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Short Review

RIFM fragrance ingredient safety assessment, *p*-menth-8-en-2-one, CAS Registry Number 7764-50-3

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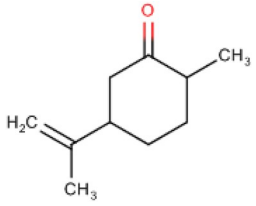
Version: 051018. This version replaces any previous versions.
Name: *p*-Menth-8-en-2-one
CAS Registry Number: 7764-50-3
Additional CAS Numbers*:
5524-05-0 Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, (2R,5R)-
*This material was included in this assessment because the materials are isomers.



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
GLP - Good Laboratory Practice

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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.

p-Menth-8-en-2-one was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data on read-across analog pulegone (CAS # 89-82-7) and weight-of-evidence *l*-carvone (CAS # 6485-40-1) show that *p*-menth-8-en-2-one is not expected to be genotoxic. The repeated dose, reproductive, and local respiratory toxicity endpoints were completed using the Threshold of Toxicological Concern (TTC) for a Cramer Class II material (0.009 mg/kg/day, 0.009 mg/kg/day, and 0.47 mg/day, respectively). The phototoxicity/photoallergenicity endpoint was completed based on UV spectra; *p*-menth-8-en-2-one is not expected to be phototoxic/photoallergenic. The skin sensitization endpoint was completed using DST for non-reactive materials (900 μg/cm^2); exposure is below the DST. The environmental endpoints were evaluated; *p*-menth-8-en-2-one was not found to be PBT as per 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 genotoxic. (NTP, 2011)
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 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: 2.8 (BIOWIN 3) (EPI Suite v4.1; US EPA, 2012a)
Bioaccumulation: Screening-level: 35.27 L/kg (EPI Suite v4.1; US EPA, 2012a)
Ecotoxicity: Screening-level: Fish LC50: 37.41 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: Fish LC50: 37.41 mg/L (RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.03741 μg/L
- **Revised PEC/PNECs (2011 IFRA VoU):** North America and Europe: not applicable; cleared at screening-level

1. Identification

Chemical Name: *p*-Menth-8-en-2-one	Chemical Name: Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, (2R,5R)-
CAS Registry Number: 7764-50-3	**CAS Registry Number:** 5524-05-0
Synonyms: Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans-; Dihydrocarvone; cis-Dihydrocarvone; 3-Isopropenyl-6-methylcyclohexanone; 2-Methyl-5-(1-methyl)ethenyl)cyclohexanone; 2-Methyl-5-(1-methylvinyl) cyclohexan-1-one; 5-Isopropenyl-2-methylcyclohexanone; d-Dihydrocarvone; *p*-Menth-8-en-2-one	**Synonyms:** Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, (2R,5R)-; Dihydrocarvone
Molecular Formula: $C_{10}H_{16}O$	**Molecular Formula:** $C_{10}H_{16}O$
Molecular Weight: 152.24	**Molecular Weight:** 152.24
RIFM Number: 958	**RIFM Number:** 6986

2. Physical data*

1. **Boiling Point:** 222 °C (FMA Database), 217.88 °C (EPI Suite)
2. **Flash Point:** 189 °F; CC (FMA Database), 87 °C (GHS)
3. **Log K_{ow}:** 2.86 (EPI Suite)
4. **Melting Point:** −0.75 °C (EPI Suite)

5. **Water Solubility:** 273.1 mg/L (EPI Suite)
6. **Specific Gravity:** 0.924 (FMA Database)
7. **Vapor Pressure:** 0.123 mm Hg @ 20 °C (EPI Suite v4.0), 0.183 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:** Almost colorless or pale, straw-colored liquid with warm herbaceous odor

*Physical data for both materials in this assessment are identical.

3. Exposure

1. **Volume of Use (Worldwide Band):** 0.1–1 metric tons per year (IFRA, 2011)
2. **95**th **Percentile Concentration in Hydroalcoholics:** 0.019% (RIFM, 2017)
3. **Inhalation Exposure*:** 0.000033 mg/kg/day or 0.0023 mg/day (RIFM, 2017)
4. **Total Systemic Exposure**:** 0.00074 mg/kg/day (RIFM, 2017)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM aggregate exposure model (Comiskey et al., 2015; Safford et al., 2015; 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., 2015; Safford et al., 2017; and Comiskey et al., 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 or 97.5th percentile, 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 II, Intermediate (Expert Judgment)

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

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

2. Analogs Selected:
   a. **Genotoxicity:** Pulegone (CAS # 89-82-7), *l*-carvone (CAS # 6485-40-1)
   b. **Repeated Dose Toxicity:** None
   c. **Reproductive Toxicity:** None
   d. **Skin Sensitization:** None
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. Read-across Justification: See Appendix below

6. Metabolism

Not considered for this risk assessment.

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

p-Menth-8-en-2-one is reported to occur in the following foods by the VCF* and in some natural complex substances (NCS):

Buchu oilCaraway (*Carum carvi* L.)Citrus fruitsDill (*Anethum* species)Lemon balm (*Melissa officinalis* L.)Mentha oilsOriganum (Spanish) (*Coridothymus cap.*(L.) Rchb.)Passion fruit (*Passiflora* species)Pepper (*Piper nigrum* L.)Thyme (*Thymus* species).

Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, (2R, 5R)- is not reported to occur in foods by the VCF*.

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

8. IFRA standard

None.

9. REACH dossier

Both materials in this assessment are pre-registered for 2010; no dossier available as of 05/09/2018.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data, *p*-menth-8-en-2-one does not present a concern for genotoxicity.

10.1.1.1. Risk assessment. *p*-Menth-8-en-2-one was assessed in the BlueScreen assay and found negative for both cytotoxicity and genotoxicity, with and without metabolic activation (RIFM, 2013). There are no studies assessing the mutagenic activity of *p*-menth-8-en-2-one; however, read-across can be made to pulegone (CAS # 89-82-7; see Section V). The mutagenic activity of pulegone has been evaluated in 3 bacterial reverse mutation assays conducted in compliance with GLP and with guidelines similar to OECD TG 471 using the plate incorporation method. *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and *Escherichia coli* strain WP2uvrA were treated with pulegone at concentrations up to 3500 μg/plate in 3 separate studies. No increases in the mean number of revertant colonies were observed at any tested dose in the presence or absence of S9 in first 2 studies; however, there were statistically significant increases in TA98 and *Escherichia coli* strain WP2uvrA in the presence of S9 in a third assay, and the test material was considered positive (NTP, 2011). Based on the results of these 3 assays, pulegone was considered negative in 2 of 3 studies conducted. These increases were small when compared to the solvent control (4.1-fold maximum increases in TA98 and 3.2-fold maximum increases in WP2uvrA). These increases were only observed at the highest doses assessed and were not compared to historical control ranges. Additional weight of evidence from read-across analog *l*-carvone (CAS # 6485-40-1) can be used; this material was assessed in an Ames assay conducted in compliance with GLP and according to guidelines similar to OECD TG 471 using the standard plate

Table 1
Acceptable concentrations for *p*-menth-8-en-2-one based on non-reactive DST.

IFRA Category[a]	Description of Product Type	Acceptable Concentrations in Finished Products	95th Percentile Concentration
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%	0.00%[b]
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%	No data
9	Products with body and hand exposure, primarily rinse-off	0.75%	0.01%
10	Household care products with mostly hand contact	2.70%	0.03%
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	0.025%

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

incorporation and preincubation methods. *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, and *Escherichia coli* strain WP2uvrA were treated with *l*-carvone in dimethyl sulfoxide (DMSO) at concentrations up to 5000 μg/plate in the presence and absence of metabolic activation. No increases in the mean number of revertant colonies were observed at any tested dose in the presence or absence of S9 (ECHA REACH Dossier). Under the conditions of the study, *l*-carvone was not mutagenic in bacteria. Taken together, *l*-carvone was not considered to be mutagenic, and this can be extended to *p*-menth-8-en-2-one.

There are no studies assessing the clastogenic activity of *p*-menth-8-en-2-one; however, read-across can be made to pulegone (CAS # 89-82-7; see Section V). The clastogenic activity of pulegone was evaluated in an *in vivo* micronucleus test conducted in compliance with GLP regulations. The test material was administered in corn oil via oral gavage to groups of male and female B6C3F1 mice. Doses from 9.375 to 150 mg/kg were administered. Mice from each dose level were euthanized at the end of the 3-month study; the bone marrow was extracted and examined for polychromatic erythrocytes. The test material did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow (NTP, 2011). Under the conditions of the study, pulegone was not clastogenic in the *in vivo* micronucleus test. Additional weight of evidence is available from tests with *l*-carvone (CAS # 6485-40-1). The clastogenicity of *l*-carvone was assessed in an *in vitro* chromosome aberration study conducted in compliance with GLP regulations and in accordance with OECD TG 473. Human peripheral blood lymphocytes were treated with *l*-carvone in DMSO at concentrations up to 1502.2 μg/mL in the presence and absence of metabolic activation. No statistically significant increases in the frequency of cells with structural chromosomal aberrations or polyploid cells were observed with any dose of the test item, either with or without S9 metabolic activation (ECHA REACH Dossier). Under the conditions of the study, *l*-carvone was considered to be non-clastogenic in the *in vitro* chromosome aberration assay. Taken together, pulegone and *l*-carvone were not considered to be clastogenic, and this can be extended to *p*-menth-8-en-2-one.

Based on the data available, *p*-menth-8-en-2-one does not present a concern for genotoxic potential.

Additional References: Franzios et al., 1997.

Literature Search and Risk Assessment Completed On: 05/07/2017.

10.1.2. Repeated Dose Toxicity

There are insufficient repeated dose toxicity data on *p*-menth-8-en-2-one and any read-across materials evaluated. The total systemic exposure to *p*-menth-8-en-2-one is below the TTC for the repeated dose toxicity endpoint of a Cramer Class II material at the current level of use.

This safety assessment will be updated when exposure data becomes available.

10.1.2.1. Risk assessment. There are no repeated dose toxicity data on *p*-menth-8-en-2-one or any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to *p*-menth-8-en-2-one (0.19 μg/kg/day) is below the TTC (9 μg/kg bw/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class II material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 02/24/2017.

10.1.3. Reproductive toxicity

There are insufficient reproductive toxicity data on *p*-menth-8-en-2-one and any read-across materials evaluated. The total systemic exposure to *p*-menth-8-en-2-one is below the TTC for the reproductive toxicity endpoint of a Cramer Class II material at the current level of use.

This safety assessment will be updated when exposure data becomes available.

10.1.3.1. Risk assessment. There are no developmental toxicity data on *p*-menth-8-en-2-one or any read-across materials that can be used to support the developmental toxicity endpoint. The total systemic exposure to *p*-menth-8-en-2-one (0.19 μg/kg/day) is below the TTC (9 μg/kg bw/day; Kroes et al., 2007) for the developmental toxicity endpoint of a Cramer Class II material at the current level of use.

There are no fertility data *p*-menth-8-en-2-one or any read-across materials that can be used to support the fertility endpoint. The total systemic exposure to *p*-menth-8-en-2-one (0.19 μg/kg/day) is below the TTC (9 μg/kg bw/day; Kroes et al., 2007) for the fertility endpoint of a Cramer Class II material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 02/24/2017.

10.1.4. Skin sensitization

Based on the existing data and application of DST, *p*-menth-8-en-2-one does not present a concern for skin sensitization under the current, declared levels of use.

10.1.4.1. Risk assessment. Limited skin sensitization studies are available for *p*-menth-8-en-2-one. Based on existing data and application of DST, *p*-menth-8-en-2-one does not present a concern for skin sensitization. The chemical structure of this material indicates that it would not be expected to react with skin proteins directly (Toxtree 2.6.13; OECD Toolbox v3.4). In a human maximization test and a human repeat insult patch test, there were no reactions indicative of sensitization with *p*-menth-8-en-2-one (RIFM, 1973; RIFM, 1977). Conservatively, based on limited data, the exposure is benchmarked utilizing the non-reactive DST of 900 μg/cm^2. The current exposure from the 95th percentile concentration for non-reactive materials when evaluated in all QRA categories. Table 1 provides the acceptable concentration for *p*-menth-8-en-2-one which presents no appreciable risk for skin sensitization based on the non-reactive DST.

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/9/17.

10.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, *p*-menth-8-en-2-one would not be expected to present a concern for phototoxicity or photoallergenicity.

10.1.5.1. Risk assessment. There are no phototoxicity studies available for *p*-menth-8-en-2-one 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, *p*-menth-8-en-2-one 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} · cm^{-1} (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/20/17.

10.1.6. Local Respiratory Toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The material, *p*-menth-8-en-2-one, exposure level is below the Cramer Class III* TTC value for inhalation exposure local effects.

10.1.6.1. Risk assessment. There are no inhalation data available on *p*-menth-8-en-2-one. Based on the Creme RIFM Model, the inhalation exposure is 0.00092 mg/day. This exposure is 511 times lower than the Cramer Class III* TTC value of 0.47 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.

*As per Carthew et al., 2009, Cramer Class II materials default to Cramer Class III.

Additional References: None.

Literature Search and Risk Assessment Completed On: 6/27/2017.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of *p*-menth-8-en-2-one was performed following the RIFM Environmental Framework (Salvito et al., 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log K_{OW}, and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity, as discussed in Salvito et al. (2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 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, *p*-menth-8-en-2-one was identified as a fragrance material with no potential to present a possible risk to the aquatic environment (*i.e.*, its screening-level PEC/PNEC < 1).

A screening-level hazard assessment using EPI Suite v4.1 (US EPA, 2012a) did not identify *p*-menth-8-en-2-one as possibly persistent nor 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 the current Volume of Use (2011), *p*-menth-8-en-2-one does not present a risk to the aquatic compartment in the screening-level assessment.

Biodegradation: No data available.
Ecotoxicity: No data available.

10.2.2.1. Other available data. *p*-Menth-8-en-2-one 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.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	37.41			1,000,000	0.03741	

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	2.85	2.85
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 0.03741 μg/L. The revised PEC/PNECs for EU and NA: Not applicable; cleared at screening-level; therefore, the material does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 4/27/17.

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.

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.2019.03.021.

Appendix

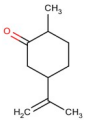
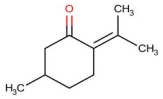
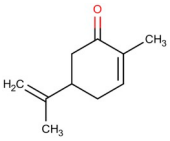
Read-across Justification

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 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 (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).

Principal Name	Target material	Read-across material	Weight of Evidence
	2-methyl-5-(1-methylethenyl)cyclo-hexanone	Pulegone	*l*-Carvone
CAS No.	7764-50-3	89-82-7	6485-40-1
Structure			
Similarity (Tanimoto Score)		0.84	0.83
Read-Across Endpoint		• Genotoxicity	• Genotoxicity
Molecular Formula	$C_{10}H_{16}O_1$	$C_{10}H_{16}O$	$C_{10}H_{14}O$
Molecular Weight	152.24	152.24	150.22
Melting Point (°C, EPI Suite)	−0.75	10.17	9.86
Boiling Point (°C, EPI Suite)	217.88	227.28	224.23
Vapor Pressure (Pa @ 25°C, EPI Suite)	24.4	21.6	17.3
Log Kow (KOWWIN v1.68 in EPI Suite)	2.85	3.08	2.71
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	1020	173.7	1310
J_{max} (mg/cm^2/h, SAM)	70.336	170.35	79.350
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.41E+001	1.05E+001	7.83E+000
Genotoxicity			
DNA Binding (OASIS v1.4 QSAR Toolbox v3.4)	• No alert found	• No alert found	• No alert found
DNA Binding by OECD QSAR Toolbox (v3.4)	• No alert found	• No alert found	• Michael addition
Carcinogenicity (Genotox and Non-genotox) Alerts ISS)	• Non-Carcinogen (moderate reliability)	• Carcinogen (moderate reliability)	• Non-carcinogen (Experimental value)
DNA Alerts for Ames, MN, CA by OASIS v1.1	• No alert found	• No alert found	• No alert found
***In Vitro* Mutagenicity (Ames test Alerts by ISS)**	• No alert found	• Alpha-beta unsaturated carbonyl	• Alpha-beta unsaturated carbonyl
***In Vivo* Mutagenicity (Micronucleus alerts by ISS)**	• No alert found	• Alpha-beta unsaturated carbonyl	• Alpha-beta unsaturated carbonyl
Oncologic Classification	• Not classified	• Not classified	• Not classified
Metabolism			
OECD QSAR Toolbox (v3.4)			
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites	See Supplemental Data 1	See Supplemental Data 2	See Supplemental Data 3

Summary

There are insufficient toxicity data on the target material *p*-menth-8-en-2-one (CAS # 7764-50-3). Hence, *in silico* evaluation was conducted by determining a read-across analog for this material. Based on structural similarity, reactivity, metabolism data, physical–chemical properties, and expert judgment, analogs pulegone (CAS # 89-82-7) and *l*-carvone (CAS # 6485-40-1) were identified as read-across materials with sufficient data for toxicological evaluation.

Conclusions

- Pulegone (CAS # 89-82-7) and *l*-carvone (CAS # 6485-40-1) were used as a read-across analog and weight of evidence, respectively, for the target material *p*-menth-8-en-2-one (CAS # 7764-50-3) for the genotoxicity endpoint.
  - o The target substance and the read-across analogs are structurally similar and belong to the structural class of cyclic unsaturated ketones.
  - o The target substance and the read-across analogs share a cyclic unsaturated ketone structure.
  - o The key difference between the target substance and the read-across analog pulegone (CAS # 89-82-7) is that the target substance has a methyl group at the 2 position whereas the read-across analog has it at the 3 position. Also, the target substance has a vinyl group, and the read-across analog has a vinylene group. The key difference between the target substance and the read-across analog *l*-carvone (CAS # 6485-40-1) is that the read-across analog has 2 sites of unsaturation while the target substance has only 1. This structural difference between the target substance and the read-across analog is toxicologically insignificant.
  - o Similarity between the target substance and the read-across analogs are indicated by the Tanimoto score in the above table. 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 analogs are sufficiently similar to enable comparison of their toxicological properties.
  - o According to the QSAR OECD Toolbox (v3.4), structural alerts for toxicological endpoints are consistent between the target substance and the read-across analogs.
  - o The read-across analog pulegone (CAS # 89-82-7) has a carcinogenicity alert by the ISS model, while the target substance is predicted to be a non-carcinogen. There is an alpha,beta-unsaturated carbonyl alert for both of the read-across analogs by ISS. There is no such alert for the

target substance. This indicates that the read-across analogs are more reactive as compared to the target substance. The data described in the genotoxicity section shows that the read-across analogs do not pose a concern for the genotoxicity endpoint. Therefore, the alert was superseded by the data.

- o The target substance and the read-across analogs 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.
- o The structural differences between the target material and the read-across analog are toxicologically insignificant.

Explanation of Cramer Classification

Due to potential discrepancies between the current *in silico* tools (Bhatia et al., 2015), the Cramer class of the target material was determined using expert judgment, based on the Cramer decision tree (Cramer et al., 1978).

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

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