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Short Review

RIFM fragrance ingredient safety assessment, 2,6-dimethylpyrazine, CAS registry number 108-50-9

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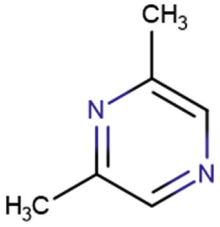

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Abbreviation/Definition List:
2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
CNIH – Confirmation of No Induction in Humans test. A human repeat insult patch test that is performed to confirm an already

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determined safe use level for fragrance ingredients (Na et al., 2021)
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015a, 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DRF - Dose Range Finding
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observed Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
Perfumery - In this safety assessment, perfumery refers to fragrances made by a perfumer used in consumer products only. The exposures reported in the safety assessment include consumer product use but do not include occupational exposures.
QRA - Quantitative Risk Assessment
QSAR - Quantitative Structure-Activity Relationship
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use
vPvB - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

The Expert Panel for Fragrance Safety* concludes that this material is safe as described in this safety assessment.

This safety assessment is based on the RIFM Criteria Document (Api et al., 2015), which should be referred to for clarifications.

Each endpoint discussed in this safety assessment includes the relevant data that were available at the time of writing (version number in the top box is indicative of the date of approval based on a 2-digit month/day/year), both in the RIFM Database (consisting of publicly available and proprietary data) and through publicly available information sources (e.g., SciFinder and PubMed). Studies selected for this safety assessment were based on appropriate test criteria, such as acceptable guidelines, sample size, study duration, route of exposure, relevant animal species, most relevant testing endpoints, etc. A key study for each endpoint was selected based on the most conservative endpoint value (e.g., PNEC, NOAEL, LOEL, and NESIL).

*The Expert Panel for Fragrance Safety is an independent body that selects its own members and establishes its own operating procedures. The Expert Panel is comprised of internationally known scientists that provide RIFM with guidance relevant to human health and environmental protection.

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Summary: The existing information supports the use of this material as described in this safety assessment. The material has not been evaluated for photoallergenicity.

2,6-Dimethylpyrazine was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, photoirritation/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analog 2,3,5-trimethylpyrazine (CAS # 14667-55-1) show that 2,6-dimethylpyrazine is not expected to be genotoxic. The repeated dose, reproductive, and local respiratory toxicity endpoints were evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class II material, and the exposure to 2,6-dimethylpyrazine is below the TTC (0.009 mg/kg/day, 0.009 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 2,6-dimethylpyrazine for skin sensitization under the current declared levels of use. The photoirritation endpoint was evaluated based on data; 2,6-dimethylpyrazine is not photoirritating. 2,6-Dimethylpyrazine has not been evaluated for photoallergenicity. The environmental endpoints were evaluated; 2,6-dimethylpyrazine 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.

Human Health Safety Assessment	
Genotoxicity: Not expected to be genotoxic.	(RIFM, 2016a; RIFM, 2016b)
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, 2017c; RIFM, 2017b)
Photoirritation/Photoallergenicity: Not photoirritating/not evaluated for photoallergy.	RIFM (2017a)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.	
Environmental Safety Assessment	
Hazard Assessment:	
Persistence: Screening-level: 2.8 (BIOWIN 3)	(EPI Suite v4.11; US EPA, 2012a)
Bioaccumulation: Screening-level: 3.16 (L/kg)	(EPI Suite v4.11; US EPA, 2012a)

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Ecotoxicity: Screening-level: Fish LC50: 1018 mg/L **Conclusion:** Not PBT or vPvB as per IFRA Environmental Standards	(RIFM Framework; Salvito et al., 2002)
Risk Assessment:	
Screening-level: PEC/PNEC (North America and Europe)	(RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: Fish LC50: 1018 mg/L	(RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 1018 μg/L	
• **Revised PEC/PNECs (2019 IFRA VoU):** North America and Europe: Not applicable; cleared at screening-level	

1. Identification

1. **Chemical Name:** 2,6-Dimethylpyrazine
2. **CAS Registry Number:** 108-50-9
3. **Synonyms:** 2,6-Dimethyl-1,4-diazine; 2,6-Dimethylparadiazine; 2,6-Dimethylpiazine; Pyrazine, 2,6-dimethyl-; 2,6-Dimethylpyrazine
4. **Molecular Formula:** $C_6H_8N_2$
5. **Molecular Weight:** 108.14 g/mol
6. **RIFM Number:** 6108
7. **Stereochemistry:** No stereocenter present and no stereoisomer possible.

2. Physical data

1. **Boiling Point:** 144 °C (Fragrance Materials Association [FMA]), 168.64 °C (EPI Suite)
2. **Flash Point:** 49 °C (Globally Harmonized System), 120 °F; closed cup (FMA)
3. **Log K_{OW}:** 1.03 (EPI Suite)
4. **Melting Point**: 41 °C (FMA), 25.52 °C (EPI Suite)
5. **Water Solubility:** 38160 mg/L (EPI Suite)
6. **Specific Gravity:** 0.920 (FMA)
7. **Vapor Pressure:** 1.11 mm Hg at 20 °C (EPI Suite v4.0), 4.0 mm Hg at 20 °C (FMA), 1.75 mm Hg at 25 °C (EPI Suite)
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 (2349, 4614, and 2238 L mol^{-1} • cm^{-1} under neutral, acidic, and basic conditions, respectively) are above the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** White crystals which have a sweet, "fried" odor, resembling that of fried potatoes.

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:** 0.039% (RIFM, 2020)
2. **Inhalation Exposure*:** 0.00010 mg/kg/day or 0.0067 mg/day (RIFM, 2020)
3. **Total Systemic Exposure**:** 0.00093 mg/kg/day (RIFM, 2020)

*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

1. **Cramer Classification:** Class II, Intermediate (Expert Judgment)

Expert Judgment	Toxtree v3.1	OECD QSAR Toolbox v4.5
II	III	III

*See the Appendix below for details.

2. Analogs Selected:
   a. **Genotoxicity:** 2,3,5-trimethylpyrazine (CAS # 14667-55-1)
   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
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

2,6-Dimethylpyrazine is reported to occur in the following foods by the VCF*:

Acerola (*Malpighia*)
Asparagus (*Asparagus officinalis* L.)
Beef
Beer
Cabbage (*Brassica oleracea*)
Cashew nut (*Anacardium occidentale*)
Cheese, various types
Chicken
Clam
Shrimps (prawn)

*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. This is a partial list.

9. REACH dossier

2,6-Dimethylpyrazine has been pre-registered for 2010; no dossier available as of 10/13/22.

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, 2,6-dimethylpyrazine does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. There are no studies assessing the mutagenic or clastogenic activity of 2,6-dimethylpyrazine; however, read-across can be made to 2,3,5-trimethylpyrazine (CAS # 14667-55-1; see Section VI).

The mutagenic activity of 2,3,5-trimethylpyrazine 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 2,3,5-trimethylpyrazine in water at concentrations up to 5000 μg/plate. Increases in the mean number of revertant colonies were observed in strain WP2uvrA in the presence or absence of S9 and in strain TA98 in the presence of S9 (RIFM, 2016a). However, the increases were not dose-responsive and were within the historical control limits. Therefore, the increases were considered to be not biologically relevant. Under the conditions of the study, 2,3,5-trimethylpyrazine was not mutagenic in the Ames test, and this can be extended to 2, 6-dimethylpyrazine.

The clastogenic activity of 2,3,5-trimethylpyrazine 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 2,3,5-trimethylpyrazine in water at concentrations up to 1220 μg/mL in the dose range finding (DRF) study; micronuclei analysis was conducted at concentrations up to 1220 μg/mL in the presence and absence of metabolic activation. 2,3,5-Trimethylpyrazine did not induce binucleated cells with micronuclei when tested up to the maximum concentration in either the presence or absence of an S9 activation system (RIFM, 2016b). Under the conditions of the study, 2,3, 5-trimethylpyrazine was considered to be non-clastogenic in the *in vitro* micronucleus test, and this can be extended to 2,6-dimethylpyrazine.

Based on the data available2,3,5-trimethylpyrazine does not present a concern for genotoxic potential, and this can be extended to 2,6-dimethylpyrazine.

Additional References: Stich et al., 1981.

Literature Search and Risk Assessment Completed On: 05/20/22.

11.1.2. Repeated dose toxicity

There are no repeated dose toxicity data on 2,6-dimethylpyrazine or any read-across materials. The total systemic exposure to 2,6-dimethylpyrazine is below the TTC for the repeated dose toxicity endpoint of a Cramer Class II material at the current level of use.

11.1.2.1. Risk assessment. There are no repeated dose toxicity data on 2,6-dimethylpyrazine or any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to 2,6-dimethylpyrazine (0.93 μg/kg/day) is below the TTC for the repeated dose toxicity endpoint of a Cramer Class II material (9 μg/kg/day; Kroes et al., 2007) at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 03/15/22.

11.1.3. Reproductive toxicity

There are no reproductive toxicity data on 2,6-dimethylpyrazine or any read-across materials. The total systemic exposure to 2,6-dimethylpyrazine is below the TTC for the reproductive toxicity endpoint of a Cramer Class II material at the current level of use.

11.1.3.1. Risk assessment. There are no reproductive toxicity data on 2,6-dimethylpyrazine or any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to 2,6-dimethylpyrazine (0.93 μg/kg/day) is below the TTC for the reproductive toxicity endpoint of a Cramer Class II material (9 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 03/15/22.

11.1.4. Skin sensitization

Based on existing data on the target material and read-across material 2-ethyl-3-methylpyrazine (CAS # 15707-23-0), 2,6-dimethylpyrazine presents no concern for skin sensitization.

11.1.4.1. Risk assessment. Limited skin sensitization data are available for 2,6-dimethylpyrazine. Therefore, read-across material 2-ethyl-3-methylpyrazine (CAS # 15707-23-0; see Section VI) was used for the risk assessment of 2,6-dimethylpyrazine. The data on the read-across material are summarized in Table 1 below (Table 1). Based on the existing data on the read-across material, 2,6-dimethylpyrazine is not considered a skin sensitizer. The chemical structure of the read-across material and the target material indicate that they would not be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.5). 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, 2017c; RIFM, 2017b).

Based on the weight of evidence (WoE) from structural analysis and *in vitro* and animal studies on the read-across material as well as the target material, 2,6-dimethylpyrazine does not present a concern for skin sensitization.

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/16/22.

11.1.5. Photoirritation/photoallergenicity

Based on the available *in vitro* study data, 2,6-dimethylpyrazine would not be expected to present a concern for photoirritation. 2,6-dimethylpyrazine has not been evaluated for photoallergenicity; however, RIFM is sponsoring an *in vitro* photoallergy research program to evaluate the photoallergy potential of 2,6-dimethylpyrazine.

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 432), 2,6-dimethylpyrazine was not predicted to have photoirritating potential (RIFM, 2017a). Based on the available *in vitro* study data, 2,6-dimethylpyrazine would not be expected to present a concern for photoirritation. 2,6-Dimethylpyrazine was not evaluated for photoallergy; however, RIFM is sponsoring an *in vitro* photoallergy research program to evaluate the photoallergy potential of 2,6-dimethylpyrazine.

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

	Human Data				Animal Data			
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]	
	NA	NA	NA	NA	NA	NA	NA	
	In vitro Data[6]				*In silico* protein binding alerts (OECD Toolbox v4.5)			
No evidence of sensitization[7]	KE 1	KE 2	KE 3		Target Material	Autoxidation simulator	Metabolism simulator	
	Negative	Negative	Negative		No alert found	No alert found	No alert found	

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

[1]WoE Skin Sensitization Potency Category is only applicable for identified sensitizers with sufficient data, based on collective consideration of all available data (Na et al., 2021).

[2]Data derived from CNIH or HMT

[3]WoE NESIL limited to 2 significant figures

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

Research Institute for Fragrance Materials, Inc.

[5]Studies conducted according to the OECD TG 406 are included in the table.

[6]Studies conducted according to the OECD TG 442, Cottrez et al. (2016), or Forreryd et al. (2016) are included in the table.

[7]Determined based on Criteria for the Research Institute for Fragrance Materials, Inc. (RIFM) safety evaluation process for fragrance ingredients (Api et al., 2015).

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 (2349, 4614, and 2238 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: 05/23/22.

11.1.6. Local respiratory toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 2,6-Dimethylpyrazine 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 2,6-dimethylpyrazine. Based on the Creme RIFM Model, the inhalation exposure is 0.0067 mg/day. This exposure is 70.1 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.

*As per Carthew et al. (2009), Cramer Class II materials default to Cramer Class III for the local respiratory toxicity endpoint.

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/19/22.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 2,6-dimethylpyrazine 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. 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, 2,6-dimethylpyrazine 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 2,6-dimethylpyrazine as possibly being 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 ≥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).

11.2.1.1. Risk assessment. Based on the current VoU (2019), 2,6-dimethylpyrazine presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2. Key studies

11.2.2.1. Biodegradation. No data available.

11.2.2.2. Ecotoxicity. No data available.

11.2.2.3. Other available data. 2,6-Dimethylpyrazine has been pre-registered for REACH with no additional data at this time.

11.2.2.4. 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	1.03	1.03
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional VoU Tonnage Band	<1	<
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 1018 μg/L. The revised PEC/PNECs for EU and NA are <1; therefore, the material does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 05/16/22.

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 10/13/22.

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

	LC50 (Fish)	EC50 (*Daphnia*)	EC50 (Algae)	AF	PNEC	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	1018 mg/L			1000000	1.018 μg/L	

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.2023.113600.

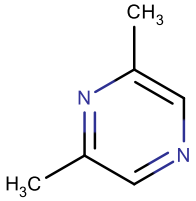
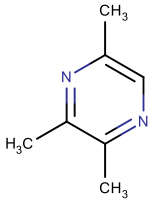
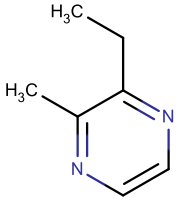
Appendix

Read-across Justification

Methods

The read-across analogs were 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 the OECD QSAR Toolbox v4.5 (OECD, 2021b).
- ER binding and repeat dose categorization were generated using the 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 the OECD QSAR Toolbox v4.5 (OECD, 2021b).
- To keep continuity and compatibility with *in silico* alerts, OECD QSAR Toolbox v4.5 was selected as the alert system.

	Target Material	Read-across Material	Read-across Material
Principal Name	2,6-Dimethylpyrazine	2,3,5-Trimethylpyrazine	2-Ethyl-3-methylpyrazine
CAS No.	108-50-9	14667-55-1	15707-23-0
Structure			
Similarity (Tanimoto Score)		0.86	0.62
SMILES	Cc1cncc(C)n1	Cc1cnc(C)c(C)n1	CCc1nccnc1C
Endpoint		Genotoxicity	Skin sensitization
Molecular Formula	$C_6H_8N_2$	$C_7H_{10}N_2$	$C_7H_{10}N_2$
Molecular Weight (g/mol)	108.144	122.171	122.171
Melting Point (°C, EPI Suite)	47.50	20.23	17.11
Boiling Point (°C, EPI Suite)	155.60	171.50	189.48
Vapor Pressure (Pa @ 25°C, EPI Suite)	2.33E+02	1.93E+02	8.11E+01
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	3.82E+04	1.52E+04	1.20E+04
Log KOW	0.54	0.95	1.07
J_{max} (μg/cm^2/h, SAM)	223.40	123.13	119.05
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	3.60E-01	3.97E-01	4.78E-01
Genotoxicity			
DNA Binding (OASIS v1.4, QSAR Toolbox v4.5)	No alert found	No alert found	
DNA Binding (OECD QSAR Toolbox v4.5)	No alert found	No alert found	
Carcinogenicity (ISS)	No alert found	No alert found	
DNA Binding (Ames, MN, CA, OASIS v1.1)	No alert found	No alert found	
***In Vitro* Mutagenicity (Ames, ISS)**	No alert found	No alert found	
***In Vivo* Mutagenicity (Micronucleus, ISS)**	H-acceptor-path3-H-acceptor		

(*continued on next page*)

(*continued*)

	Target Material	Read-across Material	Read-across Material
		H-acceptor-path3-H-acceptor	
Oncologic Classification	Not classified	Not classified	
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	See Supplemental Data 3

Summary

There are insufficient toxicity data on 2,6-Dimethylpyrazine (CAS # 108-50-9). 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, 2,3,5-trimethylpyrazine (CAS # 14667-55-1) and 2-ethyl-3-methylpyrazine (CAS # 15707-23-0) were identified as read-across analogs with sufficient data for toxicological evaluation.

Conclusions

- 2,3,5-Trimethylpyrazine (CAS # 14667-55-1) was used as a read-across analog for the target material, 2,6-dimethylpyrazine (CAS # 108-50-9), for the genotoxicity endpoint.
  - o The target material and the read-across analog are structurally similar and belong to the pyrazine group.
  - o The key difference between the target material and the read-across analog is an additional methyl substituent in the read-across analog. 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 v4.5, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - 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.
- 2,3-Dimethylpyrazine (CAS # 5910-89-4) was used as a read-across analog for the target material, 2,6-Dimethylpyrazine (CAS # 108-50-9), for the skin sensitization endpoint.
  - o The target material and the read-across analog are structurally similar and belong to the pyrazine group.
  - o The key difference between the target material and the read-across analog is the ethyl group substituent at position 2 in the read-across analog while the target material has a methyl group in this position and the presence of a methyl group at position 3 in the read-across analog while the target material has a methyl group at position 6. These structural differences are 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 v4.5, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - 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.

Explanation of Cramer Classification

Due to potential discrepancies with the current *in silico* tools (Bhatia et al., 2015), the Cramer class of the target material was determined using expert judgment based on the Cramer decision tree (Cramer et al., 1978).

Q1. Normal constituent of the body? No.
Q2. Contains functional groups associated with enhanced toxicity? No.
Q3. Contains elements other than C, H, O, N, and divalent S? No.
Q5. Simply branched aliphatic hydrocarbon or a common carbohydrate? No.
Q6. Benzene derivative with certain substituents? No.
Q7. Heterocyclic? Yes.
Q8. Lactone or cyclic diester? No.

Q10. 3-membered heterocycles? No.
Q11. Has a heterocyclic ring with complex substituents? No.
Q12. Heteroaromatic? Yes.
Q13. Does the ring bear any substituents? Yes.
Q14. More than one aromatic ring? No.
Q22. A common component of food? Yes, Class Intermediate (Class II)

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