Food and Chemical Toxicology 179 (2023) 113916

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Short Review

RIFM fragrance ingredient safety assessment, 5,6,7,8-tetrahydroquinoxaline, CAS registry number 34413-35-9

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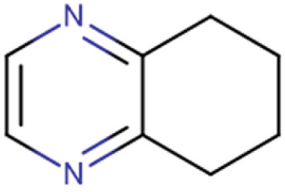
ARTICLE INFO

Handling Editor: Dr. Bryan Delaney

Version: 011923. Initial publication. All fragrance materials are evaluated on a five-year rotating basis. Revised safety assessments are published if new relevant data become available. Open access to all RIFM Fragrance Ingredient Safety Assessments is here: fragrance materialsafetyresource.elsevier.com.



Name: 5,6,7,8-Tetrahydroquinoxaline CAS Registry Number: 34413-35-9

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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., 2021)

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https://doi.org/10.1016/j.fct.2023.113916
Received 19 January 2023; Received in revised form 19 June 2023; Accepted 21 June 2023
Available online 24 June 2023

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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
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 material has not been fully evaluated for photoallergenic potential.

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. This material has not been fully evaluated for photoallergenic potential.

5,6,7,8-Tetrahydroquinoxaline was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, photoirritation/photoallergenicity, skin sensitization, and environmental safety. Data show that 5,6,7,8-tetrahydroquinoxaline is not genotoxic. The repeated dose, reproductive, and local respiratory toxicity endpoints were evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class III material, and the exposure to 5,6,7,8-tetrahydroquinoxaline is below the TTC (0.0015 mg/kg/day, 0.0015 mg/kg/day, and 0.47 mg/day, respectively). Data from read-across analog 2-ethyl-3-methylpyrazine (CAS # 15707-23-0) show that there are no safety concerns for 5,6,7,8-tetrahydroquinoxaline for skin sensitization under the current declared levels of use. The photoirritation endpoint was evaluated based on data; 5,6,7,8-tetrahydroquinoxaline does not present a concern for photoirritation. 5,6,7,8-Tetrahydroquinoxaline was not evaluated for photoallergenicity. The environmental endpoints were evaluated; 5,6,7,8-tetrahydroquinoxaline 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 (VoU) in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

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Human Health Safety Assessment

Genotoxicity: Not genotoxic. (RIFM, 2015; RIFM, 2014b)
Repeated Dose Toxicity: No NOAEL available. Exposure is below TTC.
Reproductive Toxicity: No NOAEL available. Exposure is below TTC.
Skin Sensitization: Not a concern for skin sensitization. (RIFM, 2018; RIFM, 2017b; RIFM, 2017a)
Photoirritation/Photoallergenicity: Not photoirritating/not evaluated for photoallergy. RIFM (2016)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:
Persistence:
Screening-level: 8.3 (BIOWIN 3) (EPI Suite v4.11; US EPA, 2012a)
Bioaccumulation:
Screening-level: 8.3 L/kg (EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:
Screening-level: Fish LC: 220.8 mg/L ((RIFM Framework; 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 (RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: Fish LC50: 220.8 mg/L (RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.2208 μg/L
- **Revised PEC/PNECs (2019 IFRA VoU):** North America and Europe: Not applicable; cleared at the screening-level

1. Identification

1. **Chemical Name:** 5,6,7,8-Tetrahydroquinoxaline
2. **CAS Registry Number:** 34413-35-9
3. **Synonyms:** Cyclohexapyrazine 'so called'; Quinoxaline, 5,6,7,8-tetrahydro-; Tetrahydroquinoxaline; 5,6,7,8-Tetrahydroquinoxaline
4. **Molecular Formula:** $C_8H_{10}N_2$
5. **Molecular Weight:** 134.18 g/mol
6. **RIFM Number:** 6752
7. **Stereochemistry:** No stereocenter present and no stereoisomer possible.

2. Physical data

1. **Boiling Point:** 73 °C at 4 mm Hg (Fragrance Materials Association [FMA]), 220.02 °C (EPI Suite v4.11)
2. **Flash Point:** 198 °F; closed cup (FMA)
3. **Log K_{OW}:** 1.9 (EPI Suite v4.11)
4. **Melting Point**: 37.96 °C (EPI Suite v4.11)
5. **Water Solubility:** 2091 mg/L (EPI Suite v4.11)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.0553 mm Hg at 20 °C (EPI Suite v4.0), 0.1 mm Hg at 20 °C (FMA), 0.0931 mm Hg at 25 °C (EPI Suite v4.11)
8. **UV Spectra:** Significant absorbance between 290 and 700 nm, with peak absorbance at 290 nm and returning to baseline by 330 nm. Molar absorption coefficients (3100, 3640, and 3820 L $mol^{-1} \bullet cm^{-1}$

under neutral, acidic, and basic conditions, respectively) are above the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** Not Available

3. Volume of use (worldwide band)

1. <0.1 metric ton per year (IFRA, 2019)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v3.0)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.000027% (RIFM, 2021)
2. **Inhalation Exposure*:** 0.000037 mg/kg/day or 0.0023 mg/day (RIFM, 2021)
3. **Total Systemic Exposure**:** 0.000038 mg/kg/day (RIFM, 2021)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM Aggregate Exposure Model (Comiskey et al., 2015; Safford, 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 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, 2015; Safford, 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 v4.5 (OECD, 2021b)
III	III	III

6.2. *Analogs* selected

a. **Genotoxicity:** None
b. **Repeated Dose Toxicity:** None
c. **Reproductive Toxicity:** None
d. **Skin Sensitization:** 2-Ethyl-3-methylpyrazine (CAS # 15707-23-0)
e. **Photoirritation/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. Additional **References:** None.

8. Natural occurrence

5,6,7,8-Tetrahydroquinoxaline is reported to occur in the following foods by the VCF*.

Cocoa category	Pork
Coffee	Sesame seed (roasted)
Filbert, hazelnut (*Corylus avellano*)	Wheaten bread
Peanut (*Arachis hypogaea* L.)	

*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

5,6,7,8-Tetrahydroquinoxaline has been pre-registered for 2010; no dossier available as of 07/11/22.

10. Conclusion

The existing information supports the use of this material as described in this safety assessment.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, 5,6,7,8-tetrahydroquinoxaline does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. 5,6,7,8-Tetrahydroquinoxaline was assessed in the BlueScreen assay and found positive for both cytotoxicity (positive: <80% relative cell density) and genotoxicity without metabolic activation and negative for both cytotoxicity and genotoxicity with metabolic activation (RIFM, 2014a). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures (Thakkar et al., 2022). While the BlueScreen assay on the target material showed positive results, additional assays were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

The mutagenic activity of 5,6,7,8-tetrahydroquinoxaline 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 method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with 5,6,7,8-tetrahydroquinoxaline 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, 2015). Under the conditions of the study, 5,6,7,8-tetrahydroquinoxaline was not mutagenic in the Ames test.

The clastogenic activity of 5,6,7,8-tetrahydroquinoxaline was evaluated in an *in vitro* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes were treated with 5,6,7,8-tetrahydroquinoxaline in DMSO at concentrations up to 1342 μg/mL in the dose range finding (DRF) study; micronuclei analysis was conducted at concentrations up to 1342 μg/mL in the presence and absence of metabolic activation. 5,6,7,8-Tetrahydroquinoxaline did not induce binucleated cells with micronuclei when tested up to the cytotoxic or maximum concentration in either the presence or absence of an S9 activation system (RIFM, 2014b). Under the conditions of the study, 5,6,7,8-tetrahydroquinoxaline was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the data available, 5,6,7,8-tetrahydroquinoxaline does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 07/08/22.

11.1.2. Repeated dose toxicity

There are insufficient repeated dose toxicity data on 5,6,7,8-tetrahydroquinoxaline or any read-across materials. The total systemic exposure to 5,6,7,8-tetrahydroquinoxaline is below the TTC for the repeated dose toxicity endpoint of a Cramer Class III material at the current level of use.

11.1.2.1. Risk assessment. There are limited repeated dose toxicity data on 5,6,7,8-tetrahydroquinoxaline. A single-dose, 90-day study was conducted in male and female albino rats (FDRL-strain). Groups of 15 rats/sex/dose were administered test material 5,6,7,8-tetrahydroquinoxaline at doses of 0 or 14.88 mg/kg/day in the diet. No toxicologically relevant effects were observed for behavior, growth, hematology, urine analyses, and histopathology. The NOAEL for the study was considered to be 14.88 mg/kg/day, the highest dose tested. As this was a single-dose study, the data were considered to be insufficient for the safety assessment.

The total systemic exposure to 5,6,7,8-tetrahydroquinoxaline (0.038 μg/kg/day) is below the TTC (1.5 μg/kg/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class III material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 07/07/22.

11.1.3. Reproductive toxicity

There are insufficient reproductive toxicity data on 5,6,7,8-tetrahydroquinoxaline or any read-across materials. The total systemic exposure to 5,6,7,8-tetrahydroquinoxaline is below the TTC for the reproductive toxicity endpoint of a Cramer Class III material at the current level of use.

11.1.3.1. Risk assessment. There are no reproductive toxicity data on 5,6,7,8-tetrahydroquinoxaline or on any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to 5,6,7,8-tetrahydroquinoxaline (0.038 μg/kg/day) is below the TTC (1.5 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the reproductive toxicity endpoint of a Cramer Class III material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 07/07/22.

11.1.4. Skin sensitization

Based on the existing data on the target material and read-across material 2-ethyl-3-methylpyrazine, 5,6,7,8-tetrahydroquinoxaline presents no concern for skin sensitization.

11.1.4.1. Risk assessment. Limited skin sensitization data are available for 5,6,7,8-tetrahydroquinoxaline. Therefore, read-across material 2-ethyl-3-methylpyrazine (CAS # 15707-23-0; see Section VI) was used for the risk assessment of 5,6,7,8-tetrahydroquinoxaline. The data on the read-across material are summarized in Table 1 below. Based on the existing data on the read-across material, 5,6,7,8-tetrahydroquinoxaline is not considered a skin sensitizer. The chemical structures of the read-across material and the target material indicate that they would not

Table 1
Summary of existing data on 2-ethyl-3-methylpyrazine as a read-across for 5,6,7,8-tetrahydroquinoxaline.

WoE Skin Sensitization Potency Category[1]	NOEL-CNIH (induction) μg/cm²	NOEL-HMT (induction) μg/cm²	LOEL[2] (induction) μg/cm²	WoE NESIL[3] μg/cm²	LLNA[4] Weighted Mean EC3 Value μg/cm²	GPMT[5]	Buehler[5]
No evidence of sensitization[7]	NA	NA	NA	NA	NA	NA	NA
	***In vitro* Data[6]**				***In silico* protein binding alerts (OECD Toolbox v4.5)**		
	KE 1	**KE 2**	**KE 3**		**Target Material**	**Autoxidation simulator**	**Metabolism simulator**
	Negative	Negative	Negative		No alert found	No alert found	No alert found

be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). Read-across material 2-ethyl-3--methylpyrazine is predicted *in vitro* to be a non-sensitizer when evaluated following the OECD Guideline No. 497: Defined Approaches on Skin Sensitization (OECD, 2021a). Read-across 2-ethyl-3-methylpyrazine was found to be negative in an *in vitro* direct peptide reactivity assay (DPRA), KeratinoSens, and human cell line activation test (h-CLAT) (RIFM, 2018; RIFM, 2017b; RIFM, 2017a).

Based on the weight of evidence (WoE) from structural analysis and *in vitro* studies on the read-across material as well as the target material, 5,6,7,8-tetrahydroquinoxaline does not present a concern for skin sensitization.

Additional References: None.

Literature Search and Risk Assessment Completed On: 07/06/22.

11.1.5. Photoirritation/photoallergenicity

Based on the available *in vitro* study data, 5,6,7,8-tetrahydroquinoxaline would not be expected to present a concern for photoirritation. 5,6,7,8-Tetrahydroquinoxaline has not been evaluated for photoallergenicity; however, RIFM is sponsoring an *in vitro* photoallergy research program to evaluate the photoallergy potential of 5,6,7,8-tetrahydroquinoxaline.

11.1.5.1. Risk assessment. UV/Vis absorption spectra indicate significant absorption between 290 and 700 nm. The corresponding molar absorption coefficients are above the benchmark of concern for photoirritation and photoallergenicity (Henry et al., 2009). In an *in vitro* 3T3-Neutral Red uptake photoirritation assay (OECD TG 432), 5,6,7,8-tetrahydroquinoxaline was not predicted to have photoirritating potential (RIFM, 2016). Based on the available *in vitro* study data, 5,6,7,8-tetrahydroquinoxaline would not be expected to present a concern for photoirritation. 5,6,7,8-Tetrahydroquinoxaline was not evaluated for photoallergy; however, RIFM is sponsoring an *in vitro* photoallergy research program to evaluate the photoallergy potential of 5,6,7,8-tetrahydroquinoxaline.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate significant absorbance between 290 and 700 nm, with peak absorbance at 290 nm and returning to baseline by 330 nm. Molar absorption coefficients (3100, 3640, and 3820 L mol^{-1} • cm^{-1} under neutral, acidic, and basic conditions, respectively) are above the benchmark of concern for photoirritating effects, 1000 L mol^{-1} • cm^{-1} (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/26/22.

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 5,6,7,8-tetrahydroquinoxaline is below the Cramer Class III TTC value for inhalation exposure local effects.

11.1.6.1. Risk assessment. There are no inhalation data available on 5,6,7,8-tetrahydroquinoxaline. Based on the Creme RIFM Model, the inhalation exposure is 0.0023 mg/day. This exposure is 204.3 times lower than the Cramer Class III TTC value of 0.47 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: None.

Literature Search and Risk Assessment Completed On: 06/27/22.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 5,6,7,8-tetrahydroquinoxaline 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 VoU Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, 5,6,7,8-tetrahydroquinoxaline 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 v4.11 (US EPA, 2012a) did not identify 5,6,7,8-tetrahydroquinoxaline as possibly 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 (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, 2017a). 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 $\geq$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).

11.2.2. Risk assessment

Based on the current VoU (2019), 5,6,7,8-tetrahydroquinoxaline presents a risk to the aquatic compartment in the screening-level assessment.

Key Studies:

Biodegradation:

No data available.

Ecotoxicity:

No data available.

11.2.2.1. Other available data. 5,6,7,8-Tetrahydroquinoxaline has been pre-registered for REACH with no additional data at this time.

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

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	220.8			1000000	0.2208	

Environmental Framework: Salvito et al., 2002).

Exposure	Europe	North America
Log K_{ow} Used	1.9	1.9
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional VoU Tonnage Band	<1	<1
Risk Characterization: PEC/PNEC	**<1**	**<1**

Based on available data, the RQ for this material is < 1. No further assessment is necessary.

The RIFM PNEC is 0.2208 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:** 06/09/22.

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
- **PubChem:** https://pubchem.ncbi.nlm.nih.gov/
- **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 ChemView:** https://chemview.epa.gov/chemview/
- **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 01/19/23.

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.2023.113916.

Appendix

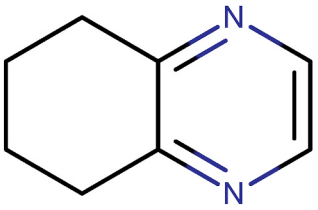
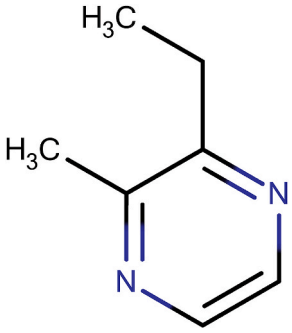
Read-across Justification

Methods

The read-across analog was identified using RIFM fragrance chemicals inventory clustering and read-across search criteria (Date et al., 2020). These criteria are in compliance with the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015) and are 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, 2017b).

- 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 (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 v4.5 (OECD, 2021b).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v4.5 (OECD, 2021b).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010), and skin sensitization was predicted using Toxtree v2.6.13.
- Protein binding was predicted using OECD QSAR Toolbox v4.5 (OECD, 2021b).
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.5 (OECD, 2021b).
- To keep continuity and compatibility with *in silico* alerts, OECD QSAR Toolbox v4.2 was selected as the alert system.

	Target Material	Read-across Material
Principal Name	5,6,7,8-Tetrahydroquinoxaline	2-Ethyl-3-methylpyrazine
CAS No.	34413-35-9	15707-23-0
Structure		
Similarity (Tanimoto Score)		0.62
SMILES	C1CCc2nccnc2C1	CCc1nccnc1C
Endpoint		Skin sensitization
Molecular Formula	$C_8H_{10}N_2$	$C_7H_{10}N_2$
Molecular Weight (g/mol)	134.182	122.171
Melting Point (°C, EPI Suite)	37.96	17.11
Boiling Point (°C, EPI Suite)	220.02	189.48
Vapor Pressure (Pa @ 25°C, EPI Suite)	1.24E+01	8.11E+01
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	2.09E+03	1.20E+04
Log K_{OW}	1.9	1.07
J_{max} (μg/cm^2/h, SAM)	58.28	119.05
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	2.80E-01	4.78E-01
Skin Sensitization		
Protein Binding (OASIS v1.1)	No alert found	No alert found
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)	No alert found	No alert found
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	No skin sensitization reactivity domain alerts were identified	No skin sensitization reactivity domain alerts were identified
Metabolism		
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.5)	See Supplemental Data 1	See Supplemental Data 2

Summary

There are insufficient toxicity data on 5,6,7,8-tetrahydroquinoxaline (CAS # 34413-35-9). 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-ethyl-3-methylpyrazine (CAS # 15707-23-0) was identified as a read-across analog with sufficient data for toxicological evaluation.

Conclusions

- 2-Ethyl-3-methylpyrazine (CAS # 15707-23-0) was used as a read-across analog for the target material, 5,6,7,8-tetrahydroquinoxaline (CAS # 34413-35-9), for the skin sensitization endpoint.
  - o The target material and the read-across analog are structurally similar and belong to the pyrazine functional group.
  - o The key difference between the target material and the read-across analog is that the target material has an attached cyclohexane ring, while the read-across analog has an ethyl and methyl group in the same positions. 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 a 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 Neither the target material nor the read-across analog has structural alerts for skin sensitization. The data on the read-across analog confirms that the material does not present a concern for skin sensitization. Therefore, based on the structural similarity between the target material and the read-across analog and the data on the read-across analog, *in silico* alerts are consistent with the data.
- 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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