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RIFM fragrance ingredient safety assessment, *cis*-3-hexenyl isovalerate, CAS Registry Number 35154-45-1

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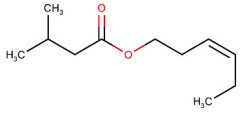

Name: *cis*-3-Hexenyl isovalerate
CAS Registry Number: 35154-45-1
Abbreviation/Definition List:
2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor

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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., 2020)
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., 2015, 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

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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 Observable 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 isovalerate 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-hexenyl acetate (CAS # 3681-71-8) show that *cis*-3-hexenyl isovalerate is not expected to be genotoxic and provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose and reproductive toxicity endpoints. Data from analogs hex-3-enyl acetate (CAS # 1708-82-3) and *cis*-3-hexen-1-yl acetate (CAS # 3681-71-8) provided a No Expected Sensitization Induction Level (NESIL) of 1000 μg/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on data and ultraviolet/visible (UV/Vis) spectra; *cis*-3-hexenyl isovalerate is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material; exposure is below the TTC (1.4 mg/day). Environmental endpoints were evaluated; *cis*-3-hexenyl isovalerate was found not to be Persistent, Bioaccumulative, and Toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

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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: 1000 mg/kg/day. Fertility: 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 phototoxic/not expected to be photoallergenic.	(UV/Vis Spectra; RIFM Database; Weeks and DeSena, 1978)

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: 206.6 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: Fish LC50: 4.43 mg/L	(RIFM Framework; Salvito et al., 2002)

Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:

Screening-level: PEC/PNEC (North America and Europe) < 1	(RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: Fish LC50: 4.43 mg/L	(RIFM Framework; Salvito et al., 2002)

RIFM PNEC is: 0.00443 μg/L

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

1. Identification

1. **Chemical Name**: *cis*-3-Hexenyl isovalerate
2. **CAS Registry Number**: 35154-45-1
3. **Synonyms**: (Z)-Hex-3-enyl isovalerate; Isovaleric acid, 3-hexenyl ester, (z)-; ｱﾙｶﾝ酸(C = 1–16)ｱﾙｹﾆﾙ(C = 4–8); Hex-3-en-1-yl 3-methylbutanoate; *cis*-3-Hexenyl isovalerate
4. **Molecular Formula**: $C_{11}H_{20}O_2$
5. **Molecular Weight**: 184.27
6. **RIFM Number**: 974
7. **Stereochemistry**: *Cis* isomer specified.

2. Physical data

1. **Boiling Point**: 105 °C at 20 mm Hg (Fragrance Materials Association [FMA]), 224.17 °C (EPI Suite)
2. **Flash Point**: 185 °F; CC (FMA)
3. **Log K_{OW}**: 4.01 (EPI Suite)
4. **Melting Point**: −9.97 °C (EPI Suite)
5. **Water Solubility**: 19.61 mg/L (EPI Suite)
6. **Specific Gravity**: 0.88 (FMA)
7. **Vapor Pressure**: 0.0693 mm Hg at 20 °C (EPI Suite v4.0), 0.07 mm Hg at 20 °C (FMA), 0.106 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**: Arctander, Volume I, 1969: Colorless liquid. Relatively powerful, sweet-green, apple-like odor with buttery-creamy notes. Powerful, green-fruity, buttery, and sweet taste, reminiscent of apples. Pleasant concentration level: less than 5 ppm.

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 v1.0)

1. **95**th **Percentile Concentration in Fine Fragrance**: 0.0015% (RIFM, 2017b)
2. **Inhalation Exposure***: 0.00014 mg/kg/day or 0.0095 mg/day (RIFM, 2017b)
3. **Total Systemic Exposure****: 0.00087 mg/kg/day (RIFM, 2017b)

*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; and 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; and Comiskey et al., 2017).

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

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

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

2. **Analogs Selected**:
   a. **Genotoxicity**: *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 *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 isovalerate is reported to occur in the following foods by the VCF*:

Capsicum species	Nectarine
Cherimoya (*Annona cherimolia* Mill.)	*Salvia* species
Elderberry (*Sambucus nigra* L.)	Sea buckthorn (*Hippophae rhamnoides* L.)
Lamb's lettuce (*Valerianella locusta*)	Tea
Mastic (*Pistacia lentiscus*)	Turpentine oil (*Pistacia terebinthus*)
Mentha oils	

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*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

cis-3-Hexenyl isovalerate has been pre-registered for 2010; no dossier available as of 10/01/20.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for *cis*-3-hexenyl isovalerate 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)	1.8
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 isovalerate, the basis was the 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).

[c]Calculations by Creme RIFM Aggregate Exposure Model v3.1.1.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, *cis*-3-hexenyl isovalerate does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. *cis*-3-Hexenyl isovalerate was assessed in the BlueScreen assay and found negative for both cytotoxicity (positive: <80% relative cell density) and genotoxicity, with and without metabolic activation (RIFM, 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 *cis*-3-hexenyl isovalerate; 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 isovalerate.

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 isovalerate.

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 isovalerate.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/15/20.

11.1.2. Repeated dose toxicity

The MOE for *cis*-3-hexenyl isovalerate is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are no repeated dose data on the target material. Read-across material *cis*-3-hexenyl acetate (CAS # 3681-71-8; see Section VI) has sufficient data for the repeated dose toxicity endpoint. In an OECD 422/GLP oral gavage combined repeated dose toxicity study with a reproduction/developmental screening test, groups of 11 Wistar rats/sex/dose were administered the test material 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-responsive, treatment-related adverse effects observed on body weight, hematological and clinical chemistry parameters, or organ weights. Macroscopic and microscopic findings were not attributed to the 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 isovalerate 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 isovalerate, 333/0.00087 or 382759.

In addition, the total systemic exposure to *cis*-3-Hexenyl isovalerate (0.87 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.

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, 2020) and a reference dose of 3.33 mg/kg/day.

11.1.2.1.1. Derivation of reference dose (RfD). 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 isovalerate 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: 03/19/20.

11.1.3. Reproductive toxicity

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

11.1.3.1. Risk assessment. There are no reproductive toxicity data on the target material. Read-across material *cis*-3-hexenyl acetate (CAS # 3681-71-8; see Section VI) has sufficient data for the reproductive toxicity endpoint. An OECD 422/GLP oral gavage combined repeated dose toxicity study with a reproduction/developmental screening test was conducted in Wistar rats. Groups of 11 rats/sex/dose were administered the test material 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. In addition to systemic toxicity parameters, the fertility and developmental toxicity parameters were also assessed. There were no effects observed in 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 the 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 first litter check or on day 4 post-partum. 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 isovalerate MOE for the fertility and developmental 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 isovalerate, 1000/0.00087 or 1149425.**

In addition, the total systemic exposure to *cis*-3-hexenyl isovalerate (0.87 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: 03/31/20.

11.1.4. Skin sensitization

Based on the existing data and read-across materials hex-3-enyl acetate (CAS # 1708-82-3) and *cis*-3-hexen-1-yl acetate (CAS # 3681-71-8), *cis*-3-hexenyl isovalerate is considered a skin sensitizer with a defined NESIL of 1000 μg/cm^2.

11.1.4.1. Risk assessment. Limited skin sensitization studies are available for *cis*-3-hexenyl isovalerate. Based on the existing data and read-across materials hex-3-enyl acetate (CAS # 1708-82-3; see Section VI) and *cis*-3-hexen-1-yl acetate (CAS # 3681-71-8; see Section VI), *cis*-3-hexenyl isovalerate is considered a 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). 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, 2017a; RIFM, 2016a). In the Draize skin sensitization test, *cis*-3-hexenyl isovalerate did not produce any sensitization reactions (Weeks and DeSena, 1978). Additionally, 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 human maximization tests, no skin sensitization reactions were observed with *cis*-3-hexenyl isovalerate and read-across material *cis*-3-hexen-1-yl acetate (RIFM, 1977; RIFM, 1974). Additionally, in a Confirmation of No Induction in Humans test (CNIH) with 1112 μg/cm^2 of read-across material *cis*-3-hexen-1-yl acetate in 1:3 ethanol (EtOH):diethyl phthalate (DEP), a reaction indicative of sensitization was observed in 1 of the 104 volunteers (RIFM, 2012). However, in 2 additional CNIHs with 1003 μg/cm^2 and 969 μg/cm^2 of read-across material *cis*-3-hexen-1-yl acetate in 1:3 EtOH:DEP and EtOH, respectively, no reactions indicative of sensitization were observed in any of the 110 or 38 volunteers, respectively (RIFM, 2018; RIFM, 1965).

Based on weight of evidence (WoE) from structural analysis and animal and human studies of *cis*-3-hexenyl isovalerate and read-across materials hex-3-enyl acetate and *cis*-3-hexen-1-yl acetate, *cis*-3-hexenyl isovalerate is considered to be a weak 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, 2020) and a reference dose of 3.33 mg/kg/day.

Additional References: None.

Literature Search and Risk Assessment Completed On: 03/31/20.

Table 1
Data Summary for hex-3-enyl acetate and *cis*-3-hexen-1-yl acetate as read-across material for *cis*-3-hexenyl isovalerate.

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 study) 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.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra and *in vivo* phototoxicity study data, *cis*-3-hexenyl isovalerate would not be expected to present a concern for phototoxicity or photoallergenicity.

11.1.5.1. Risk assessment. 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). In a phototoxicity study conducted in rabbits, 25% *cis*-3-hexenyl isovalerate did not cause phototoxicity (Weeks and DeSena, 1978). Based on the *in vivo* phototoxicity study data and the lack of absorbance, *cis*-3-hexenyl isovalerate does not present a concern for phototoxicity. Based on the lack of absorbance, *cis*-3-hexenyl isovalerate does not present a concern for 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: 03/20/20.

11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for *cis*-3-hexenyl isovalerate is below the Cramer Class I TTC value for inhalation exposure local effects.

11.1.6.1. Risk assessment. There are no inhalation data available on *cis*-3-hexenyl isovalerate. Based on the Creme RIFM Model, the inhalation exposure is 0.0095 mg/day. This exposure is 147.4 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: 04/20/20.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of *cis*-3-hexenyl isovalerate was performed following the RIFM Environmental Framework (Salvito et al., 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log K_{OW}, and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity, as discussed in Salvito et al. (2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 2012b), which provides chemical class-specific ecotoxicity estimates. Finally, if necessary, Tier 3 is conducted using measured biodegradation and ecotoxicity data to refine the RQ, thus allowing for lower PNEC uncertainty factors. The data for calculating the PEC and PNEC for this safety assessment are provided in the table below. For the

PEC, the range from the most recent IFRA Volume of Use Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, *cis*-3-hexenyl isovalerate 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 *cis*-3-hexenyl isovalerate as possibly persistent or bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11).

11.2.2. Risk assessment

Based on the current Volume of Use (2015), *cis*-3-hexenyl isovalerate presents no risk to the aquatic compartment in the screening-level assessment.

11.2.3. Key studies

11.2.3.1. Biodegradation. No data available.

11.2.3.2. Ecotoxicity. No data available.

11.2.4. Other available data

cis-3-Hexenyl isovalerate has been pre-registered for REACH, with no additional information available at this time.

11.2.5. 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	4.01	4.01
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	<1	<1
Risk Characterization: PEC/PNEC	**<1**	**<1**

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

The RIFM PNEC is 0.00443 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 volumes of use.

Literature Search and Risk Assessment Completed On: 03/17/20.

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
- **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

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	4.43			1000000	0.00443	

links listed above were active as of 09/30/20.

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.2021.112533.

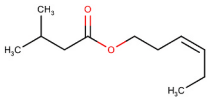
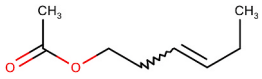
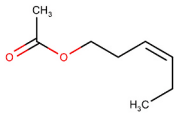
Appendix

Read-across Justification

Methods

The read-across analogs were identified following the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015). The strategy is also consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemicals 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, 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).
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018), 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, 2018).

	Target Material	Read-across	Read-across
Principal Name	*cis*-3-Hexenyl isovalerate	Hex-3-enyl acetate	*cis*-3-Hexen-1-yl acetate
CAS No.	35154-45-1	1708-82-3	3681-71-8
Structure			
Similarity (Tanimoto Score)		0.72	0.72
Read-across Endpoint		• Skin sensitization	• Repeated Dose • Reproductive toxicity • Genotoxicity • Skin sensitization
Molecular Formula	$C_{11}H_{20}O_2$	$C_8H_{14}O_2$	$C_8H_{14}O_2$
Molecular Weight	184.27000000	142.19000000	142.19000000
Melting Point (°C, EPI Suite)	−9.97000	−33.28000	−33.28000
Boiling Point (°C, EPI Suite)	224.17000	176.55000	176.55000
Vapor Pressure (Pa @ 25°C, EPI Suite)	14.13213	151.98708	151.98708
Log K_{ow} (KOWWIN v1.68 in EPI Suite)	4.01000	2.61000	2.61000
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	19.61000	480.50000	480.50000
Jmax (mcg/cm^2/h, SAM)	2.37	30.25	30.25
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	150.97425	64.44270	64.44270
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

(*continued on next page*)

(*continued*)

	Target Material	Read-across	Read-across
			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 identified.	• No skin sensitization reactivity domain alerts identified.	• No skin sensitization reactivity domain alerts identified.
Metabolism			
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	See Supplemental Data 1	See Supplemental Data 2	See Supplemental Data 3

Summary

There are insufficient toxicity data on *cis*-3-hexenyl isovalerate (CAS # 35154-45-1). 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, hex-3-enyl acetate (CAS # 1708-82-3) and *cis*-3-hexen-1-yl acetate (CAS # 3681-71-8) were identified as read-across analogs with sufficient data for toxicological evaluation.

Conclusions

- Hex-3-enyl acetate (CAS # 1708-82-3) and *cis*-3-hexen-1-yl acetate (CAS # 3681-71-8) were used as a read-across analog for the target material *cis*-3-hexenyl isovalerate (CAS # 35154-45-1) for the skin sensitization endpoint.
  - o The target material and the read-across analogs are structurally similar and belong to a class of aliphatic esters. They are isomers of each other.
  - o The target material and the read-across analogs share a 3-hexenyl fragment.
  - o The key difference between the target material and the read-across analog is that the target material has a branched acid, whereas the read-across analog has a straight-chain acid position. This structural difference is toxicologically insignificant.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - o According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - o *In silico* alerts for the target material and the read-across analog are consistent with 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.
- *cis*-3-Hexen-1-yl acetate (CAS # 3681-71-8) was used as a read-across analog for the target material *cis*-3-hexenyl isovalerate (CAS # 35154-45-1) for the genotoxicity, repeated dose toxicity, reproductive toxicity, and skin sensitization endpoints.
  - o The target material and the read-across analog are structurally similar and belong to a class of aliphatic esters.
  - o The target material and the read-across analog share a 3-hexenyl fragment.
  - o The key difference between the target material and the read-across analog is that the target material has a branched acid, whereas the read-across analog has a straight-chain acid position. This structural difference is toxicologically insignificant.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.

- The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
- According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
- *In silico* alerts for the target material and the read-across analog are consistent with 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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