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Short Review

RIFM fragrance ingredient safety assessment, methyl isovalerate, CAS Registry Number 556-24-1

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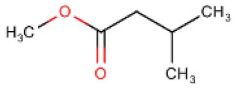
Version: 073118. This version replaces any previous versions.
Name: Methyl isovalerate
CAS Registry Number: 556-24-1



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
GLP - Good Laboratory Practice

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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
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 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 isovalerate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that methyl isovalerate is not genotoxic. Data from read-across analog ethyl-2-methylbutyrate (CAS # 7452-79-1) 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 isovalerate 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 isovalerate is below the TTC (1.4 mg/day). The phototoxicity/photoallergenicity endpoint was evaluated based on UV spectra; methyl isovalerate is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; methyl isovalerate was found not to be PBT as per the IFRA Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., PEC/PNEC), are < 1.

Human Health Safety Assessment

Genotoxicity: Not genotoxic.	(RIFM, 2017a; RIFM, 2017b)
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 Database)
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: 7.38 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Screening-level: Fish LC50: 248.40 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: 248.40 mg/L	(RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.24840 µg/L	

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

I. Identification

1. **Chemical Name:** Methyl isovalerate
2. **CAS Registry Number:** 556-24-1
3. **Synonyms:** Butanoic acid, 3-methyl-, methyl ester; Methyl isopentanoate; Methyl isovalerianate; Methyl 3-methylbutyrate; Methyl 3-methylbutanoate; ﾍﾟﾝﾀﾝ酸ｱﾙｷﾙ(C = 1 ~ 5); Methyl isovalerate
4. **Molecular Formula:** $C_6H_{12}O_2$
5. **Molecular Weight:** 116.16
6. **RIFM Number:** 6152
7. **Stereochemistry:** Isomer not specified. No stereocenters and no stereoisomers possible.

2. Physical data

1. **Boiling Point:** 117 °C (FMA database), 111.74 °C (USEPA, 2012a)
2. **Flash Point:** 26 °C (GHS), 79 °F; CC (FMA database)
3. **Log K_{OW}:** 1.77 (US EPA, 2012a)
4. **Melting Point**: 68.43 °C (US EPA, 2012a)
5. **Water Solubility:** 2892 mg/L (US EPA, 2012a)
6. **Specific Gravity:** 0.88 (FMA database), 0.9015 (EOA, 1976 Sample 76–187)
7. **Vapor Pressure:** 13.7 mm Hg @ 20 °C (US EPA, 2012a), 14 mm Hg 20 °C (FMA database), 18.3 mm Hg @ 25 °C (US EPA, 2012a)
8. **UV Spectra:** No significant absorbance between 290 and 700 nm;

molar absorption coefficient is below the benchmark (1000 L mol^{-1} · cm^{-1})
9. **Appearance/Organoleptic:** colorless liquid, pungent, ethereal, fruity apple-like odor; sweet ethereal and apple fruity taste (Arctander, 2264, Volume II, 1969)

3. Exposure to fragrance ingredient

1. **Volume of Use (Worldwide Band):** < 0.1 metric ton per year (IFRA, 2015)
2. **95th Percentile Concentration in Hydroalcoholics:** 0.0038% (RIFM, 2017c)
3. **Inhalation Exposure*:** 0.000017 mg/kg/day or 0.0012 mg/day (RIFM, 2017c)
4. **Total Systemic Exposure**:** 0.00011 mg/kg/day (RIFM, 2017c)

*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

1. **Dermal:** Assumed 100%
2. **Oral:** Assumed 100%
3. **Inhalation:** Assumed 100%

5. Computational toxicology evaluation

1. Cramer Classification: Class I, Low

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

2. Analogs Selected:
   a. Genotoxicity: None
   b. Repeated Dose Toxicity: Ethyl-2-methylbutyrate (CAS # 7452-79-1)
   c. Reproductive Toxicity: Ethyl-2-methylbutyrate (CAS # 7452-79-1)
   d. Skin Sensitization: Ethyl isobutyrate (CAS # 97-62-1); methyl 2-methylbutyrate (CAS # 868-57-5)
   e. Phototoxicity/Photoallergenicity: None
   f. Local Respiratory Toxicity: None
   g. 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 isovalerate is reported to occur in the following foods by the VCF* and is not found in natural complex substances (NCS).

Alpinia species.
Apple fresh (*Malus* species).
Artocarpus species.
Banana (*Musa sapientum* L.)
Blue cheeses.
Cape gooseberry (*Physalis peruviana* L.)
Capsicum species.
Cashew apple (*Anacardium occidentale*).
Cashew apple wine.
Cheese, various types.
Cherimoya (*Annona cherimolia* Mill.)
Coffee.
Custard apple, atemoya (*Annona atemoya*).
Honey.
Lamb's lettuce (*Valerianella locusta*).
Mangifera species.
Melon.
Mentha oils.
Mushroom.
Nectarine.
Olive (*Olea europaea*).
Peas (*Pisum sativum* L.)
Pepper (*Piper nigrum* L.)
Pineapple (*Ananas comosus*).
Rooibos tea (*Aspalathus linearis*).
Salvia species.
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

Pre-registered for 2010; no dossier available as of 07/31/18.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

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

10.1.1.1. Risk assessment. Methyl isovalerate was assessed in the BlueScreen assay and found negative for both cytotoxicity and genotoxicity, with and without metabolic activation (RIFM, 2013). The mutagenic activity of methyl isovalerate 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 methyl isovalerate 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, 2017a). Under the conditions of the study, methyl isovalerate was not mutagenic in the Ames test.

The clastogenic activity of methyl isovalerate 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 methyl isovalerate in DMSO at concentrations up to 1160 μg/mL in the presence and absence of metabolic activation (S9) for 4 h and in the absence of metabolic activation for 24 h. Methyl isovalerate did not induce binucleated cells with micronuclei when tested up to cytotoxic levels concentration in either the presence or absence of an S9 activation system (RIFM, 2017b). Under the conditions of the study, methyl isovalerate was considered to be non-clastogenic in the *in vitro* micronucleus test.

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

Additional References: None.

Literature Search and Risk Assessment Completed On: 12/04/17.

10.1.2. Repeated dose toxicity

The margin of exposure for methyl isovalerate 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 isovalerate. Read-across material, ethyl 2-methylbutrate (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 isovalerate 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 isovalerate, 333/0.00011 or 3027273.

In addition, the total systemic exposure to methyl isovalerate (0.11 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 isovalerate 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 isovalerate. Read-across material, ethyl 2-methylbutrate (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 isovalerate MOE for the reproductive toxicity endpoint can be calculated by dividing ethyl 2-methylbutrate NOAEL in mg/kg/day by the total systemic exposure to methyl isovalerate, 1000/0.00011 or 9090909.

In addition, the total systemic exposure to methyl isovalerate (0.11 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 isovalerate 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 isovalerate. 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 isovalerate does not present a safety concern for skin sensitization under the current, declared levels of use. The chemical structure 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 materials 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 materials ethyl isobutyrate and methyl 2-methylbutyrate (RIFM, 1982; RIFM, 1982; RIFM, 1975). Based on weight of evidence from structural analysis, animal and human studies, and read-across materials ethyl isobutyrate and methyl 2-methylbutyrate, methyl isovalerate 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 isovalerate 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 isovalerate 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 isovalerate 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 L mol^{-1} · cm^{-1} (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/20/17.

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 isovalerate 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 isovalerate. Based on the Creme RIFM model, the inhalation exposure is 0.0012 mg/day. This exposure is 1167 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 isovalerate 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 isovalerate 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 isovalerate as possibly persistent or bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

10.2.2. Risk assessment

Based on the current Volume of Use (2015), methyl isovalerate 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 isovalerate has been pre-registered for 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 μg/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)**	<u>248.40</u>			1,000,000	0.24840	

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} used	1.77	1.77
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 0.24840 μg/L. The revised PEC/PNECs for EU and NA are: not applicable. The material was cleared at the 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: 11/28/17.

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/
- 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/oppthpv/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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2018.11.032.

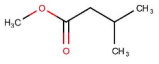
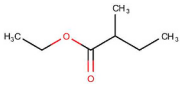
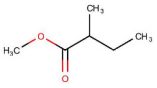
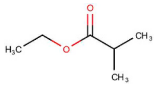
Appendix

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).

	Target Material	Read-across Material	Read-across Material	Read-across Material
Principal Name	Methyl isovalerate	Ethyl 2-methylbutyrate	Methyl 2-methylbutyrate	Ethyl isobutyrate
CAS No.	556-24-1	7452-79-1	868-57-5	97-62-1
Structure				
Similarity (Tanimoto Score)		0.73	0.81	0.70
Read-across Endpoint		• Repeated dose Toxicity • Reproductive Toxicity	• Skin sensitization	• Skin sensitization
Molecular Formula	$C_6H_{12}O_2$	$C_7H_{14}O_2$	$C_6H_{12}O_2$	$C_6H_{12}O_2$
Molecular Weight	116.16	130.19	116.16	116.16
Melting Point (°C, EPI Suite)	−68.43	−56.05	−68.43	−68.43
Boiling Point (°C, EPI Suite)	111.74	134.87	111.74	111.74
Vapor Pressure (Pa @ 25°C, EPI Suite)	2.44E+003	1.07E+003	3E+003	3E+003
Log Kow(KOWWIN v1.68 in EPI Suite)	1.82	2.26	1.77	1.77
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	2892	1070	3172	3172
J_{max} (mg/cm^2/h, SAM)	465.295	297.516	440.615	460.179
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	4.16E+001	5.52E+001	4.16E+001	4.16E+001
	Repeated Dose Toxicity			
Repeated Dose (HESS)	• Not categorized	• Not categorized		
	Reproductive Toxicity			

ER Binding (OECD QSAR Toolbox v3.4)	• Non-binder, cyclic structure	• Non-binder, 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	• 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
	Metabolism			
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 isovalerate (CAS # 556-24-1). 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 isovalerate (CAS # 556-24-1) 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 chain acid ester structures.
  - The key structural difference between the target material and the read-across analog is that the target material is the methyl ester of isovaleric 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 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 isovalerate (CAS # 556-24-1) 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 isovaleric acid, whereas the read-across analog is the methyl 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 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 isovalerate (CAS # 556-24-1) 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 esters of similar 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 isovaleric acid, whereas the read-across analog is the ethyl ester of isobutyric 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 acid 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.

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