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Short Review

RIFM fragrance ingredient safety assessment cyclohexanecarboxylic acid, CAS Registry Number 98-89-5

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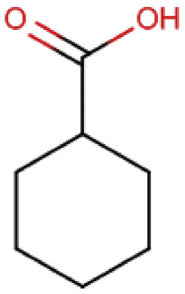
Version: 110817. This version replaces any previous versions.
Name: Cyclohexanecarboxylic acid
CAS Registry Number: 98-89-5



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

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Creme RIFM model - The Creme RIFM model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015, 2017) compared to a deterministic aggregate approach
DEREK - Derek nexus is an *in silico* tool used to identify structural alerts
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observable Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
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
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 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.

Cyclohexanecarboxilic acid was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analog 3,3,5-trimethylcyclohexaneacetic acid (CAS # 3213-73-8) show that cyclohexanecarboxilic acid is not expected to be genotoxic. The skin sensitization endpoint was completed using DST for non-reactive materials (900 μg/cm^2/day); exposure is below the DST. The repeated dose, reproductive and local respiratory toxicity endpoints were completed using the TTC for a Cramer Class I material, and the exposure to cyclohexanecarboxilic acid is below the TTC (0.03, 0.03 mg/kg/day and 1.4 mg/day, respectively). The phototoxicity/photoallergenicity endpoint was completed based on UV spectra; cyclohexanecarboxilic acid is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated, and cyclohexanecarboxilic acid 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, 2014b; RIFM, 2016a; RIFM, 2015; RIFM, 2016b)

Repeated Dose Toxicity: No NOAEL available. Exposure is below the TTC.
Reproductive Toxicity: No NOAEL available. Exposure is below the TTC.
Skin Sensitization: No safety concerns at current, declared use levels; exposure is below the DST.
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: 3.3 (Biowin 3) (EPI Suite v4.1)

Bioaccumulation: Screening-Level: 3.2 L/kg (EPI Suite v4.1)

Ecotoxicity: Screening-Level: Fish LC50: 84.06 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: Fish LC50: 84.06 mg/L (Salvito et al., 2002)

RIFM PNEC is: 0.08406 µg/L

- **Revised PEC/PNECs (2011 IFRA VoU):** North America and Europe: not applicable; cleared at the screening-level

1. Identification

1. **Chemical Name:** Cyclohexanecarboxylic acid
2. **CAS Registry Number:** 98-89-5
3. **Synonyms:** Cyclohexanecarboxylic acid
4. **Molecular Formula:** $C_7H_{12}O_2$
5. **Molecular Weight:** 128.17
6. **RIFM Number:** 6101

2. Physical data

1. **Boiling Point:** 232 °C, 235.07 °C (EPI Suite)
2. **Flash Point:** >200°F
3. **Log K_{ow}:** 2.36 (EPI Suite)
4. **Melting Point**: 43.53 °C (EPI Suite)
5. **Water Solubility:** 4919 mg/L (EPI Suite)
6. **Specific Gravity:** 1.033
7. **Vapor Pressure:** 0.0334 mm Hg @ 20 °C (EPI Suite), 0.004 mm Hg 20C, 0.0568 mm Hg @ 25 °C (EPI Suite)
8. **UV Spectra:** Minor absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} · cm^{-1})
9. **Appearance/Organoleptic:** A white crystalline solid with a fruity, acidic, metallic, cheesy, tropical, berry odor. The taste is described as fruity, woody, berry-like with green dirty nuances.*

*http://www.thegoodscentscompany.com/data/rw1036731.html#toorgano, retrieved 5/10/2017.

3. Exposure

1. **Volume of Use (worldwide band):** < 0.1 metric ton per year (IFRA, 2011)
2. **95th Percentile Concentration in Hydroalcoholics:** 0.000011% (RIFM, 2017)
3. **Inhalation Exposure*:** < 0.0001 mg/kg/day or < 0.0001 mg/day (RIFM, 2017)
4. **Total Systemic Exposure**:** 0.0000002 mg/kg/day (RIFM, 2017)

*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, 2017; and Comiskey et al., 2017).

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

4. Derivation of systemic absorption

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

5. Computational toxicology evaluation

1. **Cramer Classification:** Class I, Low

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

2. **Analogs Selected:**
   a. **Genotoxicity:** 3,3,5-Trimethylcyclohexaneacetic acid (CAS # 3213-73-8)
   b. **Repeated Dose Toxicity:** None
   c. **Reproductive Toxicity:** None
   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)

Cyclohexanecarboxylic acid 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 that 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 5/10/2017.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the existing data, cyclohexanecarboxylic acid does not present a concern for genotoxicity.

10.1.2. Risk assessment

Cyclohexanecarboxylic acid was tested in the BlueScreen assay and found positive for genotoxicity at cytotoxic concentrations (reduced the relative cell density to less than 80%) in the presence of metabolic S9-activation and negative without S9 (RIFM, 2014a). BlueScreen is a screening assay which assesses genotoxic stress through human-derived gene expression. Additional assays on a more reactive read-across material were considered to fully assess the potential mutagenic or clastogenic effects on the target material. There are no studies assessing the mutagenicity of cyclohexanecarboxylic acid. The mutagenic activity of read-across material 3,3,5-trimethylcyclohexaneacetic acid (CAS # 3213-73-8; see Section V) has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP and OECD TG 471 (OECD, 1997) using the standard plate incorporation method. *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, and *Escherichia coli* strains WP2uvrA were treated with 3,3,5-trimethylcyclohexaneacetic acid in DMSO (dimethyl sulfoxide) at concentrations up to 5000 μg/plate. No increases in the mean number of revertant colonies were observed at any tested dose with or without S9 (RIFM, 2014b). Under the conditions of the study, 3,3,5-trimethylcyclohexaneacetic acid was not mutagenic in the Ames test.

There are no studies assessing the clastogenicity of cyclohexanecarboxylic acid. The clastogenic activity of 3,3,5-trimethylcyclohexaneacetic acid was evaluated in an in vitro micronucleus test conducted in compliance with GLP and OECD TG 487 (OECD, 2016b). Human peripheral blood lymphocytes were treated with 3,3,5-trimethylcyclohexaneacetic acid in DMSO up to 1840 μg/mL with and without S9 at the 3-h and 24-h timepoints. 3,3,5-Trimethylcyclohexaneacetic acid did not induce binucleated cells with micronuclei when tested up to cytotoxic levels in either 3- or 24-h non-activated test systems; however, a statistically significant increase in binucleated cells with micronuclei was observed in the 3-h treatment with S9 metabolic activation (RIFM, 2016a). Under the conditions of the study, 3,3,5-trimethylcyclohexaneacetic acid was considered to be clastogenic in the in vitro micronucleus test. To further assess these adverse results in vitro, two studies were conducted to clarify the results of 3,3,5-trimethylcyclohexaneacetic acid: a 3D reconstructed skin micronucleus assay and an in vivo micronucleus assay. A GLP compliant 3D reconstructed skin micronucleus assay (RSMN) was conducted to evaluate the genotoxic potential of 3,3,5-trimethylcyclohexaneacetic acid in EpiDerm™. Acetone was used as the vehicle. EpiDerm™ tissues were treated with 3,3,5-trimethylcyclohexaneacetic acid at 24-h intervals for 48 and 72 h up to 40 mg/mL 3,3,5-Trimethylcyclohexaneacetic acid did not induce binucleated cells with micronuclei when tested up to cytotoxic levels, and therefore was concluded to be negative for induction of micronuclei in the RSMN in EpiDerm™ (RIFM, 2015). Since there were conflicting results in the two in vitro assays conducted to determine the clastogenic potential of 3,3,5-trimethylcyclohexaneacetic acid, an in vivo study was conducted. The clastogenic activity of 3,3,5-trimethylcyclohexaneacetic acid was evaluated in an in vivo micronucleus test conducted in compliance with GLP and OECD TG 474 (OECD, 2016a). The test material was administered in corn oil via oral gavage to groups of male and female Hsd:ICR mice (5/sex/dose). Doses of 125, 250, or 500 mg/kg were administered. Mice from each dose level were euthanized at 48 h; the bone marrow was extracted and examined for polychromatic erythrocytes. The test material did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow (RIFM, 2016b). Under the conditions of the study, 3,3,5-trimethylcyclohexaneacetic acid was not clastogenic in the in vivo micronucleus test.

Based on the data available, 3,3,5-trimethylcyclohexaneacetic acid does not present a concern for genotoxic potential, and this can be applied to cyclohexanecarboxylic acid.

Additional References: None.

Literature Search and Risk Assessment Completed on: 5/10/2017.

10.1.3. Repeated dose toxicity

There are insufficient repeated dose toxicity data on cyclohexanecarboxylic acid or any read-across materials. The total systemic exposure to cyclohexanecarboxylic acid is below the TTC for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

10.1.4. Risk assessment

There are no repeated dose toxicity data on cyclohexanecarboxylic acid or any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to cyclohexanecarboxylic acid (0.0002 μg/kg/day) is below the TTC (30 μg/kg bw/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed on: 04/28/2017.

10.1.5. Reproductive toxicity

There are insufficient reproductive toxicity data on cyclohexanecarboxylic acid or any read-across materials evaluated. The total systemic exposure to cyclohexanecarboxylic acid is below the TTC for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

10.1.6. Risk assessment

There are no developmental toxicity or fertility data on cyclohexanecarboxylic acid or any read-across materials that can be used to support the evaluation. The total systemic exposure to cyclohexanecarboxylic acid (0.0002 μg/kg/day) is below the TTC (30 μg/kg bw/

Table 1
Acceptable concentrations for cyclohexanecarboxylic acid based on DST non-reactive.

IFRA Category[a]	Description of Product Type	Acceptable Concentrations in Finished Products	95th Percentile Concentration
1	Products applied to the lips	0.069%	0.00%
2	Products applied to the axillae	0.021%	0.00%
3	Products applied to the face using fingertips	0.41%	0.00%
4	Fine fragrance products	0.39%	0.00%[b]
5	Products applied to the face and body using the hands (palms), primarily leave-on	0.10%	0.00%
6	Products with oral and lip exposure	0.23%	0.00%
7	Products applied to the hair with some hand contact	0.79%	0.00%
8	Products with significant ano-genital exposure	0.04%	0.00%
9	Products with body and hand exposure, primarily rinse off	0.75%	0.00%
10	Household care products with mostly hand contact	2.70%	0.00%
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate	1.50%	0.00%
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted	0.00%

Note.
[a] For a description of the categories, refer to the IFRA/RIFM Informational Booklet.
[b] Negligible exposure (< 0.01%).

day; Kroes et al., 2007; Laufersweiler et al., 2012) for developmental toxicity and fertility for a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed on: 04/28/2017.

10.1.7. Skin sensitization

Based on the application of DST, cyclohexanecarboxylic acid does not present a safety concern for skin sensitization under the current, declared levels of use.

10.1.8. Risk assessment

The chemical structure of this material indicates that it would not be expected to react with skin proteins (Roberts et al., 2007; Toxtree 2.6.13; OECD Toolbox v3.4 (OECD, 2012)). No predictive or human confirmatory skin sensitization studies are available for cyclohexanecarboxylic acid. Due to the insufficient data, the reported exposure was compared to the non-reactive Dermal Sensitization Threshold (DST) of 900 μg/cm2. The current exposure from the 95th percentile concentration is below the DST for non-reactive materials when evaluated in all QRA categories. Table 1 provides the acceptable concentration for cyclohexanecarboxylic, which presents no appreciable risk for skin sensitization based on the non-reactive DST.

Additional References: None.

Literature Search and Risk Assessment Completed on: 5/4/17.

10.1.9. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, cyclohexanecarboxylic acid would not be expected to present a concern for phototoxicity or photoallergenicity.

10.1.10. Risk assessment

There are no phototoxicity studies available for cyclohexanecarboxylic acid in experimental models. UV/Vis absorption spectra indicate minor absorbance between 290 and 700 nm. Corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). Based on lack of significant absorbance in the critical range, cyclohexanecarboxylic acid does not present a concern for phototoxicity or photoallergenicity.

10.1.11. UV spectra analysis

UV/Vis absorption spectra (OECD test guideline 101 (OECD, 1981) for cyclohexanecarboxylic acid were obtained. The spectra indicate minor absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark of concern for phototoxic effects, 1000 $L\,mol^{-1} \cdot cm^{-1}$ (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed on: 04/20/17.

10.1.12. Local respiratory toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The material, cyclohexanecarboxylic acid, exposure level is below the Cramer Class I TTC value for inhalation exposure local effects.

10.1.13. Risk assessment

There are no inhalation data available on cyclohexanecarboxylic acid. Based on the Creme RIFM model, the inhalation exposure is < 0.0001 mg/day. This exposure is at least 14000 times lower than the Cramer Class I TTC value of 1.4 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: 5/8/2017.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of cyclohexanecarboxylic acid 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, which is considered proprietary information, 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, cyclohexanecarboxylic acid was identified as a fragrance material with no potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC < 1).

A screening-level hazard assessment using EPI SUITE v4.1 did not identify cyclohexanecarboxylic acid as either persistent or 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. As noted in the Criteria Document, the screening criteria applied are the same criteria used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite models BIOWIN 2 or BIOWIN 6 < 0.5 and BIOWIN 3 < 2.2, then the material is considered as 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. Should additional assessment be required, based on these model outputs (Step 1), a weight-of-evidence based review is 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 v4.1).

10.2.1.1. Risk assessment. Based on the current Volume of Use (2011), cyclohexanecarboxylic acid does not present a risk to the aquatic compartment in the screening-level assessment.

10.2.1.2. Biodegradation. No data available.

10.2.1.3. Ecotoxicity. No data available.

10.2.1.4. Other available data. Cyclohexanecarboxylic acid has been registered under REACH with no additional data at this time.

10.2.1.5. 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 Framework: Salvito et al., 2002).

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	2.36	2.36
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 class of material is < 1. No further assessment is necessary.

The RIFM PNEC is 0.08406 µg/L. The revised PEC/PNECs for EU and NA: not applicable; cleared at the 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: 5/4/17.

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=KMSoUpiQKarsQS324GwBg&ved=0CBQQ1S4

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list.

	LC50 (Fish)	EC50 (Daphnia)	EC50 (Algae)	AF	PNEC	Chemical Class
RIFM Framework Screening-Level **(Tier 1)**	84.06 mg/L			1,000,000	0.08406 µg/L	

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.fct.2018.03.026.

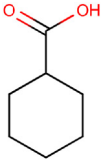
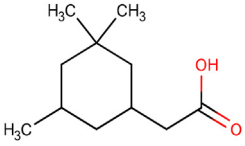
Appendix

Read-across justification

Methods

The read-across analogs were identified following the strategy for structuring and reporting a read-across prediction of toxicity described in Schultz et al. (2015) and is consistent with the guidance provided by OECD on the reporting of defined approached used within Integrated Approaches for Testing and Assessment or IATA (OECD, 2015) and the European Chemical Agency (ECHA) read-across assessment framework or RAAF (ECHA, 2016).

- In essence, materials were first clustered based on their structural similarity. In the second step, data availability and data quality on the selected cluster was examined. Finally, appropriate read-across analogs from the cluster were confirmed by using expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physicochemical properties of the target substance and the read-across analog were calculated using EPI Suite™ EPA (EPI Suite, 2012).
- Jmax were calculated using RIFM skin absorption model (SAM), the parameters were calculated using consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts and oncologic classification were generated using OECD QSAR Toolbox (v3.4) (OECD, 2012).
- ER binding and repeat dose categorization were estimated using OECD QSAR Toolbox (v3.4) (OECD, 2012).
- Developmental toxicity and skin sensitization were estimated using CAESAR v2.1.7 and 2.1.6 respectively (Cassano et al., 2010).
- Protein binding was estimated using OECD QSAR Toolbox (v3.4) (OECD, 2012).
- The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox (v3.4) (OECD, 2012).

	Target material	Read-across material
Principal Name	Cyclohexanecarboxylic acid	3,3,5-Trimethylcyclohexaneacetic acid
CAS No.	98-89-5	3213-73-8
Structure		
Similarity (Tanimoto Score)		0.626
Read-across Endpoint		• Genotoxicity
Molecular Formula	$C_7H_{12}O_2$	$C_{11}H_{20}O_2$
Molecular Weight	128.17	184.28
Melting Point (°C, EPI SUITE)	43.53	76.13
Boiling Point (°C, EPI SUITE)	235.07	284.08
Vapor Pressure (Pa @ 25 °C, EPI SUITE)	7.58	0.197
Log Kow (KOWWIN v1.68 in EPI SUITE)	1.96	4.14
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPI SUITE)	4919	38.25
J_{max} (mg/cm^2/h, SAM)	162.057	46.376
Henry's Law (Pa·m^3/mol, Bond Method, EPI SUITE)	9.96E-007	3.09E-006
Genotoxicity		
DNA Binding (OASIS v 1.4 QSAR Toolbox v3.4)	• No alert found	• No alert found
DNA Binding by OECD QSAR Toolbox (v3.4)	• No alert found	• No alert found
Carcinogenicity (Genotox and Non-genotox Alerts by ISS)	• Non-Carcinogen (low reliability)	• Non-Carcinogen (low reliability)
DNA Alerts for Ames, MN, CA by OASIS v 1.1	• No alert found	• No alert found
In Vitro Mutagenicity (Ames test Alerts by ISS)	• No alert found	• No alert found
In Vivo Mutagenicity (Micronucleus Alerts by ISS)	• No alert found	• No alert found
Oncologic Classification	• Not classified	• Not classified
Metabolism		
OECD QSAR Toolbox (3.4) Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites	See Supplemental Data 1	See Supplemental Data 2

Summary

There are insufficient toxicity data on cyclohexanecarboxylic acid (CAS # 98-89-5). Hence, *in silico* evaluation was conducted by determining a read-across analog for this material. Based on structural similarity, reactivity, metabolism data, physicochemical properties and expert judgment, analog 3,3,5-trimethylcyclohexaneacetic acid (CAS # 3213-73-8) was identified as a read-across material with data for its respective toxicological endpoints.

Conclusion/Rationale

- 3,3,5-Trimethylcyclohexaneacetic acid (CAS # 3213-73-8) was used as a read-across analog for target material cyclohexanecarboxylic acid (CAS # 98-89-5) for the genotoxicity endpoint.
  - o The target substance and the read-across analog are structurally similar and belong to the structural class of acids.
  - o The target substance and the read-across analog share a common cyclohexane fragment.
  - o The key difference between the target substance and the read-across analog is that the read-across analog has 3 methyl substitutions on the cyclohexane ring and the target does not. This structural difference between the target substance and the read-across analog is not toxicologically significant.
  - o Similarity between the target substance and the read-across analog is indicated by the Tanimoto score in the above table. The Tanimoto score is mainly driven by the cyclohexane fragment. Differences between the structures that affect the Tanimoto score are not toxicologically significant.
  - o The physical-chemical properties of the target substance and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - o According to the QSAR OECD Toolbox (v3.4), structural alerts for the genotoxicity endpoint are consistent between the target substance and the read-across analog.
  - o The target substance and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - o The structural alerts for the genotoxicity endpoint are consistent between the metabolites of the read-across analog and the target material.
  - o The structural differences between the target material and the read-across analog are not toxicologically significant.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fct.2018.03.026.

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