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Short Review

RIFM fragrance ingredient safety assessment, isobutyric acid, CAS Registry Number 79-31-2

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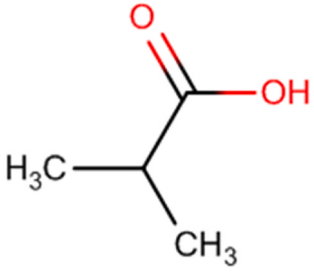

Abbreviation/Definition List:

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

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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., 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 Observable Effect Level
MOE - Margin of Exposure

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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
QRA - Quantitative Risk Assessment
QSAR - Quantitative Structure-Activity Relationship
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food

VoU - Volume of Use **vPvB** - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

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

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

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

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

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

Isobutyric acid was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that isobutyric acid is not genotoxic. Data on read-across analog propionic acid (CAS # 79-09-4) provide a calculated MOE >100 for the repeated dose toxicity and fertility endpoints. Data on read-across analog isovaleric acid (CAS # 503-74-2) provide a calculated MOE >100 for the developmental toxicity endpoint. The skin sensitization endpoint was completed using the DST for non-reactive materials (900 μg/cm^2); exposure is below the DST. The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; isobutyric acid is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was evaluated using the TTC for a Cramer Class I material, and the exposure to isobutyric acid is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; isobutyric acid was found not to be PBT as per the 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, 1982; RIFM, 2014)
Repeated Dose Toxicity: NOAEL = 1832 mg/kg/day.	OECD (2007)
Reproductive Toxicity: Developmental toxicity: 600 mg/kg/day. Fertility: 1832 mg/kg/day.	(ECHA REACH Dossier: Isovaleric Acid; ECHA, 2015; OECD, 2007)
Skin Sensitization: No safety concerns at current, declared use levels; Exposure is below the DST.	
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic.	(UV Spectra; RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.	

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Environmental Safety Assessment

Hazard Assessment:	
Persistence:	
Screening-level: 3.37 (BIOWIN 3)	(EPI Suite v4.11; US EPA, 2012a)
Bioaccumulation:	
Screening-level: 3.162 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: Fish LC50: 881 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: 881 mg/L	(RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.881 μg/L	

- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: not applicable; cleared at screening-level

1. Identification

1. **Chemical Name:** Isobutyric acid
2. **CAS Registry Number:** 79-31-2
3. **Synonyms:** Isopropylformic acid; 2-Methylpropanoic acid; Propanoic acid, 2-methyl-,; Dimethylacetic acid; Isobutanoic acid; Propanoic acid, 2-methyl-; ｱﾙｶﾝ酸(C = 4 ~ 30); Isobutyric acid
4. **Molecular Formula:** $C_4H_8O_2$
5. **Molecular Weight:** 88.1
6. **RIFM Number:** 6082
7. **Stereochemistry:** No stereocenter present and no stereoisomer possible.

2. Physical data

1. **Boiling Point:** 154 °C (Fragrance Materials Association [FMA]), 153.79 °C (EPI Suite)
2. **Flash Point:** 134 °F; CC (FMA), 62 °C (Globally Harmonized System)
3. **Log K_{OW}:** 1 (EPI Suite)
4. **Melting Point**: −8.29 °C (EPI Suite)
5. **Water Solubility:** 49180 mg/L (EPI Suite)
6. **Specific Gravity:** 0.946 (FMA)
7. **Vapor Pressure:** 2.35 mm Hg @ 20 °C (EPI Suite v4.0), 3.27 mm Hg @ 25 °C (EPI Suite)
8. **UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} · cm^{-1})
9. **Appearance/Organoleptic:** Colorless oily liquid with a pungent, strong, penetrating, rancid odor

3. Volume of use (worldwide band)

1. 0.1–1 metric ton per year (IFRA, 2015)

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

1. **95**th **Percentile Concentration in Hydroalcoholics:** 0.0039% (RIFM, 2017)
2. **Inhalation Exposure*:** 0.000031 mg/kg/day or 0.0022 mg/day (RIFM, 2017)
3. **Total Systemic Exposure**:** 0.00071 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., 2015a; 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., 2015a; 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 I, Low

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

2. Analogs Selected:
   a. **Genotoxicity:** None
   b. **Repeated Dose Toxicity:** Propionic acid (CAS # 79-09-4)
   c. **Reproductive Toxicity:** Propionic acid (CAS # 79-09-4); isovaleric acid (CAS # 503-74-2)
   d. **Skin Sensitization:** None
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. Read-across Justification: See Appendix below

7. Metabolism

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

8. Natural occurrence (discrete chemical) or composition (NCS)

Isobutyric acid is reported to occur in the following foods by the VCF*:

Apple brandy (Calvados)
Apple fresh (*Malus* species)
Apple processed (*Malus* species)
Apricot (*Prunus armeniaca* L.)
Arctic bramble (*Rubus arcticus* 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

Available; accessed 08/09/19.

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, isobutyric acid does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. The mutagenic activity of isobutyric acid has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and equivalent OECD TG 471 using the standard plate incorporation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 were treated with isobutyric acid in dimethyl sulfoxide (DMSO) at concentrations up to 150 μL/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 1982). Under the conditions of the study, isobutyric acid was not mutagenic in the Ames test.

The clastogenic activity of isobutyric acid 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 isobutyric acid in minimum essential medium at concentrations up to 880 μg/mL in the dose range finding (DRF) study; micronuclei analysis was conducted at concentrations up to 880 μg/mL in the presence and absence of metabolic activation. Isobutyric acid 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, 2014). Under the conditions of the study, isobutyric acid was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the data available, isobutyric acid does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/17/19.

11.1.2. Repeated dose toxicity

The MOE for isobutyric acid is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are no repeated dose toxicity data on isobutyric acid. Read-across material propionic acid (CAS 79-09-4; see section VI) has sufficient data to support the repeated dose toxicity endpoint. A 90-day dietary study was conducted on groups of 20 Sprague Dawley rats/sex. The animals were treated with 0%, 0.62%, 1.25%, 2.5%, or 5% propionic acid in a pulverized diet for 91 days. The concentrations are equal to approximately 0, 312, 625, 1250, or 2500 mg/kg/day (as per the conversion factors for old rats, available in the JECFA guidelines for the preparation of toxicological working papers on food additives). In parallel, 10 animals were included in the control, 0.62%, and 5% groups assigned to the post-exposure recovery groups for respective doses and fed the control diet for 6 weeks. There was a 12% decrease in the relative kidney weights among high-dose males. In high-dose females, there were 5% and 9% increases in the relative weights of the heart and liver, respectively. Examination of tissues revealed no lesions except local changes of the mucosa of the forestomach in rats in the 5% treatment group, which included acanthosis, hyperkeratosis, and proliferation of the epithelium. The changes observed in the forestomach were not observed in the recovery group, and there were no differences in the relative or absolute organ weights. There were no adverse effects on the reproductive organs. The forestomach is a species-specific organ and is not found among humans; therefore, the effects observed in the rat forestomach were considered to be of no relevance to humans. In addition, since the changes in the liver and kidney weights were not associated with any histopathological alterations, they were

not considered to be adverse. The NOAEL for systemic toxicity was considered to be 5% or 2500 mg/kg/day, the highest dose tested (OECD, 2007; ECHA, 2011b).

In an OECD 409 study, propionic acid was fed in the diet to groups of 8 male and 8 female beagle dogs for approximately 100 days. The dogs received 0%, 0.3%, 1.0%, or 3.0% propionic acid (0, 196, 660, and 1848 mg/kg/day for males and 0, 210, 696, and 1832 mg/kg/day for females) in the diet. An additional 8 animals (4/sex) were assigned to the control and high-dose groups to be maintained for an additional 6-week recovery interval. There were no effects of treatment on the dogs, except for local diffuse epithelial hyperplasia of the mucosa of the esophagus in 3 dogs in the highest-dose group. At the end of the recovery interval, the incidence of lesions of the esophagus was the same in the control and high-dose group animals. The incidence of focal epithelial hyperplasia in lower-dose animals was comparable to controls. The NOAEL for systemic toxicity was considered to be 3% propionic acid (1848 mg/kg/day for males and 1832 mg/kg/day for females) in the diet, the highest dose tested (OECD, 2007; ECHA, 2011b).

The most conservative NOAEL for the repeated dose toxicity endpoint was considered to be 1832 mg/kg/day, from the study conducted on dogs.

Therefore, the propionic acid MOE for the repeated dose toxicity endpoint can be calculated by dividing the propionic acid NOAEL in mg/kg/day by the total systemic exposure to isobutyric acid, 1832/0.00071 or 2580282.

In addition, the total systemic exposure to isobutyric acid (0.71 μg/kg/day) is below the TTC (30 μg/kg/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: 09/04/19.

11.1.3. Reproductive toxicity

The MOE for isobutyric acid is adequate for the fertility and developmental toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are no fertility or developmental toxicity data on isobutyric acid. Read-across material isovaleric acid (CAS # 503-74-2) and propionic acid (CAS 79-09-4) (see section VI) has sufficient data to support the endpoint.

There are sufficient developmental toxicity data on isovaleric acid that can be used to support the developmental toxicity endpoint. In a developmental toxicity study (GLP-compliant and similar to OECD 414), which was performed on Wistar rats (10/group), isovaleric acid (purity: 99.9%) was administered through oral gavage at a dose level of 600 mg/kg/day. All animals were treated during gestation period days 6–19. Salivation in all dams after treatment and local irritation of the larynx and upper and lower respiratory tract were reported; these findings were significant but not severe. No treatment-related effects were reported in any parameter for both dams and fetuses. The NOAEL for maternal toxicity, developmental toxicity, and teratogenicity was considered to be 600 mg/kg/day, based on the absence of any treatment-related effects (ECHA, 2015). Since this was a single-dose study with no treatment-related adverse effects observed, a NOAEL of 600 mg/kg/day was considered for the developmental toxicity endpoint.

There are sufficient fertility data on propionic acid. A 90-day dietary study was conducted on groups of 20 Sprague Dawley rats/sex. The animals were treated with 0%, 0.62%, 1.25%, 2.5%, or 5% propionic acid in a pulverized diet for 91 days. The concentrations are equal to approximately 0, 312, 625, 1250, or 2500 mg/kg/day (as per the conversion factors for old rats, available in the JECFA guidelines for the preparation of toxicological working papers on food additives). There were no effects of propionic acid treatment on the male or female reproductive organ weights or histopathology up to the highest dose tested. The NOAEL for fertility effects was considered to be 5% or 2500 mg/kg/day (OECD, 2007; ECHA, 2011b).

In an OECD 409 study, propionic acid was fed in the diet to groups of 8 male and female beagle dogs for approximately 100 days. The dogs received 0%, 0.3%, 1.0%, or 3.0% propionic acid (0, 196, 660, and 1848 mg/kg/day for males and 0, 210, 696, and 1832 mg/kg/day for females) in the diet. An additional 8 animals (4/sex) were assigned to the control and high-dose groups to be maintained for an additional 6-week recovery interval. There were no significant changes in the relative or absolute weight of the testes or ovaries in the treatment group animals relative to controls, and there were no histopathological alterations in the male and female reproductive organs in animals fed propionic acid in the diet for 90 days. The NOAEL for fertility effects was considered to be 3% propionic acid (1848 mg/kg/day for males and 1832 mg/kg/day for females) in the diet, the highest dose tested (OECD, 2007; ECHA, 2011b). The most conservative NOAEL of 1832 mg/kg/day from female dogs was considered for fertility effects.

Therefore, the isobutyric acid MOE for the developmental toxicity endpoint can be calculated by dividing the isovaleric acid NOAEL in mg/kg/day by the total systemic exposure to isobutyric acid, 600/0.00071, or 845070.

Therefore, the isobutyric acid MOE for the fertility endpoint can be calculated by dividing the propionic acid NOAEL in mg/kg/day by the total systemic exposure to isobutyric acid, 1832/0.00071, or 2580282.

In addition, the total systemic exposure to isobutyric acid (0.71 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012**) for the fertility and developmental toxicity endpoint of a Cramer Class I material at the current level of use.**

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/14/19.

11.1.4. Skin sensitization

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

11.1.4.1. 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 3.1.0; OECD Toolbox v4.3). No predictive skin sensitization studies are available for isobutyric acid. No predictive tests in animals were found for this material, and also there were no confirmatory human studies available. Due to insufficient data, the reported exposure was benchmarked utilizing the non-reactive DST of 900 μg/cm^2 (Safford, 2008; Safford et al., 2011; Roberts et al., 2015; Safford et al., 2015b). 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 maximum acceptable concentrations for isobutyric acid that present no appreciable risk for skin sensitization based on the non-reactive DST. These levels represent maximum acceptable concentrations based on the DST approach. However, additional studies may show it could be used at higher levels.

Additional References: None.

Literature Search and Risk Assessment Completed On: 09/13/19.

11.1.5. Phototoxicity/photoallergenicity

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

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

Table 1
Maximum acceptable concentrations for isobutyric acid that present no appreciable risk for skin sensitization based on non-reactive DST.

IFRA Category[a]	Description of Product Type	Maximum Acceptable Concentrations in Finished Products Based on Non-reactive DST	Reported 95th Percentile Use Concentrations in Finished Products
1	Products applied to the lips	0.069%	NRU[b]
2	Products applied to the axillae	0.021%	0.0066%
3	Products applied to the face using fingertips	0.41%	0.0024%
4	Fine fragrance products	0.39%	0.0038%
5	Products applied to the face and body using the hands (palms), primarily leave-on	0.10%	0.0043%
6	Products with oral and lip exposure	0.23%	0.010%
7	Products applied to the hair with some hand contact	0.79%	0.0022%
8	Products with significant ano-genital exposure	0.041%	No Data[c]
9	Products with body and hand exposure, primarily rinse-off	0.75%	0.012%
10	Household care products with mostly hand contact	2.7%	0.0088%
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate	1.5%	No Data[c]
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted	0.5%

[a]Note.
[a] For a description of the categories, refer to the IFRA/RIFM Information Booklet.
[b] No reported use.
[c] Fragrance exposure from these products is very low. These products are not currently in the Creme RIFM Aggregate Exposure Model.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate no significant 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: 08/13/19.

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for isobutyric acid is below the Cramer Class I TTC value for inhalation exposure local effects.

11.1.6.1. Risk assessment. There are limited inhalation data available on isobutyric acid. Based on the Creme RIFM Model, the inhalation exposure is 0.0022 mg/day. This exposure is 636.4 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: Smyth et al., 1962; ECHA, 2011a.

Literature Search and Risk Assessment Completed On: 09/11/19.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of isobutyric acid was performed following the RIFM Environmental Framework (Salvito et al., 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log K_{OW}, and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity, as discussed in Salvito et al. (2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 2012b), which provides chemical class-specific ecotoxicity estimates. Finally, if necessary, Tier 3 is conducted using measured biodegradation and ecotoxicity data to refine the RQ, thus allowing for lower PNEC uncertainty factors. The data for calculating the PEC and PNEC for this safety assessment are provided in the table below. For the PEC, the range from the most recent IFRA Volume of Use Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, isobutyric 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 v4.11 (US EPA, 2012a) did not identify isobutyric acid 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, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF $\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 Volume of Use (2015), isobutyric acid presents no risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Key studies

11.2.2.1.1. Biodegradation. No data available.

11.2.2.1.2. Ecotoxicity. No data available.

11.2.2.1.3. Other available data. Isobutyric acid has been registered for REACH with following additional data available at this time:

The short term fish (*Leuciscus idus*) toxicity test was conducted according to the DIN 38 412, part L15 guidelines under static conditions. The 96-h LC50 value based on nominal concentrations was reported to be 146.6 mg/L.

The *Daphnia* acute immobilization test was conducted according to the DIN 38 412, part 11 guidelines under static conditions. The 48-h EC50 value based on nominal concentrations was reported to be 51.25 mg/L (95% CI: 38.28–64.22 mg/L).

The algae growth inhibition test was conducted according to the DIN 38 412, part 9 guidelines under static conditions. The 72-h EC50 value based on nominal concentrations was reported to be 44.7 mg/L for cell

density recorded by fluorescence (ECHA, 2011a).

11.2.3. Risk assessment refinement

Since isobutyric acid has passed the screening criteria, measured data is included for completeness only and has not been used in PNEC derivation.

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

Endpoints used to calculate PNEC are underlined.

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

Exposure	Europe (EU)	North America (NA)
Log K_{OW} Used	1	1
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 available data, the RQ for this material is < 1. No further assessment is necessary.

The RIFM PNEC is 0.881 μg/L. The revised PEC/PNECs for EU and NA are not applicable. The material was cleared at the screening-level; therefore, it does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 10/21/19.

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**
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
- **National Library of Medicine's Toxicology Information Services:** https://toxnet.nlm.nih.gov/
- **IARC:** https://monographs.iarc.fr
- **OECD SIDS:** https://hpvchemicals.oecd.org/ui/Default.aspx
- **EPA ACToR:** https://actor.epa.gov/actor/home.xhtml
- **US EPA HPVIS:** https://ofmpub.epa.gov/oppthpv/public_search.publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&EndPointRpt=Y#submission
- **Japanese NITE:** https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com
- **ChemIDplus:** https://chem.nlm.nih.gov/chemidplus/

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 01/31/20.

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.2020.111673.

Appendix

Read-across Justification

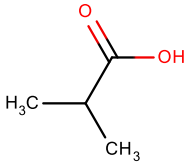
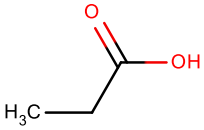
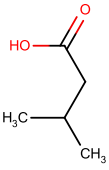
Methods

The read-across analogs were identified following the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015). The strategy is also consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	881			1000000	0.881	

(OECD, 2015) and the European Chemicals Agency read-across assessment framework (ECHA, 2016).

- First, materials were clustered based on their structural similarity. Second, data availability and data quality on the selected cluster were examined. Third, appropriate read-across analogs from the cluster were confirmed by expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physical–chemical properties of the target material and the read-across analogs were calculated using EPI Suite v4.11 (US EPA, 2012a).
- J_{max} values were calculated using RIFM's Skin Absorption Model (SAM). The parameters were calculated using the consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts, and oncologic classification predictions were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010).
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018), and skin sensitization was predicted using Toxtree.
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).

	Target Material	Read-across Material	Read-across Material
Principal Name	Isobutyric acid	Propionic acid	Isovaleric acid
CAS No.	79-31-2	79-09-4	503-74-2
Structure			
Similarity (Tanimoto Score)		0.81	0.61
Read-across Endpoint		• Repeated Dose Toxicity • Reproductive Toxicity	• Reproductive Toxicity
Molecular Formula	$C_4H_8O_2$	$C_3H_6O_2$	$C_5H_{10}O_2$
Molecular Weight	88.10	74.07	102.13
Melting Point (°C, EPI Suite)	−46.0	−21.1	−29.3
Boiling Point (°C, EPI Suite)	154.4	141.1	176.5
Vapor Pressure (Pa @ 25°C, EPI Suite)	2.41E+02	4.71E+02	5.87E+01
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	0.94	0.33	1.16
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	1.67E+05	1.00E+06	4.07E+04
J_{max} (μg/cm^2/h, SAM)	3228.89	10127.816	785.313
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	8.97E-02	4.51E-02	8.44E-02
Repeated Dose Toxicity			
Repeated Dose (HESS)	• Carboxylic acids (Hepatotoxicity) No rank	• Carboxylic acids (Hepatotoxicity) No rank| Glycolic acid (Renal Toxicity) Alert	
Reproductive Toxicity			
ER Binding (OECD QSAR Toolbox v4.2)	• Non-binder, non-cyclic structure	• Non-binder, non-cyclic structure	• Non-binder, non-cyclic structure
Developmental Toxicity (CAESAR v2.1.6)	• Toxicant (low reliability)	• Toxicant (low reliability)	• Toxicant (good reliability)
Metabolism			
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	• No metabolites	• No metabolites	• See Supplemental Data 1

Summary

There are insufficient toxicity data on isobutyric acid (CAS # 79-31-2). 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, propionic acid (CAS # 79-09-4) and isovaleric acid (CAS # 503-74-2) were identified as read-across analogs with sufficient data for toxicological evaluation.

Conclusions

- Propionic acid (CAS # 79-09-4) was used as a read-across analog for the target material isobutyric acid (CAS # 79-31-2) for the repeated dose toxicity and reproductive toxicity endpoints.
  - o The target material and the read-across analog are structurally similar and belong to a class of aliphatic acids.
  - o The target material and the read-across analog share a carboxylic acid functionality.
  - o The key difference between the target material and the read-across analog is that the target material is a branched isobutyric acid, whereas the read-across analog is a straight chain propionic acid. 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.

- The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
- According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
- Both the target material and read-across analog have a repeated dose (HESS) alert for carboxylic acids (hepatotoxicity). Some carboxylic acids induce adverse effects in the liver. The data described in the repeated dose toxicity section show that the MOE is adequate at the current level of use. The read-across analog has an additional alert because of its structural similarity with glycolic acid. This alert can be ignored because propionic acid is not part of the training set for such alerts. Therefore, the predictions are superseded by the data.
- Both the target material and the read-across analog are classified as toxicants within the developmental toxicity (CAESAR) classification scheme. The data described in the reproductive toxicity section show that the MOE is adequate at the current level of use. The predictions are superseded by the data.
- The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
- The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

- Isovaleric acid (CAS # 503-74-2) was used as a read-across analog for the target material isobutyric acid (CAS # 79-31-2) for the reproductive toxicity endpoint.
  - The target material and the read-across analog are structurally similar and belong to a class of branched aliphatic acids.
  - The target material and the read-across analog share a carboxylic acid functionality.
  - The key difference between the target material and the read-across analog is that the target material is a C4 acid, whereas the read-across analog is a C5 acid. This structural difference is toxicologically insignificant.
  - 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.
  - The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - Both the target material and the read-across analog are classified as toxicants within the developmental toxicity (CAESAR) classification scheme. The data described in the reproductive toxicity section show that the MOE is adequate at the current level of use. The predictions are superseded by the data.
  - The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

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