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Short review

RIFM fragrance ingredient safety assessment, benzenepropanenitrile, 4-ethyl-.α.,.α.-dimethyl, CAS Registry Number 134123-93-6

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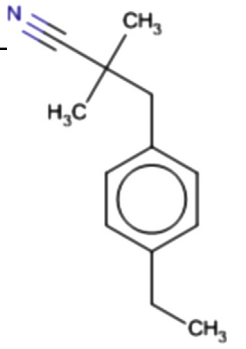

1. Identification

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

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(*continued*)

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; Safford et al., 2015; Safford et al., 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
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
RIFM- Research Institute for Fragrance Materials
RQ- Risk Quotient
TTC- Threshold of Toxicological Concern
UV/Vis Spectra- Ultra Violet/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 under the limits 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 reviews 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 two-digit month/day/year), both in the RIFM database (consisting of publicly available and proprietary data) and through publicly available information sources (*i.e.*, 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 guidance relevant to human health and environmental protection.

Summary: The use of this material under current conditions is supported by existing information.

This material was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, as well as environmental safety. Data show that this material is not genotoxic, it does not have skin sensitization potential and provided a MOE > 100 for the repeated dose toxicity endpoint. The local respiratory toxicity endpoint was completed using the TTC (Threshold of Toxicological Concern) for a Cramer Class III material (0.47 mg/day); exposure < TTC (acceptable). The developmental and reproductive toxicity endpoint was completed using 2-phenylhexanenitrile (CAS# 3508-98-3) as a read-across analog, which provided a MOE > 100. The phototoxicity/photoallergenicity endpoint was completed based on UV spectra; the material is not phototoxic/photoallergenic. The environmental endpoints were evaluated and the material was not found to be a PBT; its risk quotients, based on current volume of use in Europe and North America, were acceptable (PEC/PNEC < 1).

Human Health Safety Assessment

Genotoxicity: Not genotoxic. (RIFM, 1992a; RFIM, 1996)
Repeated Dose Toxicity: NOAEL = 150 mg/kg/day. (RIFM, 1993a)
Developmental and Reproductive Toxicity: NOAEL = 70 mg/kg/day. (ECHA REACH Dossier: 2-phenylhexanenitrile)

(*continued*)

Skin Sensitization: Not sensitizing. (RIFM, 1992b; RIFM, 1992d; RIFM, 1992e)
Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic. (UV Spectra, RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure < TTC (acceptable).

Environmental Safety Assessment

Hazard Assessment:
Persistence: Critical Measured Value: 6% (OECD 301D) (RIFM, 1993b)
Bioaccumulation: Screening Level: 191.3 l/kg (EPI SUITE ver 4.1)
Ecotoxicity: Screening Level: 48-hr *Daphnia magna* LC50: 1.808 mg/l (EPI SUITE ver 4.1)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:
Screening-Level: PEC/PNEC (North America and Europe) > 1 (Salvito et al., 2002)
Critical Ecotoxicity Endpoint: 48-hr *Daphnia magna* LC50: 1.808 mg/l (EPI SUITE ver. 4.1)
RIFM PNEC is: 0.1808 μg/l
• **Revised PEC/PNECs (2011 IFRA Volume of Use):** North America and Europe <1

1. **Chemical Name:** Benzenepropanenitrile, 4-ethyl-α, α-dimethyl-
2. **CAS Registry Number:** 134123-93-6
3. **Synonyms:** Benzenepropanenitrile, 4-ethyl-.α..,α.-dimethyl-; 4-Ethyl-α,α-dimethylbenzenepropanenitrile; Fleuranil
4. **Molecular Formula:** $C_{13}H_{17}N$
5. **Molecular Weight:** 187.29
6. **RIFM Number:** 6342

2. Physical data

1. **Boiling Point:** 262.5–278.0 °C @ atmospheric pressure (1024mbar) (RIFM, 1992f), (calculated) 296.64 °C [EPI Suite]
2. **Flash Point:** 132 °C (RIFM, 1992f)
3. **Log K_{OW}:** 3.46 at 22 °C (RIFM, 1992f), 3.96 [EPI Suite]
4. **Melting Point:** < = −36.5 °C (RIFM, 1992f), (calculated) 64.5 °C [EPI Suite]
5. **Water Solubility:** 8.34×10^{-2} +- 3.75×10^{-3} g/l at 20 °C (RIFM, 1992f), (calculated) 11.37 mg/l [EPI Suite]
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 1.67 Pa at 25 °C (RIFM, 1992f), (calculated) 0.000996 mm Hg @ 25 °C [EPI Suite], (calculated) 0.000533 mm Hg @ 20 °C [EPI Suite 4.0]
8. **UV Spectra:** No significant absorbance in the region of 290–700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} cm^{-1})
9. **Appearance/Organoleptic:** Colorless clear liquid with an ozone, marine, seashell, anisic, and herbal like odor (Luebke, William tgsc, 2007)*

*http://www.thegoodscentscompany.com/data/rw1059261.htmlretrieved 6/27/14.

3. Exposure

1. **Volume of Use (worldwide band):** 1–10 metric tons per year (IFRA, 2011)
2. **95**th **Percentile Concentration in Hydroalcoholics:** 0.15% (RIFM, 2014)
3. **Inhalation Exposure*:** 0.00025 mg/kg/day or 0.017 mg/day (RIFM, 2014)
4. **Total Systemic Exposure**:** 0.0037 mg/kg/day (RIFM, 2014)

*95th percentile calculated exposure derived from

concentration survey data in the Creme RIFM exposure model (Comiskey et al., 2015; Safford et al., 2015 and Safford 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 and Safford et al., 2017).

4. Derivation of systemic absorption

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

5. Computational toxicology evaluation

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

Expert Judgment	ToxTree v2.6	OECD QSAR Toolbox
III	III	III

2. Analogs Selected:
   a. Genotoxicity: None
   b. Repeated Dose Toxicity: None
   c. Developmental and Reproductive Toxicity: 2-phenylhexanenitrile (CAS# 3508-98-3)
   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.

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

Benzenepropanenitrile, 4-ethyl-α, α-dimethyl- is not reported to occur in food by the VCF*.

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

8. IFRA standard

None.

9. REACH Dossier

No; not on pre-registration list as of 04/14/2017.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current data, benzenepropanenitrile, 4-ethyl-α, α-dimethyl- does not present a concern for genetic toxicity.

10.1.2. Risk assessment

Benzenepropanenitrile, 4-ethyl-α, α-dimethyl- was tested in the BlueScreen assay and found positive for genotoxicity at cytotoxic concentrations in the presence of metabolic activation and negative without S9 indicating lack of genotoxic potential (RIFM, 2013). The mutagenic activity of benzenepropanenitrile, 4-ethyl-α, α-dimethyl- has been assessed in an Ames assay in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation method. *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 and *Escherichia coli* strain WP2uvrA were treated with benzenepropanenitrile, 4-ethyl-α, α-dimethyl- in DMSO (dimethyl sulfoxide) at the concentrations 125, 250, 500, 1000, and 2000 μg/plate in the presence and absence of S-9 mix. No increase in the number of revertant colonies was observed in any of the tester strains at any of the concentrations tested (RIFM, 1992a). Under the conditions of the study, benzenepropanenitrile, 4-ethyl-α, α-dimethyl- was considered not mutagenic in the Ames test.

The clastogenic activity of benzenepropanenitrile, 4-ethyl-α, α-dimethyl-was assessed in an *in vivo* micronucleus assay conducted in compliance with GLP regulations and in accordance with OECD TG 474. Groups of male and female Crl:CD-1(ICR)BR mice were treated with benzenepropanenitrile, 4-ethyl-α, α-dimethyl- in 10 ml via a single intraperitoneal injection for 4 h at 250, 500 and 1000 mg/kg bodyweight. Benzenepropanenitrile, 4-ethyl-alpha, alpha-dimethyl- induced no significant increases in micronucleated polylchromatic erythrocytes over the levels observed in the vehicle controls in either sex or at any of the harvest times (RFIM, 1996). Under the conditions of the study, benzenepropanenitrile, 4-ethyl-α, α-dimethyl- was considered negative for clastogenicity in the *in vivo* micronucleus test.

Based on the available data, benzenepropanenitrile, 4-ethyl-α, α-dimethyl- does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed on: 05/02/14.

10.1.3. Repeated dose toxicity

The margin of exposure for benzenepropanenitrile, 4-ethyl-α, α-dimethyl- is adequate for the repeated dose toxicity endpoint at the current level of use.

10.1.4. Risk assessment

There are sufficient repeated dose toxicity data on benzenepropanenitrile, 4-ethyl-α, α-dimethyl- to complete the safety assessment. An OECD 407 gavage 28-day subchronic toxicity study was conducted on 5 Sprague-Dawley rats/sex/dose gavaged daily with 0, 15, 150, and 500 mg/kg benzenepropanenitrile, 4-ethyl-α, α-dimethyl- suspended in a corn oil vehicle at concentrations of 0, 0.3%, 3%, and 10%. Hunched posture and abnormal gait were reported among animals of the 500 mg/kg/day and the 150 mg/kg/day (last few days of the study) dose groups. Macroscopic observations revealed enlarged livers among the animals of the high dose group. Microscopic examination included adaptive liver hepatocyte enlargement among the animals of the 500 and 150 mg/kg/day dose groups. The NOAEL was determined to be 150 mg/kg/day (RIFM, 1993a).

A default safety factor of 3 was used because the NOAEL was derived from the 28-day study. The safety factor has been approved

by The Expert Panel for Fragrance Safety*.

Thus, the derived NOAEL for the repeated dose toxicity data is 150/3 or 50 mg/kg/day.

* The Expert Panel for Fragrance Safety is composed of scientific and technical experts in their respective fields. This group provides advice and guidance.

Therefore, the benzenepropanenitrile, 4-ethyl-α, α-dimethyl- MOE for the repeated dose toxicity endpoint can be calculated by dividing the benzenepropanenitrile, 4-ethyl-α, α-dimethyl- NOAEL in mg/kg/day by the total systemic exposure to benzenepropanenitrile, 4-ethyl-α, α-dimethyl-, 50/0.0037 or 13513.

Additional References: None.

Literature Search and Risk Assessment Completed on: 09/30/16.

10.1.5. Developmental and reproductive toxicity

The margin of exposure for benzenepropanenitrile, 4-ethyl-α, α-dimethyl- is adequate for the developmental and reproductive toxicity endpoints at the current level of use.

10.1.6. Risk assessment

There are no developmental or reproductive data on benzenepropanenitrile, 4-ethyl-α, α-dimethyl-. There are sufficient developmental and reproductive toxicity data on read-across material, 2-phenylhexanenitrile (CAS # 3508-98-3; see section 5). In a dietary OECD/GLP 421 developmental and reproductive toxicity screening conducted on Wistar Han rats, groups were administered via gavage test material, 2-phenylhexanenitrile at nominal values of 0, 200, 400 and 1000 ppm (equivalent doses of 0, 15, 30 and 70–73 mg/kg bw/day for the males and 79–124 mg/kg bw/day for the females). There were no adverse developmental effects observed among the pups of female animals fed the diet containing the test material up to the highest dose levels of 79–124 mg/kg/day. Thus, the NOAEL for developmental toxicity was determined to be 80 mg/kg/day (ECHA REACH Dossier: 2-phenylhexanenitrile, accessed on 11/17/2015). There was no adverse effect of test material administration towards the male and female reproductive organs up to the highest dose tested. Thus, the NOAEL for male and female reproductive toxicity was determined to be 70 mg/kg/day, the highest dose tested in male rats (ECHA REACH Dossier: 2-phenylhexanenitrile, accessed on 11/17/2015). Therefore, the benzenepropanenitrile, 4-ethyl-α, α-dimethyl- MOE for the developmental and reproductive toxicity endpoints can be calculated by dividing the 2-phenylhexanenitrile NOAEL in mg/kg/day by the total systemic exposure to benzenepropanenitrile, 4-ethyl-α, α-dimethyl-, 70/0.0037 or 18919.

Additional References: None.

Literature Search and Risk Assessment Completed on: 09/30/16.

10.1.7. Skin sensitization

Based on the existing data, benzenepropanenitrile, 4-ethyl-α, α-dimethyl- does not present a concern for skin sensitization.

10.1.8. Risk assessment

Based on existing data, benzenepropanenitrile, 4-ethyl-α, α-dimethyl- does not present a concern for skin sensitization. The chemical structure indicates that this material would not be expected to react with skin proteins (Roberts et al., 2007; Toxtree 2.5.0; OECD toolbox v3.1). In a guinea pig maximization test, this material was reported to be a non-sensitizer (RIFM, 1992b). In human confirmatory studies no sensitization reactions indicative of sensitization to benzenepropanenitrile, 4-ethyl-α, α-dimethyl- were observed (RIFM, 1992d; RIFM, 1992e).

Additional References: None.

Literature Search and Risk Assessment Completed on: 09/28/16.

10.1.9. Phototoxicity/photoallergenicity

Based on the available UV spectra, benzenepropanenitrile, 4-ethyl-α, α-dimethyl- does not present a concern for phototoxicity or photoallergenicity.

10.1.10. Risk assessment

There are no phototoxicity studies available for benzenepropanenitrile, 4-ethyl-α, α-dimethyl- in experimental models. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. Corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity, 1000 L mol^{-1} cm^{-1} (Henry et al., 2009). Based on lack of absorbance, benzenepropanenitrile, 4-ethyl-α, α-dimethyl- does not present a concern for phototoxicity or photoallergenicity.

Additional References: None.

Literature Search and Risk Assessment Completed on: 09/07/16.

10.1.11. Local respiratory toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The material, benzenepropanenitrile, 4-ethyl-α, α-dimethyl-, exposure level is below the Cramer Class III TTC value for inhalation exposure local effects.

10.1.12. Risk assessment

There are no inhalation data available on benzenepropanenitrile, 4-ethyl-α, α-dimethyl-. Based on the Creme RIFM model, the inhalation exposure is 0.017 mg/day. This exposure is 27.6 times lower than the Cramer Class III TTC value of 0.47 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: None.

Literature Search and Risk Assessment Completed on: 9/2016.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening level risk assessment of benzenepropanenitrile, 4-ethyl-α, α-dimethyl was performed following the RIFM Environmental Framework (Salvito et al., 2002) which provides for 3 levels of screening for aquatic risk. In Tier 1, only the material's volume of use in a region, its log K_{ow} and molecular weight are needed to estimate a conservative risk quotient (RQ; Predicted Environmental Concentration/Predicted No Effect Concentration or 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). At Tier 2, the model ECOSAR (providing chemical class specific ecotoxicity estimates) is used and a lower uncertainty factor is applied. Finally, if needed, at Tier 3, measured biodegradation and ecotoxicity data are used to refine the RQ (again, with lower uncertainty factors applied to calculate the PNEC). Provided in the table below are the data necessary to calculate both the PEC and the PNEC determined within this Safety Assessment. For the PEC, while the actual regional tonnage is not provided, the range from the most recent IFRA Volume of Use Survey is reported. The PEC is calculated based on the actual tonnage and not the extremes noted for the range. Following the RIFM Environmental Framework, benzenepropanenitrile, 4-ethyl-α, α-dimethyl was identified as a fragrance material with potential to present a possible risk to the aquatic environment (*i.e.*, its screening level PEC/PNEC >1).

A screening-level hazard assessment using EPISUITE ver 4.1 identified benzenepropanenitrile, 4-ethyl-α, α-dimethyl as possibly persistent but not bio-accumulative based on its structure and physical-chemical properties. This screening level hazard assessment is a weight of evidence review of a material's physical-chemical properties, available data on environmental fate (*e.g.*, OECD Guideline biodegradation studies or die-away studies) and fish bioaccumulation, and review of model outputs (*e.g.*, USEPA's BIOWIN and BCFBAF found in EPISUITE ver 4.1). Specific key data on biodegradation and fate 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 Volume of Use (2011), benzenepropanenitrile, 4-ethyl-α, α-dimethyl presents a risk to the aquatic compartment in the screening level assessment.

10.2.3. Key studies

10.2.3.1. Biodegradation. RIFM, 1992c: Biodegradability of the test material was determined using the closed bottle test in accordance with OECD Guideline 301D. The biodegradation rate was 6% after 28 days.

10.2.3.2. Ecotoxicity. RIFM, 1993b: A 48-h *Daphnia magna* acute toxicity test was conducted according to the OECD 202 method. Under the conditions of the study, the 48-h EC50 was 5.3 mg/l.

RIFM, 1993c: A 96-h semi-static acute toxicity test was conducted with juvenile rainbow trout (*Oncorhynchus mykiss*) following the OECD 203 guidelines. Under the conditions of this study, the 96-h LC50 was 6.2 mg/l.

RIFM, 1995: An Algae growth inhibition test was conducted according to the OECD 201 guidelines. Under the condition of the study the calculated EC50 was 11.2 mg/l (median effective concentration for inhibition of growth based on a comparison of maximum growth rates from 0 to 72 h).

Other available data: Not Available.

10.2.4. Risk assessment refinement

Since benzenepropanenitrile, 4-ethyl-α, α-dimethyl 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.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} used	3.46	3.46
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	1–10	<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 0.1808 μg/l. The revised PEC/PNECs for EU and NA <1 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: 04/22/14.

11. Literature search*

- **RIFM database:** target, Fragrance Structure Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** http://echa.europa.eu/
- **NTP:** http://tools.niehs.nih.gov/ntp_tox/index.cfm
- **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://www.chem.unep.ch/irptc/sids/oecdsids/sidspub.html
- **EPA Actor:** http://actor.epa.gov/actor/faces/ACToRHome.jsp;jsessionid=0EF5C212B7906229F477472A9A4D05B7
- **US EPA HPVIS:** http://www.epa.gov/hpv/hpvis/index.html
- **US EPA Robust Summary:** http://cfpub.epa.gov/hpv-s/
- **Japanese NITE:** http://www.safe.nite.go.jp/english/db.html
- **Japan Existing Chemical Data Base:** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com/webhp?tab=ww&ei=KMSoUpiQK-arsQS324GwBg&ved=0CBQQ1S4

	LC50 (Fish)	EC50 (*Daphnia*)	EC50 (Algae)	AF	PNEC	Chemical Class
RIFM Framework Screening Level **(Tier 1)**	<u>13.56 mg/l</u>			1,000,000	0.01356 μg/l	
ECOSAR Acute Endpoints **(Tier 2)** Ver 1.11	2.656 mg/l	<u>1.808 mg/l</u>	2.849 mg/l	10,000	0.1808 μg/l	Neutral Organics

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment.

This is not an exhaustive list.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fct.2017.06.026.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.fct.2017.06.026.

Appendix

Read-across justification

Methods:

- The identified read-across analogs were confirmed by using expert judgment.
- The physicochemical properties of target and analogs were calculated using EPI Suite™ v4.11 developed by US EPA (EPI SUITE ver 4.1).
- The J_{max} were calculated using RIFM skin absorption model (SAM), the parameters were calculated using consensus model (Shen et al., 2014).
- Developmental toxicity and skin sensitization were estimated using CAESAR (v2.1.6) (Cassano et al., 2010).
- The major metabolites for the target material and read-across analog were determined and evaluated using OECD QSAR Toolbox (v3.1 & v3.4) (OECD, 2012).

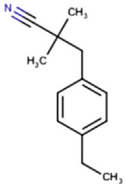
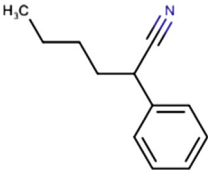
1. RIFM, 1992f

Summary

There are insufficient toxicity data on and Benzenepropanenitrile, 4-ethyl-α, α-dimethyl-(CAS # 134123-93-6). Hence, *in silico* evaluation was conducted by determining read-across analogs for this material. Based on structural similarity, reactivity, metabolism data, physicochemical properties and expert judgment, analog 2-phenylhexanenitrile (CAS # 3508-98-3) was identified as a proper read-across material with data for its respective toxicity endpoint.

Conclusion/Rationale:

- Read-across material 2-phenylhexanenitrile (CAS # 3508-98-3) could be used as structurally similar read-across analog for the target material benzenepropanenitrile, 4-ethyl-α, α-dimethyl-(CAS # 134123-93-6) for the developmental and reproductive toxicity endpoints.
  - The target substance and the read-across analog are structurally similar and belong to the structural class of aromatic nitriles.
  - The target substance and the read-across analog have the 2-phenylacetonitrile fragment common among them.
  - The key difference between the target substance and the read-across analog is that the target has a tertiary carbon at the alpha position while the read-across has a secondary carbon at the alpha position. This structural difference between the target substance and the read-across analog do not raise additional structural alerts so the structure differences are not relevant from a toxicological perspective.
  - The read-across analog has a Tanimoto score as mentioned in the above table. The Tanimoto score is mainly driven by the 2-

	Target material	Read-across material
Principal Name	Benzenepropanenitrile, 4-ethyl-α, α-dimethyl-	2-Phenylhexanenitrile
CAS No.	134123-93-6	3508-98-3
Structure		
Similarity (Tanimoto score)		0.640
Read-across endpoint		• Developmental & Reproductive
Molecular Formula	$C_{13}H_{17}N$	$C_{12}H_{15}N$
Molecular Weight	187.29	172.12
Melting Point (°C, EPI SUITE)	64.5	41.23
Boiling Point (°C, EPISUITE)	296.64	265–276.5
Vapor Pressure (Pa @ 25°C, EPI SUITE)	1.67	6.4
Log Kow (KOWWIN v1.68 in EPI SUITE)	3.46[1]	3.14
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPISUITE)	11.37	37.7
J_{max} (mg/cm^2/h, SAM)	7.64	15.402
Henry's Law (Pa·m^3/mol, Bond Method, EPI SUITE)	8.45E-006	7.66E-006
Reproductive and developmental toxicity		
ER Binding by OECD QSAR Tool Box (v3.4)	• Non-binder, without OH and NH_2 group	• Non-binder, without OH and NH_2 group
Developmental Toxicity Model by CAESAR v2.1.6	• Toxicant (low reliability)	• Toxicant (low reliability)
Metabolism		
OECD QSAR Toolbox (v3.1 & 3.4) Rat liver S9 metabolism simulator	See Supplemental data 1	See Supplemental data 2

phenylacetonitrile fragment. The differences in the structure which are responsible for Tanimoto score < 1 are not relevant from a toxicological perspective.

- The target substance and the read-across analog have similar physical chemical properties. Any differences in the physical chemical properties of the target substance and the read-across analog are estimated to be toxicologically insignificant.
- According to the QSAR OECD Toolbox (v3.1 and v3.4), structural alerts for the developmental and reproductive toxicity endpoints are consistent between the target substance and the read-across analog. The read-across analog is predicted to be a toxicant for the developmental endpoint with low reliability only by CAESAR model v2.1.6. The data described in developmental and reproductive section supports that the read-across material is safe to use within given margin of exposure and level of use for developmental toxicity endpoint; therefore, this *in silico* predictions was superseded.
- The target substance and the read-across analog are expected to be metabolized similarly as shown by metabolism simulator.
- The structural alerts for developmental and reproductive toxicity endpoint are consistent between the metabolites of the read-across analog and the target substance.
- The structural differences between the target substance and the read-across analog are deemed to be toxicologically insignificant.

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