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Short Review

RIFM fragrance ingredient safety assessment, isopentyl 2-methylcrotonate (E), CAS Registry Number 41519-18-0

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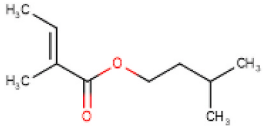
Version: 070622. Initial publication. All fragrance materials are evaluated on a five-year rotating basis. Revised safety assessments are published if new relevant data become available. Open access to all RIFM Fragrance Ingredient Safety Assessments is here: fragrancematerialsafetyresource.elsevier.com.



Name: Isopentyl 2-methylcrotonate (E)
CAS Registry Number: 41519-18-0

Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor

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CNIH – Confirmation of No Induction in Humans test. A human repeat insult patch test that is performed to confirm an already determined safe use level for fragrance ingredients (Na et al., 2021)
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 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

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LOEL - Lowest Observed Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
Perfumery - In this safety assessment, perfumery refers to fragrances made by a perfumer used in consumer products only. The exposures reported in the safety assessment include consumer product use but do not include occupational exposures.
QRA - Quantitative Risk Assessment
QSAR - Quantitative Structure-Activity Relationship
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use
vPvB - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

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

This safety assessment is based on the RIFM Criteria Document (Api 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.

Isopentyl 2-methylcrotonate (E) was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, photoirritation/photoallergenicity, skin sensitization, and environmental safety. Data show that isopentyl 2-methylcrotonate (E) is not genotoxic. The repeated dose, reproductive, and local respiratory toxicity endpoints were evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material, and the exposure to isopentyl 2-methylcrotonate (E) is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). Data from read-across material hexyl tiglate (CAS # 16930-96-4) show that there are no safety concerns for isopentyl 2-methylcrotonate (E) for skin sensitization under the current declared levels of use. The photoirritation/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; isopentyl 2-methylcrotonate (E) is not expected to be photoirritating/photoallergenic. The environmental endpoints were evaluated; for the hazard assessment based on the screening data, isopentyl 2-methylcrotonate (E) is not Persistent, Bioaccumulative, and Toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards. For the risk assessment, isopentyl 2-methylcrotonate (E) was not able to be risk screened as there were no reported volumes of use (VoU) for either North America or Europe in the 2019 IFRA Survey.

Human Health Safety Assessment

Genotoxicity: Not genotoxic. (RIFM, 2015b; RIFM, 2016)

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Repeated Dose Toxicity: No NOAEL available. Exposure is below the TTC.
Reproductive Toxicity: No NOAEL available. Exposure is below the TTC.
Skin Sensitization: No concern for skin sensitization. (RIFM, 2014; RIFM, 2015c; RIFM, 2015a)
Photoirritation/Photoallergenicity: Not expected to be photoirritating/photoallergenic. (UV/Vis Spectra; RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:
Persistence:
Screening-level: 3.0 (BIOWIN 3) (EPI Suite v4.11; US EPA, 2012a)
Bioaccumulation:
Screening-level: 106.7 L/kg (EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:
Not applicable.

Risk Assessment:
- Not applicable; no 2019 IFRA VoU reported

1. Identification

1. **Che**mical Name: Isopentyl 2-methylcrotonate (E)
2. CAS Registry Number: 41519-18-0
3. Synonyms: 2-Butenoic acid, 2-methyl-, 3-methylbutyl ester, (E)-; Isoamyl tiglate; 3-Methylbutyl 2-methylbut-2-enoate; Isopentyl 2-methylcrotonate (E)
4. Molecular Formula: $C_{10}H_{18}O_2$
5. Molecular Weight: 170.25 g/mol
6. RIFM Number: 5701
7. Stere**ochemistry:** One geometric center and 2 possible geometric isomers (E isomer specified).

2. Physical data

1. **Boiling Point:** 199.53 °C (EPI Suite)
2. **Flash Point:** Not Available
3. **Log K_{OW}:** 3.58 (EPI Suite)
4. **Melting Point**: −29.78 °C (EPI Suite)
5. **Water Solubility:** 53.9 mg/L (EPI Suite)
6. **Specific Gravity:** Not Available
7. **Vapor Pressure:** 0.25 mm Hg at 20 °C (EPI Suite v4.0), 0.369 mm Hg at 25 °C (EPI Suite)
8. **UV Spectra:** No absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** Not Available

3. Volume of use (worldwide band)

1.	No volume of use reported for 2019	IFRA (2019)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v2.0)

1.	95th Percentile Concentration in Fine Fragrance: 0.027%	RIFM (2018)
2.	Inhalation Exposure*: 0.000067 mg/kg/day or 0.0047 mg/day	RIFM (2018)
3.	Total Systemic Exposure**: 0.00061 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, 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

6.1. Cramer Classification: class I, low

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

6.2. Analogs selected

a. Genotoxicity: None
b. Repeated Dose Toxicity: None
c. Reproductive Toxicity: None
d. Skin Sensitization: Hexyl tiglate (CAS # 16930-96-4)
e. Photoirritation/Photoallergenicity: None
f. Local Respiratory Toxicity: None
g. Environmental Toxicity: None

6.3. Read-across justification

See Appendix below.

7. Metabolism

No relevant data available for inclusion in this safety assessment.
Additional References: None.

8. Natural occurrence

Isopentyl 2-methylcrotonate (E) is reported to occur in the following foods by the VCF*:

Chamomile.

*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

Pre-registered for 2010; no dossier available as of 06/26/22.

10. Conclusion

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

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, isopentyl 2-methylcrotonate (E) does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. Isopentyl 2-methylcrotonate (E) 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, 2013b). 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 potential mutagenic or clastogenic effects of the target material.

The mutagenic activity of isopentyl 2-methylcrotonate (E) 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 isopentyl 2-methylcrotonate (E) 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, 2015b). Under the conditions of the study, isopentyl 2-methylcrotonate (E) was not mutagenic in the Ames test.

The clastogenic activity of isopentyl 2-methylcrotonate (E) was

Table 1
Summary of existing data on hexyl tiglate as a read-across for isopentyl 2-methylcrotonate (E).

WoE Skin Sensitization Potency Category[a]	Human Data				Animal Data		
	NOEL-CNIH (induction) μg/cm^2	NOEL-HMT (induction) μg/cm^2	LOEL[b] (induction) μg/cm^2	WoE NESIL[c] μg/cm^2	LLNA[d] Weighted Mean EC3 Value μg/cm2	GPMT[e]	Buehler[e]
No evidence of sensitization[g]	194	8820	NA	NA	NA	NA	NA
	***In vitro* Data[f]**			***In silico* protein binding alerts (OECD Toolbox v4.2)**			
	KE 1	**KE 2**	**KE 3**	**Target Material**	**Autoxidation simulator**	**Metabolism simulator**	
	Negative	Positive	Negative	No alert found	No alert found	No alert found	

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

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

[b] Data derived from CNIH or HMT.

[c] WoE NESIL limited to 2 significant figures.

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

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

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

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

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 isopentyl 2-methylcrotonate (E) in DMSO at concentrations up to 1703 μg/mL in the dose range finding (DRF) study; micronuclei analysis was conducted at concentrations up to 350 μg/mL in the presence and absence of metabolic activation. Isopentyl 2-methylcrotonate (E) did not induce binucleated cells with micronuclei when tested in either the presence or absence of an S9 activation system (RIFM, 2016). Under the conditions of the study, isopentyl 2-methylcrotonate (E) was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the data available, isopentyl 2-methylcrotonate (E) does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 01/21/22.

11.1.2. Repeated dose toxicity

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

11.1.2.1. Risk assessment. There are no repeated dose toxicity data on isopentyl 2-methylcrotonate (E) or on any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to isopentyl 2-methylcrotonate (E) (0.61 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.

Additional References: None.

Literature Search and Risk Assessment Completed On: 01/06/22.

11.1.3. Reproductive toxicity

There are insufficient reproductive toxicity data on isopentyl 2-methylcrotonate (E) or any read-across materials. The total systemic exposure to isopentyl 2-methylcrotonate (E) is below the TTC for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

11.1.3.1. Risk assessment. There are no reproductive toxicity data on isopentyl 2-methylcrotonate (E) or on any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to isopentyl 2-methylcrotonate (E) (0.61 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: 01/06/22.

11.1.4. Skin sensitization

Based on the existing data on the target material and read-across material hexyl tiglate, isopentyl 2-methylcrotonate (E) presents no concern for skin sensitization.

11.1.4.1. Risk assessment. Limited skin sensitization data are available for isopentyl 2-methylcrotonate (E). Therefore, hexyl tiglate (CAS # 16930-96-4; see Section VI) was used for the risk assessment of isopentyl 2-methylcrotonate (E). The data on the read-across material are summarized in Table 1. Based on the existing data on the read-across material, isopentyl 2-methylcrotonate (E) is not considered a skin sensitizer. The chemical structure of the read-across material and the target material indicate that they would be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0). Read-across material hexyl tiglate was found to be negative in an *in vitro* direct peptide reactivity assay (DPRA), positive in KeratinoSens, and negative in the human cell line activation test (h-CLAT) (RIFM, 2014; RIFM, 2015c; RIFM, 2015a); therefore, the material was concluded to be non-sensitizing according to OECD TG 497 (OECD, 2021). In a human maximization test, no skin sensitization reactions were observed with read-across material hexyl tiglate at 8280 μg/cm^2 (RIFM, 1976). Additionally, in 2 Confirmation of No Induction in Humans tests (CNIHs) with 110 μg/cm^2 of hexyl tiglate in EtOH:DEP (1:3) and 194 μg/cm^2 hexyl tiglate in alcohol SDA 39c, no reactions indicative of sensitization were observed in any of the 108 or 42 volunteers, respectively (RIFM, 2013a; RIFM, 1973).

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, isopentyl 2-methylcrotonate (E) does not present a concern for skin sensitization.

Additional References: None.

Literature Search and Risk Assessment Completed On: 01/21/22.

11.1.5. Photoirritation/photoallergenicity

Based on the available UV/Vis absorption spectra, isopentyl 2-methylcrotonate (E) 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 isopentyl 2-methylcrotonate (E) 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, isopentyl 2-methylcrotonate (E) 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: 01/10/22.

11.1.6. Local Respiratory Toxicity

The margin of exposure could not be calculated due to a lack of appropriate data. The exposure level for isopentyl 2-methylcrotonate (E) 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 isopentyl 2-methylcrotonate (E). Based on the Creme RIFM Model, the inhalation exposure is 0.0047 mg/day. This exposure is 297.9 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: 01/17/22.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of isopentyl 2-methylcrotonate (E) 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. 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, isopentyl 2-methylcrotonate (E) was not assessed as no 2019 IFRA VoU was reported.

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) did not identify isopentyl 2-methylcrotonate (E) 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, 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 $\geq$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. Not applicable.

11.2.2.1. Key studies

11.2.1.2.1. Biodegradation. No data available.

11.2.1.2.2. Ecotoxicity. No data available.

11.2.1.2.3. Other available data. Isopentyl 2-methylcrotonate (E) has been pre-registered for REACH with no additional data at this time.

*11.2.2. **Risk assessment refinement***

Not applicable.

Literature Search and Risk Assessment Completed On: 07/01/22.

12. Literature Search*

- **RIFM Database:** Target, Fragrance Structure-Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** https://echa.europa.eu/
- **NTP:** https://ntp.niehs.nih.gov/
- **OECD Toolbox:** https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubChem:** https://pubchem.ncbi.nlm.nih.gov/
- **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
- **National Library of Medicine's Toxicology Information Services:** https://toxnet.nlm.nih.gov/
- **IARC:** https://monographs.iarc.fr
- **OECD SIDS:** https://hpvchemicals.oecd.org/ui/Default.aspx
- **EPA ACToR:** https://actor.epa.gov/actor/home.xhtml
- **US EPA ChemView:** https://chemview.epa.gov/chemview/
- **Japanese NITE:** https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com
- **ChemIDplus:** https://chem.nlm.nih.gov/chemidplus/

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 07/06/22.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2022.113447.

Appendix

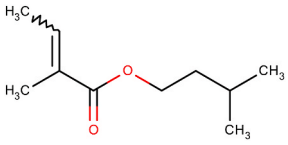
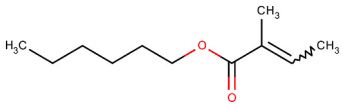
Read-across Justification

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, 2017b).

- First, materials were clustered based on their structural similarity. Second, data availability and data quality on the selected cluster were examined. Third, appropriate read-across analogs from the cluster were confirmed by expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physical–chemical properties of the target material and the read-across analogs were calculated using EPI Suite (US EPA, 2012a).

- J_{max} values were calculated using RIFM's skin absorption model (SAM). The parameters were calculated using the consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts, and oncologic classification predictions were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010), and skin sensitization was predicted using Toxtree v2.6.13.
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018).
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- To keep continuity and compatibility with *in silico* alerts, OECD QSAR Toolbox v4.2 was selected as the alert system.

	Target Material	Read-across Material
Principal Name	Isopentyl 2-methylcrotonate (E)	Hexyl tiglate
CAS No.	41519-18-0	16930-96-4
Structure		
Similarity (Tanimoto Score)		0.79
Endpoint		Skin sensitization
Molecular Formula	$C_{10}H_{18}O_2$	$C_{11}H_{20}O_2$
Molecular Weight (g/mol)	170.25	184.28
Melting Point (°C, EPI Suite)	−29.78	−7.66
Boiling Point (°C, EPI Suite)	199.53	230.32
Vapor Pressure (Pa @ 25°C, EPI Suite)	49.20	10.25
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	53.90	15.20
Log K_{OW}	3.58	4.14
J_{max} (μg/cm^2/h, SAM)	5.70	1.98
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	71.43	94.84
Skin Sensitization		
Protein Binding (OASIS v1.1)	DPRA less than 9% (DPRA 13%)|DPRA less than 9% (DPRA 13%) ≫ No protein binding alert	DPRA less than 9% (DPRA 13%)|DPRA less than 9% (DPRA 13%) ≫ No protein binding alert
Protein Binding (OECD)	Moderately reactive (GSH)|Moderately reactive (GSH) ≫ Alkenes and cycloalkenes (AN)|Slightly reactive (GSH)|Slightly reactive (GSH) ≫ Methacrylates (MA)|Slightly reactive (GSH) ≫ Tiglates (MA)	Moderately reactive (GSH)|Moderately reactive (GSH) ≫ Alkenes and cycloalkenes (AN)|Slightly reactive (GSH)|Slightly reactive (GSH) ≫ Methacrylates (MA)|Slightly reactive (GSH) ≫ Tiglates (MA)
Protein Binding Potency	Not categorized	Not categorized
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	Michael addition|Michael addition ≫ Polarised Alkenes|Michael addition ≫ Polarised Alkenes ≫ Polarised alkene - esters	Michael addition|Michael addition ≫ Polarised Alkenes|Michael addition ≫ Polarised Alkenes ≫ Polarised alkene - esters
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 isopentyl 2-methylcrotonate (E) (CAS # 41519-18-0). 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, hexyl tiglate (CAS # 16930-96-4) was identified as a read-across material with sufficient data for toxicological evaluation.

Conclusions

- Hexyl tiglate (CAS # 16930-96-4) was used as a read-across analog for the target material isopentyl 2-methylcrotonate (E) (CAS # 41519-18-0), for the skin sensitization endpoint.
  - •The target material and the read-across analog belong to the class of aliphatic crotonate esters.
  - •The key difference between the target and the read-across analog is that the target material has an isopentyl fragment on the alcohol side, whereas the read-across analog has a hexyl fragment on the alcohol side. The structural difference is toxicologically insignificant.
  - •The similarity between the target material and the read-across analog is indicated by the Tanimoto score.
  - •According to the OECD QSAR Toolbox v4.2, the structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.

•Both the target material and the read-across analog have an alert for Michael addition. This is due to the α,β-unsaturated acid portion of the ester. An initial Michael addition mechanism has been suggested to be primarily responsible for the ability of these chemicals to alkylate DNA. The data described in the skin section show that the read-across analog does not pose a concern for skin sensitization. Therefore, based on structural similarity and data for the read-across analog, the alert is superseded by the 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.

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