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Short Review

RIFM fragrance ingredient safety assessment, pulegone, CAS Registry Number 89-82-7

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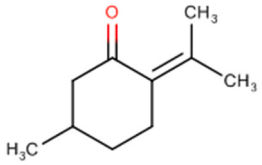
15932-80-6 (+/-)-pulegone
89-82-7 *d*-pulegone



Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration

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AF - Assessment Factor
BCF - Bioconcentration Factor
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015a, 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DRF - Dose Range Finding

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DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observable Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
Perfumery - In this safety assessment, perfumery refers to fragrances made by a perfumer used in consumer products only. The exposures reported in the safety assessment include consumer product use, but do not include occupational exposures.
QRA - Quantitative Risk Assessment
QSAR - Quantitative Structure-Activity Relationship
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use
vPvB - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

The Expert Panel for Fragrance Safety* concludes that this material is safe as described in this safety assessment.

This safety assessment is based on the RIFM Criteria Document (Api et al., 2015), which should be referred to for clarifications.

Each endpoint discussed in this safety assessment includes the relevant data that were available at the time of writing (version number in the top box is indicative of the date of approval based on a 2-digit month/day/year), both in the RIFM Database (consisting of publicly available and proprietary data) and through publicly available information sources (e.g., SciFinder and PubMed). Studies selected for this safety assessment were based on appropriate test criteria, such as acceptable guidelines, sample size, study duration, route of exposure, relevant animal species, most relevant testing endpoints, etc. A key study for each endpoint was selected based on the most conservative endpoint value (e.g., PNEC, NOAEL, LOEL, and NESIL).

*The Expert Panel for Fragrance Safety is an independent body that selects its own members and establishes its own operating procedures. The Expert Panel is comprised of internationally known scientists that provide RIFM with guidance relevant to human health and environmental protection.

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

Pulegone was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that pulegone is not genotoxic and provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and fertility endpoints. Data on read-across material *l*-carvone (CAS # 6485-40-1) provide a calculated MOE >100 for the developmental toxicity endpoint. The skin sensitization endpoint was completed using the Dermal Sensitization Threshold (DST) for non-reactive materials (900 μg/cm^2); exposure is below the DST. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet (UV) spectra; pulegone is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class II material, and the exposure to pulegone is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; pulegone was found not to be Persistent, Bioaccumulative, and Toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

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Human Health Safety Assessment

Genotoxicity: Not genotoxic.	(Andersen and Jensen, 1984; NTP, 2011; IARC, 2018; OEHHA, 2014)
Repeated Dose Toxicity: NOAEL = 1.88 mg/kg/day.	NTP (2011)
Reproductive Toxicity: Developmental toxicity: NOAEL = 12.5 mg/kg/day. Fertility: NOAEL = 75 mg/kg/day.	(ECHA REACH Dossier: L-*p*-Mentha-1(6),8-dien-2-one; ECHA, 2013)
Skin Sensitization: Not a concern for skin sensitization under the declared use levels; exposure is below the DST.	
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic.	(UV Spectra, RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.	

Environmental Safety Assessment

Hazard Assessment:	
Persistence:	
Screening-level: 83% (OECD 301 F) for CAS # 89-82-7	RIFM (2014)
Bioaccumulation:	
Screening-level: 50.02 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: Fish LC50: 18.56 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: 18.56 mg/L	(RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.01856 μg/L	
• **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: not applicable; cleared at screening-level	

1. Identification

Chemical Name: Pulegone	Chemical Name: (+/-)-Pulegone	Chemical Name: *l*-Pulegone
CAS Registry Number: 89-82-7	**CAS Registry Number:** 15932-80-6	**CAS Registry Number:** 3391-90-0
Synonyms: Cyclohexanone, 5-methyl-2-(1-methylethylidene)-, (R)-; 1-Isopropylidene-4-methyl-2-cyclohexanone; *p*-Menth-4(8)-en-3-one; δ-4(8)-*p*-Menthen-3-one; 1-Methyl-4-isopropylidene-3-cyclohexanone; 5-Methyl-2-(1-methylethylidine) cyclohexanone; *p*-ﾒﾝﾃﾝ-4(8)-ｲｿﾝ-3; 2-Isopropylidene-5-methylcyclohexanone; Pulegone Dextro; Pulegone	**Synonyms:** 2-Isopropylidene-5-methylcyclohexanone; (+/-)-Pulegone	**Synonyms:** *l*-*p*-Menth-4(8)-en-3-one; (5S)-5-methyl-2-propan-2-ylidenecyclohexan-1-one; (-)-Pulegone; Cyclohexanone,5-methyl-2-(1-methylethylidene)-, (5S)-; *l*-Pulegone
Molecular Formula: $C_{10}H_{16}O$	**Molecular Formula:** Not Available	**Molecular Formula:** $C_{10}H_{16}O$
Molecular Weight: 152.23	**Molecular Weight:** 152.23	**Molecular Weight:** 152.23
RIFM Number: 6085 (pulegone); 648 (*d*-pulegone)	**RIFM Number:** 6527	**RIFM Number:** None
Stereochemistry: One stereocenter and 2 total stereoisomers possible.	**Stereochemistry:** No isomer specified. One stereocenter and 2 total stereoisomers possible.	**Stereochemistry:** *l* isomer specified. One stereocenter and 2 total stereoisomers possible.

2. Physical data

CAS # 89-82-7 (pulegone and *d*-pulegone)	CAS # 15932-80-6	CAS # 3391-90-0
Boiling Point: 224 °C (dec.) (Fragrance Materials Association [FMA]), 227.28 °C (EPI Suite)	**Boiling Point:** 227.28 °C (EPI Suite)	**Boiling Point:** Not Available
Flash Point: 88 °C (Globally Harmonized System [GHS])	**Flash Point:** 88 °C (GHS)	**Flash Point:** Not Available
Log K_{OW}: 3.2 (EPI Suite)	**Log K_{OW}:** 3.2 (EPI Suite)	**Log K_{OW}:** Not Available
Melting Point: 10.17 °C (EPI Suite)	**Melting Point**: 10.17 °C (EPI Suite)	**Melting Point**: Not Available
Water Solubility: 173.7 mg/L (EPI Suite)	**Water Solubility:** 173.7 mg/L (EPI Suite)	**Water Solubility:** Not Available
Specific Gravity: 0.930 (FMA)	**Specific Gravity:** Not Available	**Specific Gravity:** Not Available
Vapor Pressure: 0.108 mm Hg at 20 °C (EPI Suite v4.0), 0.108 mm Hg at 20 °C (EPI Suite v4.0), 0.162 mm Hg at 25 °C (EPI Suite)	**Vapor Pressure:** 0.108 mm Hg at 20 °C (EPI Suite v4.0), 0.162 mm Hg at 25 °C (EPI Suite)	**Vapor Pressure:** Not Available
UV Spectra: Not Available	**UV Spectra:** Minor absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} · cm^{-1})	**UV Spectra:** Not Available
Appearance/ Organoleptic: Colorless to slightly yellow to yellow, oily liquid with herbaceous-minty, resinous odor, or pleasant odor, midway between peppermint and camphor	**Appearance/ Organoleptic:** Not Available	**Appearance/ Organoleptic:** Not Available

3. Volume of use (worldwide band)

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

4. Exposure*** to fragrance ingredient (Creme RIFM Aggregate Exposure Model v2.0)

1. **95**th **Percentile Concentration in Hydroalcoholics:** 0.0028% (RIFM, 2017)
2. **Inhalation Exposure*:** 0.00002 mg/kg/day or 0.0018 mg/day (RIFM, 2017)
3. **Total Systemic Exposure**:** 0.00045 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, 2017; Safford et al., 2015a, 2017).

**95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section V. It is derived from concentration survey data in the Creme RIFM Aggregate Exposure Model and includes exposure via dermal, oral, and inhalation routes whenever the fragrance ingredient is used in products that include these routes of exposure (Comiskey et al., 2015, 2017; Safford et al., 2015a, 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.

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

1. **Cramer Classification:** Class II*, Intermediate (Expert Judgment)

Expert Judgment	Toxtree v 3.1	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 the Appendix below for further details.

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

7. Metabolism

Pulegone is a monoterpene ketone that is rapidly absorbed from the gastrointestinal tract. Pulegone metabolizes through multiple pathways including hydroxylation, reduction, and conjugation (IARC, 2018). Pulegone metabolism (Fig. 1) involves the reduction of the ketone functional group to yield pulegol or oxidation of the exocyclic alkene to yield 2,8-dihydroxymenthone. The tertiary-ring carbon is hydroxylated to yield 5-hydroxypulegone and the isopropylidene substituent, which undergoes allylic oxidation to yield 9-hydroxypulegone (predominant pathway). Pulegone and its metabolites form conjugates with glucuronic acid, glutathione, and glutathionyl-glucuronide, which are ultimately excreted through urine and feces. In a secondary detoxification pathway, 9-hydroxypulegone is oxidized to 9-carboxypulegone, which cyclizes to its corresponding hydroxylactone or undergoes oxidation and hydration to yield polar hydroxy acids. The hydroxylactone moiety undergoes dehydration to yield menthofuran, ultimately forming a reactive γ-ketonal (FEMA, 1996). The formation of menthofuran is catalyzed by cytochrome P450 (CYP450). However, the reactive γ-ketonal is associated with hepatotoxicity and is known to undergo oxidation to form a reactive epoxide before forming *p*-cresol, a urinary metabolite of menthofuran. The (S)-(-) stereoisomer of pulegone is metabolized similarly to the (R)-(+) isomer of pulegone, but there are quantitative differences in the formation of metabolites following the alternate metabolic pathways. Isopulegone isomerizes to pulegone and subsequently follows similar metabolic pathways that give rise to (R)-(+) menthofuran and other metabolites. Biotransformation of pulegone results in several metabolites (14), which are excreted through urine within 24 h in rats and mice (NTP, 2011; FEMA, 1996; European Commission, 2002; EFSA, 2005; Chen et al., 2003). The metabolites identified included piperitone, menthones, and 8-hydroxymenthone when pulegone was administered to rats orally (Ferguson et al., 2007; Madyastha and Gaikwad, 1998). The authors concluded that orally administered pulegone at a dose of 0.5–1 mg/kg in humans does not yield significant amounts of menthofuran, a metabolite reported to be a potential carcinogen and responsible for the hepatotoxicity observed due to pulegone exposure. In humans, the major metabolites identified included 10-hydroxypulegone, 9-hydroxy-p-menthan-3-one, 1-hydroxy-menthan-3-one, and menthol (Anderson et al., 1996).

Overall, the data suggest that menthofuran is not a major metabolite

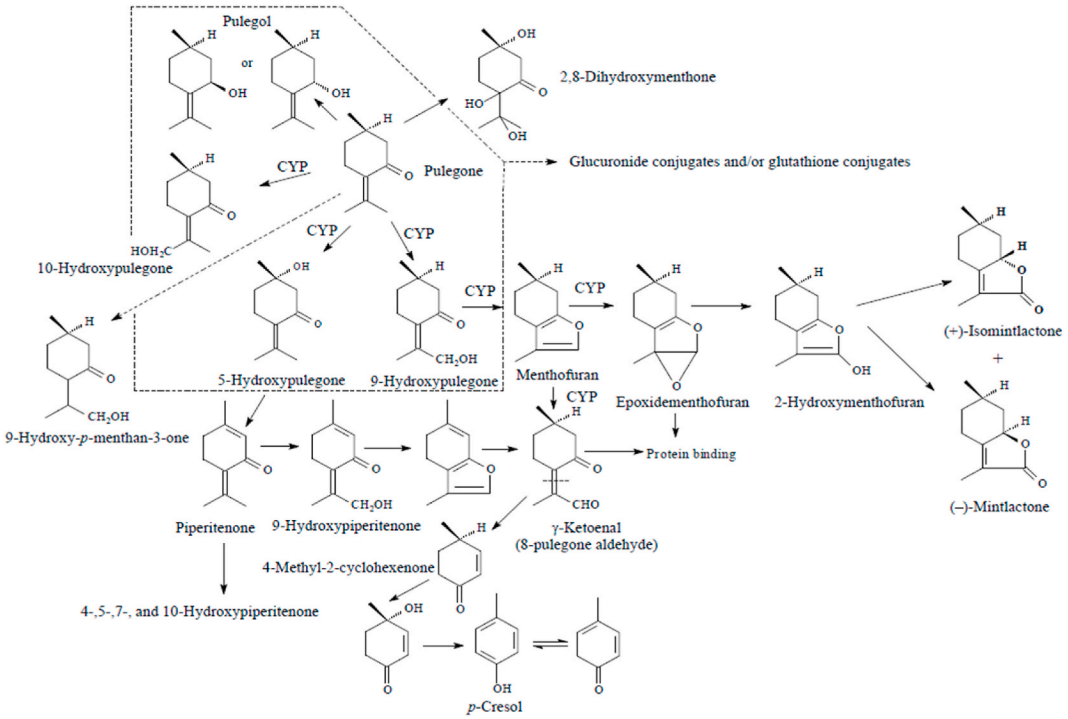


Fig. 1. Metabolism of pulegone in humans and rodents (IARC, 2018).

in rodents at doses ≤80 mg/kg; instead, it is detoxified via conjugation with glucuronic acid and glutathione. In humans, the data suggest that menthofuran is not a major metabolite of pulegone at low doses (0.5–1 mg/kg), whereas 10-hydroxypulegone, a precursor of menthofuran, is the major metabolite. In humans, detoxification is mediated via conjugation with glucuronic acid or sulfuric acid. Thus, based on the available human metabolism data, it is unlikely that menthofuran is produced in humans exposed to low doses of pulegone.

Additional References: None.

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

Pulegone is reported to occur in the following foods by the VCF*:

Black currants (*Ribes nigrum* L.)	Lemon balm (*Melissa officinalis* L.)
Calamintha nepeta oil	Licorice (*Glycyrrhiza* species)
Camomile	Mentha oils
Citrus fruits	Tea
Rosemary (*Rosmarinus officinalis* L.)	Wormwood oil (*Artemisia absinthium* L.)

(+/-)-Pulegone and *l*-pulegone are 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. This is a partial list.

9. REACH dossier

Pulegone is pre-registered for 2010; (+/-)-pulegone is pre-registered for 2013; *l*-pulegone is not pre-registered. No dossiers are available for any of these materials as of 05/06/20.

10. Conclusion

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

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, pulegone does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. The mutagenic activity of pulegone has been evaluated in several bacterial reverse mutation assays conducted in compliance with GLP regulations. *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, or TA1537 were treated with pulegone. No increases in the mean number of revertant colonies were observed at any tested concentration up to 800 μg/plate in the presence or absence of S9 (Andersen and Jensen, 1984). Under the conditions of the study, pulegone was not mutagenic in the Ames test. Three additional assays for pulegone were evaluated for mutagenic activity. In the first 2 studies, pulegone was not mutagenic with or without metabolic activation. Bacterial strains tested in the first study included *S. typhimurium* TA97, TA98, TA100, and TA1535, with and without metabolic activation. Strains tested in the second study included *S. typhimurium* strains TA98 and TA100 and *Escherichia coli* strain WP2uvrA, with and without metabolic activation (10% S9 from rat liver S9). The third study also tested pulegone in *S. typhimurium* and *E. coli*; results were positive in *Salmonella typhimurium* strain TA98 and *E. coli strain* WP2uvrA in the presence of metabolic activation (NTP, 2011). The positive results in the third study could be due to the presence of impurities, which may be causing these responses. It is well known that one impurity, menthofuran (also a metabolite of pulegone), in the presence of CYP enzymes may produce a γ-ketoenol as well as an epoxide furan ring, which could have caused in positive results in the third study (IARC, 2018). Additionally, pulegone caused glutathione depletion in the *in vitro* as well as *in vivo* studies, which may have limited glutathione conjugation of the reactive metabolite and lead to positive results at higher doses (IARC, 2018). Hence, considering the 2 negative results in the traditional Ames

tests, the positive responses in 1 study at higher doses can be considered to be biologically non-relevant. As additional weight of evidence (WoE), newer Ames studies using pulegone and peppermint oil containing pulegone were conducted according to OECD 471 guidelines at concentrations of up to 5000 μg/plate and were also concluded to be negative (Bastaki et al., 2020).

As further WoE, mutagenicity data on a more reactive structural analog, carvone, was considered, which also has negative data in an Ames study conducted using the standard plate incorporation/pre-incubation methods. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with *l*-carvone in dimethyl sulfoxide (DMSO) at concentrations up to 5000 μg/plate and was concluded to be negative (ECHA, 2013). Additionally, a carcinogenicity study showed pulegone caused cancer of the urinary bladder in female rats and cancer of the liver in male and female mice. The studies indicated that the metabolism of pulegone to menthofuran generates electrophilic species that can bind to proteins. This may result in chronic regenerative cell proliferation that may be related to the carcinogenicity in the liver and urinary bladder observed in experimental animals (IARC, 2018). Positive incidences in the carcinogenicity studies have been linked to only higher-dose exposure, which may generate electrophiles and deplete glutathione levels; therefore, the positive results may not be considered biologically relevant. For the same reasons, the classification of pulegone in the California Proposition 65 lists was not considered to be appropriate (OEHHA, 2014).

The clastogenic activity of pulegone has also been evaluated in several *in vitro* and *in vivo* assays. In an *in vivo* micronucleus test, B6C3F1 mice were administered pulegone at doses up to 150 mg/kg per day by gavage for 3 months; there was no increase in the frequency of micronucleus formation in peripheral blood erythrocytes (NTP, 2011).

Based on the data available, pulegone does not present a concern for genotoxic potential.

Additional References: None.

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

11.1.2. Repeated dose toxicity

The MOE for pulegone is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on pulegone. Toxicity data on pulegone have been extensively reviewed by the European Medical Agency (EMA, 2016), the European Food Safety Authority (EFSA, 2005), the US Food and Drug Administration (US FDA, 2018), National Industrial Chemicals Notification and Assessment Scheme (NICNAS, 2017), and the International Agency for Research on Cancer (IARC, 2018). Based on the available toxicity data, IARC classifies pulegone as a group 2B carcinogen (possible human carcinogen due to sufficient carcinogenicity evidence in experimental animals). The US FDA initially concluded that pulegone does not pose a risk to public health as a food and flavor adjuvant. However, under the Delaney Clause, the finding of carcinogenicity renders the additive "unsafe," resulting in an amendment of the previous conclusion by the US FDA. Hence, the US FDA no longer authorizes the use of pulegone as a synthetic food additive. In addition, NICNAS also updated the hazard classification for pulegone to a suspected Category 2 carcinogen based on the 2009 GHS classification criteria and advises consumers to use products containing pulegone following the safe use instructions on the product label. The EMA derived an acceptable exposure limit of 0.75 mg/kg/day based on a NOAEL of 37.5 mg/kg/day from a 90-day subchronic toxicity study in rats and using an uncertainty factor of 50 (EMA, 2016). The NTP concluded that pulegone has clear evidence of carcinogenic activity in female F344/N rats based on increased incidences of urinary bladder neoplasms and increased incidences of hepatocellular neoplasms in male and female B6C3F1 mice (NTP, 2011). A summary of relevant repeated dose toxicity data is presented below:

In a 28-day repeated dose toxicity gavage study, groups of 10 Wistar SPF rats/sex/dose were administered pulegone at doses of 0 (soybean oil), 20, 80, or 160 mg/kg/day. A significant and dose-dependent reduction in bodyweight gain among mid- (10%) and high-dose (20%) group animals was reported. Blood creatinine values also showed a dose-dependent decrease attaining statistical significance only at the highest-dose group among treated animals. In addition, an increased number of blood neutrophils among high-dose group animals was also reported. Microscopic examination revealed dose-related hepatocyte vacuolation in the zone around the central vein among the mid- and high-dose groups. Based on the decreased bodyweight gains and increased hepatocyte vacuolation at the mid and high doses, the NOAEL was concluded to be 20 mg/kg/day (Thorup et al., 1983).

In an NTP-conducted chronic/carcinogenicity study, 50 F344/N rats/sex/group were administered pulegone (purity: 96%) by gavage at doses of 0 (corn oil), 18.75, 37.5, and 75 mg/kg/day for male rats; 37.5, 75, and 150 mg/kg/day for female rats for 104 weeks (5 days/week). Due to excessive treatment-related mortality and morbidity reported in males at 75 mg/kg/day and in females at 150 mg/kg/day, pulegone administration was stopped after week 60 (stop-exposure) and the animals were instead treated with corn oil until the end of the study. An extremely high mortality rate was reported in males at 75 mg/kg/day (96%) and in females at 150 mg/kg/day (100%) towards the end of the experiment. These low survival rates suggest that maximum tolerated doses (MTD) were significantly lower than the highest administered dose in dose male and female rats. Furthermore, in the high-dose group, there was a 13%–24% decrease in average male body weight, whereas a 25%–35% decrease in average female body weight was observed. Mid-dose females were also reported to have a 12%–23% decrease in average body weight. In females, significantly increased incidences of urinary bladder papilloma and papilloma or carcinoma (combined) were reported at the highest dose. In comparison to the high-dose females, mid-dose females had higher incidences of transitional epithelium hyperplasia, which is a preneoplastic lesion. In addition, significantly increased incidences of hyaline glomerulopathy and olfactory epithelium metaplasia were reported in females of all treatment groups. Significant increases in the incidences of nephropathy, diffuse hepatocyte cellular alteration, and liver alterations, including oval cell hyperplasia, bile duct hyperplasia, and portal fibrosis were reported in animals of the mid- and high-dose groups. At 75 mg/kg/day, increased incidences of epithelial hyperplasia and perforation in the stomach were reported in males. Based on increased mortality, nephropathy, hepatotoxicity, hyperplasia, and perforation of the gastric epithelium in males, and incidences of urinary bladder papilloma and papilloma or carcinoma (combined in females), the LOAEL was considered to be 18.75 mg/kg/day (NTP, 2011; IARC, 2018).

In another NTP-conducted chronic/carcinogenicity bioassay, 50 B6C3F1 mice/sex/group were administered pulegone (purity: 96%) orally at doses of 0 (corn oil), 37.5, 75, and 150 mg/kg/day for 105 weeks (5 days/week). In comparison to the controls, mean body weights decreased at the highest dose in males (17%) and females (24%), suggesting that the MTD had been exceeded. Significantly increased incidences of hyaline glomerulopathy were reported in animals of both sexes at doses ≥75 mg/kg/day. The incidences of nephropathy and glomerular congestion were increased in both sexes at the highest dose. Olfactory epithelial degeneration was significantly increased in females (all groups) and males (≥75 mg/kg/day). At doses ≥75 mg/kg/day, a significant increase in non-neoplastic liver lesions (clear cell, eosinophilic, and mixed cell foci; focal fatty change; centrilobular hepatocyte hypertrophy; intravascular hepatocyte; necrosis; and pigmentation) was observed. Multiple hepatocellular adenoma incidences were significantly increased at all doses in males (statistical significance only in the mid-dose group). The combined incidences of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma were significantly increased with a significant positive trend in males (75 mg/kg/day) and

females (at 150 mg/kg/day). The mice strain used in this study is reported to have a high spontaneous incidence of liver neoplasms. However, increases in the incidences of combined hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma were statistically significant in male mice, with the highest incidence in the mid-dose group (84%) exceeding both the concurrent controls (58%) and the historical controls (58%–76%). Similarly, the increase in the incidence of combined hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma was statistically significant in the high-dose female mice (66%), exceeding both the concurrent controls (35%) and the historical control range (8%–35%). Thus, the observed liver neoplasms were pulegone-induced in both sexes. Based on increased hyaline glomerulopathy, nephropathy (150 mg/kg/day), non-neoplastic liver lesions (≥75 mg/kg/day), olfactory epithelial degeneration of the nose, hepatocellular adenoma (all dosed groups), and hepatocellular carcinoma or hepatoblastoma (75 mg/kg/day and 150 mg/kg/day), the LOAEL was considered to be 37.5 mg/kg/day (NTP, 2011; IARC, 2018).

The NTP study report considers pulegone to be mutagenic; hence, observations of urinary bladder neoplasms in rats were considered most likely due to the mutagenicity of pulegone. However, all extensive review of the literature in the genotoxicity section (see above) establishes pulegone as a non-genotoxic material. Hence, the carcinogenicity of pulegone is considered to be through a non-genotoxic mode of action.

A potential cytotoxic mode of action was proposed for female rat urinary bladder carcinogenicity through *in vivo* and *in vitro* studies. The *in vivo* studies were conducted with pulegone administered to female F344/N rats for 4 and 6 weeks at 0, 75, or 150 mg/kg/day. The cytotoxicity of pulegone and its metabolites piperitenone, piperitone, menthofuran, and menthone was assessed by determining the *in vitro* cell viability of MYP3 (rat) and 1T1 (human) urothelial cells. The study concluded that the mixture of pulegone and its metabolites, especially piperitenone, induce urothelial cytotoxicity and necrosis. Pulegone-induced cytotoxicity and necrosis subsequently trigger the regenerative cell proliferation resulting in tumors. The authors identified the key events for pulegone-induced urinary bladder tumors in female rats as follows: 1) chronic exposure to high concentrations of pulegone, (2) metabolism, excretion, and concentration of pulegone and cytotoxic metabolites, especially piperitenone in the urine, (3) urothelial cytotoxicity, (4) sustained regenerative urothelial cell proliferation, and (5) development of urothelial tumors. According to the authors, this mechanism of action implies a threshold effect, requiring high exposure to pulegone and its metabolites in the urine to induce cytotoxicity.

Glutathione depletion and formation of protein adducts have also been postulated as potential MOAs of pulegone, which may lead to cytotoxicity and chronic cell proliferation. *In vivo* and *in vitro* studies using inhibitors and inducers of hepatic cytochrome P450 demonstrate an association between hepatocellular damage due to menthofuran and its metabolic activation combined with covalent binding to target organ proteins. The CYP-catalyzed pulegone metabolism results in the formation of menthofuran, the latter subsequently oxidizing to form electrophilic reactive intermediates such as menthofuran epoxide and pulegone-8-aldehyde. These reactive metabolites can form covalent adducts with hepatocellular proteins, and account for significant hepatotoxicity in rodents. In addition, p-cresol, another metabolite produced only at high concentrations of pulegone, is also capable of depleting glutathione levels, which may lead to chronic regenerative cell proliferation consequently related to the hepatic carcinogenicity observed in experimental B6C3F1 mice.

Overall, the available data highlights the carcinogenic potential of pulegone, particularly in female rats, and male/female mice. However, there is evidence suggesting a threshold-based carcinogenic activity. Thus, the lowest NOAEL is derived from the LOAEL in the NTP carcinogenicity rat study of 18.75 mg/kg/day divided by a safety factor of 10. The derived NOAEL for the repeated dose toxicity endpoint is 18.75/10 = 1.88 mg/kg/day.

Therefore, the pulegone MOE for the repeated dose toxicity endpoint can be calculated by dividing the pulegone NOAEL in mg/kg/day by the total systemic exposure to pulegone, 1.88/0.00045, or 4178.

In addition, the total systemic exposure to pulegone (0.45 μg/kg/day) is below the TTC (9 μg/kg/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class II material at the current level of use.

*The Expert Panel for Fragrance Safety is composed of scientific and technical experts in their respective fields. This group provides advice and guidance.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/19/19.

11.1.3. Reproductive toxicity

The MOE for pulegone is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are no developmental toxicity data on pulegone. Read-across material *l-carvone* (CAS # 6485-40-1; see Section VI) has sufficient developmental toxicity data that can be used to support the developmental toxicity endpoint. An OECD 414 prenatal developmental toxicity study was conducted with *l*-carvone administered to 24 female pregnant Wistar CRL rats per dose by gavage at doses of 125, 250, and 500 mg/kg/day from gestation days 6–20. A statistically significant decrease in body weight in correlation to a statistically significant decrease in food consumption was observed at 500 mg/kg/day. A statistically significant decrease in the absolute weight of the uterus was observed at 500 mg/kg/day. Further, incomplete ossification of the supraoccipital bone was increased in females at the highest dose level compared to the control group. Statistically significant decrease in the mean body weights was observed for fetuses at the highest dose. Incomplete ossification of the supraoccipital bone, which could be associated with lower fetal body weight, was observed at 500 mg/kg/day. Bipartite ossification of the supraoccipital bone and unossified supraoccipital bone, which could be associated with maternal toxicity, was seen at the highest dose only. Increased incidence of transitional findings, such as a hole in the supraoccipital bone, asymmetric ossification of sternebra, and bipartite and dumbbell ossification of vertebrae, were observed in all the treatment groups as compared to the controls. These effects were considered to be adverse effects of the test material to fetuses, as no maternal toxicity was observed at the low- and mid-dose levels. The malformations of ribs (absent) were observed in all treated groups. These findings were considered to be adverse on the early prenatal development of the organism in the uterus.

In this study, the developmental toxicity LOAEL is 125 mg/kg/day. This LOAEL value is based on the occurrence of a significant decrease in mean body weights of fetuses, transitional findings of supraoccipital bone, sternebrae, vertebrae, and malformations of the ribs at a dose of 125, 250, and 500 mg/kg/day (ECHA, 2013). The NOAEL for *l*-carvone was calculated by dividing the LOAEL of 125 mg/kg/day by the uncertainty factor, 10 = 12.5 mg/kg/day.

Therefore, the pulegone MOE for the developmental toxicity endpoint can be calculated by dividing the l-carvone NOAEL in mg/kg/day by the total systemic exposure to pulegone, 12.5/0.00045, or 27778.

There are sufficient fertility data on pulegone. In an NTP subchronic toxicity study, 10 F344/N rats/sex/dose were administered pulegone via oral gavage at doses of 0, 9.375, 18.75, 37.5, 75, or 150 mg/kg/day in corn oil for 5 days per week for 14 weeks. Sperm analyses and vaginal cytology evaluations were conducted from rats of the 0, 18.75, 37.5, and 75 mg/kg/day dose groups. Reproductive organ weight (left testis, left cauda, and left epididymis) and histopathology (clitoral gland, ovary, preputial gland, prostate gland, right testis with the epididymis, and uterus) were performed. Histopathology was performed on all animals in the control and 150 mg/kg/day groups; ovary and uterus were examined up to the no effect level. No significant differences were

reported in the sperm parameters or the estrous cyclicity of the rats administered at 18.75, 37.5, and 75 mg/kg/day. Reproductive organs were examined microscopically, and no treatment-related effects were reported. Since sperm analyses and estrous cyclicity were only conducted up to 75 mg/kg/day with no treatment-related adverse effects reported, the NOAEL for effects on fertility was considered to be 75 mg/kg/day (NTP, 2011).

In another NTP-conducted subchronic toxicity study, 10 B6C3F1 mice/sex/dose were administered pulegone via oral gavage at doses of 0, 9.375, 18.75, 37.5, 75, or 150 mg/kg/day in corn oil for 5 days per week for 14 weeks. Sperm analyses and vaginal cytology evaluations were conducted from mice of the 0, 37.5, 75, and 150 mg/kg/day dose groups. Reproductive organ weight (left testis, left cauda, and left epididymis) and histopathology (clitoral gland, ovary, preputial gland, prostate gland, right testis with the epididymis, and uterus) were performed. Histopathology was performed on all animals in the control and 150 mg/kg/day dose groups. No significant differences were reported in the sperm parameters or the estrous cyclicity of the mice administered 37.5, 75, and 150 mg/kg/day. No treatment-related effects were reported for reproductive organs examined microscopically. Therefore, the NOAEL for effects on fertility was considered to be 150 mg/kg/day, the highest dose tested (NTP, 2011).

The most conservative NOAEL of 75 mg/kg/day from the rat study was selected for the fertility endpoint. **Therefore, the pulegone MOE for the fertility endpoint can be calculated by dividing the pulegone NOAEL in mg/kg/day by the total systemic exposure to pulegone, 75/0.00045, or 166667.**

In addition, the total systemic exposure to pulegone (0.45 μg/kg/day) is below the TTC (9 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the reproductive toxicity endpoint of a Cramer Class II material at the current level of use.

Additional References: NTP, 2011; IARC, 2018.

Literature Search and Risk Assessment Completed On: 04/07/20.

11.1.4. Skin sensitization

Based on the existing data and the application of DST, pulegone does not present a safety concern for skin sensitization under the current, declared levels of use.

11.1.4.1. Risk assessment. Limited skin sensitization studies are available for pulegone. The chemical structure of this material indicates that it would be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree 3.1.0; OECD Toolbox v 4.2). In a human maximization test, no skin sensitization reactions were observed (RIFM, 1975). Due to the limited data, the reported exposure was benchmarked utilizing the non-reactive DST of 900 μg/cm^2 (Safford, 2008, Safford et al., 2011, 2015b; Roberts et al., 2015). 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 maximum acceptable concentrations for pulegone that present no appreciable risk for skin sensitization calculated for each product category as described by Api et al. (RIFM, 2020) based on the non-reactive DST. These levels represent maximum acceptable concentrations based on the DST approach. However, additional studies may show it could be used at higher levels.

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/06/20.

11.1.5. Phototoxicity/photoallergenicity

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

11.1.5.1. Risk assessment. There are no phototoxicity studies available for pulegone in experimental models. UV/Vis absorption spectra indicate minor absorbance between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry, 2009). Based on the lack of significant absorbance in the critical range, pulegone does not present a concern for phototoxicity or photoallergenicity.

Table 1
Maximum acceptable concentrations for pulegone that present no appreciable risk for skin sensitization based on non-reactive DST.

IFRA Category[a]	Description of Product Type	Maximum Acceptable Concentrations in Finished Products Based on Non-reactive DST	Reported 95th Percentile Use Concentrations in Finished Products
1	Products applied to the lips	0.069%	0.0014%
2	Products applied to the axillae	0.021%	0.0022%
3	Products applied to the face using fingertips	0.41%	2.1×10^{-4}%
4	Fine fragrance products	0.39%	0.017%
5	Products applied to the face and body using the hands (palms), primarily leave-on	0.10%	0.0037%
6	Products with oral and lip exposure	0.23%	0.012%
7	Products applied to the hair with some hand contact	0.79%	6.5×10^{-4}%
8	Products with significant ano-genital exposure	0.041%	No Data[c]
9	Products with body and hand exposure, primarily rinse-off	0.75%	0.0023%
10	Household care products with mostly hand contact	2.7%	0.0031%
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate	1.5%	No Data[c]
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted	0.12%

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

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) for pulegone were obtained. The spectra indicate minor 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: 04/06/20.

11.1.6. Local respiratory toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for pulegone is below the Cramer Class III* TTC value

for inhalation exposure local effects.

11.1.6.1. Risk assessment. There are insufficient inhalation data available on pulegone. Based on the Creme RIFM Model, the inhalation exposure is 0.0018 mg/day. This exposure is 261 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 for the local respiratory toxicity endpoint.

Additional References: Ellis and Baxendale (1997); Rice and Coats (1994); Coats et al. (1991).

Literature Search and Risk Assessment Completed On: 04/03/20.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of pulegone 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, pulegone was identified as a fragrance material with no potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC <1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) did not identify pulegone 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 $\geq$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).

11.2.2. Risk assessment

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

11.2.2.1. Key studies

11.2.2.1.1. Biodegradation. For CAS # 89-82-7.

RIFM, 2014: The ready biodegradability of the test material was evaluated using the manometric respirometry test according to the OECD 301F guideline. Biodegradation of 83% was observed after 28 days under test conditions.

11.2.2.1.2. Ecotoxicity. No data available.

11.2.2.1.3. Other available data. Pulegone (CAS # 89-82-7) has been pre-registered under REACH with no additional information available at this time.

11.2.3. Risk assessment refinement

Pulegone has passed the screening criteria, measured data is included for completeness only and has not been used in PNEC derivation.

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	3.2	3.2
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band*	<1	<1
Risk Characterization: PEC/PNEC	**<1**	**<1**

*Combined Regional Volumes of Use for all CAS #s.

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

The RIFM PNEC is 0.01856 μg/L. The revised PEC/PNECs for EU and NA are not applicable. The material was cleared at the screening-level; therefore, it does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 04/03/20.

12. Literature Search*

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

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	18.56			1000000	0.01856	

links listed above were active as of 09/30/20.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2021.112092.

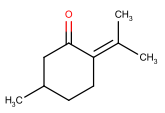
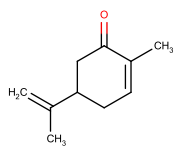
Appendix

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 Chemicals Agency read-across assessment framework (ECHA, 2017).

- 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 material 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 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).
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018), and skin sensitization was predicted using Toxtree.
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).

	Target Material	Read-across Material
Principal Name	Pulegone	Carvone mixture
CAS No.	89-82-7	99-49-0 6485-40-1 2244-16-8
Structure		
Similarity (Tanimoto Score)		0.60

(*continued on next page*)

(*continued*)

	Target Material	Read-across Material
Read-across Endpoint		• Developmental Toxicity
Molecular Formula	$C_{10}H_{16}O$	$C_{10}H_{14}O$
Molecular Weight	152.23	150.22
Melting Point (°C, EPI Suite)	10.17	9.86
Boiling Point (°C, EPI Suite)	224	228.5
Vapor Pressure (Pa @ 25°C, EPI Suite)	1.64E+001	1.37E+001
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	3.08	2.71
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	173.7	1300
J_{max} (μg/cm^2/h, SAM)	170.350	79.350
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.05E+001	7.83E+000
Developmental Toxicity		
ER Binding (OECD QSAR Toolbox v4.2)	• Non-binder, without OH or NH2 group	• Non-binder, without OH or NH2 group
Developmental Toxicity (CAESAR v2.1.6)	• Toxicant (good reliability)	• Toxicant (low reliability)
Metabolism		
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	See Supplemental Data 1	See Supplemental Data 2

Summary

There are insufficient toxicity data on pulegone (CAS # 89-82-7). 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, carvone mixture (CAS #s 99-49-0, 6485-40-1, and 2244-16-8) was identified as a read-across analog with sufficient data for toxicological evaluation.

Conclusions

- Carvone mixture (CAS #s 99-49-0, 6485-40-1, and 2244-16-8) was used as a read-across analog for the target material pulegone (CAS # 89-82-7) for the developmental toxicity endpoint.
  - o The target material and the read-across analog are structurally similar and belong to a class of α,β-unsaturated cyclic ketones.
    - oThe target material and the read-across analog share a 6-membered cyclic ketone bearing an α,β-unsaturation and a methyl group.
    - oThe key difference between the target material and the read-across analog is that the target material has a fully saturated cyclohexanone with a methyl group in position 5 and a 1-methylethylidene branch in position 2, whereas the read-across analog has an unsaturated cylohexen-1-one ring with a methyl group in position 2 and a 1-methylethenyl in position 5. This structural difference is toxicologically insignificant.
    - oSimilarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
    - oThe physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
    - oAccording to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
    - oBoth the target material and the read-across analog have a toxicant alert for developmental toxicity (CAESAR v2.1.6). The data described in the reproductive toxicity section above show that the MOE is adequate at the current level of use. The predictions are superseded by the data.
    - oThe target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
    - oThe structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

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.

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, Intermediate (Class II)

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