



Short Review



RIFM fragrance ingredient safety assessment, 2-methylpropyl 3-methylbutyrate, CAS Registry Number 589-59-3

A.M. Api^a, D. Belsito^b, D. Botelho^a, M. Bruze^c, G.A. Burton Jr.^d, M.A. Cancellieri^a, H. Chon^a, M.L. Dagli^e, M. Date^a, W. Dekant^f, C. Deodhar^a, A.D. Fryer^g, L. Jones^a, K. Joshi^a, M. Kumar^a, A. Lapczynski^a, M. Lavelle^a, I. Lee^a, D.C. Liebler^h, H. Moustakas^a, M. Na^a, T.M. Penningⁱ, G. Ritacco^a, J. Romine^a, N. Sadekar^a, T.W. Schultz^j, D. Selechnik^a, F. Siddiqi^a, I.G. Sipes^k, G. Sullivan^{a,*}, Y. Thakkar^a, Y. Tokura^l

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

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

^c Member Expert Panel for Fragrance Safety, Malmö University Hospital, Department of Occupational & Environmental Dermatology, Sodra Forstadsgatan 101, Entrance 47, Malmö, SE-20502, Sweden

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

^e Member Expert Panel for Fragrance Safety, University of São Paulo, School of Veterinary Medicine and Animal Science, Department of Pathology, Av. Prof. Dr. Orlando Marques de Paiva, 87, São Paulo, CEP 05508-900, Brazil

^f Member Expert Panel for Fragrance Safety, University of Würzburg, Department of Toxicology, Versbacher Str. 9, 97078, Würzburg, Germany

^g Member Expert Panel for Fragrance Safety, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd., Portland, OR, 97239, USA

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

ⁱ Member of Expert Panel for Fragrance Safety, 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

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

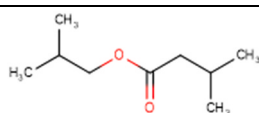
^k Member Expert Panel for Fragrance Safety, Department of Pharmacology, University of Arizona, College of Medicine, 1501 North Campbell Avenue, P.O. Box 245050, Tucson, AZ, 85724-5050, USA

^l Member Expert Panel for Fragrance Safety, The Journal of Dermatological Science (JDS), Department of Dermatology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, 431-3192, Japan

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Name: 2-Methylpropyl 3-methylbutyrate CAS Registry Number: 589-59-3

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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* Corresponding author.

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

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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; Safford et al., 2017) compared to a deterministic aggregate approach

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

DRF - Dose Range Finding

DST - Dermal Sensitization Threshold

ECHA - European Chemicals Agency

ECOSAR - Ecological Structure-Activity Relationships Predictive Model

EU - Europe/European Union

GLP - Good Laboratory Practice

IFRA - The International Fragrance Association

LOEL - Lowest Observed Effect Level

MOE - Margin of Exposure

MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition

NA - North America

NESIL - No Expected Sensitization Induction Level

NOAEC - No Observed Adverse Effect Concentration

NOAEL - No Observed Adverse Effect Level

NOEC - No Observed Effect Concentration

NOEL - No Observed Effect Level

OECD - Organisation for Economic Co-operation and Development

OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines

PBT - Persistent, Bioaccumulative, and Toxic

PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration

Perfumery - In this safety assessment, perfumery refers to fragrances made by a perfumer used in consumer products only. The exposures reported in the safety assessment include consumer product use but do not include occupational exposures.

QRA - Quantitative Risk Assessment

QSAR - Quantitative Structure-Activity Relationship

REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals

RfD - Reference Dose

RIFM - Research Institute for Fragrance Materials

RQ - Risk Quotient

Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test

TTC - Threshold of Toxicological Concern

UV/Vis spectra - Ultraviolet/Visible spectra

VCF - Volatile Compounds in Food

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

WoE - Weight of Evidence

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

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

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

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

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

2-Methylpropyl 3-methylbutyrate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, photoirritation/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analog isobutyl isobutyrate (CAS # 97-85-8) show that 2-methylpropyl 3-methylbutyrate is not expected to be genotoxic and that there are no safety concerns for 2-methylpropyl 3-methylbutyrate for skin sensitization under the current declared levels of use. Data on read-across analog isoamyl isovalerate (CAS # 659-70-1) provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose

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toxicity and reproductive toxicity endpoints. The photoirritation/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; 2-methylpropyl 3-methylbutyrate 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; exposure to 2-methylpropyl 3-methylbutyrate is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; 2-methylpropyl 3-methylbutyrate was found not to be Persistent, Bioaccumulative, and Toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards, and its risk quotients, based on its current volume of use (VoU) in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

Human Health Safety Assessment

Genotoxicity: Not expected to be genotoxic. (ECHA REACH Dossier: Isobutyl isobutyrate; ECHA, 2018)

Repeated Dose Toxicity: NOAEL = 267 mg/kg/day. RIFM (2017)

Reproductive Toxicity: NOAEL = 800 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: Screening-level: 2.98 (BIOWIN 3) (EPI Suite v4.11; US EPA, 2012a)

Bioaccumulation: Screening-level: 57.6 L/kg (EPI Suite v4.11; US EPA, 2012a)

Ecotoxicity: Screening-level: Fish LC50: 20.45 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: 20.45 mg/L (RIFM Framework; Salvito et al., 2002)

RIFM PNEC is: 0.02045 µg/L

• **Revised PEC/PNECs (2019 IFRA VoU):** North America and Europe: Not applicable; cleared at screening-level

1. Identification

- Chemical Name:** 2-Methylpropyl 3-methylbutyrate
- CAS Registry Number:** 589-59-3
- Synonyms:** Butanoic acid, 3-methyl-, 2-methylpropyl ester; Isobutyl isovalerate; 異ブチ酸アミル(C = 1 ~ 5); Isobutyl 3-methylbutanoate; 2-Methylpropyl 3-methylbutyrate
- Molecular Formula:** C₉H₁₈O₂
- Molecular Weight:** 158.24 g/mol
- RIFM Number:** 1022
- Stereochemistry:** Stereoisomer not specified. NO stereocenter present and no stereoisomers possible.

2. Physical data

- Boiling Point:** 172 °C (Fragrance Materials Association [FMA]), 165.67 °C (EPI Suite)
- Flash Point:** 50 °C (Globally Harmonized System), 122 °F; closed cup (FMA)
- Log Kow:** 3.17 (EPI Suite)
- Melting Point:** 43.28 °C (EPI Suite)
- Water Solubility:** 136.1 mg/L (EPI Suite)
- Specific Gravity:** 0.872 (FMA)
- Vapor Pressure:** 1.18 mm Hg at 20 °C (EPI Suite v4.0), 1.5 mm Hg at 20 °C (FMA), 1.68 mm Hg at 25 °C (EPI Suite)
- 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:** A colorless mobile liquid that has a fruity-ethereal Apple-Raspberry-like odor.

3. Volume of use (worldwide band)

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

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

- 1. **95th Percentile Concentration in Fine Fragrance:** 0.000011% (RIFM, 2022)
- 2. **Inhalation Exposure*:** 0.000060 mg/kg/day or 0.0045 mg/day (RIFM, 2022)
- 3. **Total Systemic Exposure**:** 0.00036 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

1. Cramer Classification: Class I, Low

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

2. Analogs Selected:

- a. **Genotoxicity:** Isobutyl isobutyrate (CAS # 97-85-8)
- b. **Repeated Dose Toxicity:** Isoamyl isovalerate (CAS # 659-70-1)
- c. **Reproductive Toxicity:** Isoamyl isovalerate (CAS # 659-70-1)
- d. **Skin Sensitization:** Isobutyl isobutyrate (CAS # 97-85-8)
- e. **Photoirritation/Photoallergenicity:** None
- f. **Local Respiratory Toxicity:** None
- g. **Environmental Toxicity:** None

3. Read-across Justification: See Appendix below

7. Metabolism

No relevant data available for inclusion in this safety assessment.

Additional References: None

8. Natural occurrence

2-Methylpropyl 3-methylbutyrate is reported to occur in the following foods by the VCF*:

Alpinia species	Lamb's lettuce (<i>Valerianella locusta</i>)
Artocarpus species	Mangifera species

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Banana (<i>Musa Sapientum</i> L.)	Tomato (<i>Lycopersicon Esculentum</i> Mill.)
Capsicum species	Wine
Fig (<i>Ficus Carica</i> 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

2-Methylpropyl 3-methylbutyrate has been pre-registered for 2010; no dossier available as of 06/10/22.

10. Conclusion

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

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, 2-methylpropyl 3-methylbutyrate does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. 2-Methylpropyl 3-methylbutyrate 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 on an appropriate read-across material were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

There are no studies assessing the mutagenic or clastogenic activity of 2-methylpropyl 3-methylbutyrate; however, read-across can be made to isobutyl isobutyrate (CAS # 97-85-8; see Section VI).

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, and this can be extended to 2-methylpropyl 3-methylbutyrate.

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, and this can be extended to 2-methylpropyl 3-methylbutyrate.

Based on the data available, isobutyl isobutyrate does not present a

concern for genotoxic potential, and this can be extended to 2-methylpropyl 3-methylbutyrate.

Additional References: None

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

11.1.2. Repeated dose toxicity

The MOE for 2-methylpropyl 3-methylbutyrate is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are insufficient repeated dose toxicity data on 2-methylpropyl 3-methylbutyrate. The 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 the 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 422/GLP 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 the 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 (a 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 groups (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 the 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. 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 2-methylpropyl 3-methylbutyrate 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 2-methylpropyl 3-methylbutyrate, 267/0.00036, or 741667.

In addition, the total systemic exposure to 2-methylpropyl 3-methylbutyrate (0.36 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 2-methylpropyl 3-methylbutyrate is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are insufficient reproductive toxicity data on 2-methylpropyl 3-methylbutyrate. The 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 422/GLP 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 the 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 (a 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 groups (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. Estrus 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 the 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 the 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. 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 2-methylpropyl 3-methylbutyrate MOE for the reproductive toxicity can be calculated by dividing the isoamyl isovalerate NOAEL in mg/kg/day by the total systemic exposure to 2-methylpropyl 3-methylbutyrate, 800/0.00036, or 2222222.

In addition, the total systemic exposure to 2-methylpropyl 3-methylbutyrate (0.36 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 on the target material and read-across material isobutyl isobutyrate, 2-methylpropyl 3-methylbutyrate presents no concern for skin sensitization.

11.1.4.1. Risk assessment. Limited skin sensitization data are available for 2-methylpropyl 3-methylbutyrate. Therefore, read-across material isobutyl isobutyrate (CAS # 97-85-8; see Section VI) was used for the risk assessment of 2-methylpropyl 3-methylbutyrate. The data on the read-across material are summarized in Table 1. Based on the existing data on the read-across material, 2-methylpropyl 3-methylbutyrate is not considered a skin sensitizer. The chemical structure of the read-across material and the target material indicate that they would not be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). Read-across material isobutyl isobutyrate was predicted not to be sensitizing in an *in vitro* direct peptide reactivity assay (DPRA) and KeratinoSens (ECHA, 2018). In a human maximization test, no skin sensitization reactions were observed with 2-methylpropyl 3-methylbutyrate (RIFM, 1978).

Based on the weight of evidence (WoE) from structural analysis and *in vitro* and human studies on the read-across material as well as the target material, 2-methylpropyl 3-methylbutyrate 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, 2-methylpropyl 3-methylbutyrate 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 2-methylpropyl 3-methylbutyrate 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, 2-methylpropyl 3-methylbutyrate 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, $1000 \text{ L mol}^{-1} \cdot \text{cm}^{-1}$ (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 2-methylpropyl 3-methylbutyrate is below the Cramer Class I TTC value for inhalation exposure local effects.

Table 1

Summary of existing data on isobutyl isobutyrate as a read-across for 2-methylpropyl 3-methylbutyrate.

WoE Skin Sensitization Potency Category ¹	Human Data				Animal Data		
	NOEL-CNIH (induction) $\mu\text{g}/\text{cm}^2$	NOEL-HMT (induction) $\mu\text{g}/\text{cm}^2$	LOEL ² (induction) $\mu\text{g}/\text{cm}^2$	WoE NESIL ³ $\mu\text{g}/\text{cm}^2$	LLNA ⁴ Weighted Mean EC3 Value $\mu\text{g}/\text{cm}^2$	GPMT ⁵	Buehler ⁵
No evidence of sensitization ⁷	NA	NA	NA	NA	NA	NA	NA
	<i>In Vitro</i> Data ⁶				<i>In Silico</i> 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.

¹WoE 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).

²Data derived from CNIH or HMT.

³WoE NESIL limited to 2 significant figures.

⁴Studies conducted according to OECD TG 406 are included in the table.

⁵Studies conducted according to OECD TG 442, Cottrez et al. (2016), or Forreryd et al. (2016) are included in the table.

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

11.1.6.1. Risk assessment. There are no inhalation data available on 2-methylpropyl 3-methylbutyrate. Based on the Creme RIFM Model, the inhalation exposure is 0.0045 mg/day. This exposure is 311 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 2-methylpropyl 3-methylbutyrate was performed following the RIFM Environmental Framework (Salvito et al., 2002), which provides for 3 levels of screening for aquatic risk. In Tier 1, only the material's VoU in a region, its log K_{ow} and molecular weight are needed to estimate a conservative risk quotient (RQ; Predicted Environmental Concentration/Predicted No Effect Concentration or PEC/PNEC). In Tier 1, a general QSAR for fish toxicity is used with a high uncertainty factor, as discussed in Salvito et al. (2002). At Tier 2, the model ECOSAR (US EPA, 2012b; providing chemical class-specific ecotoxicity estimates) is used, and a lower uncertainty factor is applied. Finally, if needed, at Tier 3, measured biodegradation and ecotoxicity data are used to refine the RQ (again, with lower uncertainty factors applied to calculate the PNEC). Provided in the table below are the data necessary to calculate both the PEC and the PNEC determined within this safety assessment. For the PEC, while the actual regional tonnage, which is considered proprietary information, is not provided, the range from the most recent IFRA Volume of Use Survey is reported. The PEC is calculated based on the actual tonnage and not the extremes noted for the range. Following the RIFM Environmental Framework, 2-methylpropyl 3-methylbutyrate 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 v4.1 did not identify 2-methylpropyl 3-methylbutyrate 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, 2012b). 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 v4.1).

11.2.1.1. Risk assessment. Based on the current Volume of Use (2019), 2-methylpropyl 3-methylbutyrate does not present a risk to the aquatic compartment in the screening-level assessment.

11.2.1.2. Key studies. Biodegradation:

No data available.

Ecotoxicity:

No data available.

11.2.1.3. Other available data. 2-Methylpropyl 3-methylbutyrate has

been pre-registered for REACH with no additional data at this time.

11.2.2. 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.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	3.17	3.17
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 the available data, the RQ for this material is < 1. No further assessment is necessary.

The RIFM PNEC is 0.02045 $\mu\text{g/L}$. 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 VoU.

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

12. Literature Search*

- **RIFM Database:** Target, Fragrance Structure-Activity Group materials, other references, JECHA, 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 Technical Bulletin:** https://www.nlm.nih.gov/pubs/techbull/nd19/nd19_toxnet_new_locations.html
- **IARC:** <https://monographs.iarc.fr>
- **OECD SIDS:** <https://hpvchemicals.oecd.org/ui/Default.aspx>
- **EPA ACToR:** <https://actor.epa.gov/actor/home.xhtml>
- **US EPA ChemView:** <https://chemview.epa.gov/chemview/>
- **Japanese NITE:** https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** <https://www.google.com>
- **ChemIDplus:** <https://pubchem.ncbi.nlm.nih.gov/source/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 05/31/22.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. RIFM staff are employees of the Research Institute for Fragrance Materials, Inc. (RIFM). The Expert Panel receives a small honorarium for time spent reviewing the subject work.

	LC50 (Fish)	EC50 (Daphnia)	EC50 (Algae)	AF	PNEC	Chemical Class
RIFM Framework Screening-level (Tier 1)	23.56 mg/L			1000000	0.02356	

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fct.2023.114274>.

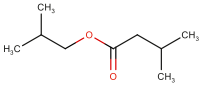
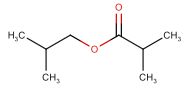
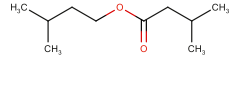
Appendix

Read-across Justification

Methods

The read-across analogs were identified using RIFM fragrance chemicals inventory clustering and read-across search criteria (Date et al., 2020). These criteria are in compliance with the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015) and are consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemical Agency read-across assessment framework (ECHA, 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	Read-across Material
Principal Name	2-Methylpropyl 3-methylbutyrate	Isobutyl isobutyrate	Isoamyl isovalerate
CAS No.	589-59-3	97-85-8	659-70-1
Structure			
Similarity (Tanimoto Score)		0.72	0.86
SMILES	CC(C)COC(=O)CC(C)C	CC(C)COC(=O)C(C)C	CC(C)CCOC(=O)CC(C)C
Endpoint		Genotoxicity Skin sensitization	Repeated dose toxicity Reproductive toxicity
Molecular Formula	C ₉ H ₁₈ O ₂	C ₈ H ₁₆ O ₂	C ₁₀ H ₂₀ O ₂
Molecular Weight (g/mol)	158.241	144.214	172.268
Melting Point (°C, EPI Suite)	−43.28	−80.70	−31.53
Boiling Point (°C, EPI Suite)	168.50	148.60	190.40
Vapor Pressure (Pa @ 25°C, EPI Suite)	2.45E+02	5.77E+02	1.18E+02
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	1.36E+02	1.00E+03	4.46E+01
Log KOW	3.17	2.68	3.66
J_{\max} (μg/cm²/h, SAM)	11.94	65.89	4.88
Henry's Law (Pa·m³/mol, Bond Method, EPI Suite)	9.73E+01	8.33E+01	1.29E+02
Genotoxicity			

(continued on next page)

(continued)

	Target Material	Read-across Material	Read-across Material
DNA Binding (OASIS v1.4, QSAR Toolbox v4.2)	No alert found	No alert found	
DNA Binding (OECD QSAR Toolbox v4.2)	No alert found	No alert found	
Carcinogenicity (ISS)	No alert found	No alert found	
DNA Binding (Ames, MN, CA, OASIS v1.1)	No alert found	No alert found	
<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	
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)	Non-toxicant (low reliability)		Non-toxicant (low reliability)
Skin Sensitization			
Protein Binding (OASIS v1.1)	No alert found	No alert found	
Protein Binding (OECD)	No alert found	No alert found	
Protein Binding Potency	Not possible to classify according to these rules (GSH)	Not possible to classify according to these rules (GSH)	
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	No alert found	No alert found	
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	No skin sensitization reactivity domain alerts were identified.	No skin sensitization reactivity domain alerts were identified.	
Metabolism			
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	See Supplemental Data 1	See Supplemental Data 2	See Supplemental Data 3

Summary

There are insufficient toxicity data on 2-methylpropyl 3-methylbutyrate (CAS # 589-59-3). 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, isobutyl isobutyrate (CAS # 97-85-8) and isoamyl isovalerate (CAS # 659-70-1) were identified as read-across materials with sufficient data for toxicological evaluation.

Conclusions

- Isobutyl isobutyrate (CAS # 97-85-8) was used as a read-across analog for the target material, 2-methylpropyl 3-methylbutyrate (CAS # 589-59-3), for the genotoxicity and skin sensitization 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 share similar branched saturated ester structures.
 - The key structural difference between the target material and the read-across analog is in the branching pattern on acid and alcohol portions. 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 and the read-across analog. *In silico* alerts are consistent with 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.
- Isoamyl isovalerate (CAS # 659-70-1) was used as a read-across analog for the target material, 2-methylpropyl 3-methylbutyrate (CAS # 589-59-3), for the 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 share similar branched saturated ester structures.
 - The key structural difference between the target material and the read-across analog is in the branching pattern on acid and alcohol portions. 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 saturated 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 and the read-across analog. *In silico* alerts are consistent with data.

- o The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
- o The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

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