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Short Review

# RIFM fragrance ingredient safety assessment, 2-methylbutyl 3-methylbutanoate, CAS Registry Number 2445-77-4

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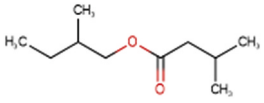
Version: 061322. Initial publication. All fragrance materials are evaluated on a five-year rotating basis. Revised safety assessments are published if new relevant data become available. Open access to all RIFM Fragrance Ingredient Safety Assessments is here: fragrancematerialsafetyresource.elsevier.com.



**Name**: 2-Methylbutyl 3-methylbutanoate
**CAS Registry Number**: 2445-77-4

**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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**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; Safford et al., 2015a; Safford et al., 2017; Comiskey 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

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**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, 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-Methylbutyl 3-methylbutanoate 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-methylbutyl 3-methylbutanoate is not genotoxic and that there are no safety concerns for 2-methylbutyl 3-methylbutanoate 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 toxicity and reproductive toxicity endpoints. The photoirritation/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; 2-methylbutyl 3-methylbutanoate 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 2-methylbutyl 3-methylbutanoate is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; 2-methylbutyl 3-methylbutanoate 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**

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| | |
|---|---|
| **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: 3.0 (BIOWIN 3) | (EPI Suite v4.11; US EPA, 2012a) |
| **Bioaccumulation:** | |
| Screening-level: 121.4 L/kg | (EPI Suite v4.11; US EPA, 2012a) |
| **Ecotoxicity:** | |
| Screening-level: Fish LC50: 8.36 mg/L | (RIFM Framework; Salvito, 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, 2002) |
| **Critical Ecotoxicity Endpoint:** Fish LC50: 8.36 mg/L | (RIFM Framework; Salvito, 2002) |
| **RIFM PNEC is:** 0.00836 μg/L | |
| • **Revised PEC/PNECs (2019 IFRA VoU):** North America and Europe: Not applicable; cleared at screening-level | |

## 1. Identification

1. **Chemical Name:** 2-Methylbutyl 3-methylbutanoate
2. **CAS Registry Number:** 2445-77-4
3. **Synonyms:** Butanoic acid, 3-methyl-, 2-methylbutyl ester; 2-Methylbutyl isovalerate; 2-Methylbutyl isopentanoate; 2-Methylbutyl isovalerianate; 2-Methylbutyl 3-methylbutanoate
4. **Molecular Formula:** $C_{10}H_{20}O_2$
5. **Molecular Weight:** 172.26 g/mol
6. **RIFM Number:** 6198
7. **Stereochemistry:** Stereoisomer not specified. One chiral center is present, and 2 total enantiomers are possible.

## 2. Physical data

1. **Boiling Point:** 194 °C (Fragrance Materials Association [FMA]), 186.63 °C (EPI Suite)
2. **Flash Point:** 150 °F; closed cup (FMA)
3. **Log $K_{OW}$:** 3.66 (EPI Suite)
4. **Melting Point**: −31.53 °C (EPI Suite)
5. **Water Solubility:** 44.59 mg/L (EPI Suite)
6. **Specific Gravity:** 0.855 (FMA)
7. **Vapor Pressure:** 0.482 mm Hg at 20 °C (EPI Suite v4.0), 0.699 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:** Not Available

## 3. Volume of use (Worldwide Band)

1. <0.01 metric ton 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:** 0.08% (RIFM, 2022)

2. **Inhalation Exposure*:** 0.00034 mg/kg/day or 0.021 mg/day (RIFM, 2022)
3. **Total Systemic Exposure**:** 0.0031 mg/kg/day (RIFM, 2022)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM Aggregate Exposure Model (Comiskey, 2015; Safford et al., 2015; Safford, 2017; Comiskey, 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, 2015; Safford et al., 2015; Safford, 2017; Comiskey, 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-Methylbutyl 3-methylbutanoate is reported to occur in the following foods by the VCF*:

*Artocarpus* species.
Camomile.
Cheddar cheese.
Hop (*Humulus lupulus*)
Mastic (*Pistacia lentiscus*)
Mentha oils.
Quince, marmelo (*Cydonia oblonga* Mill.)
Sherry.
Tomato (*Lycopersicon esculentum* Mill.)

*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-Methylbutyl 3-methylbutanoate has been pre-registered for 2010; no dossier available as of 06/13/22.

## 10. Conclusion

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

## 11. Summary

### *11.1. Human health endpoint summaries*

#### *11.1.1. Genotoxicity*

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

*11.1.1.1. Risk assessment.* 2-Methylbutyl 3-methylbutanoate 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-methylbutyl 3-methylbutanoate; 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-methylbutyl 3-methylbutanoate.

**Additional References:** None.

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

#### *11.1.2. Repeated dose toxicity*

The MOE for 2-methylbutyl 3-methylbutanoate 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-methylbutyl 3-methylbutanoate. 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/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 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.

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

Therefore, the 2-methylbutyl 3-methylbutanoate 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-methylbutyl 3-methylbutanoate, 267/0.0031, or 86129.

In addition, the total systemic exposure to 2-methylbutyl 3-methylbutanoate (3.1 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:** 11/22/21.

### *11.1.3. Reproductive toxicity*

The MOE for 2-methylbutyl 3-methylbutanoate 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-methylbutyl 3-methylbutanoate. 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/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 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-methylbutyl 3-methylbutanoate 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-methylbutyl 3-methylbutanoate, 800/0.0031, or 258064.**

In addition, the total systemic exposure to 2-methylbutyl 3-methylbutanoate (3.1 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:** 11/22/21.

### *11.1.4. Skin sensitization*

Based on the existing data on the target material and read-across material isobutyl isobutyrate, 2-methylbutyl 3-methylbutanoate presents no concern for skin sensitization.

*11.1.4.1. Risk assessment.* Limited skin sensitization data are available for 2-methylbutyl 3-methylbutanoate. Therefore, read-across material isobutyl isobutyrate (CAS # 97-85-8; see Section VI) was used for the risk assessment of 2-methylbutyl 3-methylbutanoate. The data on the read-across material are summarized in Table 1. Based on the existing data on the read-across material, 2-methylbutyl 3-methylbutanoate 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

**Table 1**
Summary of existing data on isobutyl isobutyrate as a read-across for 2-methylbutyl 3-methylbutanoate.

| 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[d] Weighted Mean EC3 Value μg/cm$^2$ | GPMT[e] | Buehler[e] |
| No evidence of sensitization[g] | NA | NA | NA | NA | NA | NA | NA |
| | ***In Vitro* Data[f]** | | | | ***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] Based on animal data using classification defined in ECETOC, Technical Report No. 87, 2003.

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

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

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

peptide reactivity assay (DPRA) and KeratinoSens (ECHA, 2018).

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-methylbutyl 3-methylbutanoate 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-methylbutyl 3-methylbutanoate 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-methylbutyl 3-methylbutanoate in experimental models. UV/Vis absorption spectra indicate no absorption between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for photoirritation and photoallergenicity (Henry et al., 2009). Based on the lack of absorbance, 2-methylbutyl 3-methylbutanoate 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 2-methylbutyl 3-methylbutanoate 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 2-methylbutyl 3-methylbutanoate. Based on the Creme RIFM Model, the inhalation exposure is 0.021 mg/day. This exposure is 66.7 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-methylbutyl 3-methylbutanoate was performed following the RIFM Environmental Framework (Salvito, 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 VoU 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-methylbutyl 3-methylbutanoate 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-methylbutyl 3-methylbutanoate 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, 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2017a). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then

performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.1).

*11.2.1.1. Risk assessment.* Based on the current VoU (2019), 2-methylbutyl 3-methylbutanoate does not present a risk to the aquatic compartment in the screening-level assessment.

*11.2.1.2. Key studies*

*11.2.1.2.1. Biodegradation.* No data available.

*11.2.1.2.2. Ecotoxicity.* No data available.

*11.2.1.2.3. Other available data.* 2-Methylbutyl 3-methylbutanoate has been pre-registered for REACH with no additional data at this time.

*11.2.1.3. 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 Environmental Framework: Salvito, 2002)

| Exposure | Europe (EU) | North America (NA) |
|---|---|---|
| Log $K_{ow}$ Used | 3.66 | 3.66 |
| Biodegradation Factor Used | 0 | 0 |
| Dilution Factor | 3 | 3 |
| Regional VoU 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 0.00836 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 ChemView:** https://chemview.epa.gov/chemview/
  - **Japanese NITE:** https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com
- **ChemIDplus:** https://chem.nlm.nih.gov/chemidplus/

Search keywords: CAS number and/or material names.

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

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2022.113460.

## Appendix

*Read-across Justification*

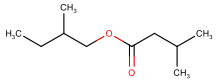
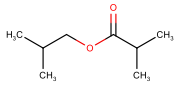
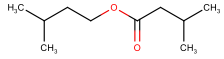
*Methods*

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)

| | LC50 (Fish) | EC50 (*Daphnia*) | EC50 (Algae) | AF | PNEC | Chemical Class |
|---|---|---|---|---|---|---|
| RIFM Framework Screening-level **(Tier 1)** | 8.36 mg/L | | | 1000000 | 0.00836 | |

and are consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemical Agency read-across assessment framework (ECHA, 2017b).

- First, materials were clustered based on their structural similarity. Second, data availability and data quality on the selected cluster were examined. Third, appropriate read-across analogs from the cluster were confirmed by expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physical–chemical properties of the target material and the read-across analogs were calculated using EPI Suite (US EPA, 2012a).
- $J_{max}$ values were calculated using RIFM's skin absorption model (SAM). The parameters were calculated using the consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts, and oncologic classification predictions were generated using 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-Methylbutyl 3-methylbutanoate | Isobutyl isobutyrate | Isoamyl isovalerate |
| **CAS No.** | 2445-77-4 | 97-85-8 | 659-70-1 |
| **Structure** |  |  |  |
| **Similarity (Tanimoto Score)** | | 0.70 | 0.89 |
| **SMILES** | CCC(C)COC(=O)CC(C)C | CC(C)COC(=O)C(C)C | CC(C)CCOC(=O)CC(C)C |
| **Endpoint** | | Genotoxicity<br>Skin sensitization | Repeated dose toxicity<br>Reproductive toxicity |
| **Molecular Formula** | $C_{10}H_{20}O_2$ | $C_8H_{16}O_2$ | $C_{10}H_{20}O_2$ |
| **Molecular Weight (g/mol)** | 172.268 | 144.214 | 172.268 |
| **Melting Point (°C, EPI Suite)** | −31.53 | −80.70 | −31.53 |
| **Boiling Point (°C, EPI Suite)** | 186.63 | 148.60 | 190.40 |
| **Vapor Pressure (Pa @ 25°C, EPI Suite)** | 9.32E+01 | 5.77E+02 | 1.18E+02 |
| **Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)** | 4.46E+01 | 1.00E+03 | 4.46E+01 |
| **Log KOW** | 3.66 | 2.68 | 3.66 |
| **$J_{max}$ (μg/cm$^2$/h, SAM)** | 4.88 | 65.89 | 4.88 |
| **Henry's Law (Pa·m$^3$/mol, Bond Method, EPI Suite)** | 1.29E+02 | 8.33E+01 | 1.29E+02 |
| ***Genotoxicity*** | | | |
| **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)** | Structural alert for nongenotoxic carcinogenicity\| Substituted n-alkylcarboxylic acids (Nongenotox) | No alert found | |
| **DNA Binding (Ames, MN, CA, OASIS v1.1)** | No alert found | No alert found | |
| ***In Vitro* Mutagenicity (Ames, ISS)** | No alert found | No alert found | |
| ***In Vivo* Mutagenicity (Micronucleus, ISS)** | 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-methylbutyl 3-methylbutanoate (CAS # 2445-77-4). 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-methylbutyl 3-methylbutanoate (CAS # 2445-77-4) for the genotoxicity and skin sensitization endpoints.
  - o The target material and the read-across analog are structurally similar and belong to the class of branched-chain saturated esters.
  - o The target material and the read-across analog share similar branched saturated ester structures.
  - o 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.
  - o 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.
  - o The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - o According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - o There are no *in silico* alerts for the target material and the read-across analog. *In silico* alerts are consistent with the data.
  - o The target material has a non-genotoxic carcinogenicity alert. The data on the read-across analog confirms that the substance does not pose a concern for genotoxicity. Therefore, based on the structural similarity between the read-across analog and target material and the data on the read-across analog, the *in silico* alert is superseded.
  - 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.
- Isoamyl isovalerate (CAS # 659-70-1) was used as a read-across analog for the target material 2-methylbutyl 3-methylbutanoate (CAS # 2445-77-4) for the repeated dose and reproductive toxicity endpoints.
  - o The target material and the read-across analog are structurally similar and belong to the class of branched-chain saturated esters.
  - o The target material and the read-across analog share similar branched saturated ester structures.
  - o 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.
  - o 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.
  - o The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - o According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - o There are no *in silico* alerts for the target material and the read-across analog. *In silico* alerts are consistent with the 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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