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RIFM fragrance ingredient safety assessment, carveol, CAS Registry Number 99-48-9

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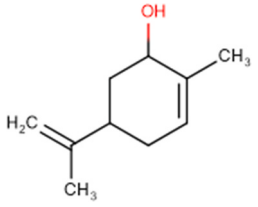
Name: Carveol
CAS Registry Number: 99-48-9
2102-59-2 *laevo*-Carveol



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1197-07-5 *trans*-Carveol (No Reported Use)
*Included 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

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CNIH – Confirmation of No Induction in Humans test. A human repeat insult patch test that is performed to confirm an already determined safe use level for fragrance ingredients (Na et al., 2021)
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
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 Observed 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.

Carveol was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that carveol is not genotoxic. Data on read-across material *d*-carvone (CAS # 2244-16-8) provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and reproductive toxicity endpoints. 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/visible (UV/Vis) spectra; carveol 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 I material, and the exposure to carveol is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; carveol 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. (Mortelmans et al., 1986; RIFM, 2014)
Repeated Dose Toxicity: NOAEL = 30 mg/kg/day. (EFSA, 2014)
Reproductive Toxicity: Developmental toxicity: NOAEL = 12.5 mg/kg/day. Fertility: NOAEL = 90 mg/kg/day.
(ECHA REACH Dossier: L-p-mentha-1(6),8-dien-2-one; ECHA, 2013; EFSA, 2014)
Skin Sensitization: No safety concerns at current, declared use levels. Exposure is below the DST.
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic. (UV/Vis Spectra; RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:
Persistence:
Screening-level: 3.02 (BIOWIN 3) (EPI Suite v4.11; US EPA, 2012a)
Bioaccumulation:
Screening-level: 53.16 L/kg (EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:
Critical Ecotoxicity Endpoint: Fish LC50: 15.49 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: 15.49 mg/L (RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.01549 μg/L
- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: Not applicable; cleared at screening-level

1. Identification

Chemical Name: Carveol	Chemical Name: *laevo*-Carveol	Chemical Name: *trans*-Carveol
CAS Registry Number: 99-48-9	**CAS Registry Number:** 2102-59-2	**CAS Registry Number:** 1197-07-5
Synonyms: 2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-; *p*-Mentha-6,8-dien-2-ol; 1-Methyl-4-isopropenyl-6-cyclohexen-2-ol; 1-メチル-4-イソプロペニル-6-シクロヘキセン-2-オール; 5-Isopropenyl-2-methylcyclohex-2-en-1-ol; Carveol	**Synonyms:** 6-Cyclohexen-2-ol, 1-methyl-4-isopropenyl-, l-; *l-p*-Mentha-6,8-dien-2-ol; *l*-1-Methyl-4-isopropenyl-6-cyclohexen-2-ol; (1R-*cis*)-2-Methyl-5-(1-methylvinyl)cyclohex-2-en-1-ol; 5-Isopropenyl-2-methylcyclohex-2-en-1-ol; *laevo*-Carveol	**Synonyms:** 2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, *trans*-; *p*-Mentha-6,8-dien-2-ol, *trans*; *trans*-Carveol
Molecular Formula: $C_{10}H_{16}O$	**Molecular Formula:** $C_{10}H_{16}O$	**Molecular Formula:** $C_{10}H_{16}O$
Molecular Weight: 152.23 g/mol	**Molecular Weight:** 152.23 g/mol	**Molecular Weight:** 152.23 g/mol
RIFM Number: 6271	**RIFM Number:** 290	**RIFM Number:** None
Stereochemistry: Isomer not specified. Two total chiral centers and 4 distereoisomers possible.	**Stereochemistry:** 1S, 3S isomer specified. Two total chiral centers and 4 distereoisomers possible.	**Stereochemistry:** 1R, 3S isomer specified. Two total chiral centers and 4 distereoisomers possible.

2. Physical data

CAS # 99-48-9	CAS # 2102-59-2	CAS # 1197-07-5
Boiling Point: 227 °C (Fragrance Materials Association [FMA] Database), 230.02 °C (EPI Suite)	**Boiling Point:** 230.02 °C (EPI Suite)	**Boiling Point:** 230.02 °C (EPI Suite)
Flash Point: >93 °C (Globally Harmonized System [GHS]), 200 °F; CC (FMA Database)	**Flash Point:** 92 °C (GHS)	**Flash Point:** Not Available
Log K_{OW}: 3.29 (EPI Suite)	**Log K_{OW}:** 3.29 (EPI Suite)	**Log K_{OW}:** 3.29 (EPI Suite)
Melting Point: 5.73 °C (EPI Suite)	**Melting Point:** 5.73 °C (EPI Suite)	**Melting Point:** 5.73 °C (EPI Suite)
Water Solubility: 519.7 mg/L (EPI Suite)	**Water Solubility:** 519.7 mg/L (EPI Suite)	**Water Solubility:** 519.7 mg/L (EPI Suite)
Specific Gravity: 1.496 (FMA Database)	**Specific Gravity:** Not Available	**Specific Gravity:** Not Available
Vapor Pressure: 0.00787 mm Hg at 20 °C (EPI Suite v4.0), 0.0132 mm Hg at 25 °C (EPI Suite)	**Vapor Pressure:** 0.00787 mm Hg at 20 °C (EPI Suite v4.0), 0.0132 mm Hg at 25 °C (EPI Suite)	**Vapor Pressure:** 0.0132 mm Hg at 25 °C (EPI Suite)
UV Spectra: No significant absorbance between 290 and 700 nm; the molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})	**UV Spectra:** No significant absorbance between 290 and 700 nm; the molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})	**UV Spectra:** Not Available
Appearance/ Organoleptic: Not available	**Appearance/ Organoleptic:** A colorless liquid that has an odor more Caraway-like than Spearmint-like.	**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 Fine Fragrance:** 0.0024% (RIFM, 2019)
2. **Inhalation Exposure******:** 0.0000077 mg/kg/day or 0.00050 mg/day (RIFM, 2019)
3. **Total Systemic Exposure*******:** 0.00031 mg/kg/day (RIFM, 2019)

*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 fine fragrance, inhalation exposure, and total exposure.

**95th percentile calculated exposure derived from concentration survey data in the Creme RIFM Aggregate Exposure Model (Comiskey et al., 2015; Safford, 2015a, 2017; and Comiskey et al., 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; Safford, 2015a, 2017; and Comiskey et al., 2017).

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 I, Low

Expert Judgment	Toxtree v3.1	OECD QSAR Toolbox v4.2
I	I	I

2. Analogs Selected:

a. **Genotoxicity:** None
b. **Repeated Dose Toxicity:** *d*-Carvone (CAS # 2244-16-8)
c. **Reproductive Toxicity:** *d*-Carvone (CAS # 2244-16-8)
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

Mascher (2001): The pharmacokinetic profile of carvone was determined in 15 healthy human volunteers. Essential oil, caraway oil consisting of 50%–65% *d*-carvone, was administered either as enteric-coated (50 mg) or non-enteric-coated (20 mg) capsules. The volunteers were part of an open, randomized, 2-period cross-over study with a washout period of 1 week before administration of the second formulation. The volunteers first received the 2 enteric-coated capsules equivalent to 100 mg/person dose of caraway oil (50%–65% *D*-carvone). The capsules were administered after a 10-h fast and with 250 mL of water. Blood was withdrawn between 0 and 15 h time intervals. The plasma samples were examined by GC/MS to determine the following pharmacokinetic parameters: C_{max}, T_{max}, AUC, and $t_{1/2}$. The same protocol was followed for the non-enteric-coated capsules following a 1-week washout period, wherein the volunteers received 5 capsules (100 mg/person) of caraway oil followed by plasma sampling as described above. The pharmacokinetic parameters determined are summarized in the table below (Table 1). The parameters indicated that *d*-carvone has a plasma half-life of 2.4 h and is more readily absorbed from non-enteric-coated capsules.

Engel (2001): Metabolism of *d*- and *l*-carvone was investigated in 6 (3/sex) healthy human volunteers. Carvone was administered at doses of 1 mg/kg body weight, and urine was collected 24 h before and after administration. The metabolites were identified by MS using synthetic standards and NMR analysis. The urine samples were treated with sulphatase and glucuronidase. The metabolites identified included 3 side-chain oxidation products as the main primary unconjugated metabolites of *d*- and *l*-carvone: dihydrocarvonic acid, carvonic acid, and uroterpenolone, with 10-hydroxycarvone as the proposed intermediate metabolic step (see Fig. 1). However, 10-hydroxycarvone was not detected in humans, and the authors suggested this was due to efficient oxidation of it to produce carvonic acid. The authors also identified minor metabolites in the form of reduction products of carvone: carveol and dihydrocarveol. The authors concluded there were no differences

Table 1
Pharmacokinetic parameters in human study (Mascher, 2001).

Carvone	Enteric-coated mean ± SD (geometric mean)	Non-enteric-coated mean ± SD (geometric mean)
AUC (0-∞) (ng/mL × h)	40.8 ± 74.6 (24.28)	28.9 ± 20 (25.12)
C_{max} (ng/mL)	14.9 ± 23.2 (9.92)	14.8 ± 10.4 (12.57)
T_{max} (h)	2.5 ± 0.7 (2.41)	1.3 ± 0.6(1.24)
$t_{1/2}$ (h)	2.5 ± 0.7 (2.4)	2.4 ± 1.2 (2.0)

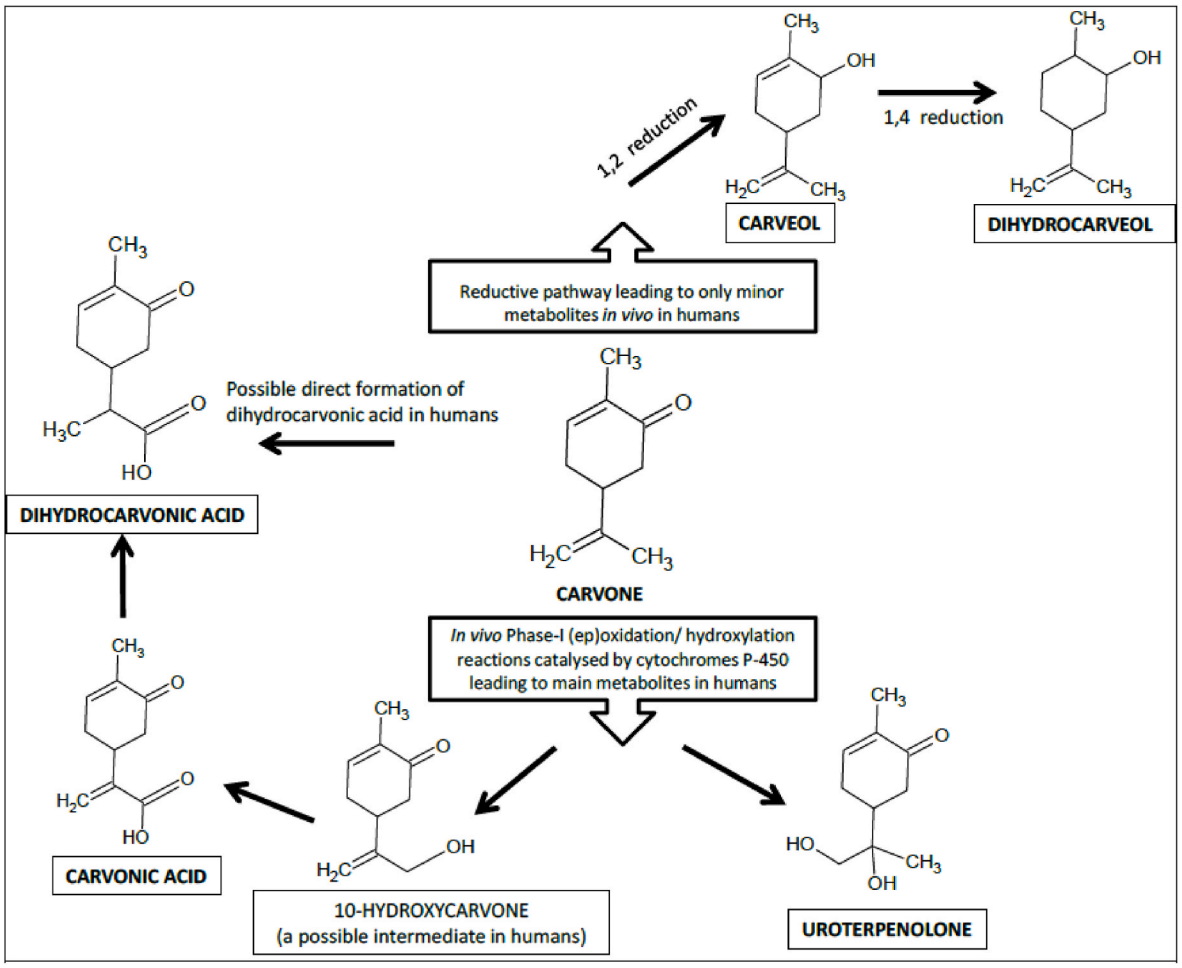


Fig. 1. (Adapted from EFSA report on carvone; EFSA, 2014).

observed between the metabolisms of *l*- and *d*-carvone.

Jager et al., 2000: *In vitro* metabolism of R-(−)- and S-(+)-carvone (*l*- and *d*-carvone, respectively) was studied in liver microsomes from the rat (Sprague Dawley males) and humans (undergoing liver resection; 1 female, 2 males) using chiral gas chromatography. The results indicated the sole metabolite formed from *l*-carvone was 4R, 6S-(−)-carveol, whereas the sole metabolite from *d*-carvone was 4S, 6S-(+)-carveol. In both rat and human microsomes, a significantly lower apparent Michaelis-Menten Constant (Km) was observed for 4R, 6S-(−)-carveol compared to 4S, 6S-(+)-carveol. The maximal formation rate (Vmax) was almost twice as high with human liver microsomes when compared to rat microsomes. When the rat and human liver microsomes were incubated in the presence of UDPGA (uridine 5′-diphosphoglucuronic acid), only the glucuronidation of 4R, 6S-(−)-carveol was observed, and the Vmax for glucuronide formation was more than 4-fold higher in the rat liver compared with human liver preparations (no species-related differences were observed for Km values). This *in vitro* study demonstrated stereoselective phase-I and phase-II metabolism for *l*- and *d*-carvone.

Shimada et al., 2002: *In vitro* metabolism of *d*-carvone ((+)-carvone), *d*-carveol ((+)-carveol), and other structurally related terpenoids was investigated using liver microsomes from mice, rats, guinea pigs, rabbits, dogs, monkeys, and humans. Microsomes were obtained from male liver samples for all of the species except for the rat, in which microsomal preparations of livers from both male and female Sprague Dawley rats were assessed. When *d*-carveol and *d*-carvone were used as substrates, dogs, rabbits, and guinea pigs metabolized them to *d*-carvone and *d*-carveol, respectively. In contrast, humans, monkeys, rats, and mice did not convert *d*-carveol to *d*-carvone but metabolized *d*-carvone to *d*-carveol, with liver microsomes from male rats having the highest rates. Hepatic CYP2C enzymes were suggested to play a major role in metabolizing *d*-carveol to *d*-carvone and *d*-carvone to *d*-carveol since the activities were inhibited significantly by anti-human CYP2C9 antibodies. Studies with recombinant P450 enzymes suggested that CYP2C9 and CYP2C19 in humans had the highest activities. CYP2C11 and CYP2B1 in male rats were the major enzymes in metabolizing (+)-carvone. Female-specific CYP2C12 had very low activity, suggesting that the metabolism of carvone by female rats may be slower than males. These results suggest that there are species-related differences in the metabolism of *d*-carvone, and for rats, potentially sex-related differences.

Conclusions: Human toxicokinetic studies on *d*-carvone suggest rapid clearance from plasma with a plasma half-life of 2.4 h. No such data are available on *l*-carvone (Mascher, 2001). Data from *in vitro* and *in vivo* metabolic studies indicate species differences (Shimada et al., 2002; Jager et al., 2000). Since rats have a tendency to undergo enterohepatic recirculation, and no such recirculation has been demonstrated via human *in vivo* studies, this makes rats more susceptible to liver effects from carvone or its metabolites.

8. Natural occurrence

Carveol is reported to occur in the following foods by the VCF*:
Black currants (*Ribes nigrum* L.)
Celery (*Apium graveolens* L.)
Dill (*Anethum* species)

Eucalyptus oil (*Eucalyptus globulus* Labill)
Lamb's lettuce (*Valerianella locusta*)
Mangifera species
Mentha oils
Pistacia atlantica
Tea
Thyme (*Thymus* species)

laevo-carveol and *trans*-carveol 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.

9. REACH dossier

All 3 materials are pre-registered for 2010; no dossiers available as of 12/16/21

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, carveol does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. Carveol was assessed in the BlueScreen assay and found negative for genotoxicity, with and without metabolic activation (RIFM, 2016). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures.

The mutagenic activity of carveol has been evaluated in a bacterial reverse mutation assay conducted by the National Toxicology Program (NTP) according to guidelines similar to OECD TG 471 using the standard preincubation. *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 were treated with carveol in dimethyl sulfoxide (DMSO) at concentrations up to 560 μg/plate. No increases in the mean number of revertant colonies were observed at any tested dose in the presence or absence of S9 (Mortelmans et al., 1986). Under the conditions of the study, carveol was not mutagenic in the Ames test.

The clastogenicity of carveol was assessed in an *in vitro* micronucleus assay conducted in compliance with GLP regulations and in accordance with OECD 487. Human peripheral blood lymphocytes were treated with carveol in DMSO at concentrations up to 1523 μg/mL in the dose range finding (DRF) study; micronuclei analysis was conducted at concentrations up to 360 μg/mL for 4 h with and without S9 and 24 h without S9. No increase in the frequency of cells with micronuclei compared to vehicle control was observed (RIFM, 2014). Under the conditions of the study, carveol was not clastogenic in the *in vitro* micronucleus assay.

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

Additional References: None

Literature Search and Risk Assessment Completed On: 09/24/21

11.1.2. Repeated dose toxicity

There is an adequate MOE for carveol for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are no repeated dose toxicity data for carveol. Read-across material *d*-carvone (CAS 2244-16-8; see Section VI) has sufficient repeated dose toxicity data to support the repeated dose toxicity endpoint. In an NTP 90-day gavage study in Fischer 344 rats reported in 1982, groups of 10 rats/sex/group were administered *d*-carvone by gavage at 0, 93, 187, 375, 750, and 1500 mg/kg/day. Mortality and clinical signs were reported among animals of the 2 highest dose groups. Body weights were reduced among males only of the doses of 187 and 575 mg/kg/day, but not at the 2 higher doses. Organ weight analysis showed an increase in relative liver weights among all treated animals and an increase in relative kidney weights among 375 mg/kg/day group animals and the 187 mg/kg/day males. For males administered 375 mg/kg bw/day, depressed sperm motility and a mild decrease in sperm concentration were observed only at the end of the study (sperm analysis was conducted at weeks 2, 4, 6, and 8 of the study). For animals at 750 mg/kg/day, testicular degeneration and relative aspermia were observed. Similar effects were not observed among animals in the 93 and 187 mg/kg/day groups. Liver, kidney, and male reproductive organs were identified as the major organs affected by treatment with *d*-carvone. A NOAEL was not derived from the study since the adversity of a significant increase in relative liver weights could not be confirmed with related alterations in clinical chemistry parameters. However, there were no alterations in liver histopathology reported during the microscopic examination. Microscopic alterations in the kidney were reported only among males of the 375 mg/kg/day dose groups, which could be related to male rat–specific alterations related to α-2u-globulin accumulation; however, this was not confirmed by appropriate staining techniques (EFSA, 2014)*. Overall, the lack of appropriate study details and limitations in study design precluded the derivation of a NOAEL.

In another study, groups of 10 Wistar Crl:(WI)BR rats/sex/dose administered test material *d*-carvone in corn oil by gavage at doses of 0, 5, 30, and 180 mg/kg/day. The study was conducted according to OECD 408/GLP guidelines. Hematological analysis showed significantly reduced prothrombin time (PT) among high-dose males and a dose-related increase in partial thromboplastin time (PTT) among mid- and high-dose females. Organ weight analysis revealed a significant increase in relative liver weights among animals of the high-dose group and mid-dose females. The relative kidney weights were significantly increased among male and female animals of the high- and mid-dose groups in a dose-related manner. Microscopic examination revealed tubular necrosis of the kidney in males only and basophilic tubules in both males and females of the high-dose group. A follow-up study examining the kidney slides from all treated and control group animals confirmed the tubular necrosis in male rats to be due to renal accumulation of α 2u-globulin (confirmed by highly positive staining of the treated high-dose group rats with antibody against α-2u-globulin), which is species-specific to male rats in response to treatment with some hydrocarbons. This effect is not considered a hazard to human health (Lehman-McKeeman and Caudill, 1992 and Lehman-McKeeman et al., 1990). The follow-up study did not report any histopathological alteration among female kidneys. Thus, the kidney weight alterations were not considered to be an adverse effect in relation to treatment with carvone. The hematological alterations were not considered to be an adverse effect following treatment with carvone, thus the NOAEL was considered to be 30 mg/kg/day, based on a decrease in body weight and food consumption among high-dose males (EFSA, 2014)*.

In another study, an OECD/GLP 416, 2-generation reproductive toxicity study was conducted on groups of 25 Wistar Crl:(WI)Br rats/sex/dose administered test material *d*-carvone by gavage at doses of 0, 3, 10, or 30 mg/kg/day. For the F1 generation, the dose levels were changed to 0, 10, 30, and 90 mg/kg/day. Males of the F0 generation were dosed at 30 mg/kg/day. The 30 and 90 mg/kg/day dose groups in

the F1 generation had increased relative kidney weights. Histopathological results showed the kidney weight increases to be related to well-documented changes of α 2u-globulin nephropathy, which is species-specific to male rats in response to treatment with some hydrocarbons. This effect is not considered a hazard to human health (Lehman-McKeeman and Caudill, 1992 and Lehman-McKeeman et al., 1990). Statistically significant increases in the relative liver weights (up to approximately 15%) were observed in males of the F1 generation dosed at 30 and 90 mg/kg/day. Histopathological evaluations of these livers were not performed. No differences were seen in females and no other histopathological changes were reported. Since there was no histopathological examination associated with the increased relative liver weights, a NOAEL for systemic toxicity was considered to be 90 mg/kg/day (EFSA, 2014)*.

In another study, groups of 40 B6C3F1 mice (30 males and 10 females) were administered test material *d*-carvone in corn oil at doses of 0, 93, 375, and 1,500 mg/kg/day, 5 days per week, for 13 weeks. An additional group of 20 B6C3F1 mice (10 per sex) were administered 187 and 750 mg/kg/day. All 30 males and 9/10 females treated with 1500 mg/kg/day and 1/10 males treated with 93 mg/kg/day died before the end of the study. The final mean body weight of the only high-dose female survivor was 12% lower than that of the controls. The relative liver weights for the animals treated with 750 mg/kg/day were significantly greater than the controls, but no treatment-related lesions were observed during microscopy. The NOAEL for systemic toxicity was considered to be 375 mg/kg/day, based on mortality and increase in relative liver weights among higher dose group animals (National Toxicology Program, 1990). Subsequently, a 2-year carcinogenicity study was conducted on groups of 100 B6C3F1 mice/dose (50 per sex) administered 0, 375, or 750 mg/kg of *d*-carvone in corn oil by gavage, 5 days per week, for 103 weeks. The control group of 100 animals (50 per sex) was treated with the vehicle only. Under the conditions of this study, there was no evidence of carcinogenic activity of *d*-carvone for male or female mice (National Toxicology Program, 1990).

As described in the metabolism section (see Section VI), rats are suspected to be more sensitive to the effects of treatment with carvone as compared to humans due to enterohepatic recirculation of its metabolites among rats but not humans. This may explain the liver weight increase in rats as seen during the 90-day rat studies, thus making the rats the more sensitive species to the effects of carvone treatment. It is to be noted that the 2-year carcinogenicity study conducted in mice did not show any evidence of tumors up to doses of 750 mg/kg/day (National Toxicology Program, 1990). However, the most conservative NOAEL of 30 mg/kg/day was considered for the repeated dose toxicity study based on a decrease in body weights among rats during the OECD 408, 90-day gavage study (EFSA, 2014)*.

Therefore, the carveol MOE for the repeated dose toxicity endpoint can be calculated by dividing the *d*-carvone NOAEL in mg/kg/day by the total systemic exposure to carveol, 30/0.00031 or 96774.

In addition, the total systemic exposure to carveol (0.31 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

*The original study reports were not available for review.

Additional References: None

Literature Search and Risk Assessment Completed On: 09/17/21

11.1.3. Reproductive toxicity

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

11.1.3.1. Risk assessment. There are no reproductive toxicity data on carveol. Read-across material *d*-carvone (CAS # 2244-16-8; see Section VI) has sufficient reproductive toxicity data that can be used to support the reproductive toxicity endpoint.

An OECD 414 prenatal developmental toxicity study was conducted with *l*-carvone administered to 24 females 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. A 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 was observed in all the treatment groups as compared to the control. 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 only in all treated groups. Thus, these transitional findings were considered to be adverse to the early prenatal development of the organism in the uterus. Thus, 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 carveol MOE for the developmental toxicity endpoint can be calculated by dividing the *d*-carvone NOAEL in mg/kg/day by the total systemic exposure to carveol, 12.5/0.00031 or 40322.**

An OECD/GLP 416 2-generation reproductive toxicity study was conducted in Wistar Crl:(WI)Br rats. Groups of 25 rats/sex/dose were administered d-carvone via oral gavage at doses of 0, 3, 10, or 30 mg/kg/day for 10 weeks prior to mating until termination. For the F1 generation, the dose levels were changed to 0, 10, 30, and 90 mg/kg/day. There were no differences between the controls and treated animals in any of the reproductive performance parameters, sperm morphology and motility, and estrous cycle. Thus, the fertility NOAEL was considered to be 90 mg/kg/day, the highest dose tested (EFSA, 2014)*. **Therefore, the carveol MOE for the fertility endpoint can be calculated by dividing the *d*-carvone NOAEL in mg/kg/day by the total systemic exposure to carveol, 90/0.00031, or 290322.**

In addition, the total systemic exposure to carveol (0.31 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

*The original study reports were not available for review.

Additional References: None

Literature Search and Risk Assessment Completed On: 09/17/21

11.1.4. Skin sensitization

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

11.1.4.1. Risk assessment. The chemical structure of this material indicates that it would be expected to react with skin proteins (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). No predictive skin sensitization studies are available for carveol. In a Freund's complete adjuvant test (FCAT), additional material *laevo*-carveol did not present reactions indicative of sensitization (Karlberg et al., 1992). In a human maximization test, no skin sensitization reactions were observed at 4%

(2760 μg/cm^2) of additional material *laevo*-carveol (RIFM, 1972). Acting conservatively due to the limited data, the reported exposure was benchmarked utilizing the reactive DST of 64 μg/cm^2 (Roberts et al., 2015; Safford, 2008, 2011, 2015b). The current exposure from the 95th percentile concentration is below the DST for non-reactive materials when evaluated in all QRA categories. Table 2 provides the maximum acceptable concentrations for carveol that present no appreciable risk for skin sensitization based on the 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: 06/04/21

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis absorption spectra, carveol 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 carveol in experimental models. UV/Vis absorption spectra indicate no absorption between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). Based on the lack of absorbance, carveol does not present a concern for phototoxicity or photoallergenicity.

Table 2
Maximum acceptable concentrations for carveol that present no appreciable risk for skin sensitization based on reactive DST.

IFRA Category[a]	Description of Product Type	Maximum Acceptable Concentrations in Finished Products Based on Reactive DST	Reported 95th Percentile Use Concentrations in Finished Products
1	Products applied to the lips	0.0049%	5.6×10^{-6}%
2	Products applied to the axillae	0.0015%	6.9×10^{-4}%
3	Products applied to the face using fingertips	0.029%	5.1×10^{-5}%
4	Fine fragrance products	0.027%	0.0024%
5	Products applied to the face and body using the hands (palms), primarily leave-on	0.0070%	4.6×10^{-4}%
6	Products with oral and lip exposure	0.016%	0.0021%
7	Products applied to the hair with some hand contact	0.056%	1.5×10^{-5}%
8	Products with significant ano-genital exposure	0.0029%	No Data[b]
9	Products with body and hand exposure, primarily rinse-off	0.054%	0.028%
10	Household care products with mostly hand contact	0.19%	6.6×10^{-4}%
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate	0.11%	0.033%
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not restricted	0.0024%

Note:
[b]No reported use.
[a] For a description of the categories, refer to the IFRA/RIFM Information Booklet.
[b] 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) were obtained. The spectra indicate no 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: 09/17/21

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for carveol is below the Cramer Class I TTC value for inhalation exposure local effects.

11.1.6.1. Risk assessment. There are limited inhalation data available on carveol. Based on the Creme RIFM Model, the inhalation exposure is 0.00050 mg/day. This exposure is 2800 times lower than the Cramer Class I TTC value of 1.4 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: Rice and Coats, 1994

Literature Search and Risk Assessment Completed On: 09/17/21

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of carveol 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, carveol 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 carveol as possibly being persistent or bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above

screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11).

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

11.2.1.2. Key studies

11.2.1.2.1. Biodegradation. No data available.

11.2.2. Ecotoxicity

No data available.

11.2.2.1. Other available data. Carveol has been pre-registered for REACH with no additional available data at this time.

11.2.2.2. Risk assessment refinement. Ecotoxicological data and PNEC derivation (all endpoints reported in mg/L; PNECs in μg/L)

Endpoints used to calculate PNEC are underlined.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	3.29	3.29
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 Volume of Use.

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

The RIFM PNEC is 0.01549 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: 09/17/21

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 links listed above were active as of 12/16/21.

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.2022.113269.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	15.49			1000000	0.01549	

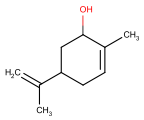
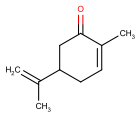
Appendix

Read-across Justification

Methods

The read-across analog was identified following the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015). The strategy is also consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemical Agency read-across assessment framework (ECHA, 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).
- 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	Carveol	*d*-Carvone
CAS No.	99-48-9	2244-16-8
Structure		
Similarity (Tanimoto Score)		0.51
Read-across Endpoint		• Reproductive Toxicity • Repeated Dose Toxicity
Molecular Formula	$C_{10}H_{16}O$	$C_{10}H_{14}O$
Molecular Weight (g/mol)	152.23	150.22
Melting Point (°C, EPI Suite)	5.73	9.86
Boiling Point (°C, EPI Suite)	228	228.5
Vapor Pressure (Pa @ 25°C, EPI Suite)	1.76	17.3
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	3.12	2.71
Water Solubility (μg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	519.7	1300
J_{max} (μg/cm^2/h, SAM)	959.138	79.35
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.41E+000	7.83E+000
Repeated Dose Toxicity		
Repeated Dose (HESS)	• Not categorized	•Not categorized
Reproductive Toxicity		
ER Binding (OECD QSAR Toolbox v4.2)	•Weak binder, OH 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 carveol (CAS # 99-48-9). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, metabolism data, physical–chemical properties, and expert judgment, *d*-carvone (CAS # 2244-16-8) was identified as a read-across analog with sufficient data for toxicological evaluation.

Metabolism

The metabolism of the target material carveol (CAS # 99-48-9) was predicted using the Rat Liver S9 Metabolism Simulator (OECD QSAR Toolbox v4.2). The target material is predicted to be metabolized to *d*-carvone (CAS # 2244-16-8) in the first step with a 0.95 probability. Hence, *d*-carvone (CAS # 2244-16-8) can be used as a read-across analog for the target material. Read-across material *d*-carvone (CAS # 2244-16-8) was in domain for the *in vivo* and *in vitro* rat S9 simulator (OASIS TIMES v2.27.19).

Conclusions

- *d*-Carvone (CAS # 2244-16-8) was used as a read-across analog for the target material carveol (CAS # 99-48-9) for the repeated dose toxicity and reproductive toxicity endpoints.
  - o The target material and the read-across analog are structurally similar and belong to a class of cyclic α,β unsaturated secondary alcohol and cyclic α,β unsaturated ketone.
  - o The read-across is the ketone resulting from the oxidation of the target secondary alcohol.
  - o The key difference between the target material and the read-across analog is that the target material is a secondary alcohol and the read-across analog is the resulting ketone from its oxidation. This structural difference is toxicologically insignificant.
  - o Similarity 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.
  - o The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - o According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - o The target material has an ER Binding (OECD QSAR Toolbox v4.2) alert for weak binder due to the hydroxy group attached to a 6 membered ring and a molecular weight below 170. Additionally, both target and read-across materials have a developmental toxicity alert (CAESAR v2.1.6) as toxicants. The data described in the developmental toxicity section show that the MOE is adequate at the current level of use. The predictions are superseded by the data.
  - o The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - o The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

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