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RIFM fragrance ingredient safety assessment, α-cyclohexylidene benzeneacetonitrile, CAS Registry Number 10461-98-0

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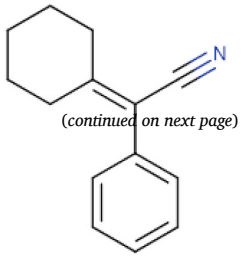

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Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
CNIH – Confirmation of No Induction in Humans test. A human repeat insult patch test that is performed to confirm an already determined safe use level for fragrance ingredients (Na et al., 2020)
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, 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 Observed 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
Perfumery - In this safety assessment, perfumery refers to fragrances made by a perfumer used in consumer products only. The exposures reported in the safety assessment include consumer product use but do not include occupational exposures.
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.

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α-Cyclohexylidene benzeneacetonitrile was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that α-cyclohexylidene benzeneacetonitrile is not genotoxic. Data on α-cyclohexylidene benzeneacetonitrile provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and reproductive toxicity endpoints. Data from read-across analog 2-cyclohexylidene-2-*o*-tolylacetonitrile (CAS # 916887-53-1) provided α-cyclohexylidene benzeneacetonitrile a No Expected Sensitization Induction Level (NESIL) of 1200 μg/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on data and ultraviolet (UV) spectra; α-cyclohexylidene benzeneacetonitrile is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class III material, and the exposure to α-cyclohexylidene benzeneacetonitrile is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; α-cyclohexylidene benzeneacetonitrile 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, 1996b; RIFM, 2008a)
Repeated Dose Toxicity: NOAEL = 40 mg/kg/day.	RIFM (2009)
Reproductive Toxicity: NOAEL = 40 mg/kg/day.	RIFM (2009)
Skin Sensitization: NESIL = 1200 μg/cm^2.	RIFM (2010b)
Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic.	(UV Spectra; RIFM Database)
Local Respiratory Toxicity:: No NOAEC available. Exposure is below the TTC.	

Environmental Safety Assessment

Hazard Assessment:	
Persistence:	
Critical Measured Value: 33% (OECD 310)	RIFM (2010a)
Bioaccumulation:	
Critical Measured Value: 285–373 L/kg (OECD 305)	RIFM (2008c)
Ecotoxicity:	
Critical Ecotoxicity Endpoint: 21-day *Daphnia magna* NOEC: 0.07 mg/L	RIFM (2002b)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards	
Risk Assessment:	
Screening-level: PEC/PNEC (North America and Europe) > 1	RIFM Framework; Salvito (2002)
Critical Ecotoxicity Endpoint: 21-day *Daphnia magna* NOEC: 0.07 mg/L	RIFM (2002b)
RIFM PNEC is: 7.0 μg/L	
• **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe <1	

1. Identification

1. **Chemical Name:** α-Cyclohexylidene benzeneacetonitrile
2. **CAS Registry Number:** 10461-98-0
3. **Synonyms:** Peonile; Benzeneacetonitrile, α-cyclohexylidene-;.δ.1,.α.-Cyclohexaneacetonitrile,.α.-phenyl-; α-Cyclohexylidene benzeneacetonitrile
4. **Molecular Formula:** $C_{14}H_{15}N$
5. **Molecular Weight:** 197.28
6. **RIFM Number:** 6355
7. **Stereochemistry:** No stereoisomer possible

2. Physical data

1. **Boiling Point:** 118 C at 1 hPa (RIFM, 1999), 332.24 °C (EPI Suite)
2. **Flash Point:** 154 °C (Globally Harmonized System)
3. **Log K_{OW}:** 4.0 at 30 °C (RIFM, 1996d), 4.29 (EPI Suite)
4. **Melting Point**: 25 °C (RIFM, 1999), 77.07 °C (EPI Suite)
5. **Water Solubility:** 5.283 mg/L (EPI Suite)
6. **Specific Gravity:** 1.031 (RIFM, 1997e), 1.031 (RIFM, 1997c), 1.031 (RIFM, 1997d)

7. **Vapor Pressure:** 0.0000566 mm Hg at 20 °C (EPI Suite v4.0), 0.000111 mm Hg at 25 °C (EPI Suite)
8. **UV Spectra:** No absorbance between 290 and 500 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** Not Available

3. Volume of use (worldwide band)

1. 100–1000 metric tons per year (IFRA, 2015)

4. Exposure to fragrance ingredient (Creme RIFM Aggregate Exposure Model v1.0)

1. **95**th **Percentile Concentration in Hydroalcoholics:** 0.29% (RIFM, 2016)
2. **Inhalation Exposure*:** 0.00084 mg/kg/day or 0.062 mg/day (RIFM, 2016)
3. **Total Systemic Exposure**:** 0.0058 mg/kg/day (RIFM, 2016)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM Aggregate Exposure Model (Comiskey, 2015, 2017; Safford, 2015, 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, 2015, 2017; Safford, 2015, 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 III, High

Expert Judgment	Toxtree v3.1	OECD QSAR Toolbox v4.2
III	III	III

2. Analogs Selected:
   a. **Genotoxicity:** None
   b. **Repeated Dose Toxicity:** None
   c. **Reproductive Toxicity:** None
   d. **Skin Sensitization:** 2-Cyclohexylidene-2-*o*-tolylacetonitrile (CAS # 916887-53-1)
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. Read-across Justification: See Appendix below

7. Metabolism

No relevant data available for inclusion in this safety assessment.
Additional References:
None.

8. Natural occurrence

α-Cyclohexylidene benzeneacetonitrile is not reported to occur in foods 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 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

Available; accessed on 07/26/21 (ECHA, 2016).

10. Conclusion

The maximum acceptable concentrations[a] in finished products for α-cyclohexylidene benzeneacetonitrile are detailed below.

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
1	Products applied to the lips (lipstick)	0.52
2	Products applied to the axillae	0.027
3	Products applied to the face/body using fingertips	0.47
4	Products related to fine fragrances	0.52
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.13
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.13
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.13
5D	Baby cream, oil, talc	0.043
6	Products with oral and lip exposure	0.052
7	Products applied to the hair with some hand contact	0.94
8	Products with significant ano-genital exposure (tampon)	0.043
9	Products with body and hand exposure, primarily rinse-off (bar soap)	1.0
10A	Household care products with mostly hand contact (hand dishwashing detergent)	2.9
10B	Aerosol air freshener	3.6
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.043
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	No Restriction

Note: [a]Maximum acceptable concentrations for each product category are based on the lowest maximum acceptable concentrations (based on systemic toxicity, skin sensitization, or any other endpoint evaluated in this safety assessment). For α-cyclohexylidene benzeneacetonitrile, the basis was the reference dose of 0.40 mg/kg/day, a predicted skin absorption value of 40%, and a skin sensitization NESIL of 1200 μg/cm^2.
[b]For a description of the categories, refer to the IFRA RIFM Information Booklet (https://www.rifm.org/downloads/RIFM-IFRA%20Guidance-for-the-use-of-IFRA-Standards.pdf).
cCalculations by Creme RIFM Aggregate Exposure Model v3.0.5.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, α-cyclohexylidene benzeneacetonitrile does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. α-Cyclohexylidene benzeneacetonitrile was assessed in the BlueScreen assay and found positive cytotoxicity (positive: <80% relative cell density) and negative for genotoxicity, with and without metabolic activation (RIFM, 2013b). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. Additional assays were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

The mutagenic activity of α-cyclohexylidene benzeneacetonitrile 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 method. *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and TA102, were treated with α-cyclohexylidene benzeneacetonitrile in dimethyl sulfoxide (DMSO) at concentrations up to 333 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, 1996b). Under the conditions of the study, α-cyclohexylidene benzeneacetonitrile was not mutagenic in the Ames test.

The clastogenic activity of α-cyclohexylidene benzeneacetonitrile was evaluated in an *in vivo* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 474. The test material was administered in corn oil via the oral route to groups of male and female NMRI mice. Doses of 125, 250, or 500 mg/kg were administered. Mice from each dose level were euthanized at 24 and 48 h, and the bone marrow was extracted and examined for polychromatic erythrocytes. The test material did not induce a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow (RIFM, 2008a). Under the conditions of the study, α-cyclohexylidene benzeneacetonitrile was considered to be not clastogenic in the *in vivo* micronucleus test.

Based on the available data, α-cyclohexylidene benzeneacetonitrile does not present a concern for genotoxic potential.

Additional References: RIFM, 1997b; RIFM, 2013a; RIFM, 2008b.

Literature Search and Risk Assessment Completed On: 06/01/21.

11.1.2. Repeated dose toxicity

The MOE for α-cyclohexylidene benzeneacetonitrile is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on α-cyclohexylidene benzeneacetonitrile. A combined 13-week repeated dose toxicity and 1-generation reproductive toxicity study was conducted with test material, peonile, administered to Crl:CD(SD) rats. The study was divided into 2 subgroups: A and B. 12 rats/sex/dose were administered test material at doses of 0, 10, 40, and 160 mg/kg/day in subgroup A (toxicity phase). 24 female rats/dose in subgroup B were administered the test material at doses of 0, 10, and 40 mg/kg/day (reproductive phase). Males in subgroup A were treated for 14–16 weeks, whereas females were treated for 13 weeks. Females in subgroup B received the test material for 21 days before pairing with subgroup A males, with continued treatment until day 20 of lactation. Subgroup B females were allowed to litter and rear their offspring to the weaning period. Control group animals in both subgroups received vehicle corn oil throughout the treatment. The study was conducted according to GLP. The study was designed to meet the requirements of the OECD 408 and OECD 415 protocols. High-dose toxicity phase animals were reported to have alterations in clinical signs (underactivity, partially closed eyelids, abnormal gait, flat tilted posture, chin rubbing, salivation, forepaw paddling, weak sensory reactivity effects, and motor activity effects and piloerection) and body weight decreases with females being more sensitive to bodyweight reduction than males. Overall, mean bodyweight gain was lower in males (weeks 0–14) and in females (weeks 0–6). Four high-dose females had to be euthanized due to animal welfare reasons during weeks 5–7 of treatment. One of the females displayed prolonged and frequent convulsions and marked bodyweight loss, while the other 3 displayed poor clinical conditions and marked bodyweight losses; however, there were no histopathological findings in these animals that were considered to be related to treatment. Thus, surviving females were retained to serve as treatment-free recovery-group animals. Clinical signs reported among high-dose females were no longer evident among recovery-group females. Body weights and body weight gains were statistically significantly reduced in males and females at the high dose. At the mid dose, transiently statistically significant bodyweight gains were reduced in females during weeks 0–6; after this, bodyweight gains were slightly but not statistically significantly lower in females at the mid dose. Hematological alterations included high lymphocyte counts among high-dose males. Alterations in clinical chemistry parameters included a significant reduction in mean ALP, ALT, AST, and sodium levels among high-dose males. The mean plasma levels of urea and potassium were reported to be significantly higher among high-dose males. Potassium levels were also significantly elevated in both sexes of the mid-dose group, and urea levels were high among females. Organ weight analysis revealed high liver, heart, and kidney weights among males. Histopathological alterations among high-dose males included centrilobular hepatocyte hypertrophy among males, which was considered to be an adaptive change and not adverse. Mid-dose females were reported to have a significant increase in relative spleen weights (not dose dependent) and absolute liver weights. **Thus, the NOAEL for systemic toxicity was considered to be 40 mg/kg/day based on alterations in clinical signs and body weight decreases reported among high-dose animals (RIFM, 2009).**

Thus, the MOE for α-cyclohexylidene benzeneacetonitrile is equal to the NOAEL for α-cyclohexylidene benzeneacetonitrile divided by the total systemic exposure to α-cyclohexylidene benzeneacetonitrile, 40/0.0058 or 6897.

Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020) and a reference dose (RfD) of 0.40 mg/kg/day.

11.1.2.1.1. Derivation of RfD. The RIFM Criteria Document (Api, 2015) calls for a default MOE of 100 (10 × 10), based on uncertainty factors applied for interspecies (10 ×) and intraspecies (10 ×) differences. The RfD for α-cyclohexylidene benzeneacetonitrile was calculated by dividing the lowest NOAEL (from the Repeated Dose and Reproductive Toxicity sections) of 40 mg/kg/day by the uncertainty factor, 100 = 0.40 mg/kg/day.

Additional References: RIFM, 1996e; RIFM, 1997a.

Literature Search and Risk Assessment Completed On: 05/20/21.

11.1.3. Reproductive toxicity

The MOE for α-cyclohexylidene benzeneacetonitrile is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are sufficient reproductive toxicity data on α-cyclohexylidene benzeneacetonitrile for the reproductive toxicity endpoint. A combined OECD 408 and 415 oral gavage 13-week subchronic toxicity study and 1-generation reproductive toxicity study was conducted in Crl:CD(SD) rats. Groups of rats were administered via oral gavage daily with peonile (α-cyclohexylidene benzeneacetonitrile) at doses of 0, 10, 40, or 160 mg/kg/day in corn oil. Toxicity phase males (12/dose, subgroup A) were treated for 10 weeks before pairing up until necropsy (total treatment period of approximately 14 or 16 weeks); toxicity phase females (12/dose, subgroup A) were treated for 13 weeks. Females in the 160 mg/kg/day dose group only received treatment for 6 weeks, due to marked toxicity manifested as clinical signs and reduced body weights and bodyweight gains, and 4 females were euthanized for welfare reasons during weeks 5–7 of treatment. The surviving high-dose

group dams that only received 6 weeks of treatment had a 4-week treatment-free recovery period. As a result of adverse effects observed at 160 mg/kg/day, it was decided that 160 mg/kg/day would not be included in the reproductive phase of the study. Therefore, F0 reproductive phase females (24/dose at 0, 10, or 40 mg/kg/day, subgroup B) were treated for 21 days before pairing with subgroup A males, during gestation, and until day 20 of lactation. F1 pups did not receive any direct administration of the test material; any exposure was *in utero* or via the milk. Mating, fertility, reproductive performance, survival, growth, and development of pups were not affected by the treatment at 10 or 40 mg/kg/day. The NOAEL for fertility and on the development of pups was considered to be 40 mg/kg/day (RIFM, 2009).

Therefore, the α-cyclohexylidene benzeneacetonitrile MOE for the reproductive toxicity endpoint can be calculated by dividing the α-cyclohexylidene benzeneacetonitrile NOAEL in mg/kg/day by the total systemic exposure to α-cyclohexylidene benzeneacetonitrile, 40/0.0058 or 6897.

Additional References: RIFM, 1997e.

Literature Search and Risk Assessment Completed On: 05/31/21.

11.1.4. Skin sensitization

Based on the existing data and data from read-across material 2-cyclohexylidene-2-*o*-tolylacetonitrile (CAS # 916887-53-1), α-cyclohexylidene benzeneacetonitrile is considered a skin sensitizer with a defined NESIL of 1200 μg/cm^2.

11.1.4.1. Risk assessment. Limited skin sensitization studies are available for α-cyclohexylidene benzeneacetonitrile. Based on the existing data and read-across material 2-cyclohexylidene-2-*o*-tolylacetonitrile (CAS # 916887-53-1; see Section VI), α-cyclohexylidene benzeneacetonitrile is considered a skin sensitizer. The chemical structure of these materials indicate that they would be expected to react with skin proteins (Roberts, 2007; OECD Toolbox v4.2) In a murine local lymph node assay (LLNA), read-across material 2-cyclohexylidene-2-o-tolylacetonitrile was found to be sensitizing with an EC3 value of 9.3% (2325 μg/cm^2) (RIFM, 2005). However, in a guinea pig maximization test, α-cyclohexylidene benzeneacetonitrile did not present reactions indicative of sensitization at 100% (RIFM, 1996a). In 2 Confirmation of No Induction in Humans tests (CNIHs) with 5% (2500 μg/cm^2) or 30% (15000 μg/cm^2) of α-cyclohexylidene benzeneacetonitrile in dimethyl phthalate, no reactions indicative of sensitization were observed in any of the 54 and 47 volunteers, respectively (RIFM, 1998; RIFM, 2003). In another CNIH with 2.5% (1250 μg/cm^2) read-across material 2-cyclohexylidene-2-*o*-tolylacetonitrile in 1:3 ethanol:diethyl phthalate, no reactions indicative of sensitization was observed in any of the 101 volunteers (RIFM, 2010b).

Table 1
Data Summary for 2-cyclohexylidene-2-*o*-tolylacetonitrile as read-across material for α-cyclohexylidene benzeneacetonitrile.

LLNA Weighted Mean EC3 Value μg/cm^2 (No. Studies)	Potency Classification Based on Animal Data[a]	Human Data			
		NOEL-CNIH (Induction) μg/cm^2	NOEL-HMT (Induction) μg/cm^2	LOEL[b] (Induction) μg/cm^2	WoE NESIL[c] μg/cm^2
2325 (1)	Moderate	1250	NA	NA	1200

NOEL = No observed effect level; CNIH = Confirmation of No Induction in Humans test; HMT = Human Maximization Test; LOEL = lowest observed effect level; NA = Not Available.

[a] Based on animal data using classification defined in ECETOC, Technical Report No. 87, 2003.

[b] Data derived from CNIH or HMT.

[c] WoE NESIL limited to 2 significant figures.

Based on the available data on read-across material 2-cyclohexylidene-2-*o*-tolylacetonitrile, summarized in Table 1, α-cyclohexylidene benzeneacetonitrile is considered to be a moderate skin sensitizer with a defined NESIL of 1200 μg/cm^2. Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020) and a reference dose of 0.40 mg/kg/day.

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/27/21.

11.1.5. Phototoxicity/photoallergenicity

Based on available UV spectra and data α-cyclohexylidene benzeneacetonitrile does not present a concern for phototoxicity or photoallergenicity.

11.1.5.1. Risk assessment. The available UV spectra indicate no absorbance in the region of 290–500 nm. The corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity (Henry, 2009). In an *in vivo* photoxicity/photoallergenicity study, topical application of α-cyclohexylidene benzeneacetonitrile and UV irradiation did not result in any skin reactions in female guinea pigs; α-cyclohexylidene benzeneacetonitrile was not considered phototoxic or photoallergenic (RIFM, 1997f). Based on the *in vivo* study data and the lack of absorbance, α-cyclohexylidene benzeneacetonitrile does not present a concern for phototoxicity or photoallergenicity.

11.1.5.2. UV spectra analysis. The available UV spectra indicate no significant absorbance in the range of 290–500 nm. The molar absorption coefficient is below the benchmark of concern for phototoxic effects, 1000 L mol^{-1} • cm^{-1} (Henry, 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/19/21.

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for α-cyclohexylidene benzeneacetonitrile is below the Cramer Class III TTC value for inhalation exposure local effects.

11.1.6.1. Risk assessment. There are insufficient inhalation data available on α-cyclohexylidene benzeneacetonitrile. Based on the Creme RIFM Model, the inhalation exposure is 0.062 mg/day. This exposure is 7.6 times lower than the Cramer Class III TTC value of 0.47 mg/day (based on human lung weight of 650 g; Carthew, 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: RIFM, 1999.

Literature Search and Risk Assessment Completed On: 05/28/21.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of α-cyclohexylidene benzeneacetonitrile was performed following the RIFM Environmental Framework (Salvito, 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, α-cyclohexylidene benzeneacetonitrile was identified as a fragrance material with the 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 α-cyclohexylidene benzeneacetonitrile as possibly being 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, 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.

11.2.2. Risk assessment

Based on the current Volume of Use (2015), α-cyclohexylidene benzeneacetonitrile presents a risk to the aquatic compartment in the screening-level assessment.

11.2.3. Key studies

11.2.3.1. Biodegradation. RIFM, 1996c**:** Biodegradability was evaluated using the manometric respirometry test according to the OECD 301F guideline. No biodegradation occurred after 28 days.

RIFM, 2010a**:** A 43-day ready biodegradability test was conducted using the headspace test according to the OECD 310 guideline. At 1.0 mg/L, no signs of degradation were reported. At 0.1 mg/L, primary biodegradation was observed at a mean value of 33% (carbon-14 test material).

RIFM, 2008c**:** A bioaccumulation study according to the OECD 305 guideline was conducted in *Danio rerio* (zebrafish) using a flow-through system. Based on calculations with total carbon-14 residues, BCF values ranged from 285 to 373 L/kg.

11.2.3.2. Ecotoxicity. RIFM, 1997c: An algae growth inhibition test was conducted according to OECD 201 method. Following a preliminary range-finding study, algae were exposed to an aqueous dispersion of test material at several concentrations for 72 h, under constant illumination and shaking. The 72-h EC50 values based on average exposure concentration for cell growth inhibition and growth rate were reported to be 0.86 mg/L and 1.96 mg/L, respectively. The NOEC was 0.5 mg/L based on average exposure concentration.

RIFM, 1997d: A 48-h *Daphnia magna* acute toxicity test was conducted according to the OECD 202 method. Based on a measured concentration, the EC50 was reported to be 2.3 mg/L.

RIFM, 1997e: A 96-h fish (rainbow trout) acute toxicity test was conducted according to the OECD 203 method under static conditions. Based on an average exposure concentration, the LC50 was reported to be 1.4 mg/L.

RIFM, 2002a: An early-life stage fish (*Danio rerio*) chronic toxicity test was conducted according to the OECD 210 method under flow-through conditions. The 28-day NOEC value based on mean measured concertation was reported to be 0.280 mg/L.

RIFM, 2002b: A *Daphnia magna* reproduction test was conducted according to the OECD method 211 under flow-through conditions. The 21-day NOECs based on mean measured concentration for reproduction and body length were 0.07 mg/L and 0.27 mg/L, respectively.

11.2.4. Other available data

α-Cyclohexylidene benzeneacetonitrile has been registered under REACH and the following additional data is available (ECHA, 2016):

The acute fish (*Salmo gairdneri*) toxicity test was conducted according to the 92/69/EEC, C1 guideline under semi-static conditions. The 96-h LC50 value based on nominal test concentration was reported to be 0.6 mg/L.

A bivalve acute toxicity test (embryo larval) was conducted according to the EPA OPPTS 850.1055 guidelines under static conditions. The 48-h EC50 value based on the mean measured concentration was reported to be 1.93 mg/L.

A fish (*Danio rerio*) early-life stage toxicity test was conducted under flow-through conditions. The 30-day LC50 value was reported to be 0.32 mg/L, and the 14-day NOEC value was reported to be 0.28 mg/L.

11.2.5. 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.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	4.0	4.0
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	100–1000	100–1000
Risk Characterization: PEC/PNEC	**<1**	**<1**

Based on available data, the RQs for these materials are <1. No further assessment is necessary.

The RIFM PNEC is 7.0 μg/L. The revised PEC/PNECs for EU and NA are <1; therefore, the material does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 05/25/21.

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:** https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm
- **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

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	4.84			1000000	0.0048	
ECOSAR Acute Endpoints **(Tier 2)** v1.11	0.31	0.64	0.17	10000	0.017	Vinyl/Allyl Nitriles
ECOSAR Acute Endpoints **(Tier 2)** v1.11	1.41	0.99	1.77			Neutral Organics SAR
Tier 3: Measured Data including REACH data						
	LC50	EC50	NOEC	AF	PNEC	Comments
Fish	0.6		0.28			
Daphnia		2.3	0.07	10	7.0	
Algae		0.86	0.5			

- **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 07/26/21.

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 A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2021.112708.

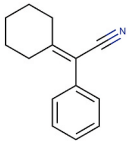
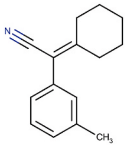
Appendix

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, 2017).

- First, the 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 material 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 material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).

	Target Material	Read-across Material
Principal Name	*α*-Cyclohexylidene benzeneacetonitrile	2-Cyclohexylidene-2-*o*-tolylacetonitrile
CAS No.	10461-98-0	916887-53-1
Structure		
Similarity (Tanimoto Score)		0.85
Read-across Endpoint		• Skin Sensitization
Molecular Formula	$C_{14}H_{15}N$	$C_{15}H_{17}N$
Molecular Weight	197.280	211.30
Melting Point (°C, EPI Suite)	77.07	92.50
Boiling Point (°C, EPI Suite)	332.24	344.33
Vapor Pressure (Pa @ 25°C, EPI Suite)	0.0148	0.00534
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	4.29	4.84
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	5.283	1.525
J_{max} (μg/cm^2/h, SAM)	8.001	4.026
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.58E+000	7.408E-001
Skin Sensitization		
Protein Binding (OASIS v1.1)	• Michael addition|Michael addition ≫ Michael addition on conjugated systems with electron-withdrawing group|Michael addition ≫ Michael addition on conjugated systems with electron-withdrawing group ≫ Cyanoalkenes	• Michael addition|Michael addition ≫ Michael addition on conjugated systems with electron-withdrawing group|Michael addition ≫ Michael addition on conjugated systems with electron-withdrawing group ≫ Cyanoalkenes
Protein Binding (OECD)	• No alert found	• No alert found
Protein Binding Potency	• Not possible to classify according to these rules (GSH)	• Not possible to classify according to these rules (GSH)
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	• Michael Addition|Michael Addition ≫ Michael addition on conjugated systems with electron-withdrawing group|Michael Addition ≫ Michael addition on conjugated systems with electron-withdrawing group ≫ Cyanoalkenes	• Michael Addition|Michael Addition ≫ Michael addition on conjugated systems with electron-withdrawing group|Michael Addition ≫ Michael addition on conjugated systems with electron-withdrawing group ≫ Cyanoalkenes
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	• No alert found	• No alert found
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 *α*-cyclohexylidene benzeneacetonitrile (CAS # 10461-98-0). 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, 2-cyclohexylidene-2-*o*-tolylacetonitrile (CAS # 916887-53-1) was identified as a read-across analog with sufficient data for toxicological evaluation.

Conclusions

- 2-Cyclohexylidene-2-*o*-tolylacetonitrile (CAS # 916887-53-1) was used as a read-across analog for the target material α-cyclohexylidene benzeneacetonitrile (CAS # 10461-98-0) for the skin sensitization endpoint.
  - o The target material and the read-across analog are structurally similar and belong to a class of aromatic nitrile.
  - o The target material and the read-across analog share a 2-cyclohexyl-2-phenylacetonitrile moiety.
  - o The key difference between the target material and the read-across analog is that the read-across analog has a methyl substituent at the 3 position on the benzyl ring. This structural difference is toxicologically insignificant.

- o The similarity between the target material 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 material 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 material and the read-across analog.
- o Both the target material and the read-across analog have Protein Binding (OASIS v1.1) and Protein Binding for Skin Sensitization (OASIS v1.1) alerts for Michael addition on conjugated systems with the electron-withdrawing group due to the cyanoalkene fragment. TIMES SS models both compounds as weak skin sensitizers, because –CN group is not a strong enough activator to make a chemical with this alert highly potent skin sensitizer. Data are consistent with *in silico* alerts.
- o The target material 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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