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RIFM fragrance ingredient safety assessment, 2-cyclohexylidene-2-o-tolylacetonitrile, CAS Registry Number 916887-53-1

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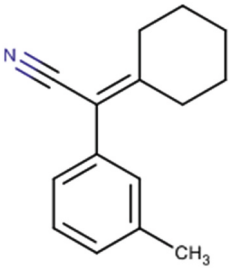

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Abbreviation/Definition List:

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; Safford et al., 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DRF - Dose Range Finding
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observable Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
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.

2-Cyclohexylidene-2-o-tolylacetonitrile was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 2-cyclohexylidene-2-o-tolylacetonitrile is not genotoxic. Data on 2-cyclohexylidene-2-o-tolylacetonitrile provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity endpoint. Data on read-across material α-cyclohexylidene benzeneacetonitrile (CAS # 10461-98-0) provide a calculated MOE >100 for the reproductive toxicity endpoint. Based on the existing data, 2-cyclohexylidene-2-o-tolylacetonitrile is considered a skin sensitizer with a defined No Expected Sensitization Induction Level (NESIL) of 1200 μg/cm^2. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet (UV) spectra; 2-cyclohexylidene-2-o-tolylacetonitrile 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 2-cyclohexylidene-2-o-tolylacetonitrile is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; 2-cyclohexylidene-2-o-tolylacetonitrile 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, 2005c; RIFM, 2008e)
Repeated Dose Toxicity: NOAEL = 16.7 mg/kg/day. (RIFM, 2008g)
Reproductive Toxicity: NOAEL = 40 mg/kg/day. RIFM (2009)
Skin Sensitization: NESIL = 1200 μg/cm^2. RIFM (2010)
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic. (UV Spectra, RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:

Persistence:

Critical Measured Value: 0% (OECD 302C) RIFM, (2005a)

(*continued on next page*)

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Bioaccumulation:	
Screening-level: 726.5 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Critical Ecotoxicity Endpoint: 21-day *Daphnia magna* NOEC: 0.28 mg/L	RIFM (2006)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards	
Risk Assessment:	
Screening-level: PEC/PNEC (North America and Europe) > 1	(RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: 21-day *Daphnia magna* NOEC: 0.28 mg/L	RIFM (2006)
RIFM PNEC is: 5.6 μg/L	
• **Revised PEC/PNECs (2015 IFRA VoU)**: North America and Europe: <1	

1. Identification

1. **Chemical Name:** 2-Cyclohexylidene-2-o-tolylacetonitrile
2. **CAS Registry Number:** 916887-53-1
3. **Synonyms:** Petalia; GR-86-6414; 2-Cyclohexylidene-2-o-tolylacetonitrile
4. **Molecular Formula:** Not Available
5. **Molecular Weight:** 211.3
6. **RIFM Number:** 10307
7. **Stereochemistry:** Stereoisomer not specified. No stereocenter present and no stereoisomer possible.

2. Physical data

1. **Boiling Point:** 344.33 °C (EPI Suite)
2. **Flash Point:** Not Available
3. **Log K_{OW}:** Log Pow = 3.3 at 23 °C (RIFM, 2008f)
4. **Melting Point**: 92.50 °C (EPI Suite)
5. **Water Solubility:** 1.29 mg/L at 20 °C (RIFM, 2008f); 1.525 mg/L at 25 °C (WSKOW v1.42 in EPI Suite)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.00534 Pa at 25 °C (EPI Suite)
8. **UV Spectra:** No significant 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. 10–100 metric tons per year (IFRA, 2015)

4. Exposure to fragrance ingredient (Creme RIFM Aggregate Exposure Model v2.0)

2. **95**th **Percentile Concentration in Fine Fragrance:** 0.43% (RIFM, 2018)
3. **Inhalation Exposure*:** 0.00060 mg/kg/day or 0.053 mg/day (RIFM, 2018)
4. **Total Systemic Exposure**:** 0.0074 mg/kg/day (RIFM, 2018)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM Aggregate Exposure Model (Comiskey et al., 2015; Safford et al., 2015; Safford et al., 2017; and Comiskey et al., 2017).

**95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section V. It is derived from concentration survey data in the Creme RIFM Aggregate Exposure Model and includes exposure via dermal, oral, and inhalation routes whenever the fragrance ingredient is used in products that include these routes of exposure (Comiskey et al., 2015; Safford et al., 2015; Safford et al., 2017; and Comiskey et al., 2017).

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

6.1. *Cramer Classification: Class III, High*

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

6.2. *Analogs Selected*

a. **Genotoxicity:** None
b. **Repeated Dose Toxicity:** None
c. **Reproductive Toxicity:** α-Cyclohexylidene benzeneacetonitrile (CAS # 10461-98-0)
d. **Skin Sensitization:** None
e. **Phototoxicity/Photoallergenicity:** None
f. **Local Respiratory Toxicity:** None
g. **Environmental Toxicity:** None

6.3. *Read-across Justification*

See Appendix below

7. Metabolism

No relevant data available for inclusion in this safety assessment.

7.1. *Additional references*

None.

8. Natural occurrence

2-Cyclohexylidene-2-o-tolylacetonitrile 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

Not pre-registered; no dossier available as of 10/06/21.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for 2-cyclohexylidene-2-o-tolylacetonitrile 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)	0.066
2	Products applied to the axillae	0.027
3	Products applied to the face/body using fingertips	0.33
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	0.066
7	Products applied to the hair with some hand contact	0.99
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)	0.066
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	66

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 2-cyclohexylidene-2-o-tolylacetonitrile, the basis was the reference dose of 0.17 mg/kg/day, a predicted skin absorption value of 10%, 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).
[c]Calculations 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, 2-cyclohexylidene-2-o-tolylacetonitrile does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. The mutagenic activity of 2-cyclohexylidene-2-o-tolylacetonitrile 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 pre-incubation methods. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA102, and *Escherichia coli* strain WP2uvrA were treated with 2-cyclohexylidene-2-o-tolylacetonitrile in dimethyl sulfoxide (DMSO) at concentrations up to 5000 μg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 2005c). Under the conditions of the study, 2-cyclohexylidene-2-o-tolylacetonitrile was not mutagenic in the Ames test.

The clastogenicity of 2-cyclohexylidene-2-o-tolylacetonitrile was assessed in an *in vitro* chromosome aberration study conducted in compliance with GLP regulations and in accordance with OECD TG 473. Chinese hamster lung cells (V79) were treated with 2-cyclohexylidene-2-o-tolylacetonitrile in acetone at concentrations up to 2100 μg/mL in the DRF study; the main test was conducted at concentrations up to 50 μg/mL in the presence and absence of metabolic activation. No statistically significant increases in the frequency of cells with structural chromosomal aberrations or polyploid cells were observed with any concentration of the test material, either with or without S9 metabolic activation (RIFM, 2008e). Under the conditions of the study, 2-cyclohexylidene-2-o-tolylacetonitrile was considered to be non-clastogenic in the *in vitro* chromosome aberration assay.

Based on the available data, 2-cyclohexylidene-2-o-tolylacetonitrile does present a concern for genotoxic potential.

11.1.1.2. Additional references. None.

11.1.1.3. Literature search and risk assessment completed on. 06/01/21.

11.1.2. Repeated dose toxicity

The margin of exposure (MOE) for 2-cyclohexylidene-2-o-tolylacetonitrile 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 2-cyclohexylidene-2-o-tolylacetonitrile. In an OECD 407/GLP compliant study, groups of 5 Wistar rats/sex/dose were administered test material 2-cyclohexylidene-2-o-tolylacetonitrile at doses of 0 (PEG300), 50, 200, or 1000 mg/kg/day for 28 days through oral gavage. There were no alterations in body weight, food consumption, hematology, locomotor activity, functional observation battery, group strength, clinical signs, or mortality among treated animals. In the mid-dose (4M, 5F) and high-dose (all animals) groups, the animals were reported to have increased relative heart weights with minimal to marked myocardial vacuolation. Since the etiology of cardiac alterations remained unexplained, these effects were considered to be adverse degenerative alterations. In addition, significant increases in liver weights and hepatocellular hypertrophy were reported in the mid- and high-dose groups. Thus, the NOAEL was considered to be 50 mg/kg/day based on pathological alterations reported in the heart and liver of treated animals at higher doses (RIFM, 2008g).

A default safety factor of 3 was used when deriving a NOAEL from the 28-day study (ECHA, 2012). The safety factor has been approved by the Expert Panel for Fragrance Safety*.

Thus, the derived NOAEL for the Repeated Dose Toxicity endpoint is 50/3 = 16.7 mg/kg/day.

Therefore, the 2-cyclohexylidene-2-o-tolylacetonitrile MOE for the repeated dose toxicity endpoint can be calculated by dividing the 2-cyclohexylidene-2-o-tolylacetonitrile NOAEL in mg/kg/day by the total systemic exposure to 2-cyclohexylidene-2-o-tolylacetonitrile, 16.7/0.0074 = 2257.

11.1.2.2. Derivation of reference dose (RfD). 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 0.17 mg/kg/day.

The RIFM Criteria Document (Api et al., 2015) calls for a default MOE of 100 (10 × 10), based on uncertainty factors applied for inter-species (10 ×) and intraspecies (10 ×) differences. The reference dose for 2-cyclohexylidene-2-o-tolylacetonitrile was calculated by dividing the lowest NOAEL (from the Repeated Dose and Reproductive Toxicity sections) of 16.7 mg/kg/day by the uncertainty factor, 100 = 0.17 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.

11.1.2.3. Additional references. None.

11.1.2.4. Literature search and risk assessment completed on. 05/20/21.

11.1.3. Reproductive toxicity

The MOE for 2-cyclohexylidene-2-o-tolylacetonitrile is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are insufficient reproductive toxicity data on 2-cyclohexylidene-2-o-tolylacetonitrile. Read-across material α-cyclohexylidene benzeneacetonitrile (CAS # 10461-98-0; see Section VI) has sufficient reproductive toxicity data that can be used to support 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 2-cyclohexylidene-2-o-tolylacetonitrile 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 2-cyclohexylidene-2-o-tolylacetonitrile, 40/0.0074 or 5405.**

Table 1
Data summary for 2-cyclohexylidene-2-*o*-tolylacetonitrile.

LLNA Weighted Mean EC3 Value μg/cm^2 (No. Studies)	Potency Classification Based on Animal Data[1]	Human Data			
		NOEL-CNIH (Induction) μg/cm^2	NOEL-HMT (Induction) μg/cm^2	LOEL[2] (Induction) μg/cm^2	WoE NESIL[3] 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.
[1] Based on animal data using classification defined in ECETOC, Technical Report No. 87, 2003.
[2] Data derived from CNIH or HMT.
[3] WoE NESIL limited to 2 significant figures.

11.1.3.2. Additional references. RIFM, 2008g.

11.1.3.3. Literature search and risk assessment completed on. 05/31/21.

11.1.4. Skin sensitization

Based on the existing data, 2-cyclohexylidene-2-*o*-tolylacetonitrile is considered a skin sensitizer with a defined NESIL of 1200 μg/cm^2.

11.1.4.1. Risk assessment. Based on the existing data, 2-cyclohexylidene-2-*o*-tolylacetonitrile is considered a skin sensitizer. The chemical structure of this material indicates that it would be expected to react with skin proteins (Roberts et al., 2007; OECD Toolbox v4.2). Furthermore, in a murine local lymph node assay (LLNA), 2-cyclohexylidene-2-*o*-tolylacetonitrile was found to be sensitizing with an EC3 value of 9.3% (2325 μg/cm^2) (RIFM, 2005d). In a Confirmation of No Induction in Humans test (CNIH) with 2.5% or 1250 μg/cm^2 of 2-cyclohexylidene-2-*o*-tolylacetonitrile in 1:3 ethanol:diethyl phthalate (EtOH:DEP), no reactions indicative of sensitization were observed in any of the 101 volunteers (RIFM, 2010).

Based on WoE from structural analysis and animal and human studies, 2-cyclohexylidene-2-*o*-tolylacetonitrile is a moderate sensitizer with a Weight of Evidence No Expected Sensitization Induction Level (WoE NESIL) of 1200 μg/cm^2 (see Table 1). 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 0.17 mg/kg/day.

11.1.4.2. Additional references. None.

11.1.4.3. Literature search and risk assessment completed on. 05/27/21.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV spectra, 2-cyclohexylidene-2-o-tolylacetonitrile would not be expected to present a concern for phototoxicity or photoallergenicity.

11.1.5.1. Risk assessment. There are no phototoxicity studies available for 2-cyclohexylidene-2-o-tolylacetonitrile in experimental models. UV absorption spectra indicate no absorption between 290 and 500 nm. As such, it is not a concern for phototoxicity or photoallergenicity (Henry et al., 2009). Based on the lack of absorbance, 2-cyclohexylidene-2-o-tolylacetonitrile does not present a concern for phototoxicity or photoallergenicity.

11.1.5.2. UV spectra analysis. The available spectra indicate no absorbance in the range of 290–500 nm. As the material does not absorb in the range of interest, it is not a concern for phototoxicity or photoallergenicity (Henry et al., 2009).

11.1.5.3. Additional references. None.

11.1.5.4. 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 2-cyclohexylidene-2-o-tolylacetonitrile 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 2-cyclohexylidene-2-o-tolylacetonitrile. Based on the Creme RIFM Model, the inhalation exposure is 0.053 mg/day. This exposure is 8.9 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.

11.1.6.2. Additional references. RIFM, 2007.

11.1.6.3. 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 2-cyclohexylidene-2-o-tolylacetonitrile was performed following the RIFM Environmental Framework (Salvito et al., 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log K_{OW}, and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity, as discussed in Salvito et al. (2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 2012b), which provides chemical class-specific ecotoxicity estimates. Finally, if necessary, Tier 3 is conducted using measured biodegradation and ecotoxicity data to refine the RQ, thus allowing for lower PNEC uncertainty factors. The data for calculating the PEC and PNEC for this safety assessment are provided in the table below. For the PEC, the range from the most recent IFRA Volume of Use Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, 2-cyclohexylidene-2-o-tolylacetonitrile 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 2-cyclohexylidene-2-o-tolylacetonitrile as possibly persistent but not bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	21.08			1000000	0.02108	
ECOSAR Acute Endpoints **(Tier 2)** v1.11	0.151	0.246	0.105	10000	0.0105	Vinyl/Allyl Nitriles
ECOSAR Acute Endpoints **(Tier 2)** v1.11	0.487	0.360	0.793			Neutral Organics
Tier 3: Measured data						
	LC50	EC50	NOEC	AF	PNEC	Comments
Fish	1.3					
Daphnia		0.82	0.28	50	5.6	
Algae		1.3	1.3			

11.2.2. Risk assessment

Based on the current Volume of Use (2015), 2-cyclohexylidene-2-o-tolylacetonitrile presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Key studies

11.2.2.1.1. Biodegradation. **RIFM, 2005a:** The inherent biodegradability was determined by the manometric respirometry test according to the OECD 302C method. No biodegradation was observed after 32 days in the test conditions.

RIFM, 2008d: The ready biodegradability was determined by the manometric respirometry test according to the OECD 301F method. No biodegradation was observed after 28 days in the test conditions.

RIFM, 2005b: The ready biodegradability was determined by the manometric respirometry test according to the OECD 301F method. No biodegradation was observed after 28 days in the test conditions.

11.2.2.1.2. Ecotoxicity. **RIFM, 2008a:** A 48-h *Daphnia magna* acute toxicity test was conducted according to the OECD 202 method under static conditions. Due to the low solubility of the test item, a dispersion of the test item with a loading rate of 100 mg/L was continuously stirred at room temperature in dark for 3 h followed by filtration. Based on the mean measured concentrations and the loading rate of 100 mg/L, the 48-h EC50 was greater than 0.82 mg/L.

RIFM, 2008b: The acute fish (zebrafish) toxicity test was conducted according to the OECD 203 method under semi-static conditions. Due to the low solubility of the test item, a dispersion of the test item with a loading rate of 100 mg/L was continuously stirred at room temperature for 3 h followed by filtration. The 96-h LC50 value based on loading rate and mean measured concentration was reported to be greater than 1.3 mg/L.

RIFM, 2008c: The algae growth inhibition test was conducted according to the OECD 201 method under static conditions. Due to the low solubility of the test item, a dispersion of the test item was prepared in test water. The dispersion was filtered and the undiluted filtrate was used as the highest concentration. The 72-h EC50 value based on measured concentration was reported greater than 1.3 mg/L the 72-h NOEC value based on measured concentration was reported to be greater than 1.3 mg/L.

RIFM, 2006: The *Daphnia magna* reproduction test was conducted according to the OECD 211 method under semi-static conditions. The 21-day NOEC value based on mean measured concentration was reported to be 0.28 mg/L and 0.87 mg/L for body length and reproduction rate, respectively.

11.2.2.1.3. Other available data. 2-Cyclohexylidene-2-o-tolylacetonitrile has not been registered under REACH.

11.2.3. Risk assessment refinement

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

Endpoints used to calculate PNEC are underlined.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	3.3	3.3
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	1–10	1–10
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 5.6 µg/L. The revised PEC/PNECs for EU and NA <1; therefore, the material does not present a risk to the aquatic environment at the current reported volumes of use.

11.2.4. 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
- **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 10/06/21.

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.2021.112681.

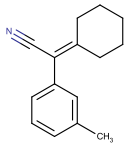
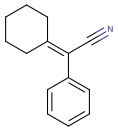
Appendix

Read-across Justification

Methods

The read-across analog was identified following the strategy for structuring and reporting a read-across prediction of toxicity as 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, 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 v4.11 (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).
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018), and skin sensitization was predicted using Toxtree.
- The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).

	Target Material	Read-across Material
Principal Name	2-Cyclohexylidene-2-*o*-tolylacetonitrile	*α*-Cyclohexylidene benzeneacetonitrile
CAS No.	916887-53-1	10461-98-0
Structure		
Similarity (Tanimoto Score)		0.85
Read-across Endpoint		• Reproductive Toxicity
Molecular Formula	$C_{15}H_{17}N$	$C_{14}H_{15}N$
Molecular Weight	211.30	197.28
Melting Point (°C, EPI Suite)	92.50	77.07
Boiling Point (°C, EPI Suite)	344.33	332.24
Vapor Pressure (Pa @ 25°C, EPI Suite)	0.00534	0.0148
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	4.84	4.29
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	1.525	55.976
J_{max} (μg/cm^2/h, SAM)	4.026	8.001
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.74E+000	5.526E-001
Reproductive Toxicity		
ER Binding (OECD QSAR Toolbox v4.2)	• Non-binder, without OH or NH2 group	• Non-binder, without OH or NH2 group
Developmental Toxicity (CAESAR v2.1.6)	• Toxicant (low reliability)	• Toxicant (low reliability)
Metabolism		
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	• See Supplemental Data 1	• See Supplemental Data 2

Summary

There are insufficient toxicity data on 2-cyclohexylidene-2-*o*-tolylacetonitrile (CAS # 916887-53-1). 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, α-cyclohexylidene benzeneacetonitrile (CAS # 10461-98-0) was identified as a read-across analog with sufficient data for toxicological evaluation.

Conclusions

- α-Cyclohexylidene benzeneacetonitrile (CAS # 10461-98-0) was used as a read-across analog for the target material 2-cyclohexylidene-2-*o*-tolylacetonitrile (CAS # 916887-53-1) for the reproductive toxicity endpoint.
  - The target material and the read-across analog are structurally similar and belong to a class of aromatic nitriles.
  - The target material and the read-across analog share a 2-cyclohexyl-2-phenylacetonitrile moiety.

- The key difference between the target material and the read-across analog is that the target material is substituted with a methyl group in the 3 position on the benzyl ring. This structural difference is toxicologically insignificant.
- 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.
- The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
- According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
- Both the target and read-across materials have a toxicant alert for Developmental Toxicity (CAESAR v2.1.6). The data described in the Developmental Toxicity section shows that the MOE is adequate at the current level of use. The predictions are superseded by the data.
- The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
- The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

References

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

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

Cassano, A., Manganaro, A., Martin, T., Young, D., Piclin, N., Pintore, M., Bigoni, D., Benfenati, E., 2010. CAESAR models for developmental toxicity. Chem. Cent. J. (4 Suppl. 1), S4.

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

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

ECHA, 2012. Guidance on Information Requirements and Chemical Safety Assessment. November 2012 v2.1. http://echa.europa.eu/.

ECHA, 2017. Read-across Assessment Framework (RAAF). Retrieved from. www.echa.europa.eu/documents/10162/13628/raaf_en.pdf.

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

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

Na, M., Ritacco, G., O'Brien, D., Lavelle, M., Api, A., Basketter, D., 2020. Fragrance Skin Sensitization Evaluation and Human Testing, Dermatitis. https://doi.org/10.1097/DER.0000000000000684. November 16, 2020. Volume Publish Ahead of Print Issue. Retrieved from.

OECD, 2015. *Guidance Document on the Reporting of Integrated Approaches to Testing and Assessment (IATA)*. ENV/JM/HA(2015)7. Retrieved from. http://www.oecd.org/.

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

RIFM (Research Institute for Fragrance Materials, Inc), 2005a. Inherent Biodegradability of 2-Cyclohexylidene-2-O-Tolylacetonitrile (Petalia). Unpublished Report from Givaudan. RIFM Report Number 60933. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2005b. Ready Bidegradability of 2-Cyclohexylidene-2-O-Tolylacetonitrile (Petalia). Unpublished Report from Givaudan. RIFM Report Number 60935. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2005c. Salmonella typhimurium and Escherichia coli Reverse Mutation Assay with 2-Cyclohexylidene-2-O-Tolylacetonitrile (Petalia). Unpublished Report from Givaudan. RIFM Report Number 60938. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2005d. 2-Cyclohexylidene-2-o-tolylacetonitrile (Petalia): Local Lymph Node Assay (LLNA) in Mice (Identification of Contact Allergens). Unpublished Report from Givaudan. RIFM Report Number 60941. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2006. 2-Cyclohexylidene-2-o-tolylacetonitrile (Petalia): Effect on Survival and Reproduction of Daphnia Magna in a Semi-static Test over Three Weeks. Unpublished Report from Givaudan. RIFM Report Number 60932. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2007. 2-Cyclohexylidene-2-o-tolylacetonitrile (Petalia): 4-Hour Acute Inhalation Toxciity Study in Rats. Unpublished Report from Givaudan. RIFM Report Number 60925. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2008a. 2-Cyclohexylidene-2-o-tolylacetonitrile (Petalia): Acute Toxicity to Daphnia Magna in a 48-hour Immobilization Test. Unpublished Report from Givaudan. RIFM Report Number 60928. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2008b. 2-Cyclohexylidene-2-o-tolylacetonitrile (Petalia): Acute Toxicity to Zebra Fish (Brachydanio Rerio) in a 96-hour Semi-static Test). Unpublished Report from Givaudan. RIFM Report Number 60929. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2008c. 2-Cyclohexylidene-2-o-tolylacetonitrile (Petalia): Toxicity to Scenedesmus Subspicatus in a 72-hour Algal Growth Inhbition Test. Unpublished Report from Givaudan. RIFM Report Number 60931. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2008d. 2-Cyclohexylidene-2-o-tolylacetonitrile (Petalia): Ready Bidegradability in a Manometric Respirometry Test. Unpublished Report from Givaudan. RIFM Report Number 60934. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2008e. In Vitro Chromosome Aberration Test in Chinese Hamster V79 Cells with 2-Cyclohexylidene-2-O-Tolylacetonitrile (Petalia). Unpublished Report from Givaudan. RIFM Report Number 60939. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2008f. 2-Cyclohexylidene-2-o-tolylacetonitrile (Petalia): Determination of the Water Solubility and the Partition Coefficient (N-octanol/water. [Amendment Attached] Unpublished Report from Givaudan. RIFM Report Number 60940. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2008g. 2-Cyclohexylidene-2-o-tolylacetonitrile (Petalia): 28-Day Oral Toxicity (Gavage) Study in the Wistar Rat. Unpublished Report from Givaudan. RIFM Report Number 60943. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2009. Combined 13 Week Toxicity Study and One-Generation Study with Alpha-Cyclohexylidene Benzeneacetonitrile (Peonile) by Oral Gavage Administration to CD Rats Unpublished Report from Givaudan. RIFM Report Number 58437. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc), 2010. Repeated Insult Patch Test with 2-Cyclohexylidene-2-O-Tolylacetonitrile (Petalia) 2.5% in ETOH/DEP 1:3. Unpublished Report from Givaudan. RIFM Report Number 60944. RIFM, Woodcliff Lake, NJ, USA.

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

RIFM (Research Institute for Fragrance Materials, Inc), 2020. Updating Exposure Assessment for Skin Sensitization Quantitative Risk Assessment for Fragrance Materials. RIFM Report Number 76775. RIFM, Woodcliff Lake, NJ, USA.

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

Rogers, D., Hahn, M., 2010. Extended-connectivity fingerprints. J. Chem. Inf. Model. 50 (5), 742–754.

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

Safford, B., Api, A.M., Barratt, C., Comiskey, D., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Rose, J., Smith, B., Tozer, S., 2017. Application of the expanded Creme RIFM consumer exposure model to fragrance ingredients in cosmetic, personal care and air care products. Regul. Toxicol. Pharmacol. 86, 148–156.

Salvito, D.T., Senna, R.J., Federle, T.W., 2002. A Framework for prioritizing fragrance materials for aquatic risk assessment. Environ. Toxicol. Chem. 21 (6), 1301–1308.

Schultz, T.W., Amcoff, P., Berggren, E., Gautier, F., Klaric, M., Knight, D.J., Mahony, C., Schwarz, M., White, A., Cronin, M.T., 2015. A strategy for structuring and reporting a read-across prediction of toxicity. Regul. Toxicol. Pharmacol. 72 (3), 586–601.

Shen, J., Kromidas, L., Schultz, T., Bhatia, S., 2014. An *in silico* skin absorption model for fragrance materials. Food Chem. Toxicol. 74, 164–176.

US EPA, 2012a. Estimation Programs Interface Suite for Microsoft Windows, v4.0–v4.11. United States Environmental Protection Agency, Washington, DC, USA.

US EPA, 2012b. The ECOSAR (ECOlogical Structure Activity Relationship) Class Program for Microsoft Windows, v1.11. United States Environmental Protection Agency, Washington, DC, USA.