Food and Chemical Toxicology 169 (2022) 113489

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Short Review

RIFM fragrance ingredient safety assessment, isobutyl isobutyrate, CAS Registry Number 97-85-8

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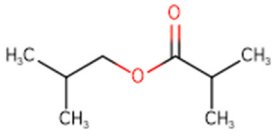
Version: 060922. Initial publication. All fragrance materials are evaluated on a five-year rotating basis. Revised safety assessments are published if new relevant data become available. Open access to all RIFM Fragrance Ingredient Safety Assessments is here: fragrancematerialsafetyresource.elsevier.com.

Name: Isobutyl isobutyrate CAS Registry Number: 97-85-8



Abbreviation/Definition List:

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

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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)
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

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https://doi.org/10.1016/j.fct.2022.113489
Received 15 June 2022; Accepted 13 October 2022
Available online 18 October 2022

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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.

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

Isobutyl isobutyrate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, photoirritation/photoallergenicity, skin sensitization, and environmental safety. Data show that isobutyl isobutyrate is not genotoxic. Data on read-across analog isoamyl isovalerate (CAS # 659-70-1) provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and reproductive toxicity endpoints. Data show that there are no safety concerns for isobutyl isobutyrate for skin sensitization under the current declared levels of use. The photoirritation/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; isobutyl isobutyrate is not expected to be photoirritating/photoallergenic. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material, and the exposure to isobutyl isobutyrate is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; isobutyl isobutyrate 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 genotoxic.	(ECHA REACH Dossier: Isobutyl isobutyrate; ECHA, 2018)

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Repeated Dose Toxicity: NOAEL = 267 mg/kg/day.	RIFM (2017)
Reproductive Toxicity: NOAEL = 267 mg/kg/day.	RIFM (2017)
Skin Sensitization: No concern for skin sensitization.	(ECHA REACH Dossier: Isobutyl isobutyrate; ECHA, 2018)

Photoirritation/Photoallergenicity: Not expected to be photoirritating/photoallergenic.
(UV/Vis Spectra; RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:

Persistence:	
Critical Measured Value: 79% (OECD 301F)	RIFM (2012)
Bioaccumulation:	
Screening-level: 27.31 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: 96-h Algae EC50: 5.692 mg/L	(EPI Suite v4.11; US EPA, 2012a)

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: 96-h Algae EC50: 5.692 mg/L	(EPI Suite v4.11; US EPA, 2012a)

RIFM PNEC is: 0.5692 μg/L
- **Revised PEC/PNECs (2019 IFRA VoU):** North America and Europe <1

1. Identification

1. **Chemical Name:** Isobutyl isobutyrate
2. **CAS Registry Number:** 97-85-8
3. **Synonyms:** Isobutyl 2-methylpropanoate; 2-Methyl-1-propyl 2-methylpropanoate; Propanoic acid, 2-methyl-, 2-methylpropyl ester; ブタン酸アルキル（C = 1 ~ 7）; Isobutyl isobutyrate
4. **Molecular Formula:** $C_8H_{16}O_2$
5. **Molecular Weight:** 144.21 g/mol
6. **RIFM Number:** 6094
7. **Stereochemistry:** Stereoisomer not specified. No stereocenter present and no stereoisomers possible.

2. Physical data

1. **Boiling Point:** 143.81 °C (EPI Suite)
2. **Flash Point:** 34 °C (Globally Harmonized System), 93 °F; closed cup (Fragrance Materials Association [FMA])
3. **Log K_{OW}:** 2.68 (EPI Suite)
4. **Melting Point**: −55.3 °C (EPI Suite)
5. **Water Solubility:** 412.1 mg/L (EPI Suite)
6. **Specific Gravity:** 0.85 (FMA)
7. **Vapor Pressure:** 3.09 mm Hg at 20 °C (EPI Suite v4.0), 3.0 mm Hg at 20 °C (FMA), 4.28 mm Hg at 25 °C (EPI Suite)
8. **UV Spectra:** No absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** Colorless liquid, which has a sweet-fruity, but also rather harsh pineapple-like, diffusive-ethereal odor.

3. Volume of use (Worldwide band)

1. 10–100 metric tons per year (IFRA, 2019)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v3.2.6)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.015% (RIFM, 2022)

2. **Inhalation Exposure*:** 0.000072 mg/kg/day or 0.0052 mg/day (RIFM, 2022)
3. **Total Systemic Exposure**:** 0.00075 mg/kg/day (RIFM, 2022)

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

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

6.2. Analogs selected

a. **Genotoxicity:** None
b. **Repeated Dose Toxicity:** Isoamyl isovalerate (CAS # 659-70-1)
c. **Reproductive Toxicity:** Isoamyl isovalerate (CAS # 659-70-1)
d. **Skin Sensitization:** None
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

Isobutyl isobutyrate is reported to occur in the following foods by the VCF*:

Apple fresh (*Malus* species).
Banana (*Musa sapientum* L.)
Camomile.
Mangifera species.
Melon.
Olive (*Olea europaea*).
Quince, marmelo (*Cydonia oblonga* Mill.)
Sherry.
Strawberry (*Fragaria* species).
Wine.

*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

Available; accessed 06/09/22 (ECHA, 2018).

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, isobutyl isobutyrate does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. Isobutyl isobutyrate was assessed in the BlueScreen assay and found negative for both cytotoxicity (positive: <80% relative cell density) and genotoxicity, with and without metabolic activation (RIFM, 2013). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. Additional assays were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

The mutagenic activity of isobutyl isobutyrate 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 isobutyl isobutyrate 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 (ECHA, 2018). Under the conditions of the study, isobutyl isobutyrate was not mutagenic in the Ames test.

The clastogenicity of isobutyl isobutyrate was assessed in an *in vitro* chromosome aberration study conducted in compliance with GLP regulations and in accordance with OECD TG 473. Human peripheral blood lymphocytes were treated with isobutyl isobutyrate in DMSO at concentrations up to 600 μg/mL in the presence and absence of metabolic activation. No statistically significant increases in the frequency of cells with structural chromosomal aberrations or polyploid cells were observed with any concentration of the test material, either with or without S9 metabolic activation (ECHA, 2018). Under the conditions of the study, isobutyl isobutyrate was considered to be non-clastogenic in the *in vitro* chromosome aberration assay.

Based on the data available, isobutyl isobutyrate does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/24/21.

11.1.2. Repeated dose toxicity

The MOE for isobutyl isobutyrate 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 isobutyl isobutyrate. Read-across material, isoamyl isovalerate (CAS # 659-70-1; see Section VI), has sufficient repeated dose toxicity data to support the repeated dose toxicity endpoint. A 90-day dietary study was conducted in CRL:COBS CD (SD) BR rats. Groups of 10–16 rats/sex/dose

were fed diets containing test material, isoamyl isovalerate, at doses of 0, 21.9, 69.2, or 219 mg/kg/day for 90 days. There were no treatment-related adverse effects observed up to the highest dose tested. Thus, the NOAEL was considered to be 219 mg/kg/day (RIFM, 1980). In another study, an OECD/GLP 422 combined repeated dose toxicity with a reproduction/developmental toxicity screening test was conducted in Sprague Dawley rats. Groups of 12 rats/sex/dose were administered via gavage test material, isoamyl isovalerate, at doses of 0, 75, 250, or 800 mg/kg/day. Males were dosed for 2 weeks prior to mating and continued through the day before euthanasia (total of 50 days), while females were dosed for 2 weeks prior to mating and continued through to lactation day 13. Additional groups of 6 rats/sex/dose were assigned to the control and high-dose group (but were not mated) to serve as the 14-day treatment-free recovery groups. One high-dose dam was euthanized on GD 24 because all pups were found dead. Prolonged parturition, irregular respiration, and skin paleness were observed during GD 23 to 24 for this dam. Macroscopic examination revealed greenish-black luminal contents in the stomach and colon and pinkish transparent thoracic fluid. The relationship between treatment and these findings was unclear since it was only observed in 1 high-dose female. However, this death was not considered to have toxicological relevance since no treatment-related adverse effects in other parameters at 800 mg/kg/day were observed during the study. At 800 mg/kg/day, salivation was observed among both males and females, but this finding was considered to be attributed to the palatability and not the systemic toxicity of the test material. Significant increases in T4 thyroid hormone levels were observed in high-dose adult males (1.24-fold of the control) and mid- and high-dose pups (up to 1.22-fold of the control). However, this was not considered to be toxicologically significant since there were no correlated microscopic findings in the thyroid (with parathyroids). There were no treatment-related adverse effects in any of the systemic toxicity parameters evaluated (body weight, food consumption, functional behavior and motor activity examination, hematology, clinical chemistry, organ weights, and macroscopic and microscopic findings). Thus, the NOAEL for systemic toxicity was considered to be 800 mg/kg/day, the highest dose tested (RIFM, 2017). Since both studies determined the NOAEL to be the highest dose tested, a NOAEL of 800 mg/kg/day from the OECD 422 was selected for this safety assessment.

A default safety factor of 3 was used when deriving a NOAEL from an OECD 422 study (ECHA, 2012). The safety factor has been approved by the Expert Panel for Fragrance Safety*.

Thus, the derived NOAEL for the repeated dose toxicity data is 800/3, or 267 mg/kg/day.

Therefore, the isobutyl isobutyrate MOE for the repeated dose toxicity can be calculated by dividing the isoamyl isovalerate NOAEL in mg/kg/day by the total systemic exposure to isobutyl isobutyrate, 267/0.00075, or 356000.

In addition, the total systemic exposure to isobutyl isobutyrate (0.75 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.

*The Expert Panel for Fragrance Safety is composed of scientific and technical experts in their respective fields. This group provides advice and guidance.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/21/21.

11.1.3. Reproductive toxicity

The MOE for isobutyl isobutyrate is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are no reproductive toxicity data on isobutyl isobutyrate. Read-across material, isoamyl isovalerate (CAS # 659-70-1; see Section VI), has sufficient reproductive toxicity data to support the reproductive toxicity endpoint. An OECD/GLP 422 combined repeated dose toxicity with a reproduction/developmental toxicity screening test was conducted in Sprague Dawley rats. Groups of 12 rats/sex/dose were administered via gavage test material, isoamyl isovalerate, at doses of 0, 75, 250, or 800 mg/kg/day. Males were dosed for 2 weeks prior to mating and continued through the day before euthanasia (total of 50 days), while females were dosed for 2 weeks prior to mating and continued through to lactation day 13. Additional groups of 6 rats/sex/dose were assigned to the control and high-dose group (but were not mated) to serve as the 14-day treatment-free recovery groups. In addition to the systemic toxicity parameters, the fertility and developmental toxicity parameters were also evaluated. Estrous cycle, precoital time, fertility data, reproductive and littering findings, F1 pup clinical signs, body weight, anogenital distance, nipple retention, and external examination were measured. Thyroid hormone (T4) level in blood was also analyzed for adult males and F1 pups. One high-dose dam was euthanized on GD 24 because all pups were found dead. Prolonged parturition, irregular respiration, and skin paleness were observed during GDs 23 to 24 for this dam. Macroscopic examination revealed greenish-black luminal contents in the stomach and colon and pinkish transparent thoracic fluid. The relationship between treatment and these findings was unclear since it was only observed in 1 high-dose female. However, this death was not considered to have toxicological relevance since no treatment-related adverse effects in other parameters at 800 mg/kg/day were observed during the study. Significant increases in T4 were observed in high-dose adult males (1.24-fold of the control) and mid- and high-dose pups (up to 1.22-fold of the control). However, this was not considered to be toxicologically significant since there were no correlated microscopic findings in the thyroid (with parathyroids). There were no treatment-related adverse effects in any of the fertility and developmental toxicity parameters evaluated. Thus, the NOAEL for fertility and developmental toxicity was considered to be 800 mg/kg/day, the highest dose tested (RIFM, 2017). **Therefore, the isobutyl isobutyrate MOE for the reproductive toxicity can be calculated by dividing the isoamyl isovalerate NOAEL in mg/kg/day by the total systemic exposure to isobutyl isobutyrate, 800/0.00075, or 1066667.**

In addition, the total systemic exposure to isobutyl isobutyrate (0.75 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/21/21.

11.1.4. Skin sensitization

Based on the existing data, isobutyl isobutyrate presents no concern for skin sensitization.

11.1.4.1. Risk assessment. Based on the existing data, isobutyl isobutyrate is not considered a skin sensitizer. The data are summarized in Table 1. The chemical structure of this material indicates that it would not be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). Isobutyl isobutyrate was predicted not to be sensitizing in an *in vitro* direct peptide reactivity assay (DPRA) and KeratinoSens tests (ECHA, 2018).

Based on the weight of evidence (WoE) from structural analysis and *in vitro* studies, isobutyl isobutyrate does not present a concern for skin sensitization.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/15/21.

11.1.5. Photoirritation/photoallergenicity

Based on the available UV/Vis absorption spectra, isobutyl

Table 1
Summary of existing data on Isobutyl isobutyrate.

WoE Skin Sensitization Potency Category[a]	Human Data				Animal Data		
	NOEL-CNIH (induction) μg/cm^2	NOEL-HMT (induction) μg/cm^2	LOEL[b] (induction) μg/cm^2	WoE NESIL[c] μg/cm^2	LLNA Weighted Mean EC3 Value μg/cm^2	GPMT[d]	Buehler[d]
No evidence of sensitization[f]	NA	NA	NA	NA	NA	NA	NA
	***In Vitro* Data[e]**				***In Silico* Protein Binding Alerts (OECD Toolbox v4.2)**		
	KE 1	**KE 2**	**KE 3**		**Target Material**	**Autoxidation simulator**	**Metabolism simulator**
	Negative	Negative	NA		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.

[a] 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).

[b] Data derived from CNIH or HMT.

[c] WoE NESIL limited to 2 significant figures.

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

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

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

isobutyrate would not be expected to present a concern for photoirritation or photoallergenicity.

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

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate no absorbance in the range of 290–700 nm. The molar absorption coefficient is below 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: 11/09/21.

11.1.6. Local Respiratory Toxicity

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

11.1.6.1. Risk assessment. There are no inhalation data available on isobutyl isobutyrate. Based on the Creme RIFM Model, the inhalation exposure is 0.0052 mg/day. This exposure is 269.2 times lower than the Cramer Class I TTC value of 1.4 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/23/21.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of isobutyl isobutyrate 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, isobutyl isobutyrate was identified as a fragrance material with the potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC >1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) did not identify isobutyl isobutyrate 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 ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

11.2.2. Risk assessment

Based on the current VoU (2019), isobutyl isobutyrate presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Key studies. ***Biodegradation:***

RIFM, 2012**:** The ready biodegradability of the test material was evaluated using the manometric respirometry test according to the OECD 301F method. Under the conditions of the study, biodegradation

of 79% was observed.

Ecotoxicity:

No data available.

Other available data:

Isobutyl isobutyrate 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 Framework: Salvito et al., 2002).

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	2.68	2.68
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional VoU Tonnage Band	<1	1–10
Risk Characterization: PEC/PNEC	**<1**	**<1**

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

The RIFM PNEC is 0.5692 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: 05/19/22.

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 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 06/09/22.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2022.113489.

	LC50 (Fish)	EC50 (*Daphnia*)	EC50 (Algae)	AF	PNEC	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	49.78 mg/L			1000000	0.04978	
ECOSAR Acute Endpoints **(Tier 2)** ***v2.0***	7.710 mg/L	14.89 mg/L	5.692 mg/L	10000	0.5692	Esters
ECOSAR Acute Endpoints **(Tier 2)** ***v2.0***	28.93 mg/L	17.49 mg/L	16.9 mg/L			Neutral Organics

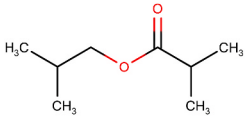
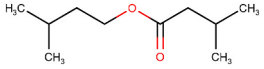
Appendix

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, 2017).

- First, materials were clustered based on their structural similarity. Second, data availability and data quality on the selected cluster were examined. Third, appropriate read-across analogs from the cluster were confirmed by expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physical–chemical properties of the target material and the read-across analogs were calculated using EPI Suite (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), and skin sensitization was predicted using Toxtree v2.6.13.
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018).
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- 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	Isobutyl isobutyrate	Isoamyl isovalerate
CAS No.	97-85-8	659-70-1
Structure		
Similarity (Tanimoto Score)		0.60
SMILES	CC(C)COC(=O)C(C)C	CC(C)CCOC(=O)CC(C)C
Endpoint		Repeated dose toxicity Reproductive toxicity
Molecular Formula	$C_8H_{16}O_2$	$C_{10}H_{20}O_2$
Molecular Weight (g/mol)	144.214	172.268
Melting Point (°C, EPI Suite)	−80.70	−31.53
Boiling Point (°C, EPI Suite)	148.60	190.40
Vapor Pressure (Pa @ 25°C, EPI Suite)	5.77E+02	1.18E+02
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	1.00E+03	4.46E+01
Log KOW	2.68	3.66
J_{max} (μg/cm^2/h, SAM)	65.89	4.88
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	8.33E+01	1.29E+02
Repeated Dose Toxicity		
Repeated Dose (HESS)	Not categorized	Not categorized
Reproductive Toxicity		
ER Binding (OECD QSAR Toolbox v4.2)	Non-binder, non-cyclic structure	Non-binder, non-cyclic structure
Developmental Toxicity (CAESAR v2.1.6)	Toxicant (low reliability)	Non-toxicant (low reliability)
Metabolism		
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	See Supplemental Data 1	See Supplemental Data 2

Summary

There are insufficient toxicity data on isobutyl isobutyrate (CAS # 97-85-8). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, metabolism, physical–chemical properties, and expert judgment, isoamyl isovalerate (CAS # 659-70-1) was identified as a read-across material with sufficient data for toxicological evaluation.

Conclusions

- Isoamyl isovalerate (CAS # 659-70-1) was used as a read-across analog for the target material isobutyl isobutyrate (CAS # 97-85-8) for the repeated dose toxicity and reproductive toxicity endpoints.
  - o The target material and the read-across analog are structurally similar and belong to the class of branched saturated esters.
  - o The target material and the read-across analog share similar branched-chain saturated ester structures.

- The key structural difference between the target material and the read-across analog is that the target material is the ester of isobutyl alcohol with isobutyric acid, whereas the read-across analog is the ester of isoamyl alcohol and isovaleric acid. This structural difference is toxicologically insignificant. The read-across analog contains the structural features of the target material that are relevant to this endpoint and is expected to have equal or greater potential for toxicity as compared to the target.
- Structural similarity between the target material and the read-across analog is indicated by the Tanimoto score. The Tanimoto score reflects the similarity of these branched-chain ester structures. 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.
- There are no *in silico* alerts for the target material or the read-across analog. *In silico* alerts are consistent with data.
- The target material has a toxicant alert by the CAESAR model for developmental toxicity. The data on the target material confirms that the MOE is adequate for the material at the current levels of use. Therefore, based on the structural similarity between the target material and the read-across analog and the data on the read-across analog, the *in silico* alert is superseded by the target 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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