Food and Chemical Toxicology 167 (2022) 113379

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Short Review

RIFM fragrance ingredient safety assessment, sclareolide, CAS Registry Number 564-20-5

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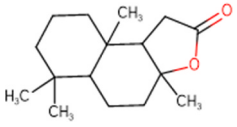
ARTICLE INFO

Handling Editor: Dr. Jose Luis Domingo

Version: 061,622. Initial publication. All fragrance materials are evaluated on a five-year rotating basis. Revised safety assessments are published if new relevant data become available. Open access to all RIFM Fragrance Ingredient Safety Assessments is here: fragrancematerialsafetyresource.elsevier.com.

Name: Sclareolide
CAS Registry Number: 564-20-5



Abbreviation/Definition List:

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

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https://doi.org/10.1016/j.fct.2022.113379
Received 16 June 2022; Received in revised form 8 August 2022; Accepted 13 August 2022
Available online 19 August 2022

(*continued*)

estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015a; Safford et al., 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

LC50 – Lethal concentration 50%

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, 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.

Sclareolide was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, photoirritation/photoallergenicity, skin sensitization, and environmental safety. Data show that sclareolide 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 III material, and the exposure to sclareolide is below the TTC (0.0015 mg/kg/day, 0.0015 mg/kg/day, and 0.47 mg/day, respectively). Data from read-across material 2(3H)-benzofuranone (CAS # 92,015-65-1) show that there are no safety concerns for sclareolide for skin sensitization under the current declared levels of use. The photoirritation/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; sclareolide is not expected to be photoirritating/photoallergenic. The environmental endpoints were evaluated; sclareolide 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 (VoU) in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

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: No concern for skin sensitization. RIFM (1994)

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:
Screening-level: 2.1 (BIOWIN 3) (EPI Suite v4.11; US EPA, 2012a)

Bioaccumulation:
Screening-level: 162.6 L/kg (EPI Suite v4.11; US EPA, 2012a)

Ecotoxicity:
Screening-level: Fish LC50: 8.135 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: 8.135 mg/L (RIFM Framework; Salvito et al, 2002)

RIFM PNEC is: 0.00814 μg/L

- **Revised PEC/PNECs (2019 IFRA VoU):** North America and Europe: Not applicable; cleared at screening-level

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1. Identification

1. **Chemical Name:** Sclareolide
2. **CAS Registry Number:** 564-20-5
3. **Synonyms:** Decahydro tetramethyl naphthofuranone; Naphtho[2,1-b]furan-2(1H)-one, decahydro-3a,6,6,9a-tetramethyl-, [3aR-(3a.α.,5a.β.,9a.α.,9b.β.]; Norambrienolide; 3a,6,6,9a-Tetramethyldecahydronaphtho[2,1-b]furan-2(1H)-one; Sclareolide
4. **Molecular Formula:** $C_{16}H_{26}O_2$
5. **Molecular Weight:** 250.38 g/mol
6. **RIFM Number:** 92
7. **Stereochemistry:** The "3aR" stereoisomer is specified. Four stereocenters are present, and 16 total stereoisomers are possible.

2. Physical data

1. **Boiling Point:** 341.9 °C (EPI Suite)
2. **Flash Point:** >93 °C (Globally Harmonized System), >200 °F; closed cup (Fragrance Materials Association)
3. **Log K_{OW}:** 3.86 (EPI Suite)
4. **Melting Point**: 107.07 °C (EPI Suite)
5. **Water Solubility:** 11.99 mg/L (EPI Suite)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.0000159 mm Hg at 20 °C (EPI Suite v4.0), 3.22e-005 mm Hg at 25 °C (EPI Suite)
8. **UV Spectra:** No absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** Not Available

3. Volume of use (Worldwide band)

1. <0.1 metric ton per year (IFRA, 2019)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v3.1.3)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.016% (RIFM, 2021)
2. **Inhalation Exposure*:** 0.0000046 mg/kg/day or 0.00028 mg/day (RIFM, 2021)
3. **Total Systemic Exposure**:** 0.00016 mg/kg/day (RIFM, 2021)

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

**95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section 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 et al., 2015; Safford et al., 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 III, High

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

2. Analogs Selected:
   a. **Genotoxicity:** None
   b. **Repeated Dose Toxicity:** None
   c. **Reproductive Toxicity:** None
   d. **Skin Sensitization:** 2(3H)-benzofuranone, hexahydro-3,6-dimethyl- (CAS # 92,015-65-1)
   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

Sclareolide is not reported to occur in foods by the VCF*.

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

9. REACH dossier

Available (ECHA, 2018); accessed on 01/27/22.

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, sclareolide does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. Sclareolide was assessed in the BlueScreen assay and found negative for both cytotoxicity and genotoxicity, with and without metabolic activation, indicating a lack of concern regarding genotoxicity (RIFM, 2014). 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 sclareolide 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 method. *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2uvrA were treated with sclareolide 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 (Research Institute for Fragrance Materials, 2017a). Under the conditions of the study, sclareolide was not mutagenic in the Ames test.

The clastogenic activity of sclareolide 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 sclareolide in DMSO at concentrations up to 1000 μg/mL in the presence and absence of S9 for 3 h and in the absence of metabolic activation for 24 h. Sclareolide did not induce binucleated cells with micronuclei when tested up to cytotoxic levels in either the presence or absence of an S9 activation system (RIFM (Research Institute for Fragrance Materials, 2017b). Under the conditions of the study, sclareolide was considered to be non-clastogenic in the *in vitro* micronucleus test.

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

Additional References: ECHA, 2018.

Literature Search and Risk Assessment Completed On: 01/21/22.

11.1.2. Repeated dose toxicity

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

11.1.2.1. Risk assessment. There are no repeated dose toxicity data on sclareolide or on any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to sclareolide (0.16 μg/kg/day) is below the TTC (1.5 μg/kg/day; Kroes et al., 2007) 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: 01/20/22.

11.1.3. Reproductive toxicity

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

11.1.3.1. Risk assessment. There are no reproductive toxicity data on sclareolide or on any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to sclareolide (0.16 μg/kg/day) is below the TTC (1.5 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the reproductive toxicity endpoint of a Cramer Class III material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 01/20/22.

11.1.4. Skin sensitization

Based on the existing data on the target material and read-across material 2(3H)-benzofuranone, hexahydro-3,6-dimethyl-, sclareolide presents no concern for skin sensitization.

11.1.4.1. Risk assessment. Limited skin sensitization data are available for sclareolide. Therefore, read-across material 2(3H)-benzofuranone (CAS # 92,015-65-1; see Section VI) was used for the risk assessment of sclareolide. The data on the read-across material are summarized in Table 1. Based on the existing data on the read-across material, sclareolide is not considered a skin sensitizer. The chemical structure of the read-across material and the target material indicate that they would not be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). In a guinea pig maximization test, read-across material 2(3H)-benzofuranone, hexahydro-3,6-dimethyl- did not present reactions indicative of sensitization (RIFM, 1994). In a human maximization test, no skin sensitization reactions were observed with sclareolide at 6900 μg/cm^2 (RIFM (Research Institute for Fragrance Materials, 1979). Additionally, in a Confirmation of No Induction in Humans test (CNIH) with 1500 μg/cm^2 of sclareolide in alcohol SDA 39C, no reactions indicative of sensitization were observed in any of the 43 volunteers (RIFM (Research Institute for Fragrance Materials, 1978).

Based on the weight of evidence (WoE) from structural analysis and animal and human studies on the read-across material as well as the target material, sclareolide does not present a concern for skin sensitization.

Additional References: None.

Literature Search and Risk Assessment Completed On: 01/17/22.

11.1.5. Photoirritation/photoallergenicity

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

11.1.5.1. Risk assessment. There are no photoirritation studies available for sclareolide 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 photoirritation and photoallergenicity (Henry et al., 2009). Based on the lack of absorbance, sclareolide 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 no absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark of concern for photoirritating effects, 1000 L mol^{-1} • cm^{-1} (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 01/10/22.

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 sclareolide is below the Cramer Class III TTC value for inhalation exposure local effects.

11.1.6.1. Risk assessment. There are no inhalation data available on sclareolide. Based on the Creme RIFM Model, the inhalation exposure is 0.00028 mg/day. This exposure is 1678.6 times lower than the Cramer Class III TTC value of 0.47 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: None.

Literature Search and Risk Assessment Completed On: 01/17/22.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of sclareolide was performed

Table 1
Summary of existing data on 2(3H)-benzofuranone, hexahydro-3,6-dimethyl- as a read-across for sclareolide.

WoE Skin Sensitization Potency Category[a]	Human Data				Animal Data		
	NOEL-CNIH (induction) μg/cm^2	NOEL-HMT (induction) μg/cm^2	LOEL[b] (induction) μg/cm^2	WoE NESIL[c] μg/cm^2	LLNA[d] Weighted Mean EC3 Value μg/cm^2	GPMT[e]	Buehler[e]
No evidence of sensitization[g]	NA	NA	NA	NA	NA	Negative	NA
	***In vitro* Data[f]**				***In silico* protein binding alerts (OECD Toolbox v4.2)**		
	KE 1	**KE 2**	**KE 3**		**Target Material**	**Autoxidation simulator**	**Metabolism simulator**
	NA	NA	NA		No alert found	Radical reactions	No alert found

NOEL = No observed effect level; CNIH = Confirmation of No Induction in Humans test; HMT = Human Maximization Test; LOEL = lowest observed effect level; KE = Key Event; NA = Not Available.

[a] WoE 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).

[b] Data derived from CNIH or HMT.

[c] WoE NESIL limited to 2 significant figures.

[d] Based on animal data using classification defined in ECETOC, Technical Report No. 87, 2003.

[e] Studies conducted according to the OECD TG 406 are included in the table.

[f] Studies conducted according to the OECD TG 442, Cottrez et al. (2016), or Forreryd et al. (2016) are included in the table.

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

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 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, sclareolide 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) identified sclareolide 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, 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 ≥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 VoU (2019), sclareolide does not present a 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.

11.2.1.2.2. Ecotoxicity. No data available.

11.2.1.3. Other available data. Sclareolide has been registered under REACH with no additional data at this time.

11.2.1.4. 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 Environmental Framework: Salvito et al, 2002)

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	3.86	3.86
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional VoU Tonnage Band	<1	<1
Risk Characterization: PEC/PNEC	**<1**	**<1**

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

The RIFM PNEC is 0.00814 μg/L. The revised PEC/PNECs for EU and NA are not applicable. The material was cleared at the screening-level; therefore, the material does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 05/24/22.

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'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 ChemView:** https://chemview.epa.gov/chemview/
  - **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 05/31/22.

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.

RIFM Framework Screening-level **(Tier 1)**	8.135			1000000	0.00814	

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2022.113379.

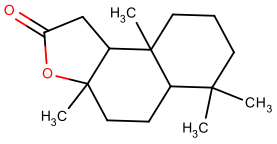
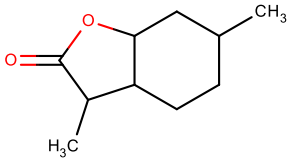
Appendix

Read-across Justification

Methods

The read-across analog was 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 Chemical 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 v4.2 (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), and skin sensitization was predicted using Toxtree v2.6.13.
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018).
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- To keep continuity and compatibility with *in silico* alerts, OECD QSAR Toolbox v4.2 was selected as the alert system.

	Target Material	Read-across Material
Principal Name	Sclareolide	2(3H)-Benzofuranone, hexahydro-3,6-dimethyl-
CAS No.	564-20-5	92,015-65-1
Structure		
Similarity (Tanimoto Score)		0.91
Endpoint		Skin sensitization
Molecular Formula	$C_{16}H_{26}O_2$	$C_{10}H_{16}O_2$
Molecular Weight (g/mol)	250.38	168.24
Melting Point (°C, EPI Suite)	107.07	21.70
Boiling Point (°C, EPI Suite)	341.90	276.66
Vapor Pressure (Pa @ 25°C, EPI Suite)	0.00	0.85
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	11.99	1518.00
Log K_{OW}	3.86	1.89
J_{max} (μg/cm^2/h, SAM)	0.40	17.72
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	60.59	25.13
Skin Sensitization		
Protein Binding (OASIS v1.1)	DPRA less than 9% (DPRA 13%)|DPRA less than 9% (DPRA 13%) ≫ Non-Conjugated carboxylic acids and esters (non-reactive)	DPRA less than 9% (DPRA 13%)|DPRA less than 9% (DPRA 13%) ≫ Non-Conjugated carboxylic acids and esters (non-reactive)
Protein Binding (OECD)	Not possible to classify according to these rules (GSH)	Not possible to classify according to these rules (GSH)
Protein Binding Potency	Not categorized	Not categorized
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	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
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 isopropyl sclareolide (CAS # 564-20-5). 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, 2(3H)-benzofuranone, hexahydro-3,6-dimethyl- (CAS # 92,015-65-1) was identified as a read-across material with sufficient data for toxicological evaluation.

Conclusions

- 2(3H)-Benzofuranone, hexahydro-3,6-dimethyl- (CAS # 92,015-65-1) was used as a read-across analog for the target material, sclareolide (CAS # 564-20-5), for the skin sensitization endpoint.
  - o The target material and the read-across analog belong to the class of lactones.
  - o The key difference between the target material and the read-across analog is that the target material has a three-ring fused system, whereas the read-across analog has a two-ring fused system. This structural difference is toxicologically insignificant.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. The Tanimoto score is mainly driven by the branched saturated acid portion. 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 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 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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