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RIFM fragrance ingredient safety assessment, 3-hexenyl 2-methylbutanoate, CAS registry number 10094-41-4

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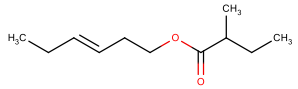

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Name: 3-Hexenyl 2-methylbutanoate
CAS Registry Number: 10094-41-4
Additional CAS Numbers*: 53398-85-9 (Z)-Hex-3-enyl 2-methylbutyrate
*This material was included in this assessment because they are a mixture of isomers.

Abbreviation/Definition List:

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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, 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, 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.

3-Hexenyl 2-methylbutanoate 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 3-hexenyl 2-methylbutanoate is not expected to be genotoxic and provide a calculated MOE >100 for the repeated dose and reproductive toxicity endpoints. Data from read-across materials 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) provided 3-hexenyl 2-methylbutanoate a NESIL of 1000 μg/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on UV/Vis spectra; 3-hexenyl 2-methylbutanoate is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was completed using the TTC for a Cramer Class I material, and the exposure to 3-hexenyl 2-methylbutanoate is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; 3-hexenyl 2-methylbutanoate was found not to be a 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: Screening-level: 3.23 (BIOWIN 3)	(EPI Suite v4.11; US EPA, 2012a)
Bioaccumulation: Screening-level: 206 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Screening-level: Fish LC50: 5.53 mg/L	(RIFM Framework; Salvito, 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, 2002)

Critical Ecotoxicity Endpoint: Fish LC50: 5.53 mg/L (RIFM Framework; Salvito, 2002)

RIFM PNEC is: 0.00553 μg/L

- **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: Not applicable; cleared at the screening-level

1. Identification

Chemical Name: 3-Hexenyl 2-methylbutanoate	Chemical Name: (Z)-Hex-3-enyl 2-methylbutyrate
CAS Registry Number: 10094-41-4	**CAS Registry Number:** 53398-85-9
Synonyms: Butanoic acid, 2-methyl-, 3-hexenyl ester; 3-Hexenyl 2-methylbutyrate; Hex-3-en-1-yl 2-methylbutanoate; 3-Hexenyl 2-methylbutanoate	**Synonyms:** *cis*-3-Hexenyl 2-methylbutyrate; Butanoic acid, 2-methyl-, 3-hexenyl ester, (Z)-; Hex-3-en-1-yl 2-methylbutanoate; ｱﾙｶﾝ酸(C = 1 ~ 6) ｱﾙｹﾆﾙ(C = 4 ~ 8)
Molecular Formula: $C_{11}H_{20}O_2$	**Molecular Formula:** $C_{11}H_{20}O_2$
Molecular Weight: 184.28	**Molecular Weight:** 184.79
RIFM Number: 975	**RIFM Number:** 5728
Stereochemistry: Isomer not specified. Two stereocenters and a total of 4 stereoisomers are possible.	**Stereochemistry:** Z isomer specified. Two stereocenters and a total of 4 stereoisomers are possible.

2. Physical data

CAS #: 10094-41-4	CAS #: 53398-85-9

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Boiling Point: >200 °C (FMA Database), 224.17 °C (US EPA, 2012a)	**Boiling Point:** 224.17 °C (US EPA, 2012a)
Flash Point: 190 °F; CC (FMA Database)	**Flash Point:** Not available
Log K_{OW}: 4.01 (US EPA, 2012a)	**Log K_{OW}:** 4.01 (US EPA, 2012a)
Melting Point: -9.97 °C (US EPA, 2012a)	**Melting Point:** -9.97 °C (US EPA, 2012a)
Water Solubility: 19.61 mg/L (US EPA, 2012a)	**Water Solubility:** 19.61 mg/L (US EPA, 2012a)
Specific Gravity: 0.877 (FMA Database)	**Specific Gravity:** Not available
Vapor Pressure: 0.0693 mm Hg at 20 °C (US EPA, 2012a), 0.106 mm Hg at 25 °C (US EPA, 2012a)	**Vapor Pressure:** 0.0693 mm Hg at 20 °C (US EPA, 2012a), 0.106 mm Hg at 25 °C (US EPA, 2012a)
UV Spectra: No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})	**UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})
Appearance/Organoleptic: Not available	**Appearance/Organoleptic:** Not available

3. Volume of use (Worldwide band)

1. 0.1–1 metric ton per year (IFRA, 2015)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v3.1.1)*

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.0070% (RIFM, 2020b)
2. **Inhalation Exposure**:** 0.00011 mg/kg/day or 0.00090 mg/day (RIFM, 2020b)
3. **Total Systemic Exposure***:** 0.00039 mg/kg/day (RIFM, 2020b)

*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, inhalation exposure, and total exposure.

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

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

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

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

2. Analogs Selected:
   a. **Genotoxicity:** *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

3-Hexenyl 2-methylbutanoate is not reported to occur in foods by the VCF*.

(Z)-Hex-3-enyl 2-methylbutyrate is reported to occur in the following foods by the VCF:

Apricot (*Prunus armeniaca* L.)	Guava and feyoa Tea
Mentha oils	Mastic (*Pistacia lentiscus*)
Capsicum species Plum *(Prunus species)*	

*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

Both materials have been pre-registered for 2010; no dossiers available as of 12/08/21.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for 3-hexenyl 2-methylbutanoate 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)	3.0
10B	Aerosol air freshener	3.0

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IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
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 3-hexenyl 2-methylbutanoate, the basis was the subchronic reference dose of 3.33 mg/kg/day, a predicted skin absorption value of 40%, 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; December 2019).

[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, 3-hexenyl 2-methylbutanoate does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. 3-hexenyl 2-methylbutanoate was assessed in the BlueScreen assay and found negative for both cytotoxicity (positive: <80% relative cell density) and genotoxicity, with and without metabolic activation (RIFM, 2013). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. Additional assays on a 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 3-hexenyl 2-methylbutanoate; 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 3-hexenyl 2-methylbutanoate.

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 3-hexenyl 2-methylbutanoate.

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 3-hexenyl 2-methylbutanoate.

Additional References: None.

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

11.1.2. Repeated dose toxicity

The margin of exposure (MOE) for 3-hexenyl 2-methylbutanoate 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 3-hexenyl 2-methylbutanoate 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 3-hexenyl 2-methylbutanoate 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 3-hexenyl 2-methylbutanoate, 333/0.00039, or 853846.

In addition, the total systemic exposure to 3-hexenyl 2-methylbutanoate (0.39 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes, 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

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, 2020c) and a subchronic reference dose (RfD) of 3.33 mg/kg/day.

11.1.2.1.1. Derivation of subchronic RfD. The RIFM Criteria Document (Api, 2015) calls for a default MOE of 100 (10 × 10), based on uncertainty factors applied for interspecies (10 ×) and intraspecies (10 ×) differences. The subchronic RfD for 3-hexenyl 2-methylbutanoate was calculated by dividing the lowest NOAEL (from the Repeated Dose and Reproductive Toxicity sections) of 333 mg/kg/day by the uncertainty factor, 100 = 3.33 mg/kg/day.

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

Additional References: None.

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

11.1.3. Reproductive toxicity

The MOE for 3-hexenyl 2-methylbutanoate 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 3-hexenyl 2-methylbutanoate 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 post-partum. Thus, the NOAEL for maternal, developmental, and reproductive toxicity was considered to be 1000 mg/kg/day, the highest dose tested (ECHA, 2013). **Therefore, the 3-hexenyl 2-methylbutanoate MOE for the developmental toxicity and fertility endpoints can be calculated by dividing the *cis*-3-hexenyl acetate NOAEL in mg/kg/day by the total systemic exposure to 3-hexenyl 2-methylbutanoate, 1000/0.00039, or 2564102.**

In addition, the total systemic exposure to 3-hexenyl 2-methylbutanoate (0.39 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes, 2007; Laufersweiler, 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 additional materials (isomers) *trans*-3-hexenyl acetate (CAS # 3681-82-1) and *cis*-3-hexenyl acetate (CAS # 3681-71-8), 3-hexenyl 2-methylbutanoate is considered a skin sensitizer with a defined No Expected Sensitization Induction Level (NESIL) of 1000 μg/cm^2.

11.1.4.1. Risk assessment. Insufficient skin sensitization studies are available for 3-hexenyl 2-methylbutanoate. Based on the existing data and read-across material hex-3-enyl acetate and 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 structure of these materials indicates that they would not be expected to react with skin proteins (Roberts, 2007; Toxtree v3.1.0; OECD Toolbox v4.2). Read-across material hex-3-enyl acetate was found to be positive in an *in vitro* direct peptide reactivity assay (DPRA) and human cell line activation test (h-CLAT) (RIFM, 2017; RIFM, 2016a). In a murine local lymph node assay (LLNA), read-across material hex-3-enyl acetate was found to be negative up to 100% (RIFM, 2016b). 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% 3-hexenyl 2-methylbutanoate (RIFM, 1977). Additionally, in a confirmatory Confirmation of No Induction in Humans test (CNIH) with 1102 μg/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 μg/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, 3-hexenyl 2-methylbutanoate is a sensitizer with a WoE NESIL of 1000 μg/cm^2 (Table 1). Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020c) and a subchronic reference dose of 3.33 mg/kg/day.

Additional References: None.

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

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 3-hexenyl 2-methylbutanoate 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 3-hexenyl 2-methylbutanoate in experimental models. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. The corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity (Henry, 2009). Based on the lack of absorbance, 3-hexenyl 2-methylbutanoate 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, 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 08/07/20.

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 3-hexenyl 2-methylbutanoate 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 3-hexenyl 2-methylbutanoate. Based on the Creme RIFM Model, the inhalation exposure is 0.00090 mg/day. This exposure is 1555.6 times lower than the Cramer Class I TTC value of 1.4 mg/day (based on human lung weight of 650 g; Carthew, 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: None.

Table 1
Data Summary for hex-3-enyl acetate as read-across material for 3-hexenyl 2-methylbutanoate.

LLNA Weighted Mean EC3 Value μg/cm^2 [No. Studies]	Potency Classification Based on Animal Data[a]	Human 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
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: 07/29/20.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 3-hexenyl 2-methylbutanoate was performed following the RIFM Environmental Framework (Salvito, 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, 3-hexenyl 2-methylbutanoate was identified as a fragrance material with no potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC <1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) did not identify 3-hexenyl 2-methylbutanoate 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, 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).

11.2.1.1. Risk assessment. Based on the current Volume of Use (2015), 3-hexenyl 2-methylbutanoate presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2. Key studies

11.2.2.1. Biodegradation. No data available.

11.2.2.2. Ecotoxicity. No data available.

11.2.2.3. Other available data. 3-Hexenyl 2-methylbutanoate has been pre-registered for REACH with no additional data at this time.

11.2.3. 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, 2002).

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	3.9	3.9
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band*	<1	<1
Risk Characterization: PEC/PNEC	**< 1**	**< 1**

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

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

The RIFM PNEC is 0.00553 μg/L. The revised PEC/PNECs for EU and NA are not applicable. The material was cleared at the screening-level; therefore, it does not present a risk to the aquatic environment at the current reported VoU.

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

12. Literature Search*

- **RIFM Database:** Target, Fragrance Structure-Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** https://echa.europa.eu/
- **NTP:** https://ntp.niehs.nih.gov/
- **OECD Toolbox:** https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **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

	LC50 (Fish)	EC50 (*Daphnia*)	EC50 (Algae)	AF	PNEC	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	<u>5.53</u>			1000000	0.00553	

- **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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2022.113060.

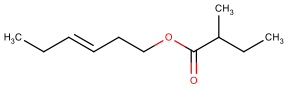
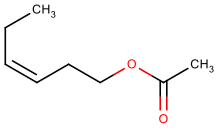
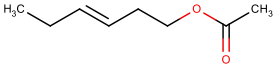
Appendix

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, 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 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 and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2020).
- To keep continuity and compatibility with the *in silico* alerts, OECD QSAR Toolbox v4.2 was selected as the alert system.

	Target Material	Read-across Material	Read-across Material
Principal Name	3-Hexenyl 2-methylbutanoate	*cis*-3-Hexen-1-yl acetate	Hex-3-enyl acetate, *trans*-3-hexenyl acetate, *cis*-3-hexen-1-yl acetate
CAS No.	10094-41-4	3681-71-8	1708-82-3, 3681-82-1
Structure			
Similarity (Tanimoto Score)		0.71	0.71
Endpoint		• Genotoxicity • Skin sensitization • Repeated dose toxicity • Reproductive toxicity	• Skin sensitization
Molecular Formula	$C_{11}H_{20}O_2$	$C_8H_{14}O_2$	$C_8H_{14}O_2$
Molecular Weight	184.279	142.198	142.198
Melting Point (°C, EPI Suite)	−9.97	−33.28	−33.28
Boiling Point (°C, EPI Suite)	224.17	176.55	176.55
Vapor Pressure (Pa @ 25°C, EPI Suite)	1.41E+01	1.52E+02	1.52E+02

(*continued on next page*)

(*continued*)

	Target Material	Read-across Material	Read-across Material
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	1.96E+01	4.81E+02	4.81E+02
Log K_{OW}	4.01	2.61	2.61
J_{max} (μg/cm^2/h, SAM)	2.37	30.25	30.25
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.51E+02	6.44E+01	6.44E+01
Genotoxicity			
DNA Binding (OASIS v1.4, QSAR Toolbox v4.2)	No alert found	AN2|AN2 ≫ Shiff base formation after aldehyde release|AN2 ≫ Shiff 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	AN2|AN2 ≫ Shiff base formation after aldehyde release|AN2 ≫ Shiff 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	No alert found
Carcinogenicity (ISS)	Structural alert for nongenotoxic carcinogenicity|Substituted n-alkylcarboxylic acids (Nongenotox)	No alert found	No alert found
DNA Binding (Ames, MN, CA, OASIS v1.1)	No alert found	No alert found	No alert found
***In Vitro* Mutagenicity (Ames, ISS)**	No alert found	No alert found	No alert found
***In Vivo* Mutagenicity (Micronucleus, ISS)**	No alert found	No alert found	No alert found
Oncologic Classification	Not classified	Not classified	Not classified
Repeated Dose Toxicity			
Repeated Dose (HESS)	Not categorized	Not categorized	Not categorized
Reproductive Toxicity			
ER Binding (OECD QSAR Toolbox v4.2)	Non-binder, non-cyclic structure	Non-binder, non-cyclic structure	Non-binder, non-cyclic structure
Developmental Toxicity (CAESAR v2.1.6)	Toxicant (low reliability)	Toxicant (good 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.
Local Respiratory Toxicity			
Respiratory Sensitization (OECD QSAR Toolbox v4.2)	No alert found	No alert found	No alert found
Metabolism			
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	See Supplemental Data 1	See Supplemental Data 2	See Supplemental Data 3

Summary

There are insufficient toxicity data on 3-hexenyl 2-methylbutanoate (CAS # 10094-41-4). 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, hex-3-enyl acetate (CAS # 1708-82-3), *trans*-3-hexenyl acetate (CAS # 3681-82-1), and *cis*-3-hexen-1-yl acetate (CAS # 3681-71-8) were identified as read-across materials 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 3-hexenyl 2-methylbutanoate (CAS # 10094-41-4) for the genotoxicity, repeated dose toxicity, and reproductive toxicity endpoints. and Hex-3-enyl acetate (CAS # 1708-82-3) and its

additional materials (isomers) *trans*-3-hexenyl acetate (CAS # 3681-82-1) and *cis*-3-hexen-1-yl acetate (CAS # 3681-71-8) were used as read-across analogs for skin sensitization endpoint.

- o The target material and the read-across analog are structurally similar and belong to a class of esters.
- o The target material and the read-across analog share a common hexenyl fragment on the alcohol portion of the ester.
- o The key difference between the target material and the read-across analog is that the target material has an ester of methyl butenoic acid, while the read-across analog is an ester of acetic acid. 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 material.
- o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. The Tanimoto score is mainly driven by the unsaturated aliphatic ester fragment. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
- o The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
- o According to the OECD QSAR Toolbox v4.2, structural alerts for the toxicological endpoints are consistent between the target material and the read-across analog.
- o The read-across analog is predicted to be a toxicant by the CAESAR model for developmental toxicity. All other alerts are negative. According to these predictions, the read-across analog is expected to be more reactive when compared to the target material. The data described in the developmental toxicity section shows that the read-across analog has an adequate MOE at the current level of use. Therefore, the predictions are superseded by the data.
- o The read-across analog has an alert for SN1, SN2, AN1, and Schiff base formation. This is because the read-across analog is an ester of acetic acid. The training set used for this alert has all acetate esters with a diverse extended fragment on the alcohol portion. The toxicity and reactivity are all from the alcohol portions of these esters. The read-across analog is completely out of the structural domain of the training set. Furthermore, the expert rule-based model confirms that necessary conditions for eliciting direct or indirect DNA interaction, described in this general mechanistic profile, are met. However, the specific structural boundaries providing sufficiency for interaction with DNA may not be identified. These specific structural boundaries are examined in the corresponding endpoint-specific profile. The data for the read-across analog confirms that the analog does not pose a concern for genetic toxicity. Therefore, the *in silico* alert is superseded by the data.
- o The read-across analog is predicted to be a toxicant by the CAESAR model for developmental toxicity, while the target material is predicted to be a non-toxicant. The data described in the developmental toxicity section above show that the read-across analog has an adequate MOE at the current level of use. Therefore, the alert will be superseded by the availability of the data.
- o The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
- o The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

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