Food and Chemical Toxicology 130 (2019) 110602

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Short Review

RIFM fragrance ingredient safety assessment, myrtenol, CAS Registry Number 515-00-4

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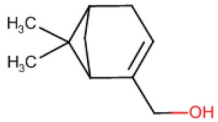
Version: 110918. This version replaces any previous versions.
Name: Myrtenol
CAS Registry Number: 515-00-4



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., 2015, 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

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https://doi.org/10.1016/j.fct.2019.110602
Received 12 November 2018; Received in revised form 28 May 2019; Accepted 19 June 2019
Available online 23 June 2019
0278-6915/

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 et al., 2015), which should be referred to for clarifications. Each endpoint discussed in this safety assessment includes the relevant data that were available at the time of writing (version number in the top box is indicative of the date of approval based on a 2-digit month/day/year), both in the RIFM database (consisting of publicly available and proprietary data) and through publicly available information sources (e.g., SciFinder and PubMed). Studies selected for this safety assessment were based on appropriate test criteria, such as acceptable guidelines, sample size, study duration, route of exposure, relevant animal species, most relevant testing endpoints, etc. A key study for each endpoint was selected based on the most conservative endpoint value (e.g., PNEC, NOAEL, LOEL, and NESIL).

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

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

Myrtenol was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that myrtenol is not genotoxic. Data from this material and the read-across analog 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene (CAS # 564-94-3) show that myrtenol is not a safety concern at the current, declared levels of use for the skin sensitization endpoint. The repeated dose, developmental and reproductive, and local respiratory toxicity endpoints were completed using the TTC for a Cramer Class I material, and the exposure to myrtenol is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). The phototoxicity/photoallergenicity endpoint was completed based on UV spectra; myrtenol is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; myrtenol 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, 2015a; RIFM, 2015b)
Repeated Dose Toxicity: No NOAEL available. Exposure is below the TTC.
Developmental and Reproductive Toxicity: No NOAEL available. Exposure is below the TTC.
Skin Sensitization: Not a concern for skin sensitization. RIFM (2012)
Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic. (UV spectra, RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:
Persistence: Screening-level: 2.8 (BIOWIN 3) (EPI Suite v4.1; US EPA, 2012a)
Bioaccumulation: Screening-level: 61.88 L/kg (EPI Suite v4.1; US EPA, 2012a)
Ecotoxicity: Screening-level: Fish LC50: 41.35 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: 41.35 mg/L (RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.04135 μg/L
- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: not applicable; cleared at screening-level

1. Identification

1. **Chemical Name:** Myrtenol
2. **CAS Registry Number:** 515-00-4
3. **Synonyms:** Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-; 6,6-Dimethylbicyclo[3.1.1]hept-2-ene-2-methanol; 6,6-Dimethyl-2-oxymethylbicyclo(1.1.3)hept-2-ene; (−)-Pin-2-ene-10-ol; 2-Pinen-10-ol; 6,6-ｼﾞﾒﾁﾙ-ﾋﾞｼｸﾛ〔3,1,1〕-ﾍﾌﾟﾀ-2-ｴﾝ-2ﾒﾀﾉｰﾙ; 6,6-ｼﾞﾒﾁﾙ-ﾋﾞｼｸﾛ〔3.1.1〕-ﾍﾌﾟﾀ-2-ｴﾝ-2; (6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methanol; Myrtenol
4. **Molecular Formula:** $C_{10}H_{16}O$
5. **Molecular Weight:** 152.24
6. **RIFM Number:** 1233

2. Physical data

1. **Boiling Point:** 224 °C (FMA Database), (calculated) 232.42 °C (EPI Suite)
2. **Flash Point:** > 200 °F; CC (FMA Database), 94 °C (201 °F) (RIFM Database)
3. **Log K_{OW}:** 2.8 (EPI Suite)
4. **Melting Point:** 38.73 °C (EPI Suite)
5. **Water Solubility:** 426.9 mg/L (EPI Suite)
6. **Specific Gravity:** 0.978–0.983 (RIFM Database)
7. **Vapor Pressure:** 0.00743 mm Hg @ 20 °C (EPI Suite v4.0), 0.006 mm Hg 20 °C (FMA Database), 0.0137 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 clear, almost colorless, liquid with a woody, cooling, minty with a medicinal nuance.

*http://www.thegoodscentscompany.com/data/rw1008881.html, retrieved on 03/23/17.

3. Exposure

1. **Volume of Use (worldwide band):** 0.1–1 metric ton per year (IFRA, 2015)
2. **95th Percentile Concentration in Hydroalcoholics:** 0.011% (RIFM, 2014)
3. **Inhalation Exposure*:** 0.000054 mg/kg/day or 0.0036 mg/day (RIFM, 2014)
4. **Total Systemic Exposure**:** 0.00037 mg/kg/day (RIFM, 2014)

*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 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 et al., 2015; Safford et al., 2015; Safford et al., 2017; and Comiskey et al., 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 I, Low

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

2. Analogs Selected:
   a. **Genotoxicity:** None
   b. **Repeated Dose Toxicity:** None
   c. **Developmental and Reproductive Toxicity:** None
   d. **Skin Sensitization:** 2-Formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene (CAS # 564-94-3)
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. Read-across Justification: See Appendix below

6. Metabolism

Not considered for this risk assessment and therefore not reviewed except where it may pertain in specific endpoint sections as discussed below.

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

Myrtenol is reported to occur in the following food by the VCF* and some natural complex substances (NCS):

Buchu Oil.
Camomile.
Cheese, Various types.
Chermimoya (*Annona cherimolia* Mill.)
Citrus Fruits.
Endive (*Cichorium endivia* L.)
Eucalyptus Oil (*Eucalyptus globulus Labill*)
Ginger (*Zingiber species*)
Grape Brandy.
Honey.
Hop (*Humulus lupulus*)
Lamb's Lettuce (*Valerianella locusta*)
Menthas Oils.
Myrtle (*Myrtus communis* L.)
Parsley (*Petroselium* species)
Pepper (*Piper nigrum* L.)
Pistachio Oil (*Pistacia vera*)
Pistacia Atlantica
Raspberry, blackberry, and Boysenberry.
Satureja Species.
Strawberry (*Fragaria* species)
Tea.
Turpentine Oil (*Pistacia terebinthus*)
Vaccinium Species.
Vanilla.
Walnut (*Juglans* species)
Wormwood Oil (*Artemisia absinthium* L.)
Xylopia 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.

8. IFRA standard

None.

9. REACH dossier

Pre-Registered for 11/30/2010; No dossier available as of 11/09/18.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

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

10.1.1.1. Risk assessment. Myrtenol tested negative in the BlueScreen assay with and without S9 metabolic activation (RIFM, 2013). The mutagenic activity of myrtenol has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG471 using the standard plate incorporation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with myrtenol 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 dose in the presence or absence of S9 (RIFM, 2015b). Under the conditions of the study, myrtenol was not mutagenic in the Ames test.

The clastogenic activity of myrtenol was evaluated in an *in vitro* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG487. Human peripheral blood lymphocytes were treated with myrtenol in solvent DMSO at concentrations up to 500 μg/mL in the presence and absence of metabolic activation (S9) for 3 and 24 h. Myrtenol did not induce binucleated cells with micronuclei when tested up to cytotoxic levels in either non-activated or S9-activated test systems (RIFM, 2015a). Under the conditions of the study, myrtenol was considered to be non-clastogenic in the *in vitro* micronucleus test.

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

Additional References: RIFM, 2013.

Literature Search and Risk Assessment Completed On: 03/09/17.

10.1.2. Repeated dose toxicity

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

10.1.2.1. Risk assessment. There are no repeated dose toxicity data on myrtenol or any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to myrtenol (0.37 μg/kg/day) is below the TTC (30 μg/kg bw/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: 03/10/17.

10.1.3. Developmental and reproductive toxicity

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

10.1.3.1. Risk assessment. There are no developmental toxicity data on myrtenol or any read-across materials that can be used to support the developmental toxicity endpoint. The total systemic exposure to myrtenol (0.37 μg/kg/day) is below the TTC (30 μg/kg bw/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the developmental toxicity endpoint of a Cramer Class I material at the current level of use.

There are no reproductive toxicity data on myrtenol or any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to myrtenol (0.37 μg/kg/day) is below the TTC (30 μg/kg bw/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: 03/10/17.

10.1.4. Skin sensitization

Based on the existing data and read-across to 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene (CAS # 564-94-3), myrtenol does not present a concern for skin sensitization.

10.1.4.1. Risk assessment. Based on the available data and read-across to 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene (CAS # 564-94-3; see Section V), myrtenol does not present a concern for skin sensitization. The chemical structure of myrtenol indicates that it would not be expected to react directly with skin proteins; however, read-across material 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene could react with skin proteins (Roberts et al., 2007; OECD toolbox v3.4). In a Buehler study in guinea pigs, no reactions indicative of sensitization were observed (RIFM, 1987a). In a local lymph node assay (LLNA), read-across material 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene was negative up to a maximum tested concentration of 50% (RIFM, 2012). Additionally, a series of human maximization tests demonstrated that myrtenol did not result in reactions indicative of sensitization, whereas prior reactions observed were attributed to impurities in the test sample (RIFM, 1985; RIFM, 1986; RIFM, 1987b). Similarly, no reactions were observed when read-across material 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene was tested in a human maximization test at 1% or 690 $\mu g/cm^2$ in petrolatum. Based on weight of evidence from structural analysis, animal and human studies, and read-across to 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene, myrtenol does not present a concern for skin sensitization.

Additional References: None.

Literature Search and Risk Assessment Completed On: 07/25/17.

10.1.5. Phototoxicity/photoallergenicity

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

10.1.5.1. Risk assessment. There are no phototoxicity studies available for myrtenol in experimental models. 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 et al., 2009). Based on lack of absorbance, myrtenol 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 $L\,mol^{-1} \cdot cm^{-1}$ (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 03/09/17.

10.1.6. Local Respiratory Toxicity

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

10.1.6.1. Risk assessment. There are no inhalation data available on myrtenol. Based on the Creme RIFM Model, the inhalation exposure is 0.0036 mg/day. This exposure is 389 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: Eriksson, 1990.

Literature Search and Risk Assessment Completed On: 03/21/17.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of myrtenol was performed following the RIFM Environmental Framework (Salvito et al., 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log K_{OW}, and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity, as discussed in Salvito et al. (2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 2012b), which provides chemical class–specific ecotoxicity estimates. Finally, if necessary, Tier 3 is conducted using measured biodegradation and ecotoxicity data to refine the RQ, thus allowing for lower PNEC uncertainty factors. The data for calculating the PEC and PNEC for this safety assessment are provided in the table below. For the PEC, the range from the most recent IFRA Volume of Use Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, myrtenol 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 v4.1 identified myrtenol as not persistent and 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 et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥ 2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.1). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

10.2.2. Risk assessment

Based on current VoU (2015), myrtenol does not present a risk to the aquatic compartment in the screening-level assessment.

10.2.3. Key studies

10.2.3.1. Biodegradation. No data available.

10.2.3.2. Ecotoxicity. No data available.

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

10.2.4. 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.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	<u>41.35</u>			1000000	0.04135	

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} used	2.8	2.8
Biodegradation Factor Used	1	1
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 this material is < 1. No additional assessment is necessary.

The RIFM PNEC is 0.04135 μg/L. The revised PEC/PNECs for EU and NA are: not applicable. The material was cleared at the screening-level and therefore does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 01/17/14.

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 10/09/2018.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Declaration of interests

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.2019.110602.

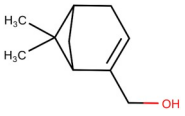
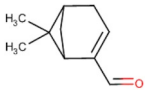
Appendix

Read-across justification

Methods

The read-across analogs were identified following the strategy for structuring and reporting a read-across prediction of toxicity described in Schultz et al. (2015). The strategy is also consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemicals Agency read-across assessment framework (ECHA, 2016).

- 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 substance and the read-across analogs were calculated using EPI Suite v4.11 (US EPA, 2012a).
- J_{max} values were calculated using RIFM's skin absorption model (SAM). The parameters were calculated using the consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts, and oncologic classification predictions were generated using OECD QSAR Toolbox v3.4 (OECD, 2018).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v3.4 (OECD, 2018).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010), and skin sensitization was predicted using Toxtree 2.6.13.
- Protein binding was predicted using OECD QSAR Toolbox v3.4 (OECD, 2018).
- The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox v3.4 (OECD, 2018).

	Target material	Read-across material
Principal Name	Myrtenol	2-Formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene
CAS No.	515-00-4	564-94-3
Structure		
Similarity (Tanimoto score)		0.86
Read-across endpoint		• Skin Sensitization
Molecular Formula	$C_{10}H_{16}O$	$C_{14}H_{24}O$
Molecular Weight	152.24	208.35
Melting Point (°C, EPI Suite)	38.73	60.19
Boiling Point (°C, EPI Suite)	224	199
Vapor Pressure (Pa @ 25 °C, EPI Suite)	0.0137	0.128
Log Kow (KOWWIN v1.68 in EPI Suite)	3.22	4.3[1]
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPI Suite)	426.9	215.9
J_{max} (μg/cm^2/h, SAM)	685.417	152.722
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	7.05E-001	7.14E+000
Skin Sensitization		
Protein binding by OASIS v1.1	• No alert found	• AN2, Michael addition • Schiff base formation

Protein binding by OECD	• No alert found	• Michael addition • Schiff base formation
Protein binding potency	• Not possible to classify (GSH)	• Moderately reactive (GSH), substituted 2-alken-1-al (Michale addition)
Protein binding alerts for skin sensitization by OASIS v1.1	• No alert found	• Michael addition • Schiff base formation
Skin Sensitization model (CAESAR) (version 2.1.6)	• Sensitizer (good reliability)	• Sensitizer (good reliability)
Metabolism		
OECD QSAR Toolbox (3.4) Rat liver S9 metabolism simulator and structural alerts for metabolites	See Supplemental Data 1	See Supplemental Data 2

1. RIFM, 1995.

Summary

There are insufficient toxicity data on the target material myrtenol (CAS # 515-00-4). Hence, *in silico* evaluation determined a read-across analog for this material. Based on structural similarity, reactivity, metabolism data, physical–chemical properties, and expert judgment, 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene (CAS # 564-94-3) was identified as a read-across material with sufficient data for toxicological evaluation.

Metabolism

Metabolism of the target material myrtenol (CAS # 515-00-4) was predicted using the Rat Liver S9 Metabolism Simulator (OECD QSAR Toolbox v3.4). The target material is predicted to be metabolized to 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene (CAS # 564-94-3) in the first step with 0.95 probability. Hence, 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene (CAS # 564-94-3) can be used as a read-across analog for the target material. Read-across material 2-formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene (CAS # 564-94-3) was in domain for the *in vivo* rat and in domain for the *in vitro* rat S9 simulator (OASIS TIMES v2.27.19).

12. Conclusions

- 2-Formyl-6,6-dimethylbicyclo(3.1.1)hept-2-ene (CAS # 564-94-3) was used as a read-across analog for the target material myrtenol (CAS # 515-00-4) for the skin sensitization endpoint.
  - o The target substance and the read-across analog are structurally similar and share the same unsaturated cyclic alkene extended fragment.
  - o The read-across analog and the target substance are metabolites of each other. The Rat Liver S9 Metabolism Simulator (OECD QSAR Toolbox v3.4) predicts the metabolic redox conversion between the primary alcohol and an aldehyde.
  - o The similarity between the target substance and the read-across analog is indicated by the Tanimoto score in the above table.
  - o The physical-chemical properties of the target substance and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - o The read-across analog has AN2, Michael addition, and Schiff base formation alerts for the skin sensitization endpoint. This is due to the fact that the read-across analog is an activated (α,β-unsaturated) aldehyde which can very well be a Michael acceptor, undergo nucleophilic attack, and form a Schiff base with a protein or a nucleic acid nitrogen atom. The target material is expected to be more reactive upon metabolic transformation from alcohol to aldehyde. The read-across analog is a direct step 1 metabolite of the target material. The alerts confirm that the read-across analog is expected to be more reactive compared to the target material. Based on the structural similarity and the predicted metabolic transformation between the target material and the read-across analog as well as existing data for the read-across material described in the skin sensitization section, which shows that the read-across analog does not present a concern for skin sensitization, the alert will be superseded by the data.
  - o The target substance and the read-across analog are predicted to be sensitizers by the CAESAR model for skin sensitization. Existing data for the read-across material described in the skin sensitization section shows that the read-across analog does not present a concern for skin sensitization. Therefore, the alert will be superseded by the data.

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