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Short Review

RIFM fragrance ingredient safety assessment, 2,2,3-trimethylcyclopent-3-enylacetonitrile, CAS Registry Number 15373-31-6

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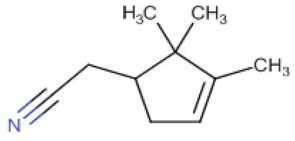
Version: 091018. This version replaces any previous versions.
Name: 2,2,3-Trimethylcyclopent-3-enylacetonitrile
CAS Registry Number: 15373-31-6



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., 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
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.

2,2,3-Trimethylcyclopent-3-enylacetonitrile was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 2,2,3-trimethylcyclopent-3-enylacetonitrile is not genotoxic. Data on read-across material citronellyl nitrile (CAS # 51566-62-2) provide a calculated MOE > 100 for the repeated dose anddevelopmental and reproductive toxicity endpoints. Data show that there are no safety concerns for 2,2,3-trimethylcyclopent-3-enylacetonitrile for skin sensitization under the current declared levels of use. The phototoxicity/photoallergenicity endpoints were evaluated based on data and UV spectra; 2,2,3-trimethylcyclopent-3-enylacetonitrile 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 2,2,3-trimethylcyclopent-3-enylacetonitrile is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; 2,2,3-trimethylcyclopent-3-enylacetonitrile 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, 2015b; RIFM, 2007)
Repeated Dose Toxicity: NOAEL = 300 mg/kg/day.	RIFM (2008)
Developmental and Reproductive Toxicity: NOAEL = 500 mg/kg/day.	RIFM (2011)
Skin Sensitization: Not sensitizing.	(RIFM, 2015a; RIFM, 1987a)
Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic.	(UV Spectra, RIFM DB; RIFM, 1987b)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.	
Environmental Safety Assessment	
Hazard Assessment:	
Persistence: Critical Measured Value: 2% OECD 301F	RIFM (2013b)
Bioaccumulation: Screening-level: 72.8 L/kg	(EPI Suite; US EPA, 2012a)
Ecotoxicity: Screening-level: LC50: 14.9 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: LC50: 14.9 mg/L	(RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.0149 μg/L	
• **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: Not applicable; Cleared at screening-level	

1. Identification

1. **Chemical Name:** 2,2,3-Trimethylcyclopent-3-enylacetonitrile
2. **CAS Registry Number:** 15373-31-6
3. **Synonyms:** Cantryl; 3-Cyclopentene-1-acetonitrile, 2,2,3-trimethyl-; α-Campholenonitrile; (2,2,3-Trimethylcyclopent-3-en-1-yl) acetonitrile; 2,2,3-Trimethylcyclopent-3-enylacetonitrile
4. **Molecular Formula:** $C_{10}H_{15}N$
5. **Molecular Weight:** 149.37
6. **RIFM Number:** 5426
7. **Stereochemistry**: Isomer not specified. One stereocenter and 2 total stereoisomers possible.

2. Physical data

1. **Boiling Point:** 235.88 °C (EPI Suite)
2. **Flash Point:** Not Available
3. **Log K_{ow}**: 3.33 (EPI Suite)
4. **Melting Point**: 31.1 °C (EPI Suite)
5. **Water Solubility:** 59.81 mg/L (EPI Suite)
6. **Specific Gravity:** Not available
7. **Vapor Pressure:** 0.0283 mm Hg @ 20 °C (EPI Suite v4.0), 0.0483 mm Hg @ 25 °C (EPI Suite)
8. **UV Spectra:** No absorbance between 290 and 500 nm
9. **Appearance/Organoleptic:** Not Available

3. Exposure

1. **Volume of Use (worldwide band):** 0.1–1 metric tons per year (IFRA, 2015)
2. **95th Percentile Concentration in Hydroalcoholics:** 0.15% (RIFM, 2015d)
3. **Inhalation Exposure*:** 0.00017 mg/kg/day or 0.014 mg/day (RIFM, 2015d)
4. **Total Systemic Exposure**:** 0.0015 mg/kg/day (RIFM, 2015d)

*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 4. 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:** 80% (predicted)

Using RIFM's *in silico* skin absorption model (RIFM, 2014) that was approved by RIFM's Independent Expert Panel (Meeting, Miami, FL, Jan. 13–14, 2014), the prediction results are:

	Parent
Name	2,2,3-Trimethylcyclopent-3-enylacetonitrile
J_{max} (μg/cm^2/h)	43.4[1]
Skin Absorption Class	80%

[1] J_{max} was calculated based on estimated log K_{ow} = 2.6 (Consensus model) and Solubility = 798 mg/L (Consensus model).

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

5. Computational toxicology evaluation

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

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

2. Analogs Selected:
   a. **Genotoxicity:** None
   b. **Repeated Dose Toxicity:** Citronellyl nitrile (CAS # 51566-62-2)
   c. **Developmental and Reproductive Toxicity:** Citronellyl nitrile (CAS # 51566-62-2)
   d. **Skin Sensitization:** None
   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)

2,2,3-Trimethylcyclopent-3-enylacetonitrile is not reported to occur in foods by 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.

8. IFRA standard

None.

9. REACH dossier

Available; accessed 04/19/18.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data, 2,2,3-trimethylcyclopent-3-enylacetonitrile does not present a concern for genetic toxicity.

10.1.1.1. Risk assessment. The mutagenic activity of 2,2,3-trimethylcyclopent-3-enylacetonitrile has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation and preincubation methods. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* WP2uvrA were treated with 2,2,3-trimethylcyclopent-3-enylacetonitrile 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, 2015b). Under the conditions of the study, 2,2,3-trimethylcyclopent-3-enylacetonitrile was not mutagenic in the Ames test.

The clastogenic activity of 2,2,3-trimethylcyclopent-3-enylacetonitrile 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 2,2,3-trimethylcyclopent-3-enylacetonitrile in DMSO at concentrations up to 1560 μg/mL in the presence and absence of metabolic activation (S9) for 4 h and in the absence of metabolic activation for 24 h 2,2,3-trimethylcyclopent-3-enylacetonitrile did not induce binucleated cells with micronuclei when tested up to cytotoxic concentrations in either the presence or absence of an S9 activation system (RIFM, 2007). Under the conditions of the study, 2,2,3-trimethylcyclopent-3-enylacetonitrile was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the available data, 2,2,3-trimethylcyclopent-3-enylacetonitrile does not present a concern for genotoxic potential.

Additional References: RIFM, 1989a; RIFM, 1989b; RIFM, 2009; RIFM, 2013a.

Literature Search and Risk Assessment Completed On: 05/16/18.

10.1.2. Repeated dose toxicity

The margin of exposure for 2,2,3-trimethylcyclopent-3-enylacetonitrile is adequate for the repeated dose toxicity endpoint at the current level of use.

10.1.2.1. Risk assessment. There are insufficient repeated dose toxicity data on 2,2,3-trimethylcyclopent-3-enylacetonitrile. Read-across material citronellyl nitrile (CAS # 51566-62-2; see Section 5) has sufficient repeated dose toxicity data that can be used to support the repeated dose toxicity endpoint. In an enhanced OECD 408 90-day oral gavage study, groups of 10 Sprague Dawley rats received doses of 0, 10, 30, 100, or 300 mg/kg/day of citronellyl nitrile in corn oil. Marginal centrilobular hepatocyte hypertrophy was observed in both sexes at 300 mg/kg/day and in 2 males and 1 female at 100 mg/kg/day and was considered to be adaptive in nature. A higher incidence of hypoplasia in the bone marrow was observed in the 300 mg/kg/day females, but was not statistically significant and was considered to be a marginal effect as there were no corresponding hematological changes. There were no other adverse findings during necropsy or histopathological

examination. The NOAEL was considered to be 300 mg/kg/day, the highest dose tested (RIFM, 2008, also available in Letizia et al., 2009). In addition, an enhanced OECD 415 oral gavage 1-generation reproductive toxicity study was conducted in groups of 25 Sprague Dawley rats/sex. The animals were treated with citronellyl nitrile at doses of 0, 75, 200, or 500 mg/kg/day in corn oil. Administration began before the cohabitation period (83 days for males; 14 days for females), through cohabitation (maximum of 14 days), until the day before euthanasia (for males only) or day 25 of presumed gestation (for females that did not deliver) or day 22 of lactation. F1 generation rats selected for continued evaluation were euthanized on day 60 ± 3 postpartum. The NOAEL for general toxicity was considered to be 200 mg/kg/day, based on a reduction in bodyweight gains and terminal body weights among the high-dose group males. No such effects were reported among the treated females. Also, there were no other treatment-related adverse effects reported up to the highest dose tested (RIFM, 2011). **Therefore, the 2,2,3-trimethylcyclopent-3-enylacetonitrile MOE for the repeated dose toxicity endpoint can be calculated by dividing the citronellyl nitrile NOAEL in mg/kg/day by the total systemic exposure to 2,2,3-trimethylcyclopent-3-enylacetonitrile, 300/0.0015 or 20000.**

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/26/18.

10.1.3. Developmental and reproductive toxicity

The margin of exposure for 2,2,3-trimethylcyclopent-3-enylacetonitrile is adequate for the developmental and reproductive toxicity endpoints at the current level of use.

10.1.3.1. Risk assessment. There are insufficient developmental toxicity data on 2,2,3-trimethylcyclopent-3-enylacetonitrile. Read-across material, citronellyl nitrile (CAS # 51566-62-2; see Section 5) has sufficient developmental toxicity data that can be used to support the developmental toxicity endpoint. In an OECD 414 oral gavage study, groups of 25 pregnant female Wistar rats received doses of 0, 50, 150, or 450 mg/kg/day of citronellyl nitrile in corn oil. Maternal effects in the high-dose group included alterations in clinical chemistry parameters and increased liver weight. There were no adverse effects on the fetuses. The NOAEL for maternal and developmental toxicity was considered to be 150 mg/kg/day and 450 mg/kg/day, respectively (RIFM, 2016a). In an enhanced OECD 415 1-generation oral gavage study, citronellyl nitrile was administered at doses of 0, 75, 200, or 500 mg/kg/day in corn oil to groups of 25 Sprague Dawley rats/sex. There were no adverse effects on the offspring. The NOAEL for developmental toxicity was considered to be 500 mg/kg/day, the highest dose tested (RIFM, 2011).

There are insufficient reproductive toxicity data on 2,2,3-trimethylcyclopent-3-enylacetonitrile. Read-across material citronellyl nitrile (CAS # 51566-62-2; see Section 5) has sufficient reproductive toxicity data that can be used to support the reproductive toxicity endpoint. In an enhanced OECD 415 1-generation oral gavage study, citronellyl nitrile was administered at doses of 0, 75, 200, or 500 mg/kg/day in corn oil to groups of 25 Sprague Dawley rats/sex. There were no apparent effects of citronellyl nitrile on mating and fertility, reproductive organs, and the sperm and estrus cycling parameters at any dose level tested. The NOAEL was considered to be 500 mg/kg/day, the highest dose tested (RIFM, 2011). In another study, citronellyl nitrile was administered via oral gavage to groups of 10 Sprague Dawley rats/sex. The study was conducted according to the OECD 408 protocol. The animals were treated with citronellyl nitrile at doses of 0, 10, 30, 100, or 300 mg/kg/day in corn oil. In addition to systemic toxicity parameters, the male (sperm analysis) and female (estrous cycling) parameters were also reported. There were no effects on the male and female reproductive parameters up to the highest dose tested (RIFM, 2008, also available in Letizia et al., 2009). The NOAEL for the reproductive toxicity endpoint was considered to be 500 mg/kg/day, the highest dose tested.

Therefore, the 2,2,3-trimethylcyclopent-3-enylacetonitrile MOE for the developmental and reproductive toxicity endpoints can be calculated by dividing the citronellyl nitrile NOAEL in mg/kg/day by the total systemic exposure to 2,2,3-trimethylcyclopent-3-enylacetonitrile, 500/0.0015 or 333333.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/27/18.

10.1.4. Skin sensitization

Based on the existing data, 2,2,3-trimethylcyclopent-3-enylacetonitrile does not present a concern for skin sensitization under the current, declared levels of use.

10.1.4.1. Risk assessment. Based on the existing data, 2,2,3-trimethylcyclopent-3-enylacetonitrile is not considered a skin sensitizer. The chemical structure of this material indicates that it would not be expected to react with skin proteins (Roberts et al., 2007; Toxtree 2.6.13; OECD toolbox v4.1). In a murine local lymph node assay (LLNA), 2,2,3-trimethylcyclopent-3-enylacetonitrile was not found to be sensitizing up to 100% (RIFM, 2015a; RIFM, 2015c). In a guinea pig maximization test, 2,2,3-trimethylcyclopent-3-enylacetonitrile did not present reactions indicative of sensitization at 10% (RIFM, 1987a).

Based on weight of evidence (WoE) from structural analysis and animal studies, 2,2,3-trimethylcyclopent-3-enylacetonitrile does not present a concern for skin sensitization under the current, declared levels of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/24/18.

10.1.5. Phototoxicity/photoallergenicity

Based on available UV absorption spectra and existing data, 2,2,3-trimethylcyclopent-3-enylacetonitrile would not be expected to present a concern for phototoxicity or photoallergenicity.

10.1.5.1. Risk assessment. The available UV absorption spectra for 2,2,3-Trimethylcyclopent-3-enylacetonitrile indicate no absorbance between 290 and 500 nm. As such, it is not a concern for phototoxicity and photoallergenicity (Henry et al., 2009). Phototoxicity and photoallergenicity of 10% 2,2,3-trimethylcyclopent-3-enylacetonitrile were evaluated in guinea pigs, and there were no reactions (RIFM, 1987b). Based on UV/Vis absorption spectra and the *in vivo* studies 2,2,3-trimethylcyclopent-3-enylacetonitrile would not be expected to present a concern for phototoxicity or photoallergenicity.

10.1.5.2. UV spectra analysis. The available spectra indicate no absorbance in the range of 290–500 nm. The material is not a concern for phototoxic effects because it does not absorb in the range of concern (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/11/18.

10.1.6. Local Respiratory Toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The exposure level for 2,2,3-trimethylcyclopent-3-enylacetonitrile is below the Cramer Class III TTC value for inhalation exposure local effects.

10.1.6.1. Risk assessment. There are no inhalation data available on 2,2,3-trimethylcyclopent-3-enylacetonitrile. Based on the Creme RIFM Model, the inhalation exposure is 0.014 mg/day. This exposure is 33.6

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.

Key Studies: None.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/23/18.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of 2,2,3-trimethylcyclopent-3-enylacetonitrile 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, 2,2,3-trimethylcyclopent-3-enylacetonitrile 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 2,2,3-trimethylcyclopent-3-enylacetonitrile 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 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 v4.11). 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 the current Volume of Use (2015), 2,2,3-trimethylcyclopent-3-enylacetonitrile does not present a risk to the aquatic compartment in the screening-level assessment.

Key Studies

Biodegradation

RIFM, 2013b: The ready biodegradability of the test material was determined in the Manometric Respirometry Test according to the OECD 301F method. After 28 days, biodegradation of 2% was observed.

Ecotoxicity

RIFM, 2016b: A *Daphnia magna* acute immobilization test was conducted according to the OECD 202 guidelines under a closed system in static conditions. The 48-h EC50 based on nominal concentrations was reported to be 24.2 mg/L.

RIFM, 2016c: An Algae growth inhibition test was conducted according to the OECD 201 method. The 72-h EC50 based on nominal concentration was reported to be 15.3 mg/L and 10.6 mg/L for growth rate and yield, respectively.

Other available data: 2,2,3-trimethylcyclopent-3-enylacetonitrile has been registered under REACH with no additional data at this time.

10.2.3. Risk assessment refinement

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

Endpoints used to calculate PNEC are highlighted.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	14.9			1000000	0.0149	

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

Exposure	Europe	North America
Log K_{ow} Used	3.3	3.3
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 0.0149 μg/L. The revised PEC/PNECs for EU and NA are: not applicable. The material was cleared at 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: 04/26/18.

11. Literature Search*

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

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 08/27/2018.

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.110571.

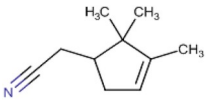
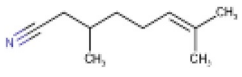
Appendix

Read-across Justification:

Methods

The read-across analog was 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 Chemical 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 (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 2.6.13.
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018).
- The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).

	Target Material	Read-across Material
Principal Name	2,2,3-Trimethylcyclopent-3-enylacetonitrile	Citronellyl nitrile
CAS No.	15373-31-6	51566-62-2
Structure		
Similarity (Tanimoto Score)		0.57
Read-across Endpoint		• Repeated dose toxicity • Reproductive toxicity • Developmental toxicity
Formula	$C_{10}H_{15}N$	$C_{10}H_{17}N$
Molecular Weight	149.24	151.25
Melting Point (°C, EPI Suite)	31.10	−8.64
Boiling Point (°C, EPI Suite)	235.88	233.15

Vapor Pressure (Pa @ 25 °C, EPI Suite)	6.44	8.84
Log Kow (KOWWIN v1.68 in EPI Suite)	3.33	3.55
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPI Suite)	59.81	37.76
J_{max} (μg/cm^2/h, SAM)	43.450	23.710
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.37E + 001	3.10E + 001
Repeated Dose Toxicity		
Repeated dose (HESS)	• Aliphatic nitriles (Hepatotoxicity) Rank B	• Aliphatic nitriles (Hepatotoxicity) Rank B
Reproductive and Developmental Toxicity		
ER Binding (OECD QSAR Toolbox v4.2)	• Non-binder, non-cyclic structure	• Non-binder, non-cyclic structure
Developmental Toxicity (CAESAR v2.1.6)	• Toxicant (low reliability)	• Non-toxicant (low reliability)
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 2,2,3-trimethylcyclopent-3-enylacetonitrile (CAS # 15373-31-6). 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, citronellyl nitrile (CAS # 51566-62-2) was identified as a read-across material with sufficient data for toxicological evaluation.

Conclusions

- Citronellyl nitrile (CAS # 51566-62-2) was used as a read-across analog for the target material 2,2,3-trimethylcyclopent-3-enylacetonitrile (CAS # 15373-31-6) for the repeated dose and reproductive toxicity endpoints.
  - o The target substance and the read-across analog are structurally similar and belong to the unsaturated aliphatic nitriles class.
  - o The key difference between the target substance and the read-across analog is that the read-across analog has a branched aliphatic fragment, whereas the target substance has a cyclic aliphatic fragment containing a similar unsaturated, branched structure. This structural difference is toxicologically insignificant for the repeated dose and reproductive toxicity endpoints.
  - o Similarity between the target substance and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The physical–chemical properties of the target substance and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - o According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target substance and the read-across analog.
  - o The target substance has an alert of toxicant with low probability by the developmental toxicity model of CAESAR. The read-across analog has an alert of non-toxicant with low probability. As described in the repeated dose and developmental and reproductive toxicity sections, the data for the read-across analog confirms that the margin of exposure is adequate at the current level of use. Based on the structural similarity between the target substance and the read-across analog, and the data for the read-across analog, the alerts are superseded by data.
  - o The target substance 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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