Food and Chemical Toxicology xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Short Review

RIFM fragrance ingredient safety assessment, caryophyllene oxide, CAS Registry Number 1139-30-6

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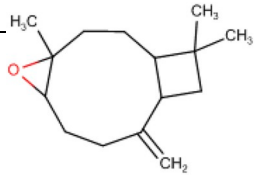
ARTICLE INFO

Keywords:
Genotoxicity
Repeated dose, developmental, and reproductive toxicity
Skin sensitization
Phototoxicity/photoallergenicity
Local respiratory toxicity
Environmental safety

Version: 100218. This version replaces any previous versions.

Name: Caryophyllene oxide
CAS Registry Number: 1139-30-6



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https://doi.org/10.1016/j.fct.2019.111102
Received 12 August 2019; Received in revised form 16 November 2019; Accepted 25 December 2019

Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015a, 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observable Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
QRA - Quantitative Risk Assessment
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use
vPvB - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

The Expert Panel for Fragrance Safety* concludes that this material is safe as described in this safety assessment.
This safety assessment is based on the RIFM Criteria Document (Api, 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.

Caryophyllene oxide was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that caryophyllene oxide is not genotoxic. Data on caryophyllene oxide provide a calculated MOE > 100 for the repeated dose toxicity and fertility endpoints. The developmental and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class III material, and the exposure to caryophyllene oxide is below the TTC (0.0015 mg/kg/day and 0.47 mg/day, respectively). The skin sensitization endpoint was completed using the DST for reactive materials (64 μg/cm^2); exposure is below the DST. The phototoxicity/photoallergenicity endpoints were evaluated based on data and UV spectra; caryophyllene oxide provide is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; caryophyllene oxide provide was found not to be PBT as per the IFRA Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., PEC/PNEC), are < 1.

Human Health Safety Assessment

Genotoxicity: Not genotoxic. (RIFM, 1979b; RIFM, 2018)
Repeated Dose Toxicity: NOAEL = 109 mg/kg/day (RIFM, 2013)
Reproductive Toxicity: Developmental toxicity: No NOAEL available. Exposure is below the TTC.
Fertility: NOAEL = 1398 mg/kg/day. (RIFM, 2013)
Skin Sensitization: Sensitizer, but does not present a concern under the current, declared levels of use.
Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic. (UV Spectra, RIFM DB; RIFM, 1979c)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:

Persistence:
Screening-level: 2.2 (BIOWIN 3) (EPI Suite v4.1; US EPA, 2012a)
Bioaccumulation:
Screening-level: 802 L/kg (EPI Suite v4.1; US EPA, 2012a)
Ecotoxicity:
Screening-level: 48-h *Daphnia magna* LC50: 0.281 mg/L (ECOSAR v1.11; US EPA, 2012b)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:
Screening-level: PEC/PNEC (North America and Europe) > 1 (RIFM Framework; Salvito, 2002)
Critical Ecotoxicity Endpoint: 0.281 mg/L (ECOSAR v1.11; US EPA, 2012b)
RIFM PNEC is: 0.0281 μg/L
• **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: < 1

1. Identification

1. **Chemical Name:** Caryophyllene oxide
2. **CAS Registry Number:** 1139-30-6
3. **Synonyms:** β-Caryophyllene oxide; 5-Oxatricyclo[8.2.0.04,6]dodecane, 4,12,12-trimethyl-9-methylene-, [1R-(1R*,4R*,6R*,10S*)]-; 5-Oxatricyclo[8.2.0.04,6]dodecane, 4,12,12-trimethyl-9-methylene-, (1R,4R,6R,10S)-; Caryophyllene epoxide; ｶﾘｵﾌｨﾚﾝﾓﾉｴﾎﾟｷｻｲﾄﾞ; 4,12,12-Trimethyl-9-methylene-5-oxatricyclo[8.2.0.0–4,6～]dodecane; β-caryophyllene epoxide; Caryophyllene oxide
4. **Molecular Formula:** $C_{15}H_{24}O$
5. **Molecular Weight:** 220.35
6. **RIFM Number:** 1050
7. **Stereochemistry:** 1R-(1R*, 4R*, 6R*, 10S*] isomer specified. Four stereocenters present and 16 stereoisomers possible.

2. Physical data

1. **Boiling Point:** 137 °C (7T) (Private communication to FEMA), 263.48 °C (EPI Suite)
2. **Flash Point:** > 93 °C (GHS), > 200 °F; CC (FMA Database)
3. **Log K_{OW}:** 4.91 (EPI Suite)
4. **Melting Point**: 61 °C (Private communication to FEMA), 62.95 °C (EPI Suite)
5. **Water Solubility:** 2.21 mg/L (EPI Suite)
6. **Specific Gravity:** 0.964 (FMA Database)
7. **Vapor Pressure:** 0.00572 mm Hg @ 20 °C (EPI Suite v4.0), 0.01 mm Hg @ 25 °C (EPI Suite)
8. **UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L $mol^{-1} \cdot cm^{-1}$)
9. **Appearance/Organoleptic:** A white solid

3. Exposure to fragrance ingredient

1. **Volume of Use (Worldwide Band):** 1–10 metric tons per year (IFRA, 2015)
2. **95**th **Percentile Concentration in Hydroalcoholics:** 0.0022% (RIFM, 2016)
3. **Inhalation Exposure*:** 0.0000083 mg/kg/day or 0.00062 mg/day (RIFM, 2016)
4. **Total Systemic Exposure**:** 0.000062 mg/kg/day (RIFM, 2016)

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

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

4. Derivation of systemic absorption

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

5. Computational toxicology evaluation

1. **Cramer Classification:** Class III, High

Expert Judgment	Toxtree v 2.6	OECD QSAR Toolbox v 3.2
III	III	III

2. **Analogs Selected**:
   a. Genotoxicity: None
   b. Repeated Dose Toxicity: None
   c. Reproductive Toxicity: None
   d. Skin Sensitization: None
   e. Phototoxicity/Photoallergenicity: None
   f. Local Respiratory Toxicity: None
   g. Environmental Toxicity: None
3. **Read-across Justification:** None

6. Metabolism

14-Hydroxycaryophyllene-5,6-oxide was reported as the major metabolite when caryophyllene oxide was administered orally to male rabbits. The major route of metabolic pathway was found to be hydroxylation, which predominantly forms primary alcohol. See Fig. 1 below.

Asakawa (1986): Six male rabbits (body weight of 2–3 kg) were orally administered a single dose of 12 g caryophyllene oxide (purity: 86.9%) after fasting for 1 day prior to testing. After dosing, rabbits were housed separately in metabolic cages with food and water *ad libitum*. Urine samples were collected daily for 3 days and fractions were analyzed by thin layer chromatography (TLC), gas chromatography (GC), and nuclear magnetic resonance (NMR). 14-Hydroxycaryophyllene-5,6-oxide was reported as the major metabolite, which occupied 60% of the peak area on the gas liquid chromatography column.

Additional References: None.

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

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

Turpentine oil (Pistacia terebinthus).
Curry (Bergera koenigii L.)
Wormwood oil (Artemisia absinthium L.)
Mastic (Pistacia lentiscus).
Curcuma species.
Lemon balm (Melissa officinalis L.)
Pepper (Piper nigrum L.)
Ocimum species.
Salvia species.
Thyme (Thymus species).

*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.

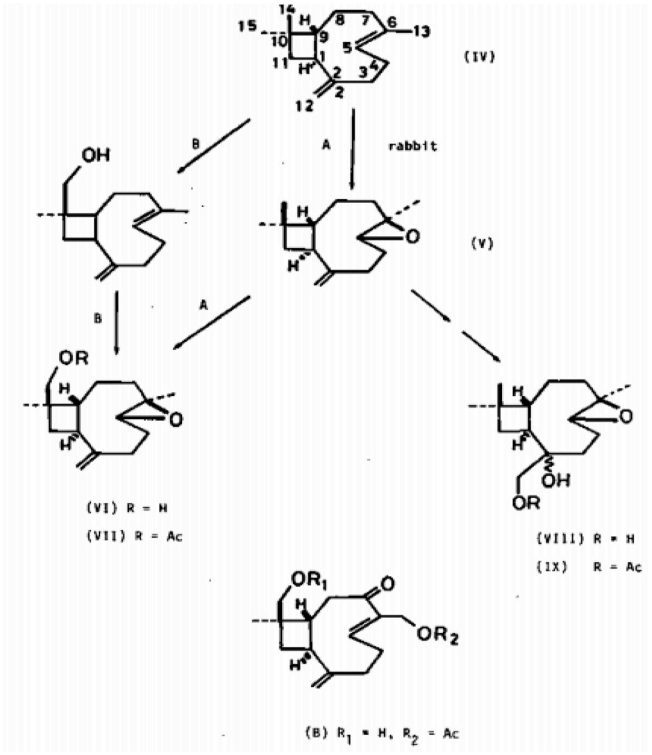

Fig. 1. Metabolism of caryophyllene oxide on gem-dimethyl and dimethyl and exo-methylene groups in rabbits (adapted from Asakawa, 1986)
Legend. V: Caryophyllene-5,6-oxide, VI: 14-Hydroxycaryophyllene-5,6-oxide, VII: 14-Acetoxycaryophyllene-5,6-oxide, VIII: caryophyllene-5,6-oxide-2,12-glycol, and IX: Caryophyllene-5,6-oxide-2,12-diol monoacetate.

8. IFRA standard

None.

9. REACH dossier

Pre-registered; no dossier available as of 10/02/18.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

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

10.1.1.1. Risk assessment. The mutagenic activity of caryophyllene oxide has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation/preincubation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 were treated with caryophyllene oxide in solvent dimethyl sulfoxide (DMSO) at concentrations up to 10000 µg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 1979b). Under the conditions of the study, caryophyllene oxide was not mutagenic in the Ames test.

The clastogenic activity of caryophyllene oxide was evaluated in an *in vitro* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes were treated with caryophyllene oxide in DMSO at concentrations up to 2000 µg/mL for a dose range finding (DRF) study. Micronuclei analysis in the main study was conducted up to 90 µg/mL in the presence and absence of metabolic activation (S9) for 4 h and in the absence of metabolic activation for 24 h. Caryophyllene oxide did not induce binucleated cells with micronuclei when tested up to cytotoxic levels concentration in either the presence or absence of an S9 activation system (RIFM, 2018). Under the conditions of the study, caryophyllene oxide was considered to be non-clastogenic in the *in vitro* micronucleus test.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/17/18.

10.1.2. Repeated dose toxicity

The margin of exposure for caryophyllene oxide is adequate for the repeated dose toxicity endpoint at the current level of use.

10.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on caryophyllene oxide. In an OECD 408 and GLP-compliant subchronic toxicity study, 10 Crl: Sprague Dawley rats/sex/dose were fed diet containing caryophyllene oxide at doses of 0 (control), 1750, 10500, and 21000 ppm (equivalent to 109, 672, and 1398 mg/kg/day for males and 137, 800, and 1660 mg/kg/day for females) for 90 days. No treatment-related effects were reported for mortality, clinical signs, ophthalmoscopic examinations, body weight, bodyweight gain, food consumption and food efficiency, hematology, coagulation, clinical chemistry, or urinalysis. A statistically significant increase in absolute and relative kidney weights in males and liver in both sexes of the mid- and high-dose groups was reported. In females, spleen weights (absolute and relative in high-dose group) and relative kidney weights (mid- and high-dose groups) were significantly decreased. However, treatment-related macroscopic alterations were not reported at any dose level. At all doses, male kidneys demonstrated α-2u-globulin nephropathy along with a dose-dependent presence of cytoplasmic droplets in proximal tubules epithelial cells. Since α-2u-globulin nephropathy is species and gender specific, this effect is not considered a human health hazard (Lehman-McKeeman, 1992 and Lehman-McKeeman et al., 1990). In both sexes at the mid and high doses, treatment-related microscopic findings were reported in the liver and mesenteric lymph nodes. The incidence and severity of these microscopic changes were dose-dependent. Liver changes were characterized by hepatocellular hypertrophy in centrilobular to midzonal regions, which correlated with the increased liver weights (< 2-fold). Due to lack of histopathological evidence for liver injury and the fact that serum activities of liver enzymes were not elevated, these liver effects were not considered treatment-related adverse events (Hall, 2012). Despite a dose-dependent presence of erythrocytes in the sinusoids of mesenteric lymph nodes reported in both sexes at the 10500 and 21000 ppm doses, histopathological changes of the stomach or intestine were not reported. Considering the effects of α-2u-globulin nephropathy and adaptive changes of liver as having minimal toxicological relevance to human health, the NOAEL for repeated dose toxicity was determined based on the observed effects in the mid- and-high dose groups. The NOAEL for repeated dose toxicity was considered to be 1750 ppm (equivalent to 109 mg/kg/day for males and 137 mg/kg/day for females). The more conservative NOAEL of 109 mg/kg/day was chosen for this risk assessment.

Therefore, the caryophyllene oxide MOE for the repeated dose toxicity endpoint can be calculated by dividing the caryophyllene oxide NOAEL in mg/kg/day by the total systemic exposure to caryophyllene oxide, 109/0.000062 or 1758065.

In addition, the total systemic exposure to caryophyllene oxide (0.062 µg/kg bw/day) is below the TTC (1.5 µg/kg bw/day; Kroes, 2007; Laufersweiler, 2012) **for the repeated dose toxicity endpoint of a Cramer Class III material at the current level of use.**

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/17/18.

10.1.3. Reproductive Toxicity

There are no developmental toxicity data on caryophyllene oxide or on any read-across materials. The total systemic exposure to caryophyllene oxide is below the TTC for the developmental toxicity endpoint of a Cramer Class III material at the current level of use.

The margin of exposure for caryophyllene oxide is adequate for the fertility endpoint at the current level of use.

10.1.3.1. Risk assessment. There are no developmental toxicity data on caryophyllene oxide or on any read-across materials that can be used to support the developmental toxicity endpoint. The total systemic exposure to caryophyllene oxide (0.062 μg/kg bw/day) is below the TTC (1.5 μg/kg bw/day; Kroes, 2007; Laufersweiler, 2012) for the developmental toxicity endpoint of a Cramer Class III material at the current level of use.

There are sufficient fertility data on caryophyllene oxide that can be used to support the fertility endpoint. An OECD 408/GLP subchronic toxicity study was conducted in Sprague Dawley rats. Groups of 10 rats/sex/dose were fed diets containing caryophyllene oxide at doses of 0, 1750, 10500, or 21000 ppm (equivalent to 0, 109, 672, and 1398 mg/kg/day for males and 0, 137, 800, and 1660 mg/kg/day for females) for 90 days. In addition to systemic toxicity parameters, effects on male and female fertility were also assessed. There were no treatment-related adverse effects on reproductive organs, estrous cycle evaluation, or spermatology up to the highest dose tested. The NOAEL for fertility was considered to be 21000 ppm (equivalent to 1398 mg/kg/day for males and 1660 mg/kg/day for females) (RIFM, 2013). The most conservative NOAEL of 1398 mg/kg/day was selected for the fertility endpoint.

Therefore, the caryophyllene oxide MOE for the fertility endpoint can be calculated by dividing the caryophyllene oxide NOAEL in mg/kg/day by the total systemic exposure to caryophyllene oxide, 1398/0.000062 or 22548387.

In addition, the total systemic exposure to caryophyllene oxide (0.062 μg/kg bw/day) is below the TTC (1.5 μg/kg bw/day; Kroes, 2007; Laufersweiler, 2012) **for the fertility endpoint of a Cramer Class III material at the current level of use.**

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/02/18.

10.1.4. Skin sensitization

Based on the existing data and the application of DST, caryophyllene oxide is considered a sensitizer, but does not present a concern for skin sensitization under the current, declared levels of use.

10.1.4.1. Risk assessment. The chemical structure of this material indicates that it would be expected to react with skin proteins (Roberts, 2007; Toxtree 3.1.0; OECD toolbox v4.2). In a guinea pig sensitization test, sensitization was observed (Skold, 2005). In a human maximization test, no skin sensitization reactions were observed (RIFM, 1979a). In a confirmatory human repeat insult patch test (HRIPT) with 3875 μg/cm^2 of caryophyllene oxide in alcohol SDA 39C, no reactions indicative of sensitization were observed in the 45 subjects (RIFM, 1971). In another HRIPT with 20% caryophyllene in white petrolatum, 1/50 subjects showed a level 2 + reaction at challenge, although the study concluded that there were no reactions indicative of skin sensitization. The description of the reaction is not provided in the report. (RIFM, 1979c).

Acting conservatively, due to the limited data, the reported exposure was benchmarked utilizing the reactive DST of 64 μg/cm^2 (Safford, 2008, 2011, 2015b; Roberts, 2015). The current exposure from the 95th percentile concentration is below the DST for reactive materials when evaluated in all QRA categories. Table 1 provides the maximum acceptable concentrations for caryophyllene oxide that present no appreciable risk for skin sensitization based on the reactive DST. These concentrations are not limits; they represent maximum acceptable concentrations based on the DST approach.

Additional References: Borje (2004).

Literature Search and Risk Assessment Completed On: 10/23/18.

10.1.5. Phototoxicity/photoallergenicity

Based on the available human study data and UV/Vis spectra, caryophyllene oxide would not be expected to present a concern for phototoxicity or photoallergenicity.

10.1.5.1. Risk assessment. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. The corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity (Henry, 2009). In a photo-HRIPT, there were no reactions to 20% caryophyllene oxide in white petrolatum during induction or challenge (RIFM, 1979c). Based on the lack of absorbance and human study data, caryophyllene oxide does not present a concern for phototoxicity or photoallergenicity.

10.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate no significant absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark of concern for phototoxic effects, 1000 L mol^{-1} · cm^{-1} (Henry, 2009).

Table 1
Maximum acceptable concentrations for caryophyllene oxide 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%	5.0 × 10^{-6}%
2	Products applied to the axillae	0.0015%	4.3 × 10^{-4}%
3	Products applied to the face using fingertips	0.029%	6.0 × 10^{-4}%
4	Fine fragrance products	0.027%	0.0037
5	Products applied to the face and body using the hands (palms), primarily leave-on	0.0070%	5.3 × 10^{-4}%
6	Products with oral and lip exposure	0.016%	0.0020
7	Products applied to the hair with some hand contact	0.056%	6.0 × 10^{-5}%
8	Products with significant ano-genital exposure	0.0029%	No Data[c]
9	Products with body and hand exposure, primarily rinse-off	0.054%	6.9 × 10^{-4}%
10	Household care products with mostly hand contact	0.19%	7.7 × 10^{-4}%
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate	0.11%	No Data[c]
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted	0.042%

Note.

[a] For a description of the categories, refer to the IFRA/RIFM Information Booklet.

[c] Fragrance exposure from these products is very low. These products are not currently in the Creme RIFM Aggregate Exposure Model.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/16/18.

10.1.6. Local Respiratory Toxicity

The margin of exposure could not be calculated due to a lack of appropriate data. The exposure level for caryophyllene oxide is below the Cramer Class III TTC value for inhalation exposure local effects.

10.1.6.1. Risk assessment. There are insufficient inhalation data available on caryophyllene oxide. Based on the Creme RIFM Model, the inhalation exposure is 0.00062 mg/day. This exposure is 758.0 times lower than the Cramer Class III TTC value of 0.47 mg/day (based on human lung weight of 650 g; Carthew, 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/23/18.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of caryophyllene oxide was performed following the RIFM Environmental Framework (Salvito, 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, Caryophyllene oxide was identified as a fragrance material with the potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC > 1).

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

10.2.2. Risk assessment

Based on the current Volume of Use (2015), caryophyllene oxide presents a risk to the aquatic compartment in the screening-level assessment.

*10.2.2.1. Key Studies***Biodegradation:**

No data available.

Ecotoxicity

RIFM, 2005: A short-term *Daphnia magna* chronic toxicity study was conducted according to the EPA/600/4–90/027 method under static conditions. The 7-day NOEC was reported to be 2.48 mg/L for survival and 0.62 mg/L for reproduction.

RIFM, 2005: **A** short-term fish (fathead minnow) chronic study was conducted according to the EPA/400/4–91/002 method under static renewal conditions. The 7-day NOEC was reported to be 2.48 mg/L for survival and 1.24 mg/L for growth.

Other available data

Caryophyllene oxide has been pre-registered for REACH with no additional data at this time.

10.2.3. Risk Assessment Refinement

Since caryophyllene oxide 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.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	0.8900			1,000,000	0.00089	
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	0.571	0.517	0.281	10,000	0.0281	Epoxides, mono
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	0.444	0.329	0.744			Neutral organic SAR

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} used	4.9	4.9
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	< 1	< 1
Risk Characterization: PEC/PNEC	**< 1**	**< 1**

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

The RIFM PNEC is 0.0281 μg/L. The revised PEC/PNECs for EU and NA are < 1; therefore, the material does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 11/19/18.

11. Literature Search*

- **RIFM Database:** Target, Fragrance Structure Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** http://echa.europa.eu/

- **NTP:** https://ntp.niehs.nih.gov/
- **OECD Toolbox**
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubMed:** http://www.ncbi.nlm.nih.gov/pubmed
- **TOXNET:** http://toxnet.nlm.nih.gov/
- **IARC:** http://monographs.iarc.fr
- **OECD SIDS:** http://webnet.oecd.org/hpv/ui/Default.aspx
- **EPA ACToR:** https://actor.epa.gov/actor/home.xhtml
- **US EPA HPVIS:** https://ofmpub.epa.gov/oppthpv/public_search.publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&EndPointRpt=Y#submission
- **Japanese NITE:** http://www.safe.nite.go.jp/english/db.html
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com
- **ChemIDplus:** https://chem.nlm.nih.gov/chemidplus/

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 5/20/2019.

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.

References

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

Asakawa, Y., Ishida, T., Toyota, M., Takemoto, T., 1986. Terpenoid biotransformation in mammals IV.Biotransformation of (+)-longifolene, (-)-caryophyllene, (-)-caryophyllene oxide,(-)-cyclocolorenone, (+)-nootkatone, (-)-elemol, (-)-abietic acid and (+)-dehydroabietic acid in rabbits. Xenobiotica 16 (8), 753–767.

Borje, A., Skold, M., Matura, M., Karlberg, A.-T., 2004. Autoxidation of the fragrance caryophllene forms contact allergens. Contact Dermatitis 50 (3), 191.

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

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

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

ECHA, 2012. Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT Assessment, November 2012 v1.1. http://echa.europa.eu/.

Hall, A.P., Elcombe, C.R., Foster, J.R., Harada, T., Kaufmann, W., et al., 2012. Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes–conclusions from the 3rd International ESTP expert workshop. Toxicol. Pathol. 40 (7), 971–994.

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

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

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

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

Lehman-McKeeman, L.D., Caudill, D., 1992. a-2u-globulin is the only member of the lipocalin protein superfamily that binds to hyaline droplet inducing agents. Toxicol. Appl. Pharmacol. 116 (2), 170–176.

Lehman-McKeeman, L.D., Rivera-Torres, M.I., Caudill, D., 1990. Lysosomal degradation of alpha2u-globulin and alpha2u-globulin-xenobiotic conjugates. Toxicol. Appl. Pharmacol. 103 (3), 539–548.

RIFM (Research Institute for Fragrance Materials, Inc), 1971. Repeated Insult Patch Test with Caryophyllene Oxide. RIFM, Woodcliff Lake, NJ, USA Unpublished report from International Flavors and Fragrances. RIFM report number 48391.

RIFM (Research Institute for Fragrance Materials, Inc), 1979. Report on human maximization studies. Report to RIFM. RIFM Report Number 1697. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 1979. Ames Metabolic Activation Test to Assess the Potential Mutagenic Effect of Caryophyllene Oxide. RIFM, Woodcliff Lake, NJ, USA Unpublished report from Firmenich Incorporated. RIFM report number 14945.

RIFM (Research Institute for Fragrance Materials, Inc), 1979. Repeated Insult Patch Test/photosensitization Study in Human Subjects on Caryophelle Oxide. RIFM, Woodcliff Lake, NJ, USA Unpublished report from Firmenich Incorporated. RIFM report number 14950.

RIFM (Research Institute for Fragrance Materials, Inc), 2005. 7-Day Chronic Toxicity Test Results with LC50 and NOEC Endpoints for 25 Fragrance Chemicals Using ceriodaphnia Dubia and Fathead Minnows. RIFM, Woodcliff Lake, NJ, USA Unpublished report from S.C.Johnson. RIFM report number 49950.

RIFM (Research Institute for Fragrance Materials, Inc), 2013. Caryophyllene Oxide (Beta-caryophyllene Epoxide): A 90-day Dietary Study in Rats. RIFM Report Number 68697. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2016. Exposure Survey 13 November 2016.

RIFM (Research Institute for Fragrance Materials, Inc), 2018. Caryophyllene Oxide: in Vitro Mammalian Cell Micronucleus Assay in Human Peripheral Blood Lymphocytes (HPBL). RIFM, Woodcliff Lake, NJ, USA RIFM report number 74249.

Roberts, D.W., Api, A.M., Safford, R.J., Lalko, J.F., 2015. Principles for identification of high potency category chemicals for which the dermal sensitization threshold (DST) approach should not be applied. Regul. Toxicol. Pharmacol. 72 (3), 683–693.

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

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

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

Safford, R.J., 2008. The dermal sensitisation threshold–A TTC approach for allergic contact dermatitis. Regul. Toxicol. Pharmacol. 51 (2), 195–200.

Safford, R.J., Api, A.M., Roberts, D.W., Lalko, J.F., 2015b. Extension of the dermal sensitization threshold (DST) approach to incorporate chemicals classified as reactive. Regul. Toxicol. Pharmacol. 72 (3), 694–701.

Safford, R.J., Aptula, A.O., Gilmour, N., 2011. Refinement of the dermal sensitisation threshold (DST) approach using a larger dataset and incorporating mechanistic chemistry domains. Regul. Toxicol. Pharmacol. 60 (2), 218–224.

Salvito, D.T., Senna, R.J., Federle, T.W., 2002. A Framework for prioritizing fragrance materials for aquatic risk assessment. Environ. Toxicol. Chem. 21 (6), 1301–1308.

Skold, M., Karlberg, A.-T., Matura, M., Borje, A., 2005. The fragrance chemical beta-caryophyllene-air oxidation and skin sensitization. Food Chem. Toxicol. 44 (4), 538–545.

US EPA, 2012a. Estimation Programs Interface Suite for Microsoft Windows, v4.0–v4.11. United States Environmental Protection Agency, Washington, DC, USA.

US EPA, 2012b. The ECOSAR (ECOlogical Structure Activity Relationship) Class Program for Microsoft Windows, v1.11. United States Environmental Protection Agency, Washington, DC, USA.