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RIFM fragrance ingredient safety assessment, *cis*-3-hexenyl isobutyrate, CAS Registry Number 41519-23-7

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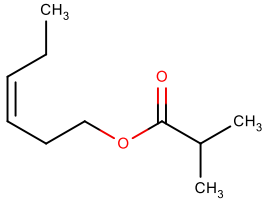
Name: *cis*-3-Hexenyl isobutyrate
CAS Registry Number: 41519-23-7



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Additional CAS #s*:
84682-20-2 (E)-Hex-3-enyl isobutyrate (No Reported Use)
*This material was included in this assessment because the materials isomers.

Abbreviation/Definition List:
2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor

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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.

cis-3-Hexenyl isobutyrate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analog *cis*-3-hexen-1-yl acetate (CAS # 3681-71-8) show that *cis*-3-hexenyl isobutyrate is not expected to be genotoxic. Data on read-across analog *cis*-3-hexen-1-yl acetate (CAS # 3681-71-8) provided an MOE >100 for the repeated dose and reproductive toxicity endpoints. Data from read-across analog hex-3-enyl acetate (CAS # 1708-82-3) and additional materials (isomers) *trans*-3-hexenyl acetate (CAS # 3681-82-1) and *cis*-3-hexenyl acetate (CAS # 3681-71-8) provided *cis*-3-hexenyl isobutyrate a NESIL of 1000 μg/cm^2 for the skin sensitization endpoint. The local respiratory toxicity endpoint was evaluated using the TTC for a Cramer Class I material, and the exposure to *cis*-3-hexenyl isobutyrate is below the TTC (1.4 mg/day). The phototoxicity/photoallergenicity endpoints were evaluated based on UV/Vis spectra; *cis*-3-hexenyl isobutyrate is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; *cis*-3-hexenyl isobutyrate was found not to be PBT as per the IFRA Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., PEC/PNEC), are <1.

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Human Health Safety Assessment

Genotoxicity: Not expected to be genotoxic.	(ECHA REACH Dossier: (Z)-hex-3-enyl acetate; ECHA, 2013)
Repeated Dose Toxicity: NOAEL = 333 mg/kg/day.	(ECHA REACH Dossier: (Z)-hex-3-enyl acetate; ECHA, 2013)
Reproductive Toxicity: Developmental toxicity: NOAEL = 1000 mg/kg/day. Fertility: NOAEL = 1000 mg/kg/day.	(ECHA REACH Dossier: (Z)-hex-3-enyl acetate; ECHA, 2013)
Skin Sensitization: NESIL = 1000 μg/cm^2.	RIFM (2018)

Phototoxicity/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:	
Critical Measured Value: 78% (OECD 301F)	RIFM (2011)
Bioaccumulation:	
Screening-level: 97.9 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: 96-h Algae EC50: 1.736 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: 96-h Algae EC50: 1.736 mg/L	(EPI Suite v4.11; US EPA, 2012a)

RIFM PNEC is: 0.1736 μg/L
- Revised PEC/PNECs (2015 IFRA VoU): North America and Europe <1

1. Identification

1. **Chemical Name:** *cis*-3-Hexenyl isobutyrate
2. **CAS Registry Number:** 41519-23-7
3. **Synonyms:** (Z)-Hex-3-enyl isobutyrate; 3-Hexenyl 2-methyl-propionate; Propanoic acid, 2-methyl-, 3-hexenyl ester, (z)-; (Z)-3-Hexenyl isobutyrate; ｱﾙｶﾝ酸(C = 1 ~ 16)ｱﾙｹﾆﾙ(C = 4 ~ 8); Hex-3-en-1-yl 2-methylpropanoate; *cis*-3-Hexenyl isobutyrate
4. **Molecular Formula:** $C_{10}H_{18}O_2$
5. **Molecular Weight:** 170.25
6. **RIFM Number:** 839
7. **Stereochemistry:** No stereocenter possible.

2. Physical data

1. **Boiling Point:** 73 °C at 6 mm Hg (Fragrance Materials Association [FMA]), 204.94 °C (EPI Suite), 199 °C (472 K) (RIFM, 2017b)
2. **Flash Point:** 164 °F; CC (FMA), 73 °C (Globally Harmonized System), 77.5 °C at 101325 Pa (average rounded off to nearest 0.5 °C) (RIFM, 2017a)
3. **Log K_{OW}:** 3.52 (EPI Suite), 3.4 (RIFM, 2016d)
4. **Melting Point**: −21.22 °C (EPI Suite)
5. **Water Solubility:** 60.2 mg/L (EPI Suite)
6. **Specific Gravity:** 0.87 (FMA)

7. **Vapor Pressure:** 0.19 mm Hg at 20 °C (EPI Suite v4.0), 0.07 mm Hg at 20 °C (FMA), 0.282 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:** Colorless liquid. Fruity-winey, sweet-green odor of considerable power and diffusion. Powerful and sweet apple-like taste (Arctander, 1969)

3. Volume of use (Worldwide band)

1. 10–100 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:** 0.020% (RIFM, 2016a)
2. **Inhalation Exposure*:** 0.000081 mg/kg/day or 0.0057 mg/day (RIFM, 2016a)
3. **Total Systemic Exposure**:** 0.00069 mg/kg/day (RIFM, 2016a)

*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).

***When a safety assessment includes multiple materials, the highest exposure out of all included materials will be recorded here for the 95th Percentile Concentration in Fine Fragrance or 95th percentile, inhalation exposure and total exposure.

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:** *cis*-3-Hexen-1-yl acetate (CAS # 3681-71-8)
   b. **Repeated Dose Toxicity:** *cis*-3-Hexen-1-yl acetate (CAS # 3681-71-8)
   c. **Reproductive Toxicity:** *cis*-3-Hexen-1-yl acetate (CAS # 3681-71-8)
   d. **Skin Sensitization:** Hex-3-enyl acetate (CAS # 1708-82-3) and additional materials (isomers) *trans*-3-hexenyl acetate (CAS # 3681-82-1) and *cis*-3-hexen-1-yl acetate (CAS # 3681-71-8)
   e. **Phototoxicity/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

cis-3-Hexenyl isobutyrate is reported to occur in the following foods by the VCF*:

Capsicum species	Macadamia nut (*Macadamia integrifolia*)
Chinese quince (*Pseudocydonia sinensis* Schneid)	Mentha oils
Guava and feyoa	

(E)-Hex-3-enyl isobutyrate is not reported to occur in food by the VCF.

*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

Available; accessed 11/22/21 (ECHA, 2018).

10. Conclusion

The maximum acceptable concentrations[a] in finished products for *cis*-3-hexenyl isobutyrate are detailed below.

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
1	Products applied to the lips (lipstick)	0.077
2	Products applied to the axillae	0.023
3	Products applied to the face/body using fingertips	0.46
4	Products related to fine fragrances	0.43
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.11
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.11
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.11
5D	Baby cream, oil, talc	0.037
6	Products with oral and lip exposure	0.25
7	Products applied to the hair with some hand contact	0.88
8	Products with significant ano-genital exposure (tampon)	0.037
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.84
10A	Household care products with mostly hand contact (hand dishwashing detergent)	1.2
10B	Aerosol air freshener	3.0
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.037
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 *cis*-3-hexenyl isobutyrate, the basis was the reference dose of 3.33 mg/kg/day, a predicted skin absorption value of 80%, and a skin sensitization NESIL of 1000 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 the current existing data, *cis*-3-hexenyl isobutyrate does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. *cis*-3-Hexenyl 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, 2013b). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. Additional assays on a more reactive 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 or clastogenic activity of *cis*-3-hexenyl isobutyrate; however, read-across can be made to *cis*-3-hexen-1-yl acetate (CAS # 3681-71-8; see Section VI).

The mutagenic activity of *cis*-3-hexen-1-yl acetate 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 methods. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with *cis*-3-hexen-1-yl acetate 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 (ECHA, 2013). Under the conditions of the study, *cis*-3-hexen-1-yl acetate was not mutagenic in the Ames test, and this can be extended to *cis*-3-hexenyl isobutyrate.

The clastogenicity of *cis*-3-hexen-1-yl acetate was assessed in an *in vitro* chromosome aberration study conducted in compliance with GLP regulations and in accordance with OECD TG 473. Human peripheral blood lymphocytes were treated with *cis*-3-hexen-1-yl acetate in DMSO at concentrations up to 1422 μg/mL in the presence and absence of metabolic activation. No statistically significant increases in the frequency of cells with structural chromosomal aberrations or polyploid cells were observed with any concentration of the test material, either with or without S9 metabolic activation (ECHA, 2013). Under the conditions of the study, *cis*-3-hexen-1-yl acetate was considered to be non-clastogenic in the *in vitro* chromosome aberration assay, and this can be extended to *cis*-3-hexenyl isobutyrate.

Based on the data available, *cis*-3-hexen-1-yl acetate does not present a concern for genotoxic potential, and this can be extended to *cis*-3-hexenyl isobutyrate.

Additional References: RIFM, 2013a.

Literature Search and Risk Assessment Completed On: 08/21/20.

11.1.2. Repeated dose toxicity

The margin of exposure (MOE) for *cis*-3-hexenyl isobutyrate is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are insufficient repeated dose toxicity data on *cis*-3-hexenyl isobutyrate for the repeated dose toxicity endpoint. Read-across material *cis*-3-hexenyl acetate (CAS # 3681-71-8; see Section VI) has an OECD/GLP 422 oral gavage combined repeated dose toxicity study with reproduction/developmental screening test conducted in Wistar rats. Groups of 11 rats/sex/dose were administered the test material *cis*-3-hexenyl acetate via gavage at doses of 0, 100, 300, or 1000 mg/kg/day in a polyethylene glycol vehicle. The males were dosed for a minimum of 4 weeks, whereas the females were dosed for approximately 7 weeks. There were no dose-response treatment-related adverse effects observed on body weights, hematological and clinical chemistry parameters, and organ weights. Macroscopic and microscopic findings were not attributed to treatment and were within the historical control range among animals of this strain and age. Thus, the NOAEL 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*.

Thus, the derived NOAEL for the repeated dose toxicity data is 1000/3 or 333 mg/kg/day.

Therefore, the *cis*-3-hexenyl isobutyrate MOE for the repeated dose toxicity endpoint can be calculated by dividing the *cis*-3-hexenyl acetate NOAEL in mg/kg/day by the total systemic exposure to *cis*-3-hexenyl isobutyrate, 333/0.00069, or 482609.

In addition, the total systemic exposure to *cis*-3-hexenyl isobutyrate (0.69 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.

11.1.2.2. Derivation of 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. (RIFM, 2020b) and a reference dose (RfD) 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 reference dose for *cis*-3-hexenyl 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.

Additional References: None.

Literature Search and Risk Assessment Completed On: 08/13/20.

11.1.3. Reproductive toxicity

The MOE for *cis*-3-hexenyl isobutyrate is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are insufficient reproductive toxicity data on *cis*-3-hexenyl isobutyrate for the reproductive toxicity endpoint. Read-across material *cis*-3-hexenyl acetate (CAS # 3681-71-8; see Section VI) has an OECD/GLP 422 oral gavage combined repeated dose toxicity study with a reproduction/developmental screening test conducted in Wistar rats. Groups of 11 rats/sex/dose were administered the test material, *cis*-3-hexenyl acetate, via gavage at doses of 0, 100, 300, or 1000 mg/kg/day in a polyethylene glycol vehicle. The males were dosed for a minimum of 4 weeks, while the females were dosed for approximately 7 weeks. In addition to systemic toxicity parameters, the fertility and developmental toxicity parameters were also assessed. There were no effects observed in the male and female reproductive function and performance (estrous cycling and sperm measures). The mean precoital time, fertility index, gestation index, conception rate, and implantation

rate were not affected by treatment with the test material. There were no toxicologically significant differences in the mean numbers of corpora lutea per dam, and no impact on the post-implantation loss was observed. There were no treatment-related alterations on the development of the pups (body weights, macroscopic or histopathological findings, birth and viability index, and sex ratio) observed at the first litter check or on day 4 postpartum. Thus, the NOAEL for developmental toxicity and fertility was considered to be 1000 mg/kg/day, the highest dose tested (ECHA, 2013). **Therefore, the *cis*-3-hexenyl isobutyrate MOE for the developmental toxicity and fertility endpoint can be calculated by dividing the *cis*-3-hexenyl acetate NOAEL in mg/kg/day by the total systemic exposure to *cis*-3-hexenyl isobutyrate, 1000/0.00069, or 1449275.**

In addition, the total systemic exposure to *cis*-3-hexenyl isobutyrate (0.69 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: 08/16/20.

11.1.4. Skin sensitization

Based on read-across material hex-3-enyl acetate (CAS # 1708-82-3) and its additional materials (isomers) *trans*-3-hexenyl acetate (CAS # 3681-82-1) and *cis*-3-hexenyl acetate (CAS # 3681-71-8), *cis*-3-hexenyl isobutyrate is considered a skin sensitizer with a defined NESIL of 1000 $\mu g/cm^2$.

11.1.4.1. Risk assessment. Insufficient skin sensitization studies are available for *cis*-3-hexenyl butyrate. Based on the existing data and read-across material hex-3-enyl acetate and its additional materials (isomers) *trans*-3-hexenyl acetate and *cis*-3-hexenyl acetate (CAS # 1708-82-3, CAS # 3681-71-8, CAS # 3681-82-1; see Section VI), *cis*-3-hexenyl isobutyrate is a skin sensitizer. The chemical structures of these materials indicate that they would not be expected to react with skin proteins (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). *cis*-3-Hexenyl isobutyrate was found to be positive in an *in vitro* direct peptide reactivity assay (DPRA) and negative in a KeratinoSens assay (RIFM, 2016e; RIFM, 2016f), while the read-across material, hex-3-enyl acetate, was found to be positive in the DPRA and human cell line activation test (h-CLAT) (RIFM, 2017e; RIFM, 2016b). In a murine local lymph node assay (LLNA), read-across material hex-3-enyl acetate was found to be negative up to 100% (RIFM, 2016c). In a guinea pig maximization test, read-across material *cis*-3-hexen-1-yl acetate led to skin sensitization reactions (RIFM, 1996; RIFM, 1997). In a human maximization test, no skin sensitization reactions were observed with additional read-across material *cis*-3-hexen-1-yl acetate (RIFM, 1974). In a human maximization test, no skin sensitization reactions were observed with the target material, 10% *cis*-3-hexenyl butyrate (RIFM, 1976). Additionally, in a Confirmation of No Induction in Humans test (CNIH) with 1102 $\mu g/cm^2$ of read-across material *cis*-3-hexen-1-yl acetate in 1:3 ethanol:DEP, a reaction indicative of sensitization was observed in 1 of the 104 volunteers (RIFM, 2012). However, in another CNIH with 1003 $\mu g/cm^2$ of additional read-across material *cis*-3-hexen-1-yl acetate in 1:3 ethanol: DEP, no reactions indicative of sensitization were observed in any of the 104 subjects (RIFM, 2018).

Based on the weight of evidence (WoE) from structural analysis, human studies, and data on the read-across material hex-3-enyl acetate, *cis*-3-hexenyl isobutyrate is a sensitizer with a Weight of Evidence No Expected Sensitization Induction Level (WoE NESIL) of 1000 $\mu g/cm^2$ (see 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. (RIFM, 2020b) and a reference dose of 3.33 mg/kg/day.

Additional References: None.

Table 1
Data Summary for hex-3-enyl acetate as read-across material for cis-3-hexenyl isobutyrate.

LLNA Weighted Mean EC3 Value $\mu g/cm^2$ [No. Studies]	Potency Classification Based on Animal Data[a]	Human Data			
		NOEL-CNIH (Induction) $\mu g/cm^2$	NOEL-HMT (Induction) $\mu g/cm^2$	LOEL[b] (Induction) $\mu g/cm^2$	WoE NESIL[c] $\mu g/cm^2$
NA [1]	Weak	1003	6900	1102	1000

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.

[a] Based on animal data (guinea pig maximization test) using classification defined in ECETOC, Technical Report No. 87, 2003.

[b] Data derived from CNIH or HMT.

[c] WoE NESIL limited to 2 significant figures.

Literature Search and Risk Assessment Completed On: 08/04/20.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, *cis*-3-hexenyl isobutyrate would not be expected to present a concern for phototoxicity or photoallergenicity.

11.1.5.1. Risk assessment. There are no phototoxicity studies available for *cis*-3-hexenyl 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 phototoxicity and photoallergenicity (Henry et al., 2009). Based on the lack of absorbance, *cis*-3-hexenyl isobutyrate does not present a concern for phototoxicity 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: 08/07/20.

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 *cis*-3-hexenyl 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 *cis*-3-hexenyl isobutyrate. Based on the Creme RIFM Model, the inhalation exposure is 0.0057 mg/day. This exposure is 246 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: 07/29/20.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of *cis*-3-hexenyl 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, *cis*-3-hexenyl isobutyrate 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 is > 1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) did not identify *cis*-3-hexenyl 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, 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 $\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). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

11.2.1.1. Risk assessment. Based on the current Volume of Use (2015), *cis*-3-hexenyl isobutyrate presents a risk to the aquatic compartment in the screening-level assessment.

11.2.1.2. Key studies

11.2.1.2.1. Biodegradation. RIFM, 2011: The ready biodegradability of the test material was evaluated using the manometric respirometry test according to the OECD 301F method. Under the conditions of the study, biodegradation of 78% was observed after 28 days.

11.2.1.3. Ecotoxicity. RIFM, 2017d: A *Daphnia magna* acute immobilization test was conducted according to the OECD 202 guidelines under semi-static conditions. The 48-h EC50 value based on mean measured concentrations was reported to be 20 mg/L (95% CI: 18–23 mg/L).

RIFM, 2017c: An algae growth inhibition test was conducted according to the OECD 201 guidelines under static conditions. The 72-h EC50 values based on mean measured concentration for growth rate and yield were reported to be 5.3 mg/L (95% CI: 4.8–5.9 mg/L) and 3.3 mg/L (95 CI: 3.2–3.5 mg/L), respectively.

11.2.1.4. Other available data. cis-3-Hexenyl isobutyrate has been registered for REACH with no additional data at this time (ECHA, 2017).

11.2.1.5. 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	3.4	3.4
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 further assessment is necessary.

*Combined Regional Volumes of Use for both CAS #s.

The RIFM PNEC is 0.1736 μg/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: 08/20/20.

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**
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **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 HPVIS:** https://ofmpub.epa.gov/oppthpv/public_search.publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&EndPointRpt=Y#submission
- **Japanese NITE:** https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com
- **ChemIDplus:** https://chem.nlm.nih.gov/chemidplus/

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 12/08/21.

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.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	13.90			1000000	0.01390	
ECOSAR Acute Endpoints **(Tier 2)** v1.11	2.939	5.190	1.736	10000	0.1736	Esters
ECOSAR Acute Endpoints **(Tier 2)** v1.11	6.005	3.924	5.227			Neutral Organic

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2022.112910.

Appendix

Read-across Justification

Methods

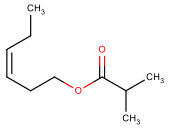
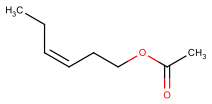
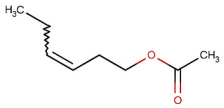
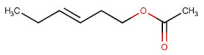
The read-across analogs were identified using RIFM fragrance materials chemical inventory clustering and read-across search criteria (RIFM, 2020a). These criteria follow the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015) and are consistent 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, 2016).

- First, materials were clustered based on their structural similarity. Second, data availability and data quality on the selected cluster were examined. Third, appropriate read-across analogs from the cluster were confirmed by expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physical–chemical properties of the target substance and the read-across analogs were calculated using EPI Suite v4.11 (US EPA, 2012a).
- J_{max} values were calculated using RIFM's Skin Absorption Model (SAM). The parameters were calculated using the consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts, 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	*cis*-3-Hexenyl isobutyrate	*cis*-3-Hexen-1-yl acetate	Hex-3-enyl acetate and *trans*-3-hexenyl acetate
CAS No.	41519-23-7	3681-71-8	1708-82-3 and 3681-82-1
Structure			

(*continued on next page*)

(*continued*)

	Target Material	Read-across Material	Read-across Material
			 
Similarity (Tanimoto Score)		0.81	0.81
Endpoint		• Genotoxicity • Skin sensitization • Repeated dose toxicity • Reproductive toxicity	• Skin sensitization
Molecular Formula	$C_{10}H_{18}O_2$	$C_8H_{14}O_2$	$C_8H_{14}O_2$
Molecular Weight	170.252	142.198	142.198
Melting Point (°C, EPI Suite)	−21.22	−33.28	−33.28
Boiling Point (°C, EPI Suite)	204.94	176.55	176.55
Vapor Pressure (Pa @ 25°C, EPI Suite)	3.76E+01	1.52E+02	1.52E+02
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	6.02E+01	4.81E+02	4.81E+02
Log K_{OW}	3.52	2.61	2.61
J_{max} (μg/cm^2/h, SAM)	6.05	30.25	30.25
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.13E+02	6.44E+01	6.44E+01
Genotoxicity			
DNA Binding (OASIS v1.4, QSAR Toolbox v4.2)	No alert found	AN2|AN2 ≫ Schiff base formation after aldehyde release|AN2 ≫ Schiff base formation after aldehyde release ≫ Specific Acetate Esters|SN1|SN1 ≫ Nucleophilic attack after carbenium ion formation|SN1 ≫ Nucleophilic attack after carbenium ion formation ≫ Specific Acetate Esters|SN2|SN2 ≫ Acylation|SN2 ≫ Acylation ≫ Specific Acetate Esters|SN2 ≫ Nucleophilic substitution at sp3 Carbon atom|SN2 ≫ Nucleophilic substitution at sp3 Carbon atom ≫ Specific Acetate Esters	
DNA Binding (OECD QSAR Toolbox v4.2)	No alert found	No alert found	
Carcinogenicity (ISS)	No alert found	No alert found	
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	Not categorized	
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)	Toxicant (good reliability)	
Skin Sensitization			
Protein Binding (OASIS v1.1)	No alert found	No alert found	No alert found
Protein Binding (OECD)	No alert found	No alert found	No alert found
Protein Binding Potency	Not possible to classify according to these rules (GSH)	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	No alert found
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	No skin sensitization reactivity domain alerts were identified.	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	See Supplemental Data 3 See Supplemental Data 4

Summary

There are insufficient toxicity data on cis-3-hexenyl isobutyrate (CAS # 41519-23-7). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, metabolism data, physical–chemical properties, and expert judgment, *cis*-3-hexen-1-yl acetate (CAS # 3681-71-8), hex-3-enyl acetate (CAS # 1708-82-3), and *trans*-3-hexenyl acetate (CAS # 3681-82-1) were identified as read-across analogs with sufficient data for toxicological evaluation.

Conclusions

- *cis*-3-Hexen-1-yl acetate (CAS # 3681-71-8) was used as a read-across analog for the target material, *cis*-3-hexenyl isobutyrate (CAS # 41519-23-7), for the genotoxicity, reproductive toxicity, repeated dose toxicity, and skin sensitization endpoints. Hex-3-enyl acetate (CAS # 1708-82-3) and *trans*-3-hexenyl acetate (CAS # 3681-82-1) were used as a read-across analogs for the target material, *cis*-3-hexenyl isobutyrate (CAS # 41519-23-7), for the skin sensitization endpoint.
  - o The target substance and the read-across analog are structurally similar and belong to a class of unsaturated aliphatic esters.
  - o The target substance and the read-across analog have a hexenyl substructure in common.
  - o The key difference between the target substance and the read-across analog is that the target substance is an ester of isobutyric acid. In contrast, the read-cross analogs are esters of acetic acid. The read-across analogs contain the structural features of the target material that are relevant to these endpoints and are expected to have equal or greater potential for toxicity as compared to the target.
  - o The similarity between the target substance and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The physical–chemical properties of the target substance 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 substance and the read-across analog.
  - o The read-across analog has an alert of AN2 reaction and Schiff base formation for genetic toxicity. This is due to the acetate portion of the read-across molecule. The structures of the read-across analogs are out of the structural domain of the training set. The data on the read-across analogs confirm that the margin of exposure is adequate at the current level of use, and the read-across analog does not pose a concern for genetic toxicity. Therefore, based on the data for the read-across analogs, the predictions are superseded by data.
  - o The target substance 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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