



Short Review

RIFM fragrance ingredient safety assessment, isoamyl isovalerate, CAS registry number 659-70-1



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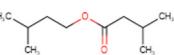
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Name: Isoamyl isovalerate CAS Registry Number: 659-70-1

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

CNIH – Confirmation of No Induction in Humans test. A human repeat insult patch test that is performed to confirm an already determined safe use level for fragrance ingredients (Na et al., 2021)

Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015a; Safford et al., 2017) compared to a deterministic aggregate approach

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

DRF - Dose Range Finding

DST - Dermal Sensitization Threshold

ECHA - European Chemicals Agency

ECOSAR - Ecological Structure-Activity Relationships Predictive Model

EU - Europe/European Union

GLP - Good Laboratory Practice

IFRA - The International Fragrance Association

LOEL - Lowest Observed Effect Level

MOE - Margin of Exposure

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

NA - North America

NESIL - No Expected Sensitization Induction Level

NOAEC - No Observed Adverse Effect Concentration

NOAEL - No Observed Adverse Effect Level

NOEC - No Observed Effect Concentration

NOEL - No Observed Effect Level

OECD - Organisation for Economic Co-operation and Development

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

PBT - Persistent, Bioaccumulative, and Toxic

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

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

QRA - Quantitative Risk Assessment

QSAR - Quantitative Structure-Activity Relationship

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

RfD - Reference Dose

RIFM - Research Institute for Fragrance Materials

RQ - Risk Quotient

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

TTC - Threshold of Toxicological Concern

UV/Vis spectra - Ultraviolet/Visible spectra

VCF - Volatile Compounds in Food

VoU - Volume of Use

vPvB - (very) Persistent, (very) Bioaccumulative

WoE - Weight of Evidence

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

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

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

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

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

Isoamyl isovalerate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, photoirritation/photoallergenicity, skin sensitization, and environmental safety. Data show that isoamyl isovalerate is not genotoxic. Data on isoamyl isovalerate provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and reproductive toxicity endpoints. Data from read-across analog isobutyl isobutyrate (CAS # 97-85-8) show that there are no safety concerns for isoamyl isovalerate for skin sensitization under the current declared levels of use. The photoirritation/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; isoamyl isovalerate is not expected to be photoirritating/photoallergenic. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material, and the exposure to isoamyl isovalerate is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; isoamyl isovalerate 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 in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

Human Health Safety Assessment

Genotoxicity: Not genotoxic.

(RIFM, 2015d; RIFM, 2016d)

Repeated Dose Toxicity: NOAEL = 267 mg/kg/day.

RIFM (2017)

Reproductive Toxicity: NOAEL = 800 mg/kg/day.

RIFM (2017)

Skin Sensitization: No concern for skin sensitization.

(ECHA REACH Dossier: Isobutyl isobutyrate; ECHA, 2018)

Photoirritation/Photoallergenicity: Not expected to be photoirritating/photoallergenic.

(UV/Vis Spectra; RIFM Database)

Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

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Environmental Safety Assessment**Hazard Assessment:****Persistence:**

Critical Measured Value: 70% (OECD 301D)

RIFM (2009b)

Bioaccumulation:

Screening-level: 121.4 L/kg

(EPI Suite v4.11; US EPA, 2012a)

Ecotoxicity:

Screening-level: 96-h Algae EC50: 1.398 mg/L

(EPI Suite v4.11; US EPA, 2012a)

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: 96-h Algae EC50: 1.398 mg/L

(EPI Suite v4.11; US EPA, 2012a)

RIFM PNEC is: 0.1398 µg/L

- Revised PEC/PNECs (2019 IFRA VoU): North America and Europe <1

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

1. Identification

- Chemical Name:** Isoamyl isovalerate
- CAS Registry Number:** 659-70-1
- Synonyms:** Amyl(iso) isovalerate; Butanoic acid, 3-methyl-, 3-methylbutyl ester; Isoamyl isopentanoate; Isoamyl isovalerianate; Isoamyl 3-methylbutanoate; Isopentyl isovalerate; 3-Methylbutyl 3-methylbutyrate; $\text{C}_1\text{H}_2\text{O}_2$ (C = 1 ~ 5); 3-Methylbutyl 3-methylbutanoate; Isoamyl isovalerate
- Molecular Formula:** $\text{C}_{10}\text{H}_{20}\text{O}_2$
- Molecular Weight:** 172.26 g/mol
- RIFM Number:** 809
- Stereochemistry:** Stereoisomer not specified. No stereocenter present and no stereoisomers possible.

2. Physical data

- Boiling Point:** 190 °C (Fragrance Materials Association [FMA] Database), 186.63 °C (EPI Suite), 192.2 °C at 1013 hPa (RIFM, 2015a)
- Flash Point:** 162 °F; CC (FMA Database), 72 °C (Globally Harmonized System), 73.0 °C (average corrected and rounded down to the nearest multiple of 0.5 °C) (RIFM, 2015b)
- Log K_{ow}:** 3.8 (RIFM, 2009a), 3.66 (EPI Suite)
- Melting Point:** 31.53 °C (EPI Suite), no melting point down to -100 °C at 1000–1015 hPa (RIFM, 2015a)
- Water Solubility:** 44.59 mg/L (EPI Suite)
- Specific Gravity:** 0.858 (FMA Database), 0.8534 (EOA, 1976 Sample 76–159)
- Vapor Pressure:** 0.398 mm Hg at 20 °C (EPI Suite v4.0), 0.6 mm Hg at 20 °C (FMA Database), 0.581 mm Hg at 25 °C (EPI Suite)
- UV Spectra:** No absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol⁻¹ • cm⁻¹)
- Appearance/Organoleptic:** Not available

3. Volume of use (Worldwide band)

- 1–10 metric tons per year (IFRA, 2019)

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

- 95th Percentile Concentration in Fine Fragrance:** 0.12% (RIFM, 2022)
- Inhalation Exposure**:** 0.00029 mg/kg/day or 0.022 mg/day (RIFM, 2022)
- Total Systemic Exposure**:** 0.0031 mg/kg/day (RIFM, 2022)

5. Derivation of systemic absorption

- Dermal:** Assumed 100%
- Oral:** Assumed 100%
- Inhalation:** Assumed 100%

6. Computational toxicology evaluation**6.1. Cramer Classification: Class I, Low**

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

6.2. Analogs Selected:

- Genotoxicity:** None
- Repeated Dose Toxicity:** None
- Reproductive Toxicity:** None
- Skin Sensitization:** Isobutyl isobutyrate (CAS # 97-85-8)
- Photoirritation/Photoallergenicity:** None
- Local Respiratory Toxicity:** None
- 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

Isoamyl isovalerate is reported to occur in the following foods by the

VCF*:

Angelica (Angelica archangelica L.)	Lamb's lettuce (Valerianella locusta)
Banana (Musa sapientum L.)	Mastic (Pistacia lentiscus)
Beer	Sherry
Cherimoya (Annona cherimolia Mill.)	Tomato (Lycopersicon esculentum Mill.)
Eucalyptus oil (Eucalyptus globulus Labill.)	Whisky

*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. This is a partial list.

9. REACH dossier

Available ([ECHA, 2016](#)); accessed 12/07/21.

10. Conclusion

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

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

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

11.1.1.1. Risk assessment. Isoamyl isovalerate 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, 2014](#)). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. Additional assays were considered to fully assess the

Table 1

Summary of existing data on isobutyl isobutyrate as a read-across for isoamyl isovalerate.

WoE Skin Sensitization Potency Category ¹	Human Data				Animal 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	LLNA ⁴ Weighted Mean EC3 Value μg/cm ²	GPM ⁵	Buehler ⁵
NA	NA	NA	NA	NA	NA	NA	NA
<i>In Vitro Data⁶</i>					<i>In Silico Protein Binding Alerts</i> (OECD Toolbox v4.2)		
No evidence of sensitization ⁷	KE 1	KE 2	KE 3	Target	Autoxidation simulator	Metabolism simulator	
	Negative	Negative	NA	No alert found	No alert found	No alert found	

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

¹WoE Skin Sensitization Potency Category is only applicable for identified sensitizers with sufficient data, based on collective consideration of all available data ([Na et al., 2021](#)).

²Data derived from CNIH or HMT.

³WoE NESIL limited to 2 significant figures.

⁴Based on animal data using classification defined in ECETOC, Technical Report No. 87, 2003.

⁵Studies conducted according to OECD TG 406 are included in the table.

⁶Studies conducted according to OECD TG 442, [Cottrez et al. \(2016\)](#), or [Forreryd et al. \(2016\)](#), are included in the table..

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

potential mutagenic or clastogenic effects of the target material.

The mutagenic activity of isoamyl 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 and preincubation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with isoamyl 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, 2015d). Under the conditions of the study, isoamyl isovalerate was not mutagenic in the Ames test.

The clastogenic activity of isoamyl 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 isoamyl isovalerate in DMSO at concentrations up to 1723 µg/mL in the dose range finding (DRF) study; micronuclei analysis was conducted at concentrations up to 1723 µg/mL in the presence and absence of metabolic activation. Isoamyl isovalerate did induce binucleated cells with micronuclei at 138 µg/mL in the 20-h treatment in the absence of an S9 activation system (RIFM, 2016d). While the increase (1.45%) was above the 95% control limit of the historical control range (0.05%–1.05%), the increase was not dose-dependent. Therefore, this increase was considered to be not biologically relevant. Under the conditions of the study, isoamyl isovalerate was considered to be non-clastogenic in the *in vitro* micronucleus test.

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

Additional References: RIFM, 2016e.

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

11.1.2. Repeated dose toxicity

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

11.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on isoamyl isovalerate. A 90-day dietary study was conducted in CRL:COBS CD (SD) BR rats. Groups of 10–16 rats/sex/dose were fed diets containing test material, isoamyl isovalerate, at doses of 0, 21.9, 69.2, or 219 mg/kg/day for 90 days. There were no treatment-related adverse effects observed up to the highest dose tested. Thus, the NOAEL was considered to be 219 mg/kg/day (RIFM, 1980). In another study, an OECD 422- and GLP-compliant combined repeated dose toxicity with a reproduction/developmental toxicity screening test was conducted in Sprague Dawley rats. Groups of 12 rats/sex/dose were administered via gavage the test material, isoamyl isovalerate, at doses of 0, 75, 250, or 800 mg/kg/day. Males were dosed for 2 weeks prior to mating and continued through the day before euthanasia (a total of 50 days), while females were dosed for 2 weeks prior to mating and continued through to lactation day 13. Additional groups of 6 rats/sex/dose were assigned to the control and high-dose group (but were not mated) to serve as the 14-day treatment-free recovery groups. One high-dose dam was euthanized on GD 24 because all pups were found dead. Prolonged parturition, irregular respiration, and skin paleness were observed during GD 23 to 24 for this dam. Macroscopic examination revealed greenish-black luminal contents in the stomach and colon and pinkish, transparent thoracic fluid. The relationship between the treatment and these findings was unclear since it was only observed in 1 high-dose female. However, this death was not considered to have toxicological relevance since no treatment-related adverse effects in other parameters at 800 mg/kg/day were observed during the study. At 800 mg/kg/day, salivation was observed among both males and females, but this finding was considered to be attributed to the palatability and not the systemic toxicity of the test material. Increases in T4 thyroid hormone levels were observed in high-dose adult males (1.24-fold of the control) and mid- and high-dose pups (up to 1.22-fold of the control).

However, this was not considered to be toxicologically significant since there were no correlated microscopic findings in the thyroid (with parathyroids). There were no treatment-related adverse effects in any of the systemic toxicity parameters evaluated (body weight, food consumption, functional behavior and motor activity examination, hematology, clinical chemistry, organ weights, and macroscopic and microscopic findings). Thus, the NOAEL for systemic toxicity was considered to be 800 mg/kg/day, the highest dose tested (RIFM, 2017). Since both studies determined the NOAEL to be the highest dose tested, a NOAEL of 800 mg/kg/day from the OECD 422 was selected for this safety assessment.

A default safety factor of 3 was used when deriving a NOAEL from an OECD 422 study (ECHA, 2012). The safety factor has been approved by the Expert Panel for Fragrance Safety*.

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

Therefore, the isoamyl isovalerate MOE for the repeated dose toxicity can be calculated by dividing the isoamyl isovalerate NOAEL in mg/kg/day by the total systemic exposure to isoamyl isovalerate, 267/0.0031, or 86129.

In addition, the total systemic exposure to isoamyl isovalerate (3.1 µg/kg/day) is below the TTC (30 µg/kg/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

*The Expert Panel for Fragrance Safety is composed of scientific and technical experts in their respective fields. This group provides advice and guidance.

Additional References: None.

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

11.1.3. Reproductive toxicity

The MOE for isoamyl isovalerate is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are sufficient reproductive toxicity data on isoamyl isovalerate. An OECD 422- and GLP-compliant combined repeated dose toxicity with a reproduction/developmental toxicity screening test was conducted in Sprague Dawley rats. Groups of 12 rats/sex/dose were administered via gavage the test material, isoamyl isovalerate, at doses of 0, 75, 250, or 800 mg/kg/day. Males were dosed for 2 weeks prior to mating and continued through the day before euthanasia (a total of 50 days), while females were dosed for 2 weeks prior to mating and continued through to lactation day 13. Additional groups of 6 rats/sex/dose were assigned to the control and high-dose groups (but were not mated) to serve as the 14-day treatment-free recovery groups. In addition to the systemic toxicity parameters, the fertility and developmental toxicity parameters were also evaluated. Estrus cycle, precoital time, fertility data, reproductive and littering findings, F1 pup clinical signs, body weight, anogenital distance, nipple retention, and external examination were measured. Thyroid hormone (T4) level in blood was also analyzed for adult males and F1 pups. One high-dose dam was euthanized on GD 24 because all pups were found dead. Prolonged parturition, irregular respiration, and skin paleness were observed during GDs 23 to 24 for this dam. Macroscopic examination revealed greenish-black luminal contents in the stomach and colon and pinkish, transparent thoracic fluid. The relationship between the treatment and these findings was unclear since it was only observed in 1 high-dose female. However, this death was not considered to have toxicological relevance since no treatment-related adverse effects in other parameters at 800 mg/kg/day were observed during the study. Increases in T4 were observed in high-dose adult males (1.24-fold of the control) and mid- and high-dose pups (up to 1.22-fold of the control). However, this was not considered to be toxicologically significant since there were no correlated microscopic findings in the thyroid (with parathyroids). There were no treatment-related adverse effects in any of

	LC50 (Fish) mg/L	EC50 (<i>Daphnia</i>) mg/L	EC50 (Algae) mg/L	AF	PNEC µg/L	Chemical Class
RIFM Framework Screening-level (Tier 1)	<u>6.328</u>			1000000	0.006328	
ECOSAR Acute Endpoints (Tier 2) Ver 1.11	2.458	4.276	<u>1.398</u>	10000	0.1398	Esters
ECOSAR Acute Endpoints (Tier 2) Ver 1.11	4.535	3.002	4.221			Neutral Organics

the fertility and developmental toxicity parameters evaluated. Thus, the NOAEL for fertility and developmental toxicity was considered to be 800 mg/kg/day, the highest dose tested (RIFM, 2017). Therefore, the isoamyl isovalerate MOE for the reproductive toxicity can be calculated by dividing the isoamyl isovalerate NOAEL in mg/kg/day by the total systemic exposure to isoamyl isovalerate, 800/0.0031, or 258064.

In addition, the total systemic exposure to isoamyl isovalerate (3.1 µg/kg/day) is below the TTC (30 µg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

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

11.1.4. Skin sensitization

Based on the existing data on the target material and read-across material isobutyl isobutyrate, isoamyl isovalerate presents no concern for skin sensitization.

11.1.4.1. Risk assessment. Limited skin sensitization data are available for isoamyl isovalerate. Therefore, read-across material isobutyl isobutyrate (CAS # 97-85-8; see Section VI) was used for the risk assessment of isoamyl isovalerate. The data on the read-across material are summarized in Table 1. Based on the existing data on the read-across material, isoamyl isovalerate is not considered a skin sensitizer. The chemical structure of the read-across material and the target material indicate that they would not be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). Isoamyl isovalerate was found to be inconclusive in *in vitro* Direct Peptide Reactivity Assay (DPRA) and negative in LuSens but positive in the human cell line activation test (h-CLAT) (RIFM, 2016b). In a guinea pig maximization test with isoamyl isovalerate, no reactions indicative of sensitization were observed (RIFM, 2016c). In a human maximization test, no skin sensitization reactions were observed with isoamyl isovalerate (RIFM, 1976). Read-across material isobutyl isobutyrate was predicted not to be sensitizing in an *in vitro* DPRA and KeratinoSens (ECHA, 2018).

Based on the weight of evidence (WoE) from structural analysis and *in vitro* and human studies on the read-across material as well as the target material, isoamyl isovalerate does not present a concern for skin

sensitization.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/23/21.

11.1.5. Photoirritation/photoallergenicity

Based on the available UV/Vis absorption spectra, isoamyl isovalerate 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 isoamyl isovalerate in experimental models. UV/Vis absorption spectra indicate no absorption between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for photoirritation and photoallergenicity (Henry et al., 2009). Based on the lack of absorbance, isoamyl isovalerate does not present a concern for photoirritation or photoallergenicity.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate no absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark of concern for photoirritating effects, 1000 L mol⁻¹ • cm⁻¹ (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/09/21.

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for isoamyl isovalerate 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 isoamyl isovalerate. Based on the Creme RIFM Model, the inhalation exposure is 0.022 mg/day. This exposure is 63.6 times lower than the Cramer Class I TTC value of 1.4 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/23/21.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of isoamyl 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, isoamyl isovalerate was identified as a fragrance material with the 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 did not identify isoamyl 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.

11.2.2. Risk assessment. Based on the current Volume of Use (2019), isoamyl isovalerate presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2.1. Key studies. Biodegradation:

RIFM, 1999: The ready biodegradability of the test material was evaluated in a Closed bottle test according to the OECD 301D method. After 28 days, biodegradation of 24% was observed.

RIFM, 2009b: The ready biodegradability of the test material was evaluated using a manometric respirometry test according to the OECD 301F method. Under the conditions of the study, biodegradation of 70% was observed after 28 days.

Ecotoxicity:

RIFM, 1999: A *Daphnia magna* acute toxicity study was conducted according to the DM 92/69/EEC C.2 method under static conditions. The 48-h geometric mean of EC0/EC100 was reported to be 6.1 mg/L.

RIFM, 2015c: An algae growth inhibition assay was conducted according to the OECD 201 method. The 72-h ErC50 (growth rate

inhibition) based on geometric mean measured concentration was reported to be 5.47 mg/L. The 72-h EbC50 (yield inhibition) based on measured concentration was reported to be 4.75 mg/L.

RIFM, 2016a: A fish (Zebrafish) acute toxicity study was conducted according to the OECD 203 method under semi-static conditions. The 96-h LC50, based on geometric mean measured test concentration, was reported to be 3.47 mg/L.

Other available data

Isoamyl isovalerate has been registered under REACH with no additional data at this time.

11.2.3. Risk assessment refinement

Since isoamyl isovalerate has passed the screening criteria, measured data is included for completeness only and has not been used in PNEC derivation.

Ecotoxicological data and PNEC derivation (all endpoints reported in mg/LL; PNECs in μ g/LL)

Endpoints used to calculate PNEC are underlined.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	3.8	3.8
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	1–10	1–10
Risk Characterization: PEC/PNEC	<1	<1

Based on available data, the RQ for this material is < 1 . No additional assessment is necessary.

The RIFM PNEC is 0.1398 μ g/L. The revised PEC/PNECs for EU and NA are <1 ; therefore, the material does not present a risk to the aquatic environment at the current reported VoU.

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

12. Literature Search*

- **RIFM Database:** Target, Fragrance Structure-Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** <https://echa.europa.eu/>
- **NTP:** <https://ntp.niehs.nih.gov/>
- **OECD Toolbox:** <https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm>
- **SciFinder:** <https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf>
- **PubChem:** <https://pubchem.ncbi.nlm.nih.gov/>
- **PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed>
- **National Library of Medicine 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_seach/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.

CRediT authorship contribution statement

G. Sullivan: Writing – review & editing.

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.2023.114400>.

*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 06/13/22.

Appendix

Read-across Justification:

Methods

The read-across analog was identified using RIFM fragrance chemicals inventory clustering and read-across search criteria (Date et al., 2020). These criteria are in compliance with the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015) and are consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemical Agency read-across assessment framework (ECHA, 2017).

- First, materials were clustered based on their structural similarity. Second, data availability and data quality on the selected cluster were examined.
- Third, appropriate read-across analogs from the cluster were confirmed by expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physical-chemical properties of the target material and the read-across analogs were calculated using EPI Suite (US EPA, 2012a).
- J_{max} values were calculated using RIFM's skin absorption model (SAM). The parameters were calculated using the consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts, and oncologic classification predictions were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010), and skin sensitization was predicted using Toxtree v2.6.13.
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018).
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- To keep continuity and compatibility with *in silico* alerts, OECD QSAR Toolbox v4.2 was selected as the alert system.

	Target Material	Read-across Material
Principal Name	Isoamyl isovalerate	Isobutyl isobutyrate
CAS No.	659-70-1	97-85-8
Structure		
Similarity (Tanimoto Score)		0.60
SMILES	CC(C)CCOC(=O)CC(C)C	CC(C)COC(=O)C(C)C
Endpoint		Skin sensitization
Molecular Formula	C ₁₀ H ₂₀ O ₂	C ₈ H ₁₆ O ₂
Molecular Weight (g/mol)	172.268	144.214
Melting Point (°C, EPI Suite)	-31.53	-80.70
Boiling Point (°C, EPI Suite)	190.40	148.60
Vapor Pressure (Pa @ 25°C, EPI Suite)	1.18E+02	5.77E+02
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	4.46E+01	1.00E+03
Log K_{ow}	3.66	2.68
J_{max} (μg/cm²/h, SAM)	4.88	65.89
Henry's Law (Pa·m³/mol, Bond Method, EPI Suite)	1.29E+02	8.33E+01
Skin Sensitization		
Protein Binding (OASIS v1.1)	No alert found	No alert found
Protein Binding (OECD)	No alert found	No alert found
Protein Binding Potency	Not possible to classify according to these rules (GSH)	Not possible to classify according to these rules (GSH)
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	No alert found	No alert found

(continued on next page)

(continued)

	Target Material	Read-across Material
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	No skin sensitization reactivity domain alerts were identified.	No skin sensitization reactivity domain alerts were identified.
Metabolism Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	See Supplemental Data 1	See Supplemental Data 2

Summary

There are insufficient toxicity data on isoamyl isovalerate (CAS # 659-70-1). 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, isobutyl isobutyrate (CAS # 97-85-8) was identified as a read-across material with sufficient data for toxicological evaluation.

Conclusions

- Isobutyl isobutyrate (CAS # 97-85-8) was used as a read-across analog for the target material, isoamyl isovalerate (CAS # 659-70-1), 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 share similar branched saturated ester structures.
 - The key structural difference between the target material and the read-across analog is in the branching pattern on the acid and alcohol portions. This structural difference is toxicologically insignificant. The read-across analog contains the structural features of the target material that are relevant to this endpoint and is expected to have equal or greater potential for toxicity as compared to the target.
- Structural similarity between the target material and the read-across analog is indicated by the Tanimoto score. The Tanimoto score reflects the similarity of these branched-chain ester structures. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
- The physical-chemical properties of the target material and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
- According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
- There are no *in silico* alerts for the target material and the read-across analog. *In silico* alerts are consistent with data.
- The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
- The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

References

Api, A.M., Belsito, D., Bruze, M., Cadby, P., Calow, P., Dagli, M.L., Dekant, W., Ellis, G., Fryer, A.D., Fukayama, M., Griem, P., Hickey, C., Kromidas, L., Lalko, J.F., Liebler, D.C., Miyachi, Y., Politano, V.T., Renskers, K., Ritacco, G., Salvito, D., Schultz, T.W., Sipes, I.G., Smith, B., Vitale, D., Wilcox, D.K., 2015. Criteria for the Research Institute for fragrance materials, Inc. (RIFM) safety evaluation process for fragrance ingredients. *Food Chem. Toxicol.* 82, S1–S19.

Carthew, P., Clapp, C., Gutsell, S., 2009. Exposure based waiving: the application of the toxicological threshold of concern (TTC) to inhalation exposure for aerosol ingredients in consumer products. *Food Chem. Toxicol.* 47 (6), 1287–1295.

Cassano, A., Manganaro, A., Martin, T., Young, D., Piclin, N., Pintore, M., Bigoni, D., Benfenati, E., 2010. CAESAR models for developmental toxicity. *Chem. Cent. J.* (4 Suppl. 1), S4.

Comiskey, D., Api, A.M., Barratt, C., Daly, E.J., Ellis, G., McNamara, C., O'Mahony, C., Robison, S.H., Safford, B., Smith, B., Tozer, S., 2015. Novel database for exposure to fragrance ingredients in cosmetics and personal care products. *Regul. Toxicol. Pharmacol.* 72 (3), 660–672.

Comiskey, D., Api, A.M., Barrett, C., Ellis, G., McNamara, C., O'Mahony, C., Robison, S. H., Rose, J., Safford, B., Smith, B., Tozer, S., 2017. Integrating habits and practices data for soaps, cosmetics and air care products into an existing aggregate exposure model. *Regul. Toxicol. Pharmacol.* 88, 144–156.

Cottrez, F., Boitel, E., Ourlin, J.C., Peiffer, J.L., et al., 2016. A 3D reconstituted epidermis based model for quantifying chemical sensitization potency: reproducibility and predictivity results from an inter-laboratory study. *Toxicol. Vitro* 32, 248–260. Apr.

Date, M.S., O'Brien, D., Botelho, D.J., Schultz, T.W., et al., 2020. Clustering a chemical inventory for safety assessment of fragrance ingredients: identifying read-across analogs to address data gaps. *Chem. Res. Toxicol.* 33 (7), 1709–1718, 2020.

ECHA, 2012. Guidance on Information Requirements and Chemical Safety Assessment. November 2012 v2.1. <http://echa.europa.eu/>.

ECHA, 2016. 3-Methylbutyl Isovalerate Registration Dossier. Retrieved from. <https://echa.europa.eu/registration-dossier/-/registered-dossier/16674/1/2>.

ECHA, 2017. Read-across Assessment Framework (RAAF). Retrieved from. https://echa.europa.eu/documents/10162/13628/raaf_en.pdf/614e5d61-891d-4154-8a47-87efebd1851a.

ECHA, 2018. Isobutyl Isobutyrate Registration Dossier. Retrieved from. <https://echa.europa.eu/registration-dossier/-/registered-dossier/24080/1/2>.

Forreryd, A., Zeller, K.S., Lindberg, T., Johansson, H., Linstedt, M., 2016. From genome-wide arrays to tailor-made biomarker readout - progress towards routine analysis of skin sensitizing chemicals with GARD. *Toxicol. Vitro* 37, 178–188.

Henry, B., Foti, C., Alsante, K., 2009. Can light absorption and photostability data be used to assess the photosafety risks in patients for a new drug molecule? *J. Photochem. Photobiol. B Biol.* 96 (1), 57–62.

IFRA (International Fragrance Association), 2019. Volume of Use Survey, January–December 2019.

Kroes, R., Renwick, A.G., Feron, V., Galli, C.L., Gibney, M., Greim, H., Guy, R.H., Lhuguenot, J.C., van de Sandt, J.J.M., 2007. Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients. *Food Chem. Toxicol.* 45 (12), 2533–2562.

Laufersweiler, M.C., Gadagbui, B., Baskerville-Abraham, I.M., Maier, A., Willis, A., et al., 2012. Correlation of chemical structure with reproductive and developmental toxicity as it relates to the use of the threshold of toxicological concern. *Regul. Toxicol. Pharmacol.* 62 (1), 160–182.

Na, M., Ritacco, G., O'Brien, D., Lavelle, M., Api, A., Basketter, D., 2021. Fragrance skin sensitization evaluation and human testing: 30-year experience. *Dermatitis* 32 (5), 339–352, 2021 Sep-Oct 01.

OECD, 2015. *Guidance Document on the Reporting of Integrated Approaches to Testing and Assessment (IATA)*. ENV/JM/HA(2015)7. Retrieved from. <http://www.oecd.org/>.

OECD, 2018. The OECD QSAR Toolbox, v3.2–4.2. Retrieved from. <http://www.qsartoolbox.org/>.

RIFM (Research Institute for Fragrance Materials, Inc.), 1976. Report on Human Maximization Studies. Report to RIFM. RIFM Report Number 1796. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 1980. 90-Day Toxicity Study on Isoamyl Isovalerate in Rats. Report to FEMA. RIFM, Woodcliff Lake, NJ, USA. Unpublished report from Damske, D.R., Mecler, F.J., Beliles, R.P. & Liverman, J.L. RIFM report number 5655.

RIFM (Research Institute for Fragrance Materials, Inc.), 1999. Investigation of the Ecological Properties of Isoamyl Isovalerate. Unpublished Report from Haarmann & Reimer GmbH. RIFM Report Number 37165. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 2009a. Partition Coefficient N-Octanol/water of Isoamyl Isovalerate. Unpublished Report from Givaudan. RIFM Report Number 57317. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 2009b. Ready Biodegradability of Isoamyl Isovalerate (Isopentyl Isovalerate). Unpublished Report from Givaudan. RIFM Report Number 59443. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 2014. Report on the Testing of Isoamyl Isovalerate in the BlueScreen HC Assay (-/+ S9 Metabolic Activation). RIFM Report Number 69260. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 2015a. Isoamyl Isovalerate: Determination of Physico-Chemical Properties Melting Point and Boiling Point. Unpublished Report from Symrise. RIFM Report Number 70384. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 2015b. Isoamyl Isovalerate: Determination of Physico-Chemical Properties Flash Point. Unpublished Report from Symrise. RIFM Report Number 70387. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 2015c. Isoamyl Isovalerate: Alga, Growth Inhibition Test with Pseudokirchneriella Subcapitata, 72 Hours. Unpublished Report from Symrise. RIFM Report Number 70389. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 2015d. Isoamyl Isovalerate: *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay. Unpublished Report from Symrise. RIFM Report Number 70391. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 2016a. Isoamyl Isovalerate: Fish (Zebrafish), Acute Toxicity Test, Semi-static, 96 Hours. Unpublished Report from Symrise. RIFM Report Number 70392. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 2016b. Isoamyl Isovalerate: in Vitro Skin Sensitization Using Direct Peptide Reactivity Assay (DPRA), LuSens (ARE-Nrf2 Luciferase Test Method) and hCLAT Human Cell Line Activation Test. Unpublished Report from RIFM Report Number 70900. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 2016d. Isoamyl Isovalerate: Micronucleus Test in Human Lymphocytes in Vitro. Unpublished Report from RIFM Report Number 71134. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 2016e. Isoamyl Isovalerate (Isoamylisovalerianat): Gene Mutation Assay in Chinese Hamster V79 Cells in Vitro (V79/HPRT). Unpublished Report from RIFM Report Number 73151. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 2017. Isoamyl Isovalerate: Combined Repeated Dose Oral Gavage Toxicity Study with the Reproduction/developmental Toxicity Screening in Sprague-Dawley Rats. Unpublished Report from Symrise. RIFM Report Number 72264. RIFM, Woodcliff Lake, NJ, USA.

RIFM (Research Institute for Fragrance Materials, Inc.), 2022. Exposure Survey 34. January 2022.

Roberts, D.W., Patlewicz, G., Kern, P.S., Gerberick, F., Kimber, I., Dearman, R.J., Ryan, C.A., Basketter, D.A., Aptula, A.O., 2007. Mechanistic applicability domain classification of a local lymph node assay dataset for skin sensitization. *Chem. Res. Toxicol.* 20 (7), 1019–1030.

Rogers, D., Hahn, M., 2010. Extended-connectivity fingerprints. *J. Chem. Inf. Model.* 50 (5), 742–754.

Safford, B., Api, A.M., Barratt, C., Comiskey, D., Daly, E.J., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Smith, B., Thomas, R., Tozer, S., 2015. Use of an aggregate exposure model to estimate consumer exposure to fragrance ingredients in personal care and cosmetic products. *Regul. Toxicol. Pharmacol.* 72, 673–682.

Safford, B., Api, A.M., Barratt, C., Comiskey, D., Ellis, G., McNamara, C., O'Mahony, C., Robison, S., Rose, J., Smith, B., Tozer, S., 2017. Application of the expanded Creme RIFM consumer exposure model to fragrance ingredients in cosmetic, personal care and air care products. *Regul. Toxicol. Pharmacol.* 86, 148–156.

Salvito, D.T., Senna, R.J., Federle, T.W., 2002. A Framework for prioritizing fragrance materials for aquatic risk assessment. *Environ. Toxicol. Chem.* 21 (6), 1301–1308.

Schultz, T.W., Amcoff, P., Berggren, E., Gautier, F., Klaric, M., Knight, D.J., Mahony, C., Schwarz, M., White, A., Cronin, M.T., 2015. A strategy for structuring and reporting a read-across prediction of toxicity. *Regul. Toxicol. Pharmacol.* 72 (3), 586–601.

Shen, J., Kromidas, L., Schultz, T., Bhatia, S., 2014. An *in silico* skin absorption model for fragrance materials. *Food Chem. Toxicol.* 74, 164–176.

US EPA, 2012a. Estimation Programs Interface Suite for Microsoft Windows, v4.0–v4.11. United States Environmental Protection Agency, Washington, DC, USA.

US EPA, 2012b. The ECOSAR (ECOlogical Structure Activity Relationship) Class Program for Microsoft Windows, v2.0. United States Environmental Protection Agency, Washington, DC, USA.