

Short Review

RIFM fragrance ingredient safety assessment, methyl isobutyrate, CAS Registry Number 547-63-7



A.M. Api^a, D. Belsito^b, D. Botelho^a, M. Bruze^c, G.A. Burton Jr^d, J. Buschmann^e, M.L. Dagli^f, M. Date^a, W. Dekant^g, C. Deodhar^a, M. Francis^a, A.D. Fryer^h, L. Jones^a, K. Joshi^a, S. La Cava^a, A. Lapczynski^a, D.C. Lieblerⁱ, D. O'Brien^a, A. Patel^a, T.M. Penning^j, G. Ritacco^a, J. Romine^a, N. Sadekar^a, D. Salvito^a, T.W. Schultz^k, I.G. Sipes^l, G. Sullivan^{a,*}, Y. Thakkar^a, Y. Tokura^m, S. Tsang^a

^a Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ, 07677, USA

^b Member RIFM Expert Panel, Columbia University Medical Center, Department of Dermatology, 161 Fort Washington Ave., New York, NY, 10032, USA

^c Member RIFM Expert Panel, Malmo University Hospital, Department of Occupational & Environmental Dermatology, Sodra Forstadsgatan 101, Entrance 47, Malmo, SE-20502, Sweden

^d Member RIFM Expert Panel, School of Natural Resources & Environment, University of Michigan, Dana Building G110, 440 Church St., Ann Arbor, MI, 48109, USA

^e Member RIFM Expert Panel, Fraunhofer Institute for Toxicology and Experimental Medicine, Nikolai-Fuchs-Strasse 1, 30625, Hannover, Germany

^f Member RIFM Expert Panel, University of Sao Paulo, School of Veterinary Medicine and Animal Science, Department of Pathology, Av. Prof. dr. Orlando Marques de Paiva, 87, Sao Paulo, CEP 05508-900, Brazil

^g Member RIFM Expert Panel, University of Wuerzburg, Department of Toxicology, Versbacher Str. 9, 97078, Wuerzburg, Germany

^h Member RIFM Expert Panel, Oregon Health Science University, 3181 SW Sam Jackson Park Rd., Portland, OR, 97239, USA

ⁱ Member RIFM Expert Panel, Vanderbilt University School of Medicine, Department of Biochemistry, Center in Molecular Toxicology, 638 Robinson Research Building, 2200 Pierce Avenue, Nashville, TN, 37232-0146, USA

^j Member of RIFM Expert Panel, University of Pennsylvania, Perelman School of Medicine, Center of Excellence in Environmental Toxicology, 1316 Biomedical Research Building (BRB) II/III, 421 Curie Boulevard, Philadelphia, PA, 19104-3083, USA

^k Member RIFM Expert Panel, The University of Tennessee, College of Veterinary Medicine, Department of Comparative Medicine, 2407 River Dr., Knoxville, TN, 37996-4500, USA

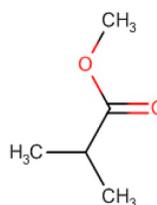
^l Member RIFM Expert Panel, Department of Pharmacology, University of Arizona, College of Medicine, 1501 North Campbell Avenue, P.O. Box 245050, Tucson, AZ, 85724-5050, USA

^m Member RIFM Expert Panel, The Journal of Dermatological Science (JDS), Editor-in-Chief, Professor and Chairman, Department of Dermatology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, 431-3192, Japan

Version: 080218. This version replaces any previous versions.

Name: Methyl isobutyrate

CAS Registry Number: 547-63-7

**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

Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015, 2017) compared to a deterministic aggregate approach

DEREK - Derek nexus is an *in silico* tool used to identify structural alerts

DST - Dermal Sensitization Threshold

ECHA - European Chemicals Agency

EU - Europe/European Union

* Corresponding author.

E-mail address: gsullivan@rifm.org (G. Sullivan).

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GLP - Good Laboratory Practice
 IFRA - The International Fragrance Association
 LOEL - Lowest Observable Effect Level
 MOE - Margin of Exposure
 MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
 NA - North America
 NESIL - No Expected Sensitization Induction Level
 NOAEC - No Observed Adverse Effect Concentration
 NOAEL - No Observed Adverse Effect Level
 NOEC - No Observed Effect Concentration
 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
 REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
 RfD - Reference Dose
 RIFM - Research Institute for Fragrance Materials
 RQ - Risk Quotient
 TTC - Threshold of Toxicological Concern
 UV/Vis Spectra - Ultraviolet/Visible spectra
 VCF - Volatile Compounds in Food
 VoU - Volume of Use vPvB - (very) Persistent, (very) Bioaccumulative
 WoE - Weight of Evidence

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

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

Each endpoint discussed in this safety assessment 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 (i.e., SciFinder and PubMed). Studies selected for this safety assessment were based on appropriate test criteria, such as acceptable guidelines, sample size, study duration, route of exposure, relevant animal species, most relevant testing endpoints, etc. A key study for each endpoint was selected based on the most conservative endpoint value (e.g., PNEC, NOAEL, LOEL, and NESIL).

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

Summary: The use of this material under current conditions is supported by existing information.

Methyl isobutyrate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analog ethyl-2-methylbutyrate (CAS # 7452-79-1) show that methyl isobutyrate is not expected to be genotoxic and provided an MOE > 100 for the repeated dose and reproductive toxicity endpoints. Read-across data from ethyl isobutyrate (CAS # 97-62-1) and methyl 2-methylbutyrate (CAS # 868-57-5) show that there are no safety concerns for methyl isobutyrate for skin sensitization under the current, declared levels of use. The local respiratory toxicity endpoint was evaluated using the TTC for a Cramer Class I material, and the exposure to methyl isobutyrate is below the TTC (1.4 mg/day). The phototoxicity/photoallergenicity endpoint was evaluated based on UV spectra; methyl isobutyrate is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; methyl isobutyrate 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 expected to be genotoxic.

(RIFM, 2000; RIFM, 2014)

Repeated Dose Toxicity: NOAEL = 333 mg/kg/day.

(ECHA Dossier: Ethyl 2-methylbutyrate; ECHA, 2013)

Reproductive Toxicity: NOAEL = 1000 mg/kg/day.

(ECHA Dossier: Ethyl 2-methylbutyrate; ECHA, 2013)

Skin Sensitization: No safety concerns at current, declared use levels.

(RIFM, 1985; ECHA Dossier: Ethyl isobutyrate; ECHA, 2017)

Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic.

(UV Spectra, RIFM DB)

Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:

Persistence: Screening-level: 3.1 (BIOWIN 3)

(EPI Suite v4.11; US EPA, 2012a)

Bioaccumulation: Screening-level: 3.26 L/kg

(EPI Suite v4.11; US EPA, 2012a)

Ecotoxicity: Screening-level: Fish LC50: 582.85 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: 582.85 mg/L

(RIFM Framework; Salvito et al., 2002)

RIFM PNEC is: 0.58285 µg/L

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

1. Identification

- Chemical Name:** Methyl isobutyrate
- CAS Registry Number:** 547-63-7
- Synonyms:** Methyl 2-methylpropanoate; Propanoic acid, 2-methyl-, methyl ester; Methyl isobutyrate
- Molecular Formula:** C₅H₁₀O₂
- Molecular Weight:** 102.13
- RIFM Number:** 6791
- Stereochemistry:** Isomer not specified. No stereocenters and no stereoisomers possible.

2. Physical data

- Boiling Point:** 93 °C (FMA database), 87.71 °C (US EPA, 2012a)
- Flash Point:** 4 °C (GHS), < 40 °F; CC (FMA database)
- Log K_{ow}:** 1.28 (US EPA, 2012a)
- Melting Point:** 81.08 °C (US EPA, 2012a)
- Water Solubility:** 9268 mg/L (US EPA, 2012a)
- Specific Gravity:** 0.888 (FMA database)
- Vapor Pressure:** 39.8 mm Hg @ 20 °C (US EPA, 2012a), 51.6 mm Hg @ 25 °C (US EPA, 2012a)
- UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol⁻¹ · cm⁻¹)
- Appearance/Organoleptic:** Colorless, mobile liquid, milder, and sweeter odor as compared to *n*-butyrate, less apple-like more apricot-like; the flavor follows the odor in that it is less apple-like more sweet apricot or peach-like (Arctander Volume II, 1969)

3. Exposure to fragrance ingredient

- Volume of Use (Worldwide Band):** 0.1–1 metric tons per year (IFRA, 2015)
- 95th Percentile Concentration in shower gel:** 0.017%

(No reported use in Hydroalcohols) (RIFM, 2016).

- Inhalation Exposure*:** 0.000068 mg/kg/day or 0.0050 mg/day (RIFM, 2016)
- Total Systemic Exposure**:** 0.00031 mg/kg/day (RIFM, 2016)

*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 IV. It is derived from concentration survey data in the Creme RIFM aggregate exposure model and includes exposure via dermal, oral, and inhalation routes whenever the fragrance ingredient is used in products that include these routes of exposure (Comiskey et al., 2015; Safford et al., 2015; Safford et al., 2017; and Comiskey et al., 2017).

4. Derivation of systemic absorption

- Dermal:** Assumed 100%
- Oral:** Assumed 100%
- Inhalation:** Assumed 100%

5. Computational toxicology evaluation

- Cramer Classification: Class I, Low

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

2. Analogs Selected:

- Genotoxicity:** Ethyl-2-methylbutyrate (CAS # 7452-79-1)
 - Repeated Dose Toxicity:** Ethyl-2-methylbutyrate (CAS # 7452-79-1)
 - Reproductive Toxicity:** Ethyl-2-methylbutyrate (CAS # 7452-79-1)
 - Skin Sensitization:** Ethyl isobutyrate (CAS # 97-62-1); methyl 2-methylbutyrate (CAS # 868-57-5)
 - Phototoxicity/Photoallergenicity:** None
 - Local Respiratory Toxicity:** None
 - Environmental Toxicity:** None
3. **Read-Across Justification:** See Appendix below

6. Metabolism

No relevant data available for inclusion in this safety assessment.

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

Methyl isobutyrate is reported to occur in the following foods by the VCF* and is not found in natural complex substances (NCS).

- Apple fresh (*Malus* species).
- Apple processed (*Malus* species).
- Banana (*Musa sapientum* L.)
- Cape gooseberry (*Physalis peruviana* L.)
- Cherimoya (*Annona cherimolia* Mill.)
- Citrus fruits.
- Cocoa category.
- Dill (*Anethum* species).
- Kiwifruit *Actinidia chinensis*, syn. *A. Deliciosa*.
- Loquat (*Eriobotrya japonica* Lindl.)
- Melon.
- Naranjilla fruit (*Solanum quitoense* Lam.)
- Papaya (*Carica papaya* L.)
- Pineapple (*Ananas comosus*).
- Potato (*Solanum tuberosum* L.)
- Shoyu (fermented soya hydrolysate).
- Starfruit (*Averrhoa carambola* L.)
- Strawberry (*Fragaria* species).
- Vaccinium species.

*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.

8. IFRA standard

None.

9. REACH dossier

Available; accessed 08/02/18.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on current existing data, methyl isobutyrate does not present a concern for genotoxicity.

10.1.1.1. Risk assessment. Methyl 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, 2015). BlueScreen is a screening assay that assesses genotoxic stress through human derived gene expression. Additional assays on a more reactive read-across material were considered to fully assess the potential mutagenic or clastogenic effects on the target material.

There are no data assessing the mutagenic activity of methyl isobutyrate; however, read-across can be made to ethyl 2-methylbutyrate (CAS # 7452-79-1; see Section 5). The mutagenic activity of ethyl 2-methylbutyrate 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 and pre-incubation methods. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102 were treated with ethyl 2-methylbutyrate in dimethyl sulfoxide (DMSO) at concentrations up to 5000 µg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 2000). Under the conditions of the study, ethyl 2-methylbutyrate was not mutagenic in the Ames test and this can be extended to methyl isobutyrate.

There are no data assessing the clastogenic activity of methyl isobutyrate; however, read-across can be made to ethyl 2-methylbutyrate (CAS # 7452-79-1; see Section V). The clastogenic activity of ethyl 2-methylbutyrate 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 ethyl 2-methylbutyrate in DMSO at concentrations up to 1300 µg/mL in the presence and absence of metabolic activation (S9) for 4 h and in the absence of metabolic activation for 24 h. Ethyl 2-methylbutyrate did not induce binucleated cells with micronuclei when tested up to cytotoxic or the maximum recommended concentrations in either the presence or absence of an S9 activation system (RIFM, 2014). Under the conditions of the study, ethyl 2-methylbutyrate was considered to be non-clastogenic in the *in vitro* micronucleus test, and this can be extended to methyl isobutyrate.

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

Additional References: RIFM, 1999.

Literature Search and Risk Assessment Completed On: 12/4/17.

10.1.2. Repeated dose toxicity

The margin of exposure for methyl isobutyrate is adequate for the repeated dose toxicity endpoint at the current level of use.

10.1.2.1. Risk assessment. There are insufficient repeated dose toxicity data on methyl isobutyrate. Read-across material, ethyl 2-methylbutyrate (CAS # 7452-79-1; see Section V) has sufficient repeated dose toxicity data to support the repeated dose toxicity endpoint. In an OECD 422 combined repeated dose toxicity study with a reproduction/developmental toxicity screening test, groups of 10 Sprague Dawley rats/sex/dose were administered ethyl 2-methylbutyrate via oral gavage at doses of 0, 250, 500, or 1000 mg/kg/day in corn oil. Males were treated for 28–41 days, and females

were treated for 40–51 days (maximum of 51 days, males and females). Males were euthanized on day 14 after mating, and females (with offspring) were euthanized on day 5 postpartum. No treatment-related adverse effects were reported for mortality, clinical signs, neurobehavior, body weight, food consumption, hematology, clinical chemistry, urinalysis, organ weights, pathological findings during necropsy, or histopathological examination. The NOAEL for repeated dose toxicity was considered to be 1000 mg/kg/day, the highest dose tested (ECHA, 2013). A default safety factor of 3 was used when deriving a NOAEL from an OECD 422 study. The safety factor has been approved by the Expert Panel for Fragrance Safety*. The derived NOAEL for the repeated dose toxicity data is 1000/3 or 333 mg/kg/day.

Therefore, the methyl isobutyrate MOE for the repeated dose toxicity endpoint can be calculated by dividing the ethyl 2-methylbutyrate NOAEL in mg/kg/day by the total systemic exposure to methyl isobutyrate, 333/0.00031 or 1074194.

In addition, the total systemic exposure to methyl isobutyrate (0.31 µg/kg bw/day) is below the TTC (30 µg/kg bw/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

*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: 12/01/17.

10.1.3. Reproductive toxicity

The margin of exposure for methyl isobutyrate is adequate for the reproductive toxicity endpoint at the current level of use.

10.1.3.1. Risk assessment. There are insufficient reproductive toxicity data on methyl isobutyrate. Read-across material, ethyl 2-methylbutyrate (CAS # 7452-79-1; see Section V) has sufficient reproductive toxicity data to support the reproductive toxicity endpoint. In an OECD 422 combined repeated dose toxicity study with a reproduction/developmental toxicity screening test, groups of 10 Sprague Dawley rats/sex/dose were administered ethyl 2-methylbutyrate via oral gavage at doses of 0, 250, 500, or 1000 mg/kg/day in corn oil. Males were treated for 28–41 days, and females were treated for 40–51 days (maximum of 51 days, males and females). Males were euthanized on day 14 after mating, and females (with offspring) were euthanized on day 5 postpartum. There were no treatment-related effects on mating performance, fertility, conception, gestation length, parturition, survival, litter size, or litter weight. In the F1 generation, no treatment-related effects were reported for mortality, clinical signs, body weight, and bodyweight changes during necropsy. Furthermore, no gross abnormalities were reported in pups. Therefore, the NOAEL for reproductive toxicity was considered to be 1000 mg/kg/day, the highest dose tested (ECHA, 2013).

Therefore, the methyl isobutyrate MOE for the reproductive toxicity endpoint can be calculated by dividing ethyl 2-methylbutyrate NOAEL in mg/kg/day by the total systemic exposure to methyl isobutyrate, 1000/0.00031 or 3225806.

In addition, the total systemic exposure to methyl isobutyrate (0.31 µg/kg bw/day) is below the TTC (30 µg/kg bw/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: 12/01/17.

10.1.4. Skin sensitization

Based on read-across ethyl isobutyrate (CAS # 97-62-1) and methyl 2-methylbutyrate (CAS # 868-57-5), methyl isobutyrate does not present a safety concern for skin sensitization under the current, declared levels of use.

10.1.4.1. Risk assessment. Insufficient skin sensitization studies are available for methyl isobutyrate. Based on existing data and read-across to ethyl isobutyrate (CAS # 97-62-1; see Section V) and methyl 2-methylbutyrate (CAS # 868-57-5; see Section V), methyl isobutyrate does not present a safety concern for skin sensitization under the current, declared levels of use. The chemical structures of these materials indicate that they would not be expected to react with skin proteins (Toxtree 2.6.13; OECD toolbox v3.4). In guinea pigs, maximization tests with read-across analogs ethyl isobutyrate and methyl 2-methylbutyrate did not present reactions indicative of sensitization (RIFM, 1985; ECHA, 2017). In human maximization tests, no skin sensitization reactions were observed with read-across analogs ethyl isobutyrate and methyl 2-methylbutyrate (RIFM, 1982; RIFM, 1975).

Based on weight of evidence from structural analysis, animal and human studies, and read-across to ethyl isobutyrate and methyl 2-methylbutyrate, methyl isobutyrate does not present a safety concern for skin sensitization under the current, declared levels of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/17/17.

10.1.5. Phototoxicity/photoallergenicity

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

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

10.1.5.2. 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, $1000 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ (Henry et al., 2009).

Additional References: None.

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

10.1.6. Local Respiratory Toxicity

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

10.1.6.1. Risk assessment. There are no inhalation data available on methyl isobutyrate. Based on the Creme RIFM Model, the inhalation exposure is 0.0050 mg/day. This exposure is 280 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: 12/01/

17.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of methyl 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 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, methyl isobutyrate 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 methyl 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 $\geq 2000 \text{ L/kg}$. 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.

10.2.2. Risk assessment

Based on the current Volume of Use (2015), methyl isobutyrate does not present a risk to the aquatic compartment in the screening-level assessment.

Biodegradation: No data available.

Ecotoxicity: No data available.

Other available data

Methyl isobutyrate has been registered under REACH with no additional data at this time.

10.2.3. Risk assessment refinement

Ecotoxicological data and PNEC derivation (all endpoints reported in mg/L; PNECs in $\mu\text{g/L}$).

Endpoints used to calculate PNEC are underlined.

| | LC50 (Fish) (mg/L) | EC50 (Daphnia) (mg/L) | EC50 (Algae) (mg/L) | AF | PNEC (µg/L) | Chemical Class |
|--|-----------------------|-----------------------------|---------------------------|-----------|-------------|----------------|
| RIFM Framework Screening-level Tier 1) | 582.85 | | | 1,000,000 | 0.58285 | |

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

| Exposure | Europe (EU) | North America (NA) |
|--|-------------|--------------------|
| Log K_{ow} used | 1.28 | 1.28 |
| 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 0.58285 µg/L. The revised PEC/PNECs for EU and NA are: not applicable. The material was cleared at screening-level and therefore does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 11/27/17.

11. Literature Search*

- **RIFM Database:** Target, Fragrance Structure Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** <http://echa.europa.eu/>
- **NTP:** <https://ntp.niehs.nih.gov/>

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fct.2018.11.026>.

Appendix

Read-across Justification

Methods

The read-across analogs were identified following the strategy for structuring and reporting a read-across prediction of toxicity described in [Schultz et al. \(2015\)](#). The strategy is also 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, 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 substance 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 v3.4 ([OECD, 2012](#)).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v3.4 ([OECD, 2012](#)).
- Developmental toxicity was predicted using CAESAR v2.1.7 ([Cassano et al., 2010](#)), and skin sensitization was predicted using Toxtree 2.6.13.
- Protein binding was predicted using OECD QSAR Toolbox v3.4 ([OECD, 2012](#)).
- The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox v3.4 ([OECD, 2012](#)).

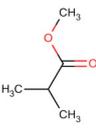
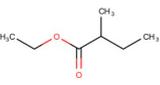
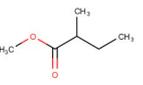
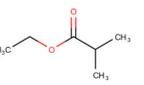
- OECD Toolbox
- **SciFinder:** <https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf>
- **PubMed:** <http://www.ncbi.nlm.nih.gov/pubmed>
- **TOXNET:** <http://toxnet.nlm.nih.gov/>
- **IARC:** <http://monographs.iarc.fr>
- **OECD SIDS:** <http://webnet.oecd.org/hpv/ui/Default.aspx>
- **EPA ACToR:** <https://actor.epa.gov/actor/home.xhtml>
- **US EPA HPVIS:** https://ofmpub.epa.gov/opthpv/public_search_publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&EndPointRpt=Y#submission
- **Japanese NITE:** <http://www.safe.nite.go.jp/english/db.html>
- **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 06/12/2018.

Conflicts of interest

The authors declare that they have no conflicts of interest.

| | Target Material | Read-across Material | Read-across Material | Read-across Material |
|--|---|---|---|--|
| Principal Name | Methyl isobutyrate | Ethyl 2-methylbutyrate | Methyl 2-methylbutyrate | Ethyl isobutyrate |
| CAS No. Structure | 547-63-7  | 7452-79-1  | 868-57-5  | 97-62-1  |
| Similarity (Tanimoto Score) Read-across Endpoint | | 0.58 ● Genotoxicity ● Repeated dose Toxicity ● Reproductive Toxicity | 0.75 ● Skin sensitization | 0.75 ● Skin sensitization |
| Molecular Formula | C ₅ H ₁₀ O ₂ | C ₇ H ₁₄ O ₂ | C ₆ H ₁₂ O ₂ | C ₆ H ₁₂ O ₂ |
| Molecular Weight | 102.13 | 130.19 | 116.16 | 116.16 |
| Melting Point (°C, EPI Suite) | −81.08 | −56.05 | −68.43 | −68.43 |
| Boiling Point (°C, EPI Suite) | 87.71 | 134.87 | 111.74 | 111.74 |
| Vapor Pressure (Pa @ 25 °C, EPI Suite) | 6.88E+003 | 1.07E+003 | 3E+003 | 3E+003 |
| Log Kow (KOWWIN v1.68 in EPI Suite) | 1.28 | 2.26 | 1.77 | 1.77 |
| Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPI Suite) | 9268 | 1070 | 3172 | 3172 |
| J _{max} (mg/cm ² /h, SAM) | 692.203 | 297.516 | 440.615 | 460.179 |
| Henry's Law (Pa·m ³ /mol, Bond Method, EPI Suite) | 3.13E+001 | 5.52E+001 | 4.16E+001 | 4.16E+001 |
| <i>Genotoxicity</i> | | | | |
| DNA Binding (OASIS v1.4, QSAR Toolbox v3.4) | ● No alert found | ● No alert found | | |
| DNA Binding (OECD QSAR Toolbox v3.4) | ● No alert found | ● No alert found | | |
| Carcinogenicity (ISS) | ● Non-carcinogen (moderate reliability) | ● Carcinogen (low reliability) | | |
| DNA Binding (Ames, MN, CA, OASIS v1.1) | ● No alert found | ● No alert found | | |
| <i>In Vitro</i> Mutagenicity (Ames, ISS) | ● No alert found | ● No alert found | | |
| <i>In Vivo</i> Mutagenicity (Micronucleus, ISS) | ● No alert found | ● No alert found | | |
| Oncologic Classification | ● Not classified | ● Not classified | | |
| <i>Repeated Dose Toxicity</i> | | | | |
| Repeated Dose (HESS) | ● Not categorized | ● Not categorized | | |
| <i>Reproductive Toxicity</i> | | | | |
| ER Binding (OECD QSAR Toolbox v3.4) | ● Non-binder, cyclic structure | ● Non-binder, cyclic structure | | |
| Developmental Toxicity (CAESAR v2.1.6) | ● Toxicant (low reliability) | ● Non-toxicant (low reliability) | | |
| <i>Skin Sensitization</i> | | | | |
| Protein Binding (OASIS v1.1) | ● No alert found | | ● No alert found | ● No alert found |
| Protein Binding (OECD) | ● No alert found | | ● No alert found | ● No alert found |
| Protein Binding Potency | ● Not possible to classify | | ● Not possible to classify | ● Not possible to classify |
| Protein Binding Alerts for Skin Sensitization (OASIS v1.1) | ● No alert found | | ● No alert found | ● No alert found |
| Skin Sensitization Reactivity Domains (Toxtree v2.6.13) | ● No alert found | | ● No alert found | ● No alert found |
| <i>Metabolism</i> | | | | |
| Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v3.4) | See Supplemental Data 1 | See Supplemental Data 2 | See Supplemental Data 3 | See Supplemental Data 4 |

Summary

There are insufficient toxicity data on methyl isobutyrate (CAS # 547-63-7). 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, ethyl 2-methylbutyrate (CAS # 7452-79-1), methyl 2-methylbutyrate (CAS # 868-57-5), and ethyl isobutyrate (CAS # 97-62-1) were identified as read-across materials with sufficient data for toxicological evaluation.

Conclusions

- Ethyl 2-methylbutyrate (CAS # 7452-79-1) was used as a read-across analog for the target material methyl isobutyrate (CAS # 547-63-7) for the genotoxicity, repeated dose, and reproductive toxicity endpoints.
 - The target material and the read-across analog are structurally similar and belong to the class of branched chain saturated esters.
 - The target material and the read-across analog are both esters of a branched chain acid and a straight chain alcohol.
 - The key structural difference between the target material and the read-across analog is that the target material is the methyl ester of isobutyric acid, whereas the read-across analog is the ethyl ester of 2-methylbutyric acid. This structural difference is toxicologically insignificant.
 - 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 comparison of their toxicological properties.
 - According to the OECD QSAR Toolbox v3.4, structural alerts for toxicological endpoints are consistent between the target material and the

read-across analog.

- The read-across analog has carcinogenicity alert by the ISS model. The target material does not have any such alert. According to these predictions, the read-across analog is expected to be more reactive compared to the target material. The data described in the genotoxicity section above shows that based on the current existing data, the read-across analog does not pose a concern for genotoxicity. Therefore, the predictions are superseded by data.
- The target is shown to be a toxicant with low reliability by CAESAR v2.1.6 while the read-across analog is shown to be a non-toxicant with low reliability by the same model. The data described for the read-across analog in the fertility and developmental toxicity sections show that the read-across analog does not pose a concern under current exposure level. ER binding alert, which is another fertility toxicity indicator is negative for both of the materials. Therefore, with the data for the read-across analog and the high structural similarity between the read-across analog and the target material, the alert for the target material will be superseded by the data for read-across analog.
- 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.
- Methyl 2-methylbutyrate (CAS # 868-57-5) was used as a read-across analog for the target material methyl isobutyrate (CAS # 547-63-7) for the skin sensitization endpoint.
 - The target material and the read-across analog are structurally similar and belong to the class of branched chain saturated esters.
 - The target material and the read-across analog are both methyl esters of branched chain acids.
 - The key structural difference between the target material and the read-across analog is that the target material is the methyl ester of isobutyric acid, whereas the read-across analog is the methyl ester of e-methylbutyric acid. This structural difference is toxicologically insignificant.
 - Structural similarity between the target material and the read-across analog is indicated by the Tanimoto score. The Tanimoto score reflects the near identity of these branched esters. 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 comparison of their toxicological properties.
 - According to the OECD QSAR Toolbox v3.4, structural alerts for the toxicological endpoint are consistent between the target material and the read-across analog.
 - 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 endpoint evaluated are consistent between the metabolites of the read-across analog and the target material.
- Ethyl isobutyrate (CAS # 97-62-1) was used as a read-across analog for the target material methyl isobutyrate (CAS # 547-63-7) for the skin sensitization endpoint.
 - The target material and the read-across analog are structurally similar and belong to the class of branched chain saturated esters.
 - The target material and the read-across are both isobutyrate esters.
 - The key structural difference between the target material and the read-across analog is that the target material is the methyl ester of isobutyric acid, whereas the read-across analog is the ethyl ester. This structural difference is toxicologically insignificant.
 - Structural similarity between the target material and the read-across analog is indicated by the Tanimoto score. The Tanimoto score reflects the near identity of these isobutyrate 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 comparison of their toxicological properties.
 - According to the OECD QSAR Toolbox v3.4, structural alerts for the toxicological endpoint are consistent between the target material and the read-across analog.
 - 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 endpoint evaluated are consistent between the metabolites of the read-across analog and the target material.

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