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Short Review

RIFM fragrance ingredient safety assessment, methyl 2-methylthiobutyrate, CAS Registry Number 42075-45-6

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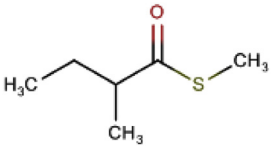
Version: 011619. This version replaces any previous versions.
Name: Methyl 2-methylthiobutyrate
CAS Registry Number: 42075-45-6



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.,

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2015, 2017; Safford et al., 2015a, 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observable Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
QRA - Quantitative Risk Assessment
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
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.

Methyl 2-methylthiobutyrate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analog methyl thiobutyrate (CAS # 2432-51-1) show that methyl 2-methylthiobutyrate is not expected to be genotoxic. The repeated dose, reproductive, and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class I material, and the exposure to methyl 2-methylthiobutyrate is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). The skin sensitization endpoint was completed using the DST for non-reactive materials (900 μg/cm^2); exposure is below the DST. The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; methyl 2-methylthiobutyrate is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; for the hazard assessment based on the screening data, methyl 2-methylthiobutyrate is not PBT as per the IFRA Environmental Standards. For the risk assessment, methyl 2-methylthiobutyrate was not able to be risk screened as there were no reported volumes of use for either North America or Europe in the 2015 IFRA Survey.

Human Health Safety Assessment

Genotoxicity: Not expected to be genotoxic. (RIFM, 2016b; RIFM, 2016a)

Repeated Dose Toxicity: No NOAEL available. Exposure is below the TTC.

Reproductive Toxicity: No NOAEL available. Exposure is below the TTC.

Skin Sensitization: No safety concerns at current, declared use levels; exposure is below the DST.

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: 2.9 (BIOWIN 3) (EPI Suite v4.11; US EPA, 2012a)

Bioaccumulation:

Screening-level: 5.472 L/kg (EPI Suite v4.11; US EPA, 2012a)

Ecotoxicity:

Screening-level: Not applicable

Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:

- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: not applicable; no volume of use in 2015 reported for Europe and North America

1. Identification

1. **Chemical Name:** Methyl 2-methylthiobutyrate
2. **CAS Registry Number:** 42075-45-6
3. **Synonyms:** Butanethioic acid, 2-methyl-, S-methyl ester; Methylthio 2-methylbutyrate; S-Methyl 2-methylbutanethioate; Methyl 2-methylthiobutyrate
4. **Molecular Formula:** $C_6H_{12}OS$
5. **Molecular Weight:** 132.22
6. **RIFM Number:** 1421
7. **Stereochemistry:** Isomer not specified. One chiral center and 1 chiral isomer possible.

2. Physical data

1. **Boiling Point**: 169.23 °C (EPI Suite)
2. **Flash Point**: Not Available
3. **Log K_{OW}**: 1.62 (EPI Suite)
4. **Melting Point**: −30.65 °C (EPI Suite)
5. **Water Solubility:** 3701 mg/L (EPI Suite)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 1.62 mm Hg @ 25 °C (EPI Suite)
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:** Not Available

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:** 0.06% (RIFM, 2018)
3. **Inhalation Exposure*:** 0.000021 mg/kg/day or 0.00014 mg/day (RIFM, 2018)
4. **Total Systemic Exposure**:** 0.000045 mg/kg/day (RIFM, 2018)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM Aggregate Exposure Model (Comiskey et al., 2015; Safford et al., 2015a; 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 4. 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.,

2015a; 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:** Methyl thiobutyrate (CAS # 2432-51-1)
   b. **Repeated Dose Toxicity:** Ethyl thioacetate (CAS # 625-60-5)
   c. **Reproductive Toxicity:** None
   d. **Skin Sensitization:** None
   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.
Additional References:
None.

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

Methyl 2-methylthiobutyrate is reported to occur in the following foods by the VCF*:

Beer.

Hop (*Humulus lupulus*).

*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 11/15/18.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data, methyl 2-methylthiobutyrate does not present a concern for genotoxicity.

10.1.1.1. Risk assessment. There are no data assessing the mutagenic and clastogenic activity of methyl 2-methylthiobutyrate; however, read-across can be made to methyl thiobutyrate (CAS # 2432-51-1; see Section 5).

The mutagenic activity of methyl thiobutyrate 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 thiobutyrate 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, 2016b). Under the conditions of the study, methyl thiobutyrate was not mutagenic in the Ames test (and this can be extended to methyl 2-methylthiobutyrate).

The clastogenic activity of methyl thiobutyrate 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 thiobutyrate in DMSO at concentrations up to 1180 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 thiobutyrate 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, 2016a). Under the conditions of the study, methyl thiobutyrate was considered to be non-clastogenic in the *in vitro* micronucleus test (and this can be extended to methyl 2-methylthiobutyrate).

Based on the data available, methyl 2-methylthiobutyrate does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 12/27/18.

10.1.2. Repeated dose toxicity

There are limited repeated dose toxicity data on methyl 2-methylthiobutyrate or any read-across materials. The total systemic exposure to methyl 2-methylthiobutyrate is below the TTC for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

10.1.2.1. Risk assessment. There are no repeated dose toxicity data on methyl 2-methylthiobutyrate. Read-across material, ethyl thioacetate (CAS # 625-60-5; see Section 5) has limited repeated dose toxicity data. Groups of 23 albino Sprague Dawley rats/sex were administered test material, ethyl thioacetate via diet at targeted dose of 6.48 mg/kg/day (6.63 and 6.7 mg/kg/day actual based on food consumption and bodyweight measurements). The test was conducted prior to GLP or standard OECD test guideline implementations; however, the parameters evaluated during the length of the study provided sufficient safety information on ethyl thioacetate at levels tested. There were no treatment-related alterations reported among treated animals up to the highest dose tested (RIFM, 1970). Thus, the NOAEL for ethyl thioacetate was considered to be 6.63 mg/kg/day, the highest dose tested among males. However, the study included only a single dose to evaluate the safety of ethyl thioacetate hence the NOAEL was not used towards the safety assessment of methyl 2-methylthiobutyrate.

The total systemic exposure to methyl 2-methylthiobutyrate (0.045 μg/kg bw/day) is below the TTC (30 μg/kg bw/day; Kroes et al., 2007: #53925) for the repeated dose toxicity endpoint for a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 12/27/18.

10.1.3. Reproductive toxicity

There are no reproductive toxicity data on methyl 2-methylthiobutyrate or on any read-across materials. The total systemic exposure to methyl 2-methylthiobutyrate is below the TTC for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

10.1.3.1. Risk assessment. There are no reproductive toxicity data on

methyl 2-methylthiobutyrate or on any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to methyl 2-methylthiobutyrate (0.045 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: 12/10/18.

10.1.4. Skin sensitization

Based on the existing data and the application of the dermal sensitization threshold (DST), methyl 2-methylthiobutyrate does not present a safety concern for skin sensitization under the current, declared levels of use.

10.1.4.1. Risk assessment. The chemical structure of this material indicates that it would not be expected to react with skin proteins (Roberts et al., 2007; Toxtree 3.1.0; OECD Toolbox v4.2). No predictive skin sensitization studies are available for methyl 2-methylthiobutyrate. Acting conservatively, due to the absence of data, the reported exposure was benchmarked utilizing the non-reactive DST of 900 μg/cm^2 (Safford, 2008; Safford et al., 2011; Roberts et al., 2015; Safford et al., 2015b). The current exposure from the 95th percentile concentration is below the DST for non-reactive materials when evaluated in all QRA categories. Table 1 provides the maximum acceptable concentrations for methyl 2-methylthiobutyrate that present no appreciable risk for skin sensitization based on the non-reactive DST. These levels represent maximum acceptable concentrations based on the DST approach. However, additional studies may show it could be used at higher levels.

Additional References: None.

Literature Search and Risk Assessment Completed On: 12/03/18.

10.1.5. Phototoxicity/Photoallergenicity

Based on the available UV/Vis spectra, methyl 2-methylthiobutyrate 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 2-methylthiobutyrate 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 2-methylthiobutyrate 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: 11/20/18.

10.1.6. Local Respiratory Toxicity

The margin of exposure could not be calculated due to a lack of appropriate data. The exposure level for methyl 2-methylthiobutyrate 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 2-methylthiobutyrate. Based on the Creme RIFM Model, the inhalation exposure is 0.00014 mg/day. This exposure is 10000 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: 12/11/18.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of methyl 2-methylthiobutyrate 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

Table 1
Maximum acceptable concentrations for methyl 2-methylthiobutyrate that present no appreciable risk for skin sensitization based on non-reactive DST.

IFRA Category[a]	Description of Product Type	Maximum Acceptable Concentrations in Finished Products Based on Non-reactive DST	Reported 95th Percentile Use Concentrations in Finished Products
1	Products applied to the lips	0.069%	1.0×10^{-5}%
2	Products applied to the axillae	0.021%	1.0×10^{-4}%
3	Products applied to the face using fingertips	0.41%	8.0×10^{-6}%
4	Fine fragrance products	0.39%	0.0020%
5	Products applied to the face and body using the hands (palms), primarily leave-on	0.10%	8.0×10^{-5}%
6	Products with oral and lip exposure	0.23%	3.0×10^{-5}%
7	Products applied to the hair with some hand contact	0.79%	1.2×10^{-5}%
8	Products with significant ano-genital exposure	0.041%	No Data[c]
9	Products with body and hand exposure, primarily rinse-off	0.75%	4.0×10^{-5}%
10	Household care products with mostly hand contact	2.7%	3.2×10^{-4}%
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate	1.5%	No Data[c]
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted	0.0020%

Note: [a]For a description of the categories, refer to the IFRA/RIFM Information Booklet.
[c]Fragrance exposure from these products is very low. These products are not currently in the Creme RIFM Aggregate Exposure Model.

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 2-methylthiobutyrate was not able to be risk screened as there were no reported volumes of use for either North America or Europe in the 2015 IFRA Survey.

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) identified methyl 2-methylthiobutyrate as possibly being 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).

10.2.2. Risk assessment

Not applicable.

10.2.2.1. Key studies

10.2.2.1.1. Biodegradation. No data available.

10.2.2.1.2. Ecotoxicity. No data available.

10.2.2.1.3. Other available data. No other data available.

10.2.3. Risk assessment refinement. Not applicable.

Literature Search and Risk Assessment Completed On: 01/03/19.

11. 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**
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
- **TOXNET:** https://toxnet.nlm.nih.gov/
- **IARC:** https://monographs.iarc.fr
- **OECD SIDS:** https://hpvchemicals.oecd.org/ui/Default.aspx
- **EPA ACToR:** https://actor.epa.gov/actor/home.xhtml
- **US EPA HPVIS:** https://ofmpub.epa.gov/oppthpv/public_search.publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&EndPointRpt=Y#submission
- **Japanese NITE:** https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com
- **ChemIDplus:** https://chem.nlm.nih.gov/chemidplus/

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 05/31/19.

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.2019.111006.

Appendix

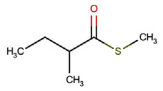
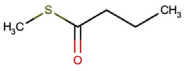
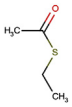
Read-across Justification

Methods

The read-across analogs were identified following the strategy for structuring and reporting a read-across prediction of toxicity as 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, the 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 v4.11 (US ECHA, 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).

- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018) and skin sensitization was predicted using Toxtree.
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).

	Target Material	Read-across Material	Read-across Material
Principal Name	Methyl 2-methylthiobutyrate	Methyl thiobutyrate	Ethyl thioacetate
CAS No.	42075-45-6	2432-51-1	625-60-5
Structure			
Similarity (Tanimoto Score)		0.83	0.44
Read-across Endpoint		• Genotoxicity	• Repeated dose toxicity
Molecular Formula	$C_6H_{12}OS$	$C_5H_{10}OS$	C_4H_8OS
Molecular Weight	132.22	118.19	104.17
Melting Point (°C, EPI Suite)	−30.65	−31.27	−43.35
Boiling Point (°C, EPI Suite)	169.23	160.71	116.4
Vapor Pressure (Pa @ 25 °C, EPI Suite)	216	324	2.45E+003
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	1.62	1.21	0.71
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPI Suite)	3701	9515	2.786e+004
J_{max} (μg/cm^2/h, SAM)	69.80	316.90	241.88
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.48E+001	4.024E+000	8.39E+000
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)	• Non-carcinogen (low reliability)	• Non-carcinogen (low reliability)	
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)	• Thiocarbamates/Sulfides (Hepatotoxicity) No rank		• Thiocarbamates/Sulfides (Hepatotoxicity) No rank
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 methyl 2-methylthiobutyrate (CAS # 42075-45-6). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, physical–chemical properties, and expert judgment, methyl thiobutyrate (CAS # 2432-51-1) and ethyl thioacetate (CAS # 625-60-5) were identified as read-across analogs with sufficient data for toxicological evaluation.

Conclusions

- Methyl thiobutyrate (CAS # 2432-51-1) was used as a read-across analog for the target material methyl 2-methylthiobutyrate (CAS # 42075-45-6) for the genotoxicity endpoint.
  - The target substance and the read-across analog are structurally similar and belong to a class of alkyl thioesters.
  - The target substance and the read-across analog are both thiomethyl esters.
  - The key difference between the target substance and the read-across analog is that the target material is a butyric acid methylthioester, whereas the read-across analog is a butyric acid methylthioester. This structural difference is toxicologically insignificant.
  - The similarity between the target substance and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - The physical–chemical properties of the target substance and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target substance and the read-across analog.
  - Data are consistent with *in silico* alerts.
  - The target substance 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.
- Ethyl thioacetate (CAS # 625-60-5) was used as a read-across analog for the target material methyl 2-methylthiobutyrate (CAS # 42075-45-6) for the repeated dose toxicity endpoint.
  - The target substance and the read-across analog are structurally similar and belong to a class of alkyl thioesters.
  - The target substance and the read-across analog share thioester structures.

- The key difference between the target substance and the read-across analog is that the target material is the thioester of methylbutyric acid and methylthiol, whereas the read-across analog is the thioester of acetic acid and ethylthiol. These structural differences are toxicologically insignificant.
- The similarity between the target substance and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
- The physical–chemical properties of the target substance and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
- According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target substance and the read-across analog.
- Both materials display an alert for repeated dose due to the thiocarboxylate group. A literature search shows however that neither the target material nor the read-across analog display hepatotoxicity. The predictions are superseded by data.
- The target substance and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
- The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

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