 47 °C (GHS), 108 °F; CC (FMA Database)
3. **Log K_{ow}:** @ pH 2.0: 4.5, 5.2, 5.3, 5.5; @ pH 7.5: 4.6,5.2,5.3,5.5 (RIFM, 1993), 4.61 (EPI Suite)
4. **Melting Point**: −13.62 °C (EPI Suite)
5. **Water Solubility:** 4.581 mg/L (EPI Suite)
6. **Specific Gravity:** 0.865 (FMA Database)
7. **Vapor Pressure:** 1.5 mm Hg @ 20 °C (EPI Suite v4.0), 2.0 mm Hg 20 °C (FMA Database), 2.09 mm Hg @ 25 °C (EPI Suite)
8. **UV Spectra:** No significant absorbance between 290 and 700 nm; the molar absorption coefficient is below the benchmark

($1000\ L\ mol^{-1} \cdot cm^{-1}$)
9. **Appearance/Organoleptic:** A colorless mobile liquid with a sweet, diffusive, and penetrating odor (Arctander, 1969)

3. Exposure

1. **Volume of Use (worldwide band):** 10–100 metric tons per year (IFRA, 2015)
2. **95**th **Percentile Concentration in Hydroalcoholics:** 0.014% (RIFM, 2016)
3. **Inhalation Exposure*:** 0.000019 mg/kg/day or 0.0014 mg/day (RIFM, 2016)
4. **Total Systemic Exposure**:** 0.00031 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; Safford et al., 2015a; Safford et al., 2017; and Comiskey et al., 2017).

**95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section 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; Safford et al., 2015a; Safford et al., 2017 and Comiskey et al., 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
I	I	I

2. Analogs Selected:
   a. **Genotoxicity:** α-Pinene (CAS # 80-56-8)
   b. **Repeated Dose Toxicity:** None
   c. **Reproductive Toxicity:** None
   d. **Skin Sensitization:** None
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** *d*-Limonene (CAS # 5989-27-5)
   g. **Environmental Toxicity:** None
3. Read-across Justification: See Appendix below

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)

δ-3-Carene is reported to occur in the following foods by the VCF*:
Alpinia species.
Angelica (*Angelica archangelica* L.)
Anise (*Pimpinella anisum* L.)
Ashanti pepper (*Piper guineense* Schum and Thom).
Aubergine, eggplant (*Solanum melongena* L.)
Banana (*Musa sapientum* L.)
Beli, bael (*Aegle marmelos* Correa).
Black currants (*Ribes nigrum* L.)
Capers (*Capparis spinoza*).
Capsicum species.
Caraway (*Carum carvi* L.)
Cardamom (*Ellettaria cardamomum* Maton.)
Carrot seed.
Cashew apple (*Anacardium occidentale*).
Celery (*Apium graveolens* L.)
Chestnut (*Castanea species*).
Cinnamomum species.
Citrus fruits.
Coriander seed (*Coriandrum sativum* L.)
Cumin seed (*Cuminum cyminum* L.)
Curcuma species.
Curry (*Bergera koenigii* L.)
Date (*Phoenix dactylifera* L.)
Dill (*Anethum* species).
Fennel (*Foeniculum vulg.,* ssp. *capillaceum;* var.)
Filbert, hazelnut (*Corylus avellano*).
Gin.
Ginger (*Zingiber* species).
Grape (*Vitis* species).
Grape brandy.
Guava and feyoa
Honey.
Hop (*Humulus lupulus*).
Juniperus communis.
Kiwifruit (*Actinidia chinensis*, syn. *A. deliciosa*).
Laurel (*Laurus nobilis* L.)
Lemon grass oil (*Cymbopogon*).
Loquat (*Eriobotrya japonica* Lindl.)
Lovage (*Levisticum officinale* Koch).
Mace (*Myristica fragrans Houttuyn*).
Mangifera species.
Mastic (*Pistacia lentiscus*).
Milk and milk products.
Mountain papaya (*C. candamarcensis, C. pubescens*).
Myrtle (*Myrtus communis* L.)
Nutmeg (*Myristica fragrans Houttuyn*).
Oats (*Avena sativa* L.)
Ocimum species.
Olive (*Olea europaea*).
Origanum (Spanish) (Cor*idothymus cap.*(L.) *Rchb.*)
Parsley (*Petroselium* species).
Passion fruit (*passiflora* species).
Pepper (Piper nigrum L.)
Pimento (allspice) (*Pimenta dioica* L. Merr.)
Pistachio oil (*Pistacia vera*).
Pistacia atlantica
Pistacia palaestina (*Pistacia terebinthus* L.)
Pork.
Potato (*Solanum tuberosum* L.)
Quince, marmelo (*Cydonia oblonga* Mill.)
Radish (*Raphanus sativus* L.)
Rice (*Oryza sativa* L.)
Rosemary (*Rosmarinus officinalis* L.)
Sapodilla fruit (*Achras sapota* L.)
Satureja species.
Soybean (*Glycine max.* L. Merr.)
Star anise.
Strawberry (*Fragaria* species).
Sweet marjoram (*Origanum majorana* L.)
Tea.
Thyme (*Thymus* species).

Tomato (*Lycopersicon esculentum* Mill.)
Udo (*Aralia cordata* Thunb.)
Vaccinium species.
Walnut (*Juglans* species).
Wild marjoram (*Origanum vulgare* L.)
Wine.

*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

Available; accessed 04/24/2018.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the currently existing data, δ-3-carene does not present a concern for genotoxicity.

10.1.1.1. Risk assessment. There are no studies assessing the mutagenic activity of δ-3-carene; however, read-across can be made to α-pinene (CAS # 80-56-8; see Section V). The mutagenic activity of α-pinene has been evaluated in a bacterial reverse mutation assay conducted according to GLP guidelines by the National Toxicology Program (NTP) using the standard plate incorporation method. *Salmonella typhimurium* strains TA98, TA100, and *Escherichia coli* strain WP2uvrA were treated with α-pinene in buffer at concentrations up to 10000 μg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 metabolic activation (NTP, 2016). Under the conditions of the study, α-pinene was not mutagenic in the Ames test. Since this study did not cover a full range of bacterial strains, additional studies were considered as weight of evidence. α-Pinene was negative in an Ames test using *S. typhimurium* strains TA98, TA100, TA1535, TA1538, and TA1537 at concentrations up to 2.5 mg/mL in the presence and absence of metabolic activation S9 mix (Heck et al., 1989). Additionally, α-pinene was negative in *S. typhimurium* strains TA98 and TA100 when tested at concentrations ranging from 10 to 500 μg/plate with or without S9 metabolic activation in a non-GLP study (Connor et al., 1985). A modified Ames test with α-pinene was negative in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 at concentrations up to 3 μM with or without S9 metabolic activation (Florin et al., 1980).

The clastogenic activity of α-pinene was evaluated in an *in vitro* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes were treated with α-pinene in dimethyl sulfoxide (DMSO) at concentrations up to 2500 μg/mL for the dose range finding (DRF) study. Micronuclei analysis in the main study was conducted up to 250 μg/mL in the presence and absence of metabolic activation (S9) for 3 h and in the absence of metabolic activation for 20 h α-Pinene did not induce binucleated cells with micronuclei when tested up to cytotoxic concentrations in either the presence or absence of an S9 activation system (ECHA Reach Dossier: Pin-2(3)-ene). Under the conditions of the study, α-pinene was considered to be non-clastogenic in the *in vitro* micronucleus test.

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/24/18.

10.1.2. Repeated dose toxicity

There are insufficient repeated dose toxicity data on δ-3-carene or on any read-across materials. The total systemic exposure to δ-3-carene 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 δ-3-carene or on any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to δ-3-carene (0.31 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: 05/01/18.

10.1.3. Reproductive toxicity

There are insufficient reproductive toxicity data on δ-3-carene or on any read-across materials. The total systemic exposure to δ-3-carene is below the TTC for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

10.1.3.1. Risk assessment. There are no reproductive toxicity data on δ-3-carene or on any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to δ-3-carene (0.31 μg/kg bw/day) is below the TTC (30 μg/kg bw/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: 05/01/18.

10.1.4. Skin sensitization

Based on existing data and the application of DST, δ-3-carene 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). However, in a guinea pig maximization test, δ-3-carene was a weak sensitizer (Shell Oil Company, 1992). In a human maximization test, no skin sensitization reactions were observed (RIFM, 1972). Acting conservatively, due to the positive data, the material is considered reactive, and the reported exposure was benchmarked utilizing the reactive DST of 64 μg/cm^2 (Safford, 2008; Safford et al., 2011; Safford et al., 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 1 provides the maximum acceptable concentrations for δ-3-carene that present no appreciable risk for skin sensitization based on the reactive DST. These concentrations are not limits; they represent maximum acceptable concentrations based on the DST approach.

Additional References: None.

Literature Search and Risk Assessment Completed On: 08/28/2018.

10.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, δ-3-carene would not be expected to present a concern for phototoxicity or photoallergenicity.

10.1.5.1. Risk assessment. There are no phototoxicity studies available

Table 1
Maximum acceptable concentrations for δ-3-carene 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	0.005	0.003%
2	Products applied to the axillae	0.001	0.001%
3	Products applied to the face using fingertips	0.029	0.00%[b]
4	Fine fragrance products	0.027	0.01%
5	Products applied to the face and body using the hands (palms), primarily leave-on	0.007	0.001%
6	Products with oral and lip exposure	0.016	0.001%
7	Products applied to the hair with some hand contact	0.056	0.000%[b]
8	Products with significant ano-genital exposure	0.003	No Data[c]
9	Products with body and hand exposure, primarily rinse-off	0.054	0.001%[b]
10	Household care products with mostly hand contact	0.192	0.005%
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate	0.107	No Data[c]
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	0.005	0.190%

Note.
[a] For a description of the categories refer to the IFRA/RIFM Information Booklet.
[b] Negligible exposure (< 0.001%).
[c] Fragrance exposure from these products is very low. These products are not currently in the Creme RIFM Aggregate Exposure Model.

for δ-3-carene in experimental models. 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). Based on lack of absorbance, δ-3-carene 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. 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 $L\,mol^{-1} \cdot cm^{-1}$ (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/11/18.

10.1.6. Local Respiratory Toxicity

There are no inhalation data available on *δ*-3-carene; however, in an acute 2-week inhalation study for the read-across analog *d*-limonene (CAS # 5989-27-5; see Section V), a NOEC of 54.3 mg/m^3 was reported (RIFM, 2013).

10.1.6.1. Risk assessment. The inhalation exposure estimated for combined exposure was considered along with toxicological data observed in the scientific literature to calculate the MOE from inhalation exposure when used in perfumery. In a 2-week acute inhalation study conducted in rats, a NOEC of 54.3 mg/m^3 was reported for read-across material *d*-limonene (RIFM, 2013). Test substance-related effects were found in the respiratory tract at the 543 mg/m^3 and 5430 mg/m^3 concentrations; they were minor and consisted of minimally increased mucus in the respiratory epithelium of nasal levels II and III, minimal to mild olfactory cell degeneration in nasal levels III and IV, minimal transitional cell degeneration in the larynx, and minimal acute inflammation and alveolar macrophage aggregates in the lung.

This NOEC expressed in mg/kg lung weight/day is:

- (54.3 mg/m^3) (1 m^3/1000 L) = 0.0543 mg/L
- Minute ventilation (MV) of 0.17 L/min for a Sprague Dawley rat × duration of exposure of 360 min per day (min/day) (according to GLP study guidelines) = 61.2 L/day
- (0.0543 mg/L) (61.2 L/day) = 3.32 mg/day
- (3.32 mg/day)/(0.0016 kg lung weight of rat*) = 2075 mg/kg lung weight/day

The 95th percentile calculated exposure was reported to be 0.0014 mg/day—this value was derived from the concentration survey data in the Creme RIFM Exposure Model (Comiskey et al., 2015 and Safford et al., 2015a). To compare this estimated exposure with the NOEC expressed in mg/kg lung weight/day, this value is divided by 0.65 kg human lung weight (Carthew et al., 2009) to give 0.0022 mg/kg lung weight/day resulting in an MOE of 943,182 (i.e., [2075 mg/kg lung weight/day]/[0.0022 mg/kg lung weight/day]).

The MOE is greater than 100. Without adjustment for specific uncertainty factors related to inter-species and intra-species variation, the material exposure by inhalation at 0.0014 mg/day is deemed to be safe under the most conservative consumer exposure scenario.

*Phalen, R.F. Inhalation Studies. Foundations and Techniques, 2 nd Ed 2009. Published by, Informa Healthcare USA, Inc., New York, NY. Chapter 9, Animal Models, in section: “Comparative Physiology and Anatomy,” subsection, “Comparative Airway Anatomy.”

Additional References: None.

Literature Search and Risk Assessment Completed On: 02/22/2018.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of δ-3-carene was performed following the RIFM Environmental Framework (Salvito et al., 2002), which provides 3 tiers 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, δ-

3-carene was identified as a fragrance material with the potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC > 1.

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) did not identify δ-3-carene as possibly persistent or bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11).

10.2.2. Risk assessment

Based on the current Volume of Use (2015), δ-3-carene presents no risk to the aquatic compartment in the screening-level assessment.

Biodegradation: No data available.

Ecotoxicity: No data available.

10.2.3. Other available data

δ-3-Carene has been registered under REACH with no additional data at this time.

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 Framework: Salvito et al., 2002).

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	4.6	4.6
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	1–10	1–10
Risk Characterization: PEC/PNEC	**< 1**	**< 1**

The RQs for these materials are < 1. No further assessment is necessary.

The RIFM PNEC is 0.0366 μg/L. The revised PEC/PNECs for EU and NA are < 1; therefore, the material does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 5/7/18.

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

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	1.003			1,000,000	0.001003	
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	0.506	0.366	0.738	10,000	0.0366	Neutral Organics

- **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. The links listed above were active as of 08/27/2018.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2018.11.049.

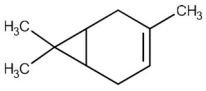
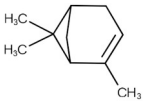
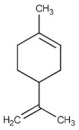
Appendix

Read-across Justification

Methods

The read-across analogs were identified following the strategy for structuring and reporting a read-across prediction of toxicity as 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 were examined. Third, appropriate read-across analogs from the cluster were confirmed by expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physical–chemical properties of the target substance and the read-across analogs were calculated using EPI Suite v4.11 (US EPA, 2012).
- 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 v4.2 (OECD, 2018).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- 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 v4.2 (OECD, 2018).
- The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).

	Target Material	Read Across Material	
Principal Name	δ-3-Carene	α-Pinene	*d*-Limonene
CAS No.	13466-78-9	80-56-8	5989-27-5
Structure			
Similarity (Tanimoto Score)		0.88	0.88
Read-across Endpoint		• Genotoxicity	• Respiratory toxicity
Molecular Formula	$C_{10}H_{16}$	$C_{10}H_{16}$	$C_{10}H_{16}$
Molecular Weight	136.24	136.24	136.24
Melting Point (°C, EPI Suite)	−13.62	−13.62	−40.76
Boiling Point (°C, EPI Suite)	157.25	157.25	167.66
Vapor Pressure (Pa @ 25 °C, EPI Suite)	279	536	193
Log Kow (KOWWIN v1.68 in EPI Suite)	4.38	4.44	4.38
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPI Suite)	4.581	2.49	13.8
Jmax (mg/cm^2/h, SAM)	138.619	0.510	2.802
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.08E + 004	1.08E + 004	3.85E + 004
Genotoxicity			
DNA Binding (OASIS v1.4, QSAR Toolbox v4.2)	• No alert found	• No alert found	
DNA Binding (OECD QSAR Toolbox v4.2)	• 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	
Local Respiratory Toxicity			
Respiratory Sensitization (OECD QSAR Toolbox v4.2)	• No alert found		• No alert found
Metabolism			
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	See Supplemental Data 1	See Supplemental Data 2	See Supplemental Data 3

Summary

There are insufficient toxicity data on δ-3-carene (CAS # 13466-78-9). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, physical–chemical properties, and expert judgment, α-pinene (CAS # 80-56-8) and *d*-limonene (CAS # 5989-27-5) were identified as read-across materials with sufficient data for toxicological evaluation.

Conclusions

- α-Pinene (CAS # 80-56-8) was used as a read-across analog for the target material δ-3-carene (CAS # 13466-78-9) for the genotoxicity endpoint.
  - The target substance and the read-across analog are structurally similar and belong to a class of unsaturated cyclic monoterpene hydrocarbons.
  - The key difference between the target substance and the read-across analog is that the target substance has fused rings while the read-across has bridged rings. This structural difference is toxicologically insignificant.
  - Similarity between the target substance and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - The physical–chemical properties of the target substance and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target substance and the read-across analog.
  - The target substance and the read-across analog do not have any alerts for Genetic toxicity. Data are consistent with *in silico* alerts.
  - The target substance and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.
- *d*-Limonene (CAS # 5989-27-5) was used as a read-across analog for the target material δ-3-carene (CAS # 13466-78-9) for the respiratory toxicity endpoint.
  - The target substance and the read-across analog are structurally similar and belong to a class of unsaturated cyclic monoterpene hydrocarbons.
  - The key difference between the target substance and the read-across analog is that the target substance has vinylene unsaturation and is bicyclic while the read-across analog has vinyl unsaturation and is monocyclic. This structural difference makes the read-across analog more reactive compared to the target substance.
  - Similarity between the target substance and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - The physical–chemical properties of the target substance and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target substance and the read-across analog.
  - The target substance and the read-across analog do not have any alerts for respiratory toxicity. Data are consistent with *in silico* alerts.
  - The target substance and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

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