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Short Review

RIFM fragrance ingredient safety assessment, germacrene D, CAS Registry Number 23986-74-5

A.M. Api [a], A. Bartlett [a], D. Belsito [b], D. Botelho [a], M. Bruze [c], A. Bryant-Freidrich [d], G.A. Burton Jr. [e], M.A. Cancellieri [a], H. Chon [a], M.L. Dagli [f], W. Dekant [g], C. Deodhar [a], K. Farrell [a], A.D. Fryer [h], L. Jones [a], K. Joshi [a], A. Lapczynski [a], M. Lavelle [a], I. Lee [a], H. Moustakas [a], J. Muldoon [a], T.M. Penning [i], G. Ritacco [a], N. Sadekar [a], I. Schember [a], T.W. Schultz [j], F. Siddiqi [a], I.G. Sipes [k], G. Sullivan [a,*], Y. Thakkar [a], Y. Tokura [l]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ, 07677, USA*
[b] *Member Expert Panel for Fragrance Safety, Columbia University Medical Center, Department of Dermatology, 161 Fort Washington Ave., New York, NY, 10032, USA*
[c] *Member Expert Panel for Fragrance Safety, Malmo University Hospital, Department of Occupational & Environmental Dermatology, Sodra Forstadsgatan 101, Entrance 47, Malmo, SE-20502, Sweden*
[d] *Member Expert Panel for Fragrance Safety, Pharmaceutical Sciences, Wayne State University, 42 W. Warren Ave., Detroit, MI, 48202, USA*
[e] *Member Expert Panel for Fragrance Safety, School of Natural Resources & Environment, University of Michigan, Dana Building G110, 440 Church St., Ann Arbor, MI, 58109, USA*
[f] *Member Expert Panel for Fragrance Safety, University of Sao Paulo, School of Veterinary Medicine and Animal Science, Department of Pathology, Av. Prof. dr. Orlando Marques de Paiva, 87, Sao Paulo, CEP 05508-900, Brazil*
[g] *Member Expert Panel for Fragrance Safety, University of Wuerzburg, Department of Toxicology, Versbacher Str. 9, 97078, Würzburg, Germany*
[h] *Member Expert Panel for Fragrance Safety, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd., Portland, OR, 97239, USA*
[i] *Member of Expert Panel for Fragrance Safety, University of Pennsylvania, Perelman School of Medicine, Center of Excellence in Environmental Toxicology, 1316 Biomedical Research Building (BRB) II/III, 421 Curie Boulevard, Philadelphia, PA, 19104-3083, USA*
[j] *Member Expert Panel for Fragrance Safety, The University of Tennessee, College of Veterinary Medicine, Department of Comparative Medicine, 2407 River Dr., Knoxville, TN, 37996- 4500, USA*
[k] *Member Expert Panel for Fragrance Safety, Department of Pharmacology, University of Arizona, College of Medicine, 1501 North Campbell Avenue, P.O. Box 245050, Tucson, AZ, 85724-5050, USA*
[l] *Member Expert Panel for Fragrance Safety, The Journal of Dermatological Science (JDS), Department of Dermatology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, 431-3192, Japan*

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* Corresponding author.
E-mail address: gsullivan@rifm.org (G. Sullivan).

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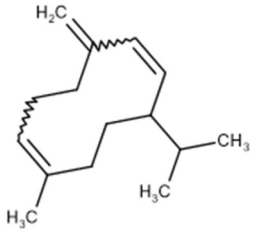
Name: Germacrene D
CAS Registry Number: 23986-74-5



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
CAESAR - Computer-Assisted Evaluation of industrial chemical Substances According to Regulations
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., 2015, 2017, 2024) 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
HESS - Hazard Evaluation Support System; a repeated dose profiler that is used to identify the toxicological profiler of chemicals
IFRA - The International Fragrance Association
ISS - Instituto Superiore di Sanita (Italian National Institute of Health)
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
OASIS - OASIS Laboratory of Mathematical Chemistry (LMC)
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
Toxtree - an *in silico* tool that can estimate toxic hazard by applying a decision tree approach
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.

(*continued on next column*)

(*continued*)

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.

Germacrene D was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, photoirritation/photoallergenicity, skin sensitization, and environmental safety. Data show that germacrene D is not genotoxic. The repeated dose, reproductive, and local respiratory toxicity endpoints were evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material, and the exposure to germacrene D is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). Data from read-across analog β-caryophyllene (CAS # 87-44-5) show that there are no safety concerns for germacrene D for skin sensitization under the current declared levels of use. The photoirritation/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; germacrene D is not expected to be photoirritating/photoallergenic. The environmental endpoints were evaluated; for the hazard assessment based on the screening data, germacrene D is not Persistent, Bioaccumulative, and Toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards. For the risk assessment, germacrene D was not able to be risk screened as there were no reported volumes of use (VoU) for either North America or Europe in the 2019 IFRA Survey.

Human Health Safety Assessment

Genotoxicity: Not genotoxic. (RIFM, 2017a; RIFM, 2017b)
Repeated Dose Toxicity: No NOAEL available. Exposure is below the TTC.
Reproductive Toxicity No NOAEL available. Exposure is below the TTC.
Skin Sensitization: Not a concern for skin sensitization. RIFM (2016)
Photoirritation/Photoallergenicity: Not expected to be photoirritating/photoallergenic. (UV/Vis Spectra; RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:
Persistence:
Critical Measured Value: 62.2% (OECD 310; day 60) RIFM (2023)
Bioaccumulation:
Screening-level: 19140 L/kg (EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:
Not applicable; no 2019 IFRA VoU reported
Risk Assessment:
- Not applicable; no 2019 IFRA VoU reported

1. Identification

1. **Chemical Name:** Germacrene D
2. **CAS Registry Number:** 23986-74-5
3. **Synonyms:** 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]-; Germacra-1(10),4(15),5-triene, (−)-; (−)-Germacrene D; Germacrene D ≥ 85%; Germacrene D
4. **Molecular Formula:** $C_{15}H_{24}$
5. **Molecular Weight:** 204.35 g/mol
6. **RIFM Number:** 6737
7. **Stereochemistry:** D-isomer specified.

2. Physical data

1. **Boiling Point:** 262.9 °C (EPI Suite v4.11)
2. **Flash Point:** Not Available

3. **Log K_{OW}:** 6.99 (EPI Suite v4.11)
4. **Melting Point**: 15.76 °C (EPI Suite v4.11)
5. **Water Solubility:** 0.01283 mg/L (EPI Suite v4.11)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.0145 mm Hg at 20 °C (EPI Suite v4.0), 0.0228 mm Hg at 25 °C (EPI Suite v4.11)
8. **UV Spectra:** Minor absorbance between 290 and 700 nm; molar absorption coefficients (176, 143, and 241 L $mol^{-1} \bullet cm^{-1}$under neutral, acidic, and basic conditions, respectively) are below the benchmark (1000 L $mol^{-1} \bullet cm^{-1}$)
9. **Appearance/Organoleptic:** Not Available

3. Volume of use (worldwide band)

1. No VoU in 2019	IFRA (2019)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v3.2.8)

1. 95th Percentile Concentration in Fine Fragrance: 0.012%	RIFM (2022)
2. Inhalation Exposure*: 0.000015 mg/kg/day or 0.0011 mg/day	RIFM (2022)
3. Total Systemic Exposure:** 0.00028 mg/kg/day	RIFM (2022)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM Aggregate Exposure Model (Comiskey, 2015, 2017; Safford, 2015, 2017, 2024).

**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, 2015, 2017; Safford, 2015, 2017, 2024).

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 v4.6
I	I	I

2. Analogs Selected:
   a. **Genotoxicity:** None
   b. **Repeated Dose Toxicity:** None
   c. **Reproductive Toxicity:** None
   d. **Skin Sensitization:** β-Caryophyllene (CAS # 87-44-5); Weight of Evidence (WoE) material: Myrcene (CAS # 123-35-3)
   e. **Photoirritation/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. Read-across Justification: See Appendix below

7. Metabolism

No relevant data available for inclusion in this safety assessment.
Additional References: None.

8. Natural occurrence

Germacrene D is reported to occur in the following foods by the VCF*.

Agastache species	Citrus fruits
Angelica (*Angelica archangelica* L.)	Ginger (*Zingiber* species)
Calamintha nepeta oil	Grape brandy
Chamomile	Pineapple (*Ananas comosus*)
Capsicum species	Salvia 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.

9. REACH dossier

No dossier available as of 11/16/23.

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, germacrene D does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. Germacrene D was assessed in the BlueScreen assay and found positive for cytotoxicity (positive: <80% relative cell density) without metabolic activation, negative for cytotoxicity with metabolic activation, and negative for genotoxicity with and without metabolic activation (RIFM, 2013). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures (Thakkar et al., 2022). Additional assays were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

The mutagenic activity of germacrene D 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 and preincubation methods. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with germacrene D in dimethyl sulfoxide (DMSO) at concentrations up to 5000 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, 2017a). Under the conditions of the study, germacrene D was not mutagenic in the Ames test.

The clastogenic activity of germacrene D 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 germacrene D in acetone at concentrations up to 2000 μg/mL in the dose range finding study; micronuclei analysis was conducted at concentrations up to 200 μg/mL in the presence and absence of metabolic activation. Germacrene D did induce binucleated cells with micronuclei when tested at 30.2 μg/mL in the 3-h treatment in the absence of an S9 activation system (RIFM, 2017b). However, the frequencies of binucleated cells with micronuclei (0.55%) at these concentrations were within the vehicle historical control ranges (0.10%–0.90%). Therefore, the statistically significant increases at these

concentrations were considered biologically non-relevant and not indicative of clastogenic effects. Under the conditions of the study, germacrene D was considered to be non-clastogenic in the *in vitro* micronucleus test.

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

Additional References: RIFM, 2020

Literature Search and Risk Assessment Completed On: 05/05/23.

11.1.2. Repeated dose toxicity

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

11.1.2.1. Risk assessment. There are no repeated dose toxicity data on germacrene D or any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to germacrene D (0.28 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.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/25/23.

11.1.3. Reproductive toxicity

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

11.1.3.1. Risk assessment. There are no reproductive toxicity data on germacrene D or any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to germacrene D (0.28 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.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/25/23.

11.1.4. Skin sensitization

Based on the existing data on the read-across material β-caryophyllene and WoE material myrcene, germacrene D presents no concern for skin sensitization, under the current declared levels of use.

11.1.4.1. Risk assessment. No skin sensitization data are available for

Table 1
Summary of existing data on β-caryophyllene as a read-across for germacrene D.

WoE Skin Sensitization Potency Category[1]	Human Data				Animal Data		
	NOEL-CNIH (induction) μg/cm²	NOEL-HMT (induction) μg/cm²	LOEL (induction) μg/cm²	WoE NESIL μg/cm²	LLNA Weighted Mean EC3 Value μg/cm²	GPMT	Buehler
No evidence of sensitization; the current use level does not present a	768	2760	N/A	N/A	N/A	N/A	N/A
	In vitro Data				*In silico* protein binding alerts (OECD Toolbox v4.6)		
concern for skin sensitization	KE 1	KE 2	KE 3		Target Material	Autoxidation simulator	Metabolism simulator
	N/A	N/A	N/A		No alert found	Radical reactions; SN2; Nucleophilic addition	Michael addition

NOEL = No observed effect level; CNIH = Confirmation of No Induction in Humans; HMT = Human Maximization Test; LOEL = lowest observed effect level; GPMT = Guinea Pig Maximization Test; KE = Key Event; N/A = Not Available.

1WoE Skin Sensitization Potency Category is only applicable for identified sensitizers with sufficient data, based on collective consideration of all available data (Na et al., 2021).

2Determined based on Criteria for the Research Institute for Fragrance Materials, Inc. (RIFM) safety evaluation process for fragrance ingredients (Api et al., 2015).

germacrene D. Therefore, β-caryophyllene (CAS # 87-44-5; see Section VI) was used for the risk assessment of germacrene D. The data on the read-across material are summarized in Table 1. Additionally, myrcene (CAS # 123-35-3; see Section VI) was used as WoE. Based on the existing data on the read-across material, germacrene D is not considered a skin sensitizer. Germacrene D, read-across material β-caryophyllene, and WoE material myrcene are predicted *in silico* to be non-reactive with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.6). In a murine local lymph node assay (LLNA), read-across material containing oxidized β-caryophyllene and caryophyllene hydroperoxides were found to be sensitizing with EC3 values of 26.2% (6550 μg/cm2) and 0.6% (150 μg/cm2), respectively (Skold, 2005). In a murine LLNA, WoE material myrcene was found to be non-sensitizing when tested up to 50% (12500 μg/cm2) (RIFM, 2010). In a human maximization test, no skin sensitization reactions were observed when read-across material β-caryophyllene was tested at 2760 μg/cm^2 (RIFM, 1971a). In human maximization tests, no skin sensitization reactions were observed when WoE material myrcene was tested at 2760 μg/cm2 (RIFM, 1972). Additionally, in 2 separate Confirmation of No Induction in Humans tests (CNIHs) with 775 and 310 μg/cm^2 of read-across material β-caryophyllene in alcohol SDA 39C, no reactions indicative of sensitization were observed in any of the 50 volunteers, respectively (RIFM, 1974b; RIFM, 1974a). In a CNIH with 3100 μg/cm2 of read-across material β-caryophyllene in alcohol SDA 39C, possible sensitization reactions were observed in 2 of the 39 volunteers (RIFM, 1971b). Additionally, in a CNIH with 768 μg/cm^2 of read-across material β-caryophyllene (with 0.1% butylated hydroxy-toluene and 0.1 mm peroxides) in 1:3 ethanol: diethyl phthalate, no reactions indicative of sensitization were observed in any of the 104 volunteers (RIFM, 2016).

The available studies were insufficient to determine hazard; however, the current exposure from the 95th percentile concentrations is below the supported concentrations when evaluated in all QRA categories (RIFM, 2022). Table 1 provides the supported concentrations for germacrene D that present no appreciable risk for skin sensitization. These levels represent the supported concentrations based on a tested exposure of 768 μg/cm^2 in the CNIH (RIFM, 2016). However, additional studies may show it could be used at higher levels.

Based on the weight of evidence (WoE) from structural analysis and human studies on the read-across and WoE material, germacrene D does not present a concern for skin sensitization under the current declared levels of use.

Additional References: Hausen et al., 1999; Skold et al., 2005; Borje et al., 2002; RIFM, 1999.

Literature Search and Risk Assessment Completed On: 05/10/23.

11.1.5. Photoirritation/photoallergenicity

Based on the available UV/Vis absorption spectra, germacrene D would not be expected to present a concern for photoirritation or photoallergenicity.

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

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate minor absorbance in the range of 290–700 nm. The molar absorption coefficients (176, 143, and 241 L mol^{-1} • cm^{-1}under neutral, acidic, and basic conditions, respectively) are below the benchmark of concern for photoirritating or photoallergenic effects, 1000 L mol^{-1} • cm^{-1} (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/02/23.

11.1.6. Local Respiratory Toxicity

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

11.1.6.1. Risk assessment. There are no inhalation data available on germacrene D. Based on the Creme RIFM Model, the inhalation exposure is 0.0011 mg/day. This exposure is 1272.7 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: None.

Literature Search and Risk Assessment Completed On: 05/02/23.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of germacrene D 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 of 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 VoU Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, germacrene D was not able to be assessed as no 2019 IFRA VoU was reported.

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) identified germacrene D 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, 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2017a). 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). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

11.2.1.1. Risk assessment. Not applicable.

11.2.1.2. Key studies

11.2.1.2.1. Biodegradation. **RIFM, 2023**: The ready biodegradability of the test material was evaluated in the Headspace test according to OECD 310. A biodegradation of 62.2% was observed after 60 days (49.3% after 28 days).

11.2.1.2.2. Ecotoxicity. No data available.

11.2.1.2.3. Other available data. Germacrene D has been pre-registered for REACH with no additional data at this time.

11.2.1.3. Risk assessment refinement. Not applicable.

Literature Search and Risk Assessment Completed On: 05/10/23.

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
- **PubChem:** https://pubchem.ncbi.nlm.nih.gov/
- **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
- **National Library of Medicine Technical Bulletin:** https://www.nlm.nih.gov/pubs/techbull/nd19/nd19_toxnet_new_locations.html
- **IARC:** https://monographs.iarc.fr
- **OECD SIDS:** https://hpvchemicals.oecd.org/ui/Default.aspx
- **EPA ACToR:** https://actor.epa.gov/actor/home.xhtml
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- **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://pubchem.ncbi.nlm.nih.gov/source/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 11/16/23.

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.2024.114542.

Appendix

Read-across Justification

Methods

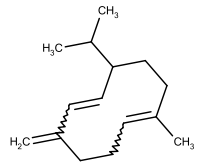
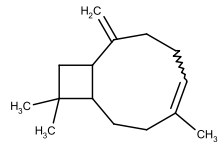
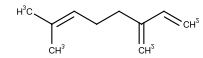
The read-across analogs were identified using RIFM fragrance chemicals inventory clustering and read-across search criteria (Date et al., 2020). These criteria are in compliance with the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015) and are 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, 2017b).

- 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 (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 v4.6 (OECD, 2023).
- ER binding and repeat dose categorization were generated using the OECD QSAR Toolbox v4.6 (OECD, 2023).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010), and skin sensitization was predicted using Toxtree v2.6.13.
- Protein binding was predicted using OECD QSAR Toolbox v4.6 (OECD, 2023).
- The major metabolites for the target material and read-across analogs were determined and evaluated using the OECD QSAR Toolbox v4.6 (OECD, 2023).
- To keep continuity and compatibility with *in silico* alerts, OECD QSAR Toolbox v4.6 was selected as the alert system.

Principal Name	Target Material	Read-across Material	WoE Material
	(−)-Germacrene D	β-Caryophyllene	Myrcene
CAS No.	23986-74-5	87-44-5	123-35-3

(*continued on next page*)

(*continued*)

Principal Name	Target Material	Read-across Material	WoE Material
	(−)-Germacrene D	β-Caryophyllene	Myrcene
Structure			
Similarity (Tanimoto Score)		0.68	0.47
SMILES	CC(C)C1CCC(C)═CCCC(=C)C═C1	CC1CCC2C(CC2(C)C)C (=C)CCC = 1	CC(C)═CCCC(=C)C═C
Endpoint		Skin sensitization	Skin sensitization
Molecular Formula	$C_{15}H_{24}$	$C_{15}H_{24}$	$C_{10}H_{16}$
Molecular Weight (g/mol)	204.357	204.357	136.238
Melting Point (°C, EPI Suite)	15.76	43.42	−64.83
Boiling Point (°C, EPI Suite)	262.90	256.80	167.00
Vapor Pressure (Pa @ 25°C, EPI Suite)	3.04E+00	4.16E+00	2.79E+02
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	1.28E-02	5.01E-02	4.00E+00
Log K_{OW}	6.99	6.3	4.33
J_{max} (μg/cm^2/h, SAM)	0.00	0.01	0.81
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.13E+05	6.99E+04	9.28E+03
Skin Sensitization			
Protein Binding (OASIS v1.1)	No alert found	No alert found	No alert found
Protein Binding (OECD)	No alert found	No alert found	No alert found
Protein Binding Potency	Not possible to classify according to these rules (GSH)	Not possible to classify according to these rules (GSH)	Not possible to classify according to these rules (GSH)
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	No alert found	No alert found	No alert found
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	No skin sensitization reactivity domain alerts were identified	No skin sensitization reactivity domain alerts were identified	No skin sensitization reactivity domain alerts were identified
Metabolism			
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.6)	See Supplemental Data 1	See Supplemental Data 2	See Supplemental Data 3

Summary

There are insufficient toxicity data on germacrene D (CAS # 23986-74-5). 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, β-caryophyllene (CAS # 87-44-5) was identified as a read-across analog, and myrcene (CAS # 123-35-3) was identified as a WoE material with sufficient data for toxicological evaluation.

Conclusions

- β-Caryophyllene (CAS # 87-44-5) was used as a read-across analog along with myrcene (CAS # 123-35-3) as WoE for the target material, germacrene D (CAS # 23986-74-5), for the skin sensitization endpoint.
  - o The target material and the read-across analog share a commonality in that they are both alkylated cyclic terpenes.
  - o The key difference between the target material and the read-across analog is that the target material contains a conjugated vinyl. Therefore, to satisfy the structural domain of the target material, myrcene (CAS # 123-35-3) is used as WoE. This chemical has a conjugated vinyl with an isolated vinylene, just like the target material. The read-across analog, combined with the WoE material, contains the structural features of the target material that are relevant to this endpoint and is expected to have equal or greater potential for toxicity as compared to the target.
  - o The 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 a comparison of their toxicological properties.
  - o Differences are predicted for J_{max}, which estimates skin absorption. J_{max} for the target material corresponds to skin absorption ≤10%, and J_{max} for the WoE material corresponds to skin absorption ≤40%. While the percentage of skin absorption estimated from J_{max} indicates exposure to the substance, it does not represent hazard or toxicity. This parameter provides context to assess the impact of bioavailability on toxicity comparisons between the materials evaluated.
  - o According to the OECD QSAR Toolbox v4.6, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - o Both the target material and read-across analog do not display *in silico* alerts for the skin sensitization endpoint. Data for the read-across analog indicates that it is not a concern for skin sensitization. Therefore, based on the structural similarity between the target material and the read-across analog and the data on the read-across analog, the *in silico* alerts are consistent with the data.
  - o The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.

- The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

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