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Short Review

RIFM fragrance ingredient safety assessment, amyl isobutyrate, CAS Registry Number 2445-72-9

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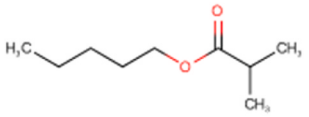

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Name: Amyl isobutyrate
CAS Registry Number: 2445-72-9

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 Confirmation of No Induction in Humans test that is performed to confirm an already determined safe use level for fragrance ingredients (Na et al., 2021)
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015a; Safford et al., 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DRF - Dose Range Finding
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observed Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
Perfumery - In this safety assessment, perfumery refers to fragrances made by a perfumer used in consumer products only. The exposures reported in the safety assessment include consumer product use but do not include occupational exposures.
QRA - Quantitative Risk Assessment
QSAR - Quantitative Structure-Activity Relationship
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use
vPvB - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

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

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

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

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

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

Amyl isobutyrate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, photoirritation/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analog ethyl 2-methylbutyrate (CAS # 7452-79-1) show that amyl isobutyrate is not expected to be genotoxic and provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and reproductive toxicity endpoints. Data from read-across analog hexyl 2-methylbutyrate (CAS # 10032-15-2) provided amyl isobutyrate a NESIL of 7000 μg/cm^2 for the skin sensitization endpoint. The photoirritation/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; amyl isobutyrate is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material; exposure is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; amyl isobutyrate 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.

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Human Health Safety Assessment	
Genotoxicity: Not expected to be genotoxic.	(RIFM, 2000a; RIFM, 2014; RIFM, 2016; RIFM, 2017)
Repeated Dose Toxicity: NOAEL = 333 mg/kg/day.	(ECHA REACH Dossier: Ethyl 2-methylbutyrate; ECHA, 2013)
Reproductive Toxicity: Developmental toxicity NOAEL: 1000 mg/kg/day. Fertility NOAEL: 1000 mg/kg/day.	(ECHA REACH Dossier: Ethyl 2-methylbutyrate; ECHA, 2013)
Skin Sensitization: NESIL = 7000 μg/cm^2.	RIFM (2018)
Photoirritation/ Photoallergenicity: Not expected to be phototoxic/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: 3.3 (BIOWIN 3)	(EPI Suite v4.11; US EPA, 2012a)
Bioaccumulation:	
Screening-level: 64.39 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: Fish LC50: 17.5 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: 17.5 mg/L	(RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.0175 μg/L	
• **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: Not applicable; cleared at screening-level	

1. Identification

1. **Chemical Name:** Amyl isobutyrate
2. **CAS Registry Number:** 2445-72-9
3. **Synonyms:** Pentyl isobutyrate; Propanoic acid, 2-methyl-, pentyl ester; ｱﾐﾙ酸ｱﾙｷﾙ(C = 1～7); Pentyl 2-methylpropanoate; Amyl isobutyrate
4. **Molecular Formula:** $C_9H_{18}O_2$
5. **Molecular Weight:** 158.24
6. **RIFM Number:** 6197
7. **Stereochemistry:** No stereocenter possible

2. Physical data

1. **Boiling Point:** 178.41 °C (EPI Suite)
2. **Flash Point:** Not Available
3. **Log K_{OW}:** 3.25 (EPI Suite)
4. **Melting Point**: −32.06 °C (EPI Suite)
5. **Water Solubility:** 117.8 mg/L (EPI Suite)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.726 mm Hg at 20 °C (EPI Suite v4.0), 1.04 mm Hg at 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. Volume of use (worldwide band)

1. 1–10 metric tons per year (IFRA, 2015)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v1.0)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.036% (RIFM, 2019)
2. **Inhalation Exposure*:** 0.00078 mg/kg/day or 0.065 mg/day (RIFM, 2019)
3. **Total Systemic Exposure**:** 0.0023 mg/kg/day (RIFM, 2019)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM Aggregate Exposure Model (Comiskey et al., 2015; Safford, 2015; Safford, 2017; Comiskey et al., 2017).

**95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section V. It is derived from concentration survey data in the Creme RIFM Aggregate Exposure Model and includes exposure via dermal, oral, and inhalation routes whenever the fragrance ingredient is used in products that include these routes of exposure (Comiskey et al., 2015; Safford, 2015; Safford, 2017; Comiskey et al., 2017).

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

1. **Cramer Classification:** Class I, Low

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

2. Analogs Selected:
   a. **Genotoxicity:** Ethyl 2-methylbutyrate (CAS # 7452-79-1)
   b. **Repeated Dose Toxicity:** Ethyl 2-methylbutyrate (CAS # 7452-79-1)
   c. **Reproductive Toxicity:** Ethyl 2-methylbutyrate (CAS # 7452-79-1)
   d. **Skin Sensitization:** Hexyl 2-methylbutyrate (CAS # 10032-15-2)
   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 (discrete chemical) or composition (NCS)

Amyl isobutyrate is reported to occur in the following foods by the VCF*:

Apple fresh (*Malus* species)	Hop (*Humulus lupulus*)
Apricot (*Prunus armeniaca* L.)	*Mangifera* species
Banana (*Musa sapientum* L.)	Plum (*Prunus* species)
Capsicum species	Spineless monkey orange (*Strychnos madagasc.*)
Date (*Phoenix dactylifera* L.)	

*VCF (Volatile Compounds in Food): Database/Nijssen, L.M.; Ingen-Visscher, C.A. van; Donders, J.J.H. (eds). – Version 15.1 – Zeist (The Netherlands): TNO Triskelion, 1963–2014. A continually updated database containing information on published volatile compounds that have been found in natural (processed) food products. Includes FEMA GRAS and EU-Flavis data.

9. REACH dossier

No dossier available as of 12/09/21.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for amyl isobutyrate are detailed below.

IFRA Category[b]	Description[a] of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
1	Products applied to the lips (lipstick)	0.54
2	Products applied to the axillae	0.16
3	Products applied to the face/body using fingertips	3.2
4	Products related to fine fragrances	3.0
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.76
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.76
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.76
5D	Baby cream, oil, talc	0.25
6	Products with oral and lip exposure	1.8
7	Products applied to the hair with some hand contact	6.1
8	Products with significant ano-genital exposure (tampon)	0.25
9	Products with body and hand exposure, primarily rinse-off (bar soap)	5.9
10A	Household care products with mostly hand contact (hand dishwashing detergent)	3.2
10B	Aerosol air freshener	19
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.25
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	No restriction

Note:
[a] Maximum acceptable concentrations for each product category are based on the lowest maximum acceptable concentrations (based on systemic toxicity, skin sensitization, or any other endpoint evaluated in this safety assessment). For amyl isobutyrate, the basis was the subchronic reference dose of 3.33 mg/kg/day, a predicted skin absorption value of 80%, and a skin sensitization NESIL of 7000 μg/cm^2.
[b] For a description of the categories, refer to the IFRA RIFM Information Booklet (https://www.rifm.org/downloads/RIFM-IFRA%20Guidance-for-the-use-of-IFRA-Standards.pdf).
[c] Calculations by Creme RIFM Aggregate Exposure Model v3.1.4.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

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

11.1.1.1. Risk assessment. Amyl isobutyrate was assessed in the BlueScreen assay and found negative for both cytotoxicity (positive: <80% relative cell density) and genotoxicity, with and without metabolic activation (RIFM, 2013). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. Additional assays on a 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 and clastogenic activity of amyl isobutyrate; however, read-across can be made to ethyl 2-methylbutyrate (CAS # 7452-79-1; see Section VI).

The mutagenic activity of ethyl 2-methylbutyrate has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102 were treated with ethyl 2-methylbutyrate in dimethyl sulfoxide (DMSO) at concentrations up to 5000 μg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 2000a). Under the conditions of the study, ethyl 2-methylbutyrate was not mutagenic in the Ames test, and this can be extended to amyl isobutyrate.

The clastogenic activity of ethyl 2-methylbutyrate was evaluated in an *in vitro* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes were treated with ethyl 2-methylbutyrate in DMSO at concentrations up to 1300 μg/mL in the dose range finding (DRF) study; micronuclei analysis was conducted at concentrations up to 1300 μg/mL in the presence and absence of metabolic activation. Ethyl 2-methylbutyrate did not induce binucleated cells with micronuclei when tested up to cytotoxic level or the maximum concentration in either the presence or absence of an S9 activation system (RIFM, 2014). Under the conditions of the study, ethyl 2-methylbutyrate was considered to be non-clastogenic in the *in vitro* micronucleus test, and this can be extended to amyl isobutyrate.

Based on the data available, ethyl 2-methylbutyrate does not present a concern for genotoxic potential, and this can be extended to amyl isobutyrate.

Additional References: RIFM, 1999.

Literature Search and Risk Assessment Completed On: 10/02/20

11.1.2. Repeated dose toxicity

The MOE for amyl isobutyrate is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are no repeated dose toxicity data on amyl isobutyrate. Read-across material ethyl 2-methylbutyrate (CAS # 7452-79-1; see Section VI) has sufficient 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 for both). 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 (ECHA, 2012). 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 amyl isobutyrate MOE for the repeated dose toxicity endpoint can be calculated by dividing the ethyl 2-methylbutyrate NOAEL in mg/kg/day by the total systemic exposure to amyl isobutyrate, 333/0.0023 or 144783.

In addition, the total systemic exposure to amyl isobutyrate (2.3 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.

11.1.2.1.1. Derivation of subchronic reference dose (RfD). Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (2020) and a subchronic reference dose of 3.33 mg/kg/day.

The RIFM Criteria Document (Api et al., 2015) calls for a default MOE of 100 (10 × 10), based on uncertainty factors applied for interspecies (10 ×) and intraspecies (10 ×) differences. The subchronic reference dose for amyl isobutyrate was calculated by dividing the lowest NOAEL (from the Repeated Dose and Reproductive Toxicity sections) of 333 mg/kg/day by the uncertainty factor, 100 = 3.33 mg/kg/day.

*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: 08/06/20

11.1.3. Reproductive toxicity

The MOE for amyl isobutyrate is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are no reproductive toxicity data on amyl isobutyrate. Read-across material ethyl 2-methylbutyrate (CAS # 7452-79-1; see Section VI) has sufficient 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 for both). 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 developmental toxicity and fertility was considered to be 1000 mg/kg/day, the highest dose tested (ECHA, 2013).

Therefore, the amyl isobutyrate MOE for the reproductive toxicity endpoint can be calculated by dividing the ethyl 2-methylbutyrate NOAEL in mg/kg/day by the total systemic exposure to amyl isobutyrate, 1000/0.0023, or 434783.

In addition, the total systemic exposure to amyl isobutyrate (2.3 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: 09/17/20

11.1.4. Skin sensitization

Based on read-across material hexyl 2-methylbutyrate (CAS # 10032-15-2), amyl isobutyrate is considered a skin sensitizer with a defined NESIL of 7000 μg/cm^2.

11.1.4.1. Risk assessment. No data on skin sensitization studies are available for amyl isobutyrate. Based on read-across material hexyl 2-methylbutyrate (CAS # 10032-15-2; see Section VI), amyl isobutyrate is considered a skin sensitizer. The chemical structure of these materials 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). The read-across material, hexyl 2-methylbutyrate, was found to be negative in the *in vitro* direct peptide reactivity assay (DPRA) and KeratinoSens assay (RIFM, 2015b; RIFM, 2015a). In a murine local lymph node assay (LLNA), read-across material hexyl 2-methylbutyrate was found to be sensitizing with an EC3 value of 54.8% (13700 μg/cm^2) (RIFM, 2000b). However, the results from this LLNA may be suboptimal since the test was conducted in the unvalidated range (>25%) of the OECD guideline (Kolle et al., 2020). In a guinea pig open epicutaneous test (OET), read-across material hexyl 2-methylbutyrate did not present reactions indicative of sensitization (Klecak, 1985). In a human maximization test, no skin sensitization reactions were observed with read-across material hexyl 2-methylbutyrate at 10% (6900 μg/cm^2) in petrolatum (RIFM, 1977). Additionally, in Confirmation of No Induction in Humans tests (CNIHs) with read-across material, hexyl 2-methylbutyrate at 7086 μg/cm^2 in 3:1 diethyl phthalate:EtOH or 967 μg/cm^2 in alcohol SDA 39C, no reactions indicative of sensitization were observed in any of the 109 or 38 volunteers, respectively (RIFM, 2018; RIFM, 1972).

Based on weight of evidence (WoE) from structural analysis and data on the read-across material hexyl 2-methylbutyrate, amyl isobutyrate is a sensitizer with a WoE NESIL of 7000 μg/cm^2 (Table 1). Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (2020) and a subchronic RfD of 3.33 mg/kg/day.

Additional References: Natsch (2007); McKim et al., 2010.

Literature Search and Risk Assessment Completed On: 09/11/20

11.1.5. Photoirritation/photoallergenicity

Based on the available UV/Vis spectra, amyl isobutyrate 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 amyl isobutyrate in experimental models. UV/Vis absorption spectra indicate no significant 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, amyl isobutyrate 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 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: 09/01/20

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for amyl isobutyrate 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 amyl isobutyrate. Based on the Creme RIFM Model, the inhalation exposure is 0.065 mg/day. This exposure is 21.5 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: 09/16/20

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of amyl isobutyrate was performed following the RIFM Environmental Framework (Salvito et al., 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log K_{OW}, and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity, as discussed in Salvito et al. (2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 2012b), which provides chemical class-specific ecotoxicity estimates. Finally, if necessary, Tier 3 is conducted using measured biodegradation and ecotoxicity data to refine the RQ, thus allowing for lower PNEC uncertainty factors. The data for calculating the PEC and PNEC for this safety assessment are provided in the table below. For the PEC, the range from the most recent IFRA Volume of Use Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, amyl isobutyrate was identified as a fragrance material with no potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC <1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) did not identify amyl isobutyrate as possibly persistent or bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA,

Table 1
Data summary for hexyl 2-methylbutyrate as read-across material for amyl isobutyrate.

LLNA Weighted Mean EC3 Value μg/cm^2 (No. Studies)	Potency Classification Based on Animal Data[1]	Human Data			
		NOEL-CNIH (Induction) μg/cm^2	NOEL-HMT (Induction) μg/cm^2	LOEL[2] (Induction) μg/cm^2	WoE NESIL[3] μg/cm^2
>13700 [1]	Weak	7086	6900	NA	7000

NOEL = No observed effect level; CNIH = Confirmation of No Induction in Humans Test; HMT = Human Maximization Test; LOEL = lowest observed effect level; NA = Not Available.

[1] Based on animal data using classification defined in ECETOC, Technical Report No. 87, 2003.

[2] Data derived from CNIH or HMT.

[3] WoE NESIL limited to two significant figures.

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 v4.11).

11.2.1.1. Risk assessment. Based on the current VoU (2015), amyl isobutyrate presents no risk to the aquatic compartment in the screening-level assessment.

11.2.1.2. Key studies. Biodegradation:

No data available.

Ecotoxicity:

No data available.

11.2.1.3. Other available data. Amyl isobutyrate has been pre-registered for REACH with no additional information available at this time.

11.2.2. 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 et al., 2002)

Exposure	Europe (EU)	North America (NA)
Log K_{OW} Used	3.25	3.25
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 0.0175 μg/L. The revised PEC/PNECs for EU and NA are not applicable. The material was cleared at the screening-level; therefore, it does not present a risk to the aquatic environment at the current reported VoU.

Literature Search and Risk Assessment Completed On: 09/16/20

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

Search keywords: CAS number and/or material names

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 12/09/21.

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.2023.114239.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	17.5			1000000	0.0175	

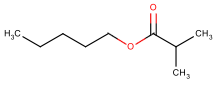
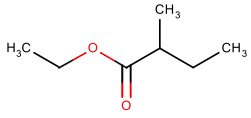
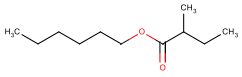
Appendix

Read-across Justification

Methods

The read-across analogs were identified using RIFM fragrance chemicals inventory clustering and read-across search criteria (Date et al., 2020). These criteria are in compliance with the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015) and are consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemical Agency read-across assessment framework (ECHA, 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 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, oncologic classification, ER binding, and repeat dose categorization predictions were generated using OECD QSAR Toolbox v4.2 (OECD, 2020).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010).
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2020), 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, 2020).
- 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	Amyl isobutyrate	Ethyl 2-methylbutyrate	Hexyl 2-methylbutyrate
CAS No.	2445-72-9	7452-79-1	10032-15-2
Structure			
Similarity (Tanimoto Score)		0.69	0.88
Endpoint		• Genotoxicity • Repeated dose toxicity • Reproductive toxicity	• Skin sensitization
Molecular Formula	$C_9H_{18}O_2$	$C_7H_{14}O_2$	$C_{11}H_{22}O_2$
Molecular Weight	158.24	130.19	186.29
Melting Point (°C, EPI Suite)	−32.06	−56.05	−9.14
Boiling Point (°C, EPI Suite)	178.41	134.87	218.34
Vapor Pressure (Pa @ 25°C, EPI Suite)	138.65	1070.58	19.07
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	117.80	1070.00	12.56
Log K_{OW}	3.25	2.26	4.23
Jmax (μg/cm^2/h, SAM)	11.17	55.11	1.68
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	97.27	55.22	171.24
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)	No alert found	Structural alert for nongenotoxic carcinogenicity| Substituted n-alkylcarboxylic acids (Nongenotox)	
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	Urethane (Renal toxicity) Alert	
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

(*continued on next page*)

(*continued*)

	Target Material	Read-across Material	Read-across Material
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 domains alerts identified.		No skin sensitization reactivity domains alerts 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 amyl isobutyrate (CAS # 2445-72-9). 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, ethyl 2-methylbutyrate (CAS # 7452-79-1) and hexyl 2-methylbutyrate (CAS # 10032-15-2) were identified as read-across analogs 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, amyl isobutyrate (CAS # 2445-72-9), for the genotoxicity, repeated dose, and reproductive toxicity endpoints.
  - o The target material and the read-across analog belong to the class of aliphatic esters.
  - o The key difference between the target material and the read-across analog is that target is a 3-carbon ester, whereas the read-across analog is a 4-carbon ester. Moreover, the target has a pentyl substituent on the alcohol end, whereas the read-across analog has an ethyl substituent on the alcohol end. These structural differences are toxicologically insignificant.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. 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 is a structural alert for nongenotoxic carcinogenicity arising due to substituted n-alkylcarboxylic acids (carcinogenicity ISS) for the read-across analog. This is a typical structural alert for n-alkyl carboxylic acids. Substances belonging to this chemical class are potentially reactive as peroxisome proliferators (PPs). PPs are a diverse group of chemicals, including hypolipidemic drugs, plasticizers, and herbicides, that were found to cause liver cancer when chronically administered to rats and mice. These chemicals are considered nongenotoxic agents, given generally negative results in genotoxicity assays.
  - 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.
- Hexyl 2-methylbutyrate (CAS # 10032-15-2) was used as a read-across analog for the target material, amyl isobutyrate (CAS # 2445-72-9), for the skin sensitization endpoint.
  - o The target material and the read-across analog belong to the class of aliphatic esters.
  - o The key difference between the target material and the read-across analog is that target is a 3-carbon ester, whereas the read-across analog is a 4-carbon ester. Moreover, the target material has a pentyl substituent on the alcohol end, whereas the read-across analog has a hexyl substituent on the alcohol end. These structural differences are toxicologically insignificant.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. 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 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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