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Short review

RIFM FRAGRANCE INGREDIENT SAFETY ASSESSMENT, 2,2,4-trimethyl-4-phenyl-butane-nitrile CAS Registry Number 75490-39-0

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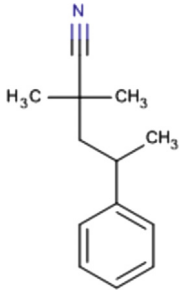

Abbreviation list:
2-Box Model- a RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF- Assessment Factor
BCF- Bioconcentration Factor
Creme RIFM model- The Creme RIFM model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, 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.

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(*continued*)

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- Ultraviolet/Visible spectra
VCF- Volatile Compounds in Food
VoU- Volume of Use
vPvB- (very) Persistent, (very) Bioaccumulative
WOE- Weight of Evidence

1. Identification

1. **Chemical Name:** 2,2,4-Trimethyl-4-phenyl-butane-nitrile
2. **CAS Registry Number:** 75490-39-0
3. **Synonyms:** Benzenebutanenitrile, α,α,γ-trimethyl-; Khusinil; 2,2,4-Trimethyl-4-phenyl-butane-nitrile
4. **Molecular Formula:** $C_{13}H_{17}N$
5. **Molecular Weight:** 187.28
6. **RIFM Number:** 6473

2. Physical data

1. **Boiling Point:** 268.5–276.0 C at 1006 mbar [RIFM, 1996j], 287.51 °C [US EPA, 2012].
   2.**Flash Point:** 126 °C at 1012 mbar [RIFM, 1996j].
   3.**Log K_{OW}:** Log P = 3.34 at 20 C [RIFM, 1996j], 3.83 [US EPA, 2012].
   4.**Melting Point:** 48.08 °C [US EPA, 2012].
   5.**Water Solubility:** 14.67 mg/l [US EPA, 2012].
   6.**Specific Gravity:** Not Available
   7.**Vapor Pressure:** 0.00232 mm Hg @ 25 °C [US EPA, 2012], 0.00127 mmHg @ 20 °C [US EPA, 2012].
8. **UV Spectra:** No significant absorption between 290 and 700 nm; molar absorption coefficient below the benchmark ($1000\ l \cdot mol^{-1} \cdot cm^{-1}$)
   9.**Appearance/Organoleptic:** colorless to pale yellow clear liquid with a medium citrus, gardenia, grapefruit, burnt autumn

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. Target data show that this material is not genotoxic and it does not have skin sensitization potential. An acceptable MOE >100 was calculated for repeated dose toxicity. The local respiratory toxicity endpoint review was completed using the TTC (Threshold of Toxicological Concern) for a Cramer Class III material (0.47 mg/day); exposure < TTC (acceptable). The developmental reproductive toxicity endpoint review was completed using 2-phenylhexanenitrile (CAS # 3508-98-3) as a read-across analog, which provided an acceptable MOE > 100. The phototoxicity/photoallergenicity endpoint was completed based on UV spectra. The environmental endpoints were evaluated and the material was not found to be PBT as per 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, 1996b; RIFM, 1996a)
Repeated Dose Toxicity: NOAEL = 50 mg/kg/day. (RIFM, 1996d)
Developmental and Reproductive Toxicity: NOAEL = 70 mg/kg/day. (ECHA REACH Dossier: 2-phenylhexanenitrile)
Skin Sensitization: Not sensitizing. (RIFM, 1996c; ECHA REACH Dossier: 2,2,4-trimethyl-4-phenyl-butane-nitrile)
Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic. (UV Spectra, RIFM DB)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:
Persistence: Screening Level: 46% (OECD 301D) (RIFM, 1996i)
Bioaccumulation: Screening Level: 157.1 l/kg (US EPA, 2012)
Ecotoxicity: Screening Level: LC50: 17.23 mg/l (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 (Salvito et al., 2002)
Critical Ecotoxicity Endpoint: LC50: 17.23 mg/l (Salvito et al., 2002)
RIFM PNEC is: 0.01723 μg/l
- **Revised PEC/PNECs (2011 IFRA Volume of Use):** North America and Europe: not applicable; cleared at screening level

leaves, vetiver at 10% in dipropylene glycol (Luebke, William tgsc, 2009)* *http://www.thegoodscentscompany.com/data/rw1104181.html, retrieved on 03/25/15

3. Exposure

1. **Volume of Use (Worldwide Band):** 0.1–1 metric tons per year (IFRA, 2011)
2. **95**th **Percentile Concentration in Hydroalcoholics:** 0.18% (RIFM, 2016)
3. **Inhalation Exposure*:** 0.00014 mg/kg/day or 0.0090 mg/day (RIFM, 2016)
4. **Total Systemic Exposure**:** 0.0029 mg/kg/day (RIFM, 2016)

*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:** Assumed 100%
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:** 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)

2,2,4-Trimethyl-4-phenyl-butane-nitrile 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

Available, accessed on 08/28/14.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current data, 2,2,4-trimethyl-4-phenyl-butane-nitrile does not present a concern for genotoxicity.

10.1.2. Risk assessment

The mutagenicity of 2,2,4-trimethyl-4-phenyl-butane-nitrile was assessed in an Ames study conducted in compliance with GLP regulations and in accordance with OECD TG 471. The *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 were treated with 2,2,4-trimethyl-4-phenyl-butane-nitrile in dimethyl sulfoxide (DMSO) at doses of 156.25, 312.5, 625, 1250, 2500, and 5000 μg/plate in the presence and absence of S9 mix. No significant increase in revertant colonies was observed in the tester strains in any of the test conditions (RIFM, 1996b). Under the conditions of the study, 2,2,4-trimethyl-4-phenyl-butane-nitrile was considered not mutagenic in bacteria.

The clastogenicity of 2,2,4-trimethyl-4-phenyl-butane-nitrile was assessed in an *in vitro* chromosomal aberrations study conducted in compliance with GLP regulations and in accordance with OECD TG 473. Cultured human lymphocytes were treated with 2,2,4-trimethyl-4-phenyl-butane-nitrile in DMSO at doses up to 1000 μg/ml. No statistically significant increases in the proportion of metaphase figures with chromosome damage were observed at any of the concentrations tested either in the presence or absence of metabolic activation (RIFM, 1996a). Under the conditions of the study, 2,2,4-trimethyl-4-phenyl-butane-nitrile was considered not clastogenic.

Based on the available data, 2,2,4-trimethyl-4-phenyl-butane-nitrile does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed on: 09/25/14.

10.1.3. Repeated dose toxicity

The margin of exposure for 2,2,4-trimethyl-4-phenyl-butane-nitrile is adequate for repeated dose toxicity at the current levels of use.

10.1.4. Risk assessment

The repeated dose toxicity data on 2,2,4-trimethyl-4-phenyl-butane-nitrile are sufficient for the repeated dose toxicity endpoint. An OECD 407 gavage 4-week subchronic toxicity study conducted in rats. Groups of 5 rats/sex/dose were gavaged with 0, 15, 150, or 500 mg/kg/day doses of 2,2,4-trimethyl-4-phenyl-butane-nitrile in corn oil for 31 days. Additional groups of 5 rats/sex/dose were gavaged with 0 or 500 mg/kg/day for 28 days followed by a two-

week treatment-free recovery period. The NOAEL was determined to be 150 mg/kg/day, based on increased water consumption, increased liver and kidney weights, centrilobular hepatocyte enlargement, and renal cortical tubules with eosinophilic inclusions (RIFM, 1996d).

A default safety factor of 3 was used when deriving a NOAEL from the OECD 407 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 2,2,4-trimethyl-4-phenyl-butane-nitrile MOE for the repeated dose toxicity endpoint can be calculated by dividing the 2,2,4-trimethyl-4-phenyl-butane-nitrile NOAEL in mg/kg/day by the total systemic exposure to 2,2,4-trimethyl-4-phenyl-butane-nitrile, 50/0.0029 or 17 241.

Additional References: RIFM, 1996e; RIFM, 1996k.

Literature Search and Risk Assessment Completed on: 09/28/2016.

10.1.5. Developmental and reproductive toxicity

The margin of exposure for 2,2,4-trimethyl-4-phenyl-butane-nitrile is adequate for developmental and reproductive toxicity at the current levels of use.

10.1.6. Risk assessment

There are no developmental or reproductive toxicity data on 2,2,4-trimethyl-4-phenyl-butane-nitrile. Read-across material 2-phenylhexanenitrile (CAS # 3508-98-3; see Section 5) has an OECD 421 dietary reproduction/developmental toxicity screening test conducted in rats. Groups of 10 rats/sex/dose were fed diets containing 0, 200, 400, or 1000 ppm 2-phenylhexanenitrile (actual ingested dosages of 0, 13, 28 or 70 mg/kg/day in males and 0, 16, 32, or 85 mg/kg/day in females). The NOAEL for developmental and reproductive toxicity was determined to be 1000 ppm, or 70 mg/kg/day, the highest dosage tested (ECHA REACH Dossier: 2-phenylhexanenitrile accessed 10/17/14). Therefore, the 2,2,4-trimethyl-4-phenyl-butane-nitrile 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 2,2,4-trimethyl-4-phenyl-butane-nitrile, 70/0.0029 or 24 138.

Additional References: RIFM, 1996e; RIFM, 1996k.

Literature Search and Risk Assessment Completed on: 09/28/2016.

10.1.7. Skin sensitization

Based on the available data, 2,2,4-trimethyl-4-phenyl-butane-nitrile does not present a concern for skin sensitization at the current levels of use.

10.1.8. Risk assessment

Based on the available data, 2,2,4-trimethyl-4-phenyl-butane-nitrile does not present a concern for skin sensitization at the current levels of use. The chemical structure indicates that this material would not be expected to react directly 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, 1996c; ECHA REACH Dossier: 2,2,4-trimethyl-4-phenyl-butane-nitrile, accessed 9/26/14). In a human confirmatory study, no sensitization reactions were observed up to 3% (1653 μg/cm^2) 2,2,4-trimethyl-4-phenyl-butane-nitrile in a 75:25 mixture of alcohol SD 39C: diethyl phthalate (RIFM, 1995).

Additional References: None.

Literature Search and Risk Assessment Completed on: 9/28/16.

10.1.9. Phototoxicity/photoallergenicity

Based on UV/Vis absorption spectra, 2,2,4-trimethyl-4-phenyl-butane-nitrile would not be expected to present a concern for phototoxicity or photoallergenicity.

10.1.10. Risk assessment

There are no phototoxicity data available for 2,2,4-trimethyl-4-phenyl-butane-nitrile. 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, 2,2,4-trimethyl-4-phenyl-butane-nitrile 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, 2,2,4-trimethyl-4-phenyl-butane-nitrile, 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 2,2,4-trimethyl-4-phenyl-butane-nitrile. Based on the Creme RIFM model, the inhalation exposure is 0.0090 mg/day. This exposure is 52 times lower than the Cramer Class I 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 2,2,4-trimethyl-4-phenyl-butane-nitrile 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, 2,2,4-trimethyl-4-phenyl-butane-nitrile 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 EPISUITE ver 4.1 identified 2,2,4-trimethyl-4-phenyl-butane-nitrile as possibly persistent but not bioaccumulative 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), 2,2,4-trimethyl-4-phenyl-butane-nitrile does not present a risk to the aquatic compartment in the screening level assessment.

10.2.3. Key studies

10.2.3.1. Biodegradation. **RIF, 1996i**: Biodegradation of the test material was evaluated by the closed bottle test according to the OECD 301D method. When corrected for total oxygen consumption, the true oxygen consumption value for carbonaceous biodegradation was 45% after 28 days.

10.2.3.2. Ecotoxicity. **RIFM, 1996f**: An acute *Daphnia magna* toxicity test was conducted according to the OECD 202 Part I method. Under the conditions of the study the 48-h EC50 was 12 mg/l.

RIFM, 1996g: A 96-h fish (rainbow trout) acute toxicity study was conducted according to the OECD 203 method under semi-static conditions. Under the conditions of this study, the 96-h LC50 was 4.6 mg/l and the NOEL was <0.86 mg/l.

RIFM, 1996h: An algae growth inhibition test was conducted according to the OECD 301 method. Median effective concentration for inhibition of growth based on a comparison of areas under growth curves after 72 h was 11 mg/l.

10.2.4. Other available data

2,2,4-Trimethyl-4-phenyl-butane-nitrile has been registered under REACH, however no additional data is available.

10.2.5. Risk assessment refinement

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

Endpoints used to calculate PNEC are underlined.

	LC50 (Fish)	EC50 (*Daphnia*)	EC50 (Algae)	AF	PNEC	Chemical Class
RIFM Framework Screening Level **(Tier 1)**	17.23 mg/l			1,000,000	0.0172 μg/l	

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} used	3.34	3.34
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 the available data, the RQ for this material is < 1. No further assessment is necessary.

The RIFM PNEC is 0.01723 μg/l. The revised PEC/PNECs for EU and NA: not applicable; 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: 9/24/14.

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

*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.2017.07.037.

Transparency document

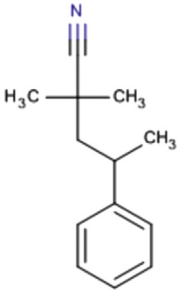
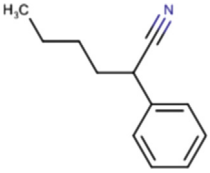
Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.fct.2017.07.037.

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 (US EPA, 2012).
- The Jmax were calculated using RIFM skin absorption model (SAM), the parameters were calculated using consensus model (Shen et al., 2014).
- Developmental toxicity was estimated using CAESAR (v.2.1.7) (Cassano et al., 2010).
- The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox (v3.4) (OECD, 2012)
- Read-across material 2-phenylhexanenitrile (CAS # 3508-98-3) could be used as structurally similar read-across analog for the target material 2,2,4-trimethyl-4-phenyl-butane-nitrile (CAS # 75490-39-0) for the reproductive and developmental 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 phenylacetonitrile fragment in common.
  - The key difference between the target substance and the read-across analog is that the target has a tertiary α carbon while the read-across has a secondary α carbon. This structural difference between the target substance and the read-across analog does not raise additional structural alerts, therefore, the structural differences are not relevant from a toxicological perspective.
  - The target substance and the read-across analog have a Tanimoto score as mentioned in the above table. The Tanimoto score is mainly driven by the 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 for reproductive and developmental toxicity.
  - According to the QSAR OECD Toolbox (v3.4), structural alerts for the reproductive and developmental toxicity endpoints are

	Target material	Read-across material
Principal Name	2,2,4-Trimethyl-4-phenyl-butane-nitrile	2-Phenylhexanenitrile
CAS No.	75490-39-0	3508-98-3
Structure		
Similarity (Tanimoto score)		0.829
Read-across endpoint		• Developmental & Reproductive
Molecular Formula	$C_{13}H_{17}N$	$C_{12}H_{15}N$
Molecular Weight	187.28	172.12
Melting Point (°C, EPISUITE)	48.08	41.23
Boiling Point (°C, EPISUITE)	287.51	265–276.5
Vapor Pressure (Pa @ 25 °C, EPISUITE)	0.75	6.4
Log Kow (KOWWIN v1.68 in EPISUITE)	3.34[a]	3.14
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPISUITE)	14.67	37.7
J_{max} (mg/cm^2/h, SAM)	9.995	15.402
Henry's Law (Pa·m^3/mol, Bond Method, EPISUITE)	1.017E-005	7.66E-006
Reproductive and developmental toxicity		
ER Binding by OECD QSAR Tool Box (3.4)	• 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 (3.4) Rat liver S9 metabolism simulator	See supplemental data 1	See supplemental data 2

[a] RIFM, 1996j.

Summary:

There are insufficient toxicity data on and 2,2,4-trimethyl-4-phenyl-butane-nitrile (CAS # 75490-39-0). 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 proper read-across material with data for the respective toxicity endpoints.

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 v.2.1.7. The data described in the developmental and reproductive sections support that the read-across material is safe to use; therefore, this *in silico* prediction 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 reproductive and developmental toxicity 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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