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Short review

RIFM fragrance ingredient safety assessment, nootkatone, CAS Registry Number 4674-50-4

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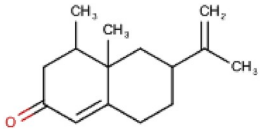

Abbreviation/Definition List:

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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
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015a; 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
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
QSAR - Quantitative Structure-Activity Relationship
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use **vPvB** - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

The Expert Panel for Fragrance Safety* concludes that this material is safe as described in this safety assessment.

This safety assessment is based on the RIFM Criteria Document (Api et al., 2015), which should be referred to for clarifications.

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

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

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

Nootkatone was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that nootkatone is not genotoxic. The repeated dose and reproductive toxicity endpoints were evaluated using the threshold of toxicological concern (TTC) for a Cramer Class II material, and the exposure to nootkatone is below the TTC (0.009 mg/kg/day and 0.009 mg/kg/day, respectively). Data show that there are no safety concerns for nootkatone for skin sensitization under the current declared levels of use. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet (UV) spectra; nootkatone is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was evaluated using the TTC for a Cramer Class III material, and the exposure to nootkatone is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; nootkatone was found not to be persistent, bioaccumulative, and toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are < 1.

Human Health Safety Assessment

Genotoxicity: Not genotoxic.	(RIFM, 1998; RIFM, 2011)
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 under the current, declared levels of use when purity is higher than 98%.	(RIFM, 2005a; RIFM, 1971a)
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic.	(UV Spectra, RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.	

Environmental Safety Assessment

Hazard Assessment:

Persistence: Critical Measured Value: 65% (OECD 301 F)	RIFM (2010b)
Bioaccumulation: Screening-level: 773.9 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Screening-level: Fish LC50: 9.78 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: 9.78 mg/L	(RIFM Framework; Salvito et al., 2002)

RIFM PNEC is: 0.00978 μg/L

- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: not applicable; cleared at the screening-level

1. Identification

1. **Chemical Name:** Nootkatone
2. **CAS Registry Number:** 4674-50-4
3. **Synonyms:** 5,6-Dimethyl-8-isopropenylbicyclo(4.4.0)dec-1-en-3-one; 4a,5-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-7-keto-3-isopropenylnaphthalene; (4R-(4α,4aα,6β))-4,4a,5,6,7,8-Hexahydro-4,4a-dimethyl-6-(1-methylvinyl)naphthalen-2(3H)-one; 4,4a,5,6,7,8-Hexahydro-6-isopropenyl-4,4a-dimethyl-2(3H)-naphthalenone; 2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-4,4a-dimethyl-6-(1-methylethenyl)-, (4R,4aS,6R)-; 4βH,5α-Eremorphila-1(10),11-dien-2-one; 4βH,5α-Eremophila-1(10),11-dien-2-one; (5,6-ｼﾞﾒﾁﾙ-8-ｲｿﾌﾛﾍﾟﾆﾙ)ﾋﾞｼｸﾛ(4.4.0)-ﾃﾞｾ-1-ｴﾝ-3-ｵﾝ; 6-Isopropenyl-4,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one; Nootkatone Crystals; Nootkatone
4. **Molecular Formula:** $C_{15}H_{22}O$
5. **Molecular Weight:** 218.34
6. **RIFM Number:** 988
7. **Stereochemistry:** Stereoisomer not specified. Two stereocenters and 1 geometric center present making a total of 8 isomers possible.

2. Physical data

1. **Boiling Point:** 125 °C at 0.5 mm Hg, 298.76 °C (EPI Suite)
2. **Flash Point:** 160 °F; CC (Fragrance Materials Association), > 212 °F
3. **Log K_{OW}:** Log Pow = 3.7 (RIFM, 2010a), 4.88 (EPI Suite)
4. **Melting Point**: 32 °C, 76.84 °C (EPI Suite)
5. **Water Solubility:** 2.375 mg/L (EPI Suite)
6. **Specific Gravity:** 0.9963 (RIFM)
7. **Vapor Pressure:** 0.000666 mm Hg @ 20 °C (EPI Suite v4.0), 0.00123 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, pale yellow to yellow liquid with an extremely powerful, fruity, sweet and citrusy odor (Arctander, 1969)

3. Volume of use (worldwide band)

1. **Volume of Use (Worldwide Band):** 0.1–1 metric ton per year (IFRA, 2015)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v2.0)

1. **95**th **Percentile Concentration in Hydroalcoholics:** 0.0025% (RIFM, 2018)
2. **Inhalation Exposure*:** 0.0000045 mg/kg/day or 0.00032 mg/day (RIFM, 2018)
3. **Total Systemic Exposure**:** 0.00012 mg/kg/day (RIFM, 2018)

*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 II, Intermediate (Expert Judgment)

Expert Judgment	Toxtree v 2.6	OECD QSAR Toolbox v 3.2
II*	III	I

*Due to potential discrepancies with the current *in silico* tools (Bhatia et al., 2015), the Cramer Class of the target material was determined using expert judgment based on the Cramer decision tree (Cramer et al., 1978). See the Appendix below for further details.

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

7. Metabolism

According to the WHO/JECFA Expert Committee, nootkatone is not expected to undergo glutathione conjugation. Rather, CYP-mediated side-chain oxidation and ketone reduction are reported to yield polar and excretable poly-oxygenated metabolites of nootkatone (see Fig. 1; WHO, 2006).

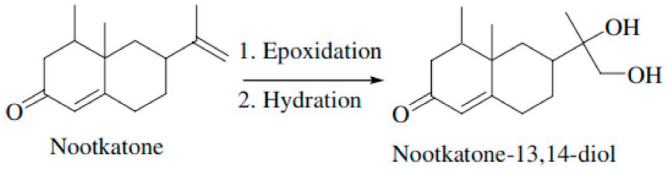

Fig. 1. Nootkatone metabolism in rabbits (WHO, 2006).

7.1. Additional References

None.

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

Nootkatone is reported to occur in the following foods by the VCF*:
Citrus fruits.
Curry (*Bergera koenigii* L.)
*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

Pre-registered for 2010; no dossier available as of 04/08/20.

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, nootkatone does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. The mutagenic activity of nootkatone has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 (OECD, 1997) using the preincubation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102 were treated with nootkatone 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, 1998). Under the conditions of the study, nootkatone was not mutagenic in the Ames test.

The clastogenic activity of nootkatone was evaluated in an *in vitro* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487 (OECD, 2010). Human peripheral blood lymphocytes were treated with nootkatone in DMSO at concentrations up to 1200 μg/mL in the dose range finding (DRF) study; micronuclei analysis was conducted at concentrations up to 185 μg/mL in the presence and absence of S9 for 3 h and in the absence of S9 for 24 h. Nootkatone 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, 2011). Under the conditions of the study, nootkatone was considered to be non-clastogenic in the *in vitro*

micronucleus test.

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

Additional References: None.

Literature Search and Risk Assessment Completed On: 06/13/19.

11.1.2. Repeated dose toxicity

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

11.1.2.1. Risk assessment. There are insufficient repeated dose toxicity data on nootkatone or any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to nootkatone (0.12 μg/kg bw/day) is below the TTC (9 μg/kg bw/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class II material at the current level of use.

Weight of Evidence (WoE):

RIFM, 2004: A GLP-compliant single-dose subchronic toxicity study was conducted following modified OECD 407 guidelines to evaluate the GRAS status of the test material. During the study, 10 Sprague Dawley Crl:CD (SD) IGS BR rats/sex/dose were administered nootkatone (purity: 70%) by gavage at doses of 0 and 10 mg/kg/day for 28 days. No treatment-related adverse effects were reported. However, at 10 mg/kg/day, globular eosinophilic material accumulations were observed in the tubular epithelium of male kidneys. This kidney finding was attributed to the α-2u-globulin nephropathy and not considered to be of toxicological significance (Lehman-McKeeman and Caudill, 1992; Lehman-McKeeman et al., 1990). Since this a single-dose study, it is considered insufficient for the current risk assessment.

Additional References: None.

Literature Search and Risk Assessment Completed On: 06/07/19.

11.1.3. Reproductive toxicity

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

11.1.3.1. Risk assessment. There are insufficient reproductive toxicity data on nootkatone or on any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to nootkatone (0.12 μg/kg bw/day) is below the TTC (9 μg/kg bw/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the reproductive toxicity endpoint of a Cramer Class II material at the current level of use.

Additional References: RIFM, 2004.

Literature Search and Risk Assessment Completed On: 05/07/19.

11.1.4. Skin sensitization

Based on the existing data, nootkatone does not present a concern for skin sensitization under the current, declared levels of use.

11.1.4.1. Risk assessment. Based on the existing data, nootkatone (purity > 98%) does not present a concern for skin sensitization under the current, declared levels of use. The chemical structure of this material indicates that it would be expected to react with skin proteins (Roberts et al., 2007; Toxtree 3.1.0; OECD Toolbox v4.2 [OECD, 2018]). In guinea pigs, an open epicutaneous test did not present skin sensitization reactions (RIFM, 1971a). In 2 human maximization tests with 10% or 6900 μg/cm^2 nootkatone, skin sensitization reactions were observed in 3/28 and 1/25 subjects (RIFM, 1978; RIFM, 1977). In these human maximization tests, the purity of the nootkatone sample was 86%. In another human maximization test with 10% or 6900 μg/cm^2 nootkatone, no reactions indicative of skin reactions were observed in the 25 subjects. In this study, the purity of nootkatone sample was 98%. Additionally, a confirmatory human repeat insult patch test (HRIPT) was conducted using nootkatone with 99% purity. No reactions indicative of sensitization were observed in any of the 103 volunteers in response to 1000 μg/cm^2 of nootkatone in 3:1 diethyl phthalate:ethanol (DEP:EtOH), (RIFM, 2005a). In another HRIPT with 2% nootkatone (unknown purity) in dimethyl phthalate (DMP), no skin sensitization reactions were observed in 53 volunteers (RIFM, 1971b).

Based on the WoE from structural analysis and animal and human studies, nootkatone with purity higher than 98% does not present a concern for skin sensitization under the current, declared levels of use. However, nootkatone at lower purity led to skin sensitization.

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/22/19.

11.1.5. Phototoxicity/photoallergenicity

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

11.1.5.1. Risk assessment. There are no phototoxicity studies available for nootkatone 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 the lack of absorbance, nootkatone does not present a concern for phototoxicity or photoallergenicity.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101; OECD, 1981) were obtained. The spectra indicate no significant absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark of concern for phototoxic effects, 1000 L mol^{-1} · cm^{-1} (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/06/19.

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 nootkatone 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 nootkatone. Based on the Creme RIFM Model, the inhalation exposure is 0.00032 mg/day. This exposure is 1468.8 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.

*As per Carthew et al. (2009), Cramer Class II materials default to Cramer Class III for the local respiratory toxicity endpoint.

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/15/19.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of nootkatone was performed following the RIFM Environmental Framework (Salvito et al., 2002) that provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional volume of use, log KOW and molecular weight are needed to estimate a conservative risk quotient (RQ) expressed as

the ratio: Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). In Tier 1, a general QSAR for fish toxicity is used with a high uncertainty factor, as discussed in Salvito et al. (2002). In Tier 2, the model ECOSAR (US EPA, 2012b; providing chemical class specific ecotoxicity estimates) is used, allowing for a lower uncertainty factor to be applied to the PNEC. 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 of the most recent IFRA Volume of Use Survey is reviewed. The PEC is then calculated based on the actual regional tonnage and not the extremes of the range. Following the RIFM Environmental Framework, nootkatone was identified as a fragrance material with no potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC < 1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) did not identify nootkatone as possibly persistent or bioaccumulative based on its structure and physical-chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same criteria used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite models BIOWIN 2 or BIOWIN 6 < 0.5 and BIOWIN 3 < 2.2, then the material is considered to be 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. Should an additional assessment be required, based on these model outputs (Step 1), a WoE-based review is 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 biodegradation, fate, and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

11.2.2. Risk assessment

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

11.2.3. Key studies

11.2.3.1. Biodegradation. **RIFM, 2010b:** The biodegradability of the test material was evaluated using the manometric respirometry test according to OECD 301F method (OECD, 1992). Biodegradation of 65% was observed after 28 days.

11.2.3.2. Ecotoxicity. **RIFM, 2005b:** The 7-day short-term chronic static-renewal toxicity tests were conducted with Fathead minnow and *Ceriodaphnia dubia*. The 7-day NOEC values of 3.56 and 7.13 mg/L were reported for Fathead minnow and *Ceriodaphnia dubia*, respectively.

11.2.4. Other available data

Nootkatone has been pre-registered under REACH, and no additional data is available at this time.

11.2.5. Risk assessment refinement

Since nootkatone has passed the screening criteria, measured data is included for completeness only and has not been used in PNEC derivation.

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

Endpoints used to calculate PNEC are underlined.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	9.78			1000000	0.00978	

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

Exposure	Europe (EU)	North America (NA)
Log K_{OW} Used	3.7	3.7
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	< 1	< 1
Risk Characterization: PEC/PNEC	< **1**	< **1**

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

The RIFM PNEC is 0.00978 μg/L. The revised PEC/PNECs for EU and NA are not applicable; the material was cleared at the screening-level; therefore, it does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 05/07/19.

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**
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
- **National Library of Medicine's Toxicology Information Services:** https://toxnet.nlm.nih.gov/
- **IARC:** https://monographs.iarc.fr
- **OECD SIDS:** https://hpvchemicals.oecd.org/ui/Default.aspx
- **EPA ACToR:** https://actor.epa.gov/actor/home.xhtml
- **US EPA HPVIS:** https://ofmpub.epa.gov/oppthpv/public_search.publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&

EndPointRpt=Y#submission

- **Japanese NITE:** https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com
- **ChemIDplus:** https://chem.nlm.nih.gov/chemidplus/

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 04/08/20.

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

Explanation of Cramer Classification

Due to potential discrepancies between the current *in silico* tools (Bhatia et al., 2015), the Cramer Class of the target material was determined using expert judgment, based on the Cramer decision tree.

Q1. A normal constituent of the body? No
Q2. Contains functional groups associated with enhanced toxicity? No
Q3. Contains elements other than C, H, O, N, and divalent S? No
Q5. Simply branched aliphatic hydrocarbon or a common carbohydrate? No
Q6. Benzene derivative with certain substituents? No
Q7. Heterocyclic? No
Q16. Common terpene (see Cramer et al., 1978 for detailed explanation)? No
Q17. Readily hydrolyzed to a common terpene? No
Q19. Open chain? No
Q23. Aromatic? No
Q24. Monocarbocyclic with simple substituents? No
Q25. Cyclopropane (see explanation in Cramer et al., 1978)? No
Q26. Monocycloalkanone or a bicyclo compound? Yes, Class II (Intermediate)

References

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

Arctander, S., 1969. Perfume and Flavor Chemicals (Aroma Chemicals), vols. I and II Published by the author: Montclair, NJ (USA).

Bhatia, S., Schultz, T., Roberts, D., Shen, J., Kromidas, L., Api, A.M., 2015. Comparison of Cramer classification between Toxtree, the OECD QSAR Toolbox and expert judgment. Regul. Toxicol. Pharmacol. 71 (1), 52–62.

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

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

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

Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard—a decision tree approach. Food Chem. Toxicol. 16 (3), 255–276.

Echa, 2012. Guidance on information requirements and chemical safety assessment Chapter R.11: PBT Assessment, November 2012 v1.1. http://echa.europa.eu/.

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

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

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

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

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

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

Oecd, 1981. *Test No. 101: UV-VIS Absorption Spectra*, OECD Guidelines for the Testing of Chemicals, Section 1. OECD Publishing, Paris. https://doi.org/10.1787/9789264069503-en. Retrieved from.

Oecd, 1992. *Test No. 301: Ready Biodegradability*, OECD Guidelines for the Testing of Chemicals, Section 3. OECD Publishing, Paris. https://doi.org/10.1787/9789264070349-en. Retrieved from.

Oecd, 1997. Test No. 471: Bacterial Reverse Mutation Test, OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris. https://doi.org/10.1787/9789264071247-en. Retrieved from.

Oecd, 2010. Test No. 487. In: Vitro Mammalian Cell Micronucleus Test. OECD Publishing, Paris. https://doi.org/10.1787/9789264091016-en. Retrieved from.

Oecd, 2018. The OECD QSAR Toolbox, v3.2–4.2. http://www.qsartoolbox.org/.

Rifm (Research Institute for Fragrance Materials, Inc), 1971. Prefung auf epikitane vertraeglicheit und sensibilitsierungsfaehigkeit am meerschweinchen. Unpublished report from Givaudan. RIFM report number 41819. RIFM, Woodcliff Lake, NJ, USA.

Rifm (Research Institute for Fragrance Materials, Inc), 1971. Sensitization and Irritation Study of Nootkatone. Unpublished report from Givaudan. RIFM report number 41820. RIFM, Woodcliff Lake, NJ, USA.

Rifm (Research Institute for Fragrance Materials, Inc), 1977. Report on Human Maximization Studies. Report to RIFM. RIFM report number 1702. RIFM, Woodcliff Lake, NJ, USA.

Rifm (Research Institute for Fragrance Materials, Inc), 1978. Report on Human Maximization Studies. Report to RIFM. RIFM report number 1698. RIFM, Woodcliff Lake, NJ, USA.

Rifm (Research Institute for Fragrance Materials, Inc), 1998. Bacterial Reverse Mutation Assay of Nootkatone (Ames Test). Unpublished report from Firmenich SA. RIFM report number 38410. RIFM, Woodcliff Lake, NJ, USA.

Rifm (Research Institute for Fragrance Materials, Inc), 2004. Nootkatone, Verbenone: Twenty-Eight Day Repeated Dose, Single Dose Level Oral Toxicity Study in the Rat. Report to FEMA. Unpublished report from Jones, L.J., McKenzie, J. & Brooks, P.N. RIFM report number 52967. RIFM, Woodcliff Lake, NJ, USA.

Rifm (Research Institute for Fragrance Materials, Inc), 2005. Repeated Insult Patch Test with Nootkatone. Unpublished report from Bedoukian Research, Inc. RIFM report number 46155. RIFM, Woodcliff Lake, NJ, USA.

Rifm (Research Institute for Fragrance Materials, Inc), 2005. 7-Day chronic toxicity test results with LC50 and NOEC endpoints for 25 fragrance chemicals using ceriodaphnia dubia and fathead minnows. Unpublished report from S.C.Johnson. RIFM report number 49950. RIFM, Woodcliff Lake, NJ, USA.

Rifm (Research Institute for Fragrance Materials, Inc), 2010. Partition Coefficient N-Octanol/water of Nootkatone (Nootkatone Crystals). Unpublished report from Givaudan. RIFM report number 60321. RIFM, Woodcliff Lake, NJ, USA.

Rifm (Research Institute for Fragrance Materials, Inc), 2010. Ready Biodegradability of Nootkatone (Crystals). Unpublished report from Givaudan. RIFM report number 60681. RIFM, Woodcliff Lake, NJ, USA.

Rifm (Research Institute for Fragrance Materials, Inc), 2011. Nootkatone: Induction of Micronuclei in Cultured Human Peripheral Blood Lymphocytes. Unpublished report from Taylor, S. & Stone, V. RIFM report number 73537. RIFM, Woodcliff Lake, NJ, USA.

Rifm(Research Institute for Fragrance Materials, Inc), 2018. Exposure Survey, vol. 21 September 2018.

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

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

Safford, B., Api, A.M., Barratt, C., Comiskey, D., Ellis, G., McNamara, C., O'Mahony, C.,

Robison, S., Rose, J., Smith, B., Tozer, S., 2017. Application of the expanded Creme RIFM consumer exposure model to fragrance ingredients in cosmetic, personal care and air care products. Regul. Toxicol. Pharmacol. 86, 148–156.
Salvito, D.T., Senna, R.J., Federle, T.W., 2002. A Framework for prioritizing fragrance materials for aquatic risk assessment. Environ. Toxicol. Chem. 21 (6), 1301–1308.
Us Epa, 2012a. Estimation Programs Interface Suite for Microsoft Windows, v4.0–v4.11. United States Environmental Protection Agency, Washington, DC, USA.
Us Epa, 2012b. The ECOSAR (ECOlogical Structure Activity Relationship) Class Program for Microsoft Windows, v1.11. United States Environmental Protection Agency, Washington, DC, USA.
Who, 2006. Evaluations of the Joint FAO/WHO expert Committee on food Additives (JECFA): safety evaluation of certain food Additives. Retrieved from. http://www.inchem.org/documents/jecfa/jecmono/v54je01.pdf.