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Short Review

RIFM fragrance ingredient safety assessment, *cis*-3-hexenyl *cis*-3-hexenoate, CAS Registry Number 61444-38-0

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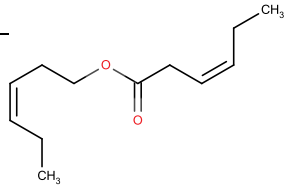
Version: 042919. This version replaces any previous versions.

Name: *cis*-3-Hexenyl *cis*-3-hexenoate
CAS Registry Number: 61444-38-0



Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
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
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency

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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
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 *cis*-3-hexenoate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analogs *cis*-3-hexenol (CAS # 928-96-1) and *trans*-2-hexenoic acid (CAS # 13419-69-7) show that *cis*-3-hexenyl *cis*-3-hexenoate is not expected to be genotoxic. The repeated dose, reproductive, and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class I material, and the exposure to *cis*-3-hexenyl *cis*-3-hexenoate is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). The skin sensitization endpoint was completed using the DST for non-reactive materials (900 μg/cm^2); exposure is below the DST. The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; *cis*-3-hexenyl *cis*-3-hexenoate is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; *cis*-3-hexenyl *cis*-3-hexenoate 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.

Human Health Safety Assessment

Genotoxicity: Not expected to be genotoxic. (Givaudan, 2000; RIFM, 2014a; RIFM, 2016)
Repeated Dose Toxicity: No NOAEL available. Exposure is below the TTC.
Reproductive Toxicity: No NOAEL available. Exposure is below the TTC.
Skin Sensitization: The exposure is below the DST.
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic. (UV Spectra, RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:
Persistence:
Critical Measured Value: 74% (OECD 301 F) RIFM (2012)
Bioaccumulation:
Screening-level: 351.3 L/kg (EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:
Screening-level: 96-h algae EC50: 0.517 mg/L (ECOSAR; US EPA, 2012b)
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: 0.517 mg/L (ECOSAR; US EPA, 2012b)
RIFM PNEC is: 0.0517 μg/L

- Revised PEC/PNECs (2015 IFRA VoU): North America and Europe: < 1

1. Identification

1. **Chemical Name:** *cis*-3-Hexenyl *cis*-3-hexenoate
2. **CAS Registry Number:** 61444-38-0
3. **Synonyms:** 3-Hexenoic acid, 3-hexenyl ester, (Z,Z)-; (Z)-3-Hexenyl (Z)-3-hexenoate; Hex-3-en-1-yl hex-3-enoate; Williams ester; *cis*-3-Hexenyl *cis*-3-hexenoate
4. **Molecular Formula:** $C_{12}H_{20}O_2$
5. **Molecular Weight:** 196.29
6. **RIFM Number:** 5015
7. **Stereochemistry:** *Cis*, *cis* isomer specified. Two geometric centers and 4 isomers possible.

2. Physical data

1. **Boiling Point:** 510 ± 2 K (237 ± 2 °C) at 97.2 kPa (RIFM, 2013b), 112 °C @ 12 mm Hg (FMA Database), 258.41 °C (EPI Suite)
2. **Flash Point:** 111 +/- 2 °C (RIFM, 2013b), 110 °C (GHS), 230 °F; CC (FMA Database)
3. **Log K_{OW}:** 1.02 × 10(4), Log_{10} Pow 4.01 (RIFM, 2013b), Log Pow = 4.1 (RIFM, 2013a), 4.36 (EPI Suite)
4. **Melting Point**: 10.62 °C (EPI Suite)
5. **Water Solubility:** < 30.78 mg/L of solution at 20 +/- 0.5 °C (RIFM, 2013b), 6.07 × 10(-3) g/L of solution in high purity water (RIFM, 2013c), 7.91 × 10(-3) g/L of sol. in Reconst. *Daphnia* med. (RIFM, 2013c), 8.588 mg/L (EPI Suite)
6. **Specific Gravity:** 0.90 (FMA Database)
7. **Vapor Pressure:** 0.039 hPa at 25 °C or 3.9 Pa at 25 °C (RIFM, 2013b), 0.0108 mm Hg @ 20 °C (EPI Suite v4.0), 0.01 mm Hg 20 °C (FMA Database), 0.0173 mm Hg @ 25 °C (EPI Suite)
8. **UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L $mol^{-1} \cdot cm^{-1}$)
9. **Appearance/Organoleptic:** Not Available

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 Hydroalcoholics:** 0.037% (RIFM, 2015)
3. **Inhalation Exposure*:** 0.00017 mg/kg/day or 0.012 mg/day (RIFM, 2015)
4. **Total Systemic Exposure**:** 0.0012 mg/kg/day (RIFM, 2015)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM Aggregate Exposure Model (Comiskey et al., 2015; Safford et al., 2015a; 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., 2015a; 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 v 2.6	OECD QSAR Toolbox v 3.2
I	I	I

2. Analogs Selected:
   a. **Genotoxicity:** *cis*-3-hexenol (CAS # 928-96-1) and *trans*-2-hexenoic acid (CAS # 13419-69-7)
   b. **Repeated Dose Toxicity:** None
   c. **Reproductive Toxicity:** None
   d. **Skin Sensitization:** None
   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 (discrete chemical) or composition (NCS)

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

Mentha oils.

*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 04/01/19.

10. Conclusion

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

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

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

11.1.1.1. Risk assessment. The mutagenic activity of *cis*-3-hexenyl *cis*-3-hexenoate 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/preincubation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102 were treated with *cis*-3-hexenyl *cis*-3-hexenoate 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 (Givaudan, 2000). Under the conditions of the study, *cis*-3-hexenyl *cis*-3-hexenoate was not mutagenic in the Ames test.

There are no data assessing the clastogenic activity of *cis*-3-hexenyl *cis*-3-hexenoate; however, read-across can be made to hydrolysis products of the target ester *cis*-3-hexenol (CAS # 928-96-1) and *trans*-2-hexenoic acid (CAS # 13419-69-7) (see Section VI). The clastogenic activity of *cis*-3-hexenol was evaluated in an *in vitro* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes were treated with *cis*-3-hexenol in DMSO at concentrations up to 1002 μg/mL in the presence and absence of metabolic activation for 3 and 24 h *cis*-3-Hexenol did not induce binucleated cells with micronuclei when tested up to the maximum dose in either non-activated or S9-activated test systems (RIFM, 2014a). Under the conditions of the study, *cis*-3-hexenol was considered to be non-clastogenic in the *in vitro* micronucleus test, and this can be extended to *cis*-3-hexenyl *cis*-3-hexenoate.

The clastogenic activity of *trans*-2-hexenoic acid was evaluated in an *in vitro* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes were treated with *trans*-2-hexenoic acid in DMSO at concentrations up to 1140 μg/mL in the presence and absence of metabolic activation (S9) for 4 h and in the absence of metabolic activation for 24 h *trans*-2-Hexenoic acid did not induce binucleated cells with micronuclei when tested up to the maximum concentration in either the presence or absence of an S9 activation system (RIFM, 2016). Under the conditions of the study, *trans*-2-hexenoic acid was considered to be non-clastogenic in the *in vitro* micronucleus test, and this can be extended to *cis*-3-hexenyl *cis*-3-hexenoate.

Based on the data available, *cis*-3-hexenyl *cis*-3-hexenoate does not present a concern for genotoxic potential.

Additional References: RIFM, 2010a; RIFM, 2010b.

Literature Search and Risk Assessment Completed On: 04/18/19.

11.1.2. Repeated dose toxicity

There are no repeated dose toxicity data on *cis*-3-hexenyl *cis*-3-hexenoate or any read-across materials. The total systemic exposure to *cis*-3-hexenyl *cis*-3-hexenoate is below the TTC for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

11.1.2.1. Risk assessment. There are no repeated dose toxicity data on *cis*-3-hexenyl *cis*-3-hexenoate or on any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to *cis*-3-hexenyl *cis*-3-hexenoate (1.2 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/04/19.

11.1.3. Reproductive toxicity

There are no reproductive toxicity data on *cis*-3-hexenyl *cis*-3-hexenoate or on any read-across materials. The total systemic exposure to *cis*-3-hexenyl *cis*-3-hexenoate is below the TTC for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

11.1.3.1. Risk assessment. There are no reproductive toxicity data on *cis*-3-hexenyl *cis*-3-hexenoate or on any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to *cis*-3-hexenyl *cis*-3-hexenoate (1.2 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: 03/27/19.

11.1.4. Skin sensitization

Based on the existing data and the application of DST, *cis*-3-hexenyl *cis*-3-hexenoate does not present a concern for skin sensitization.

11.1.4.1. Risk assessment. The chemical structure of this material indicates that it would not be expected to react with skin proteins (Roberts et al., 2007; Toxtree 3.1.0; OECD Toolbox v4.2). In a guinea pig maximization study, no reactions indicative of skin sensitization were observed in response to *cis*-3-hexenyl *cis*-3-hexenoate. However, an insufficient number of animals were used in this study (RIFM, 1999). In a human repeated insult patch test (HRIPT) with an unspecified concentration of *cis*-3-hexenyl *cis*-3-hexenoate, none of the 49 human volunteers exhibited reactions indicative of skin sensitization. Acting conservatively, due to the insufficient data, the reported exposure was benchmarked utilizing the non-reactive DST of 900 μg/cm^2 (Safford, 2008; Safford et al., 2011; Roberts et al., 2015; Safford et al., 2015b). The current exposure from the 95th percentile concentration is below the DST for non-reactive materials when evaluated in all QRA categories. Table 1 provides the maximum acceptable concentrations for *cis*-3-hexenyl *cis*-3-hexenoate that present no appreciable risk for skin sensitization based on the non-reactive DST. These levels represent maximum acceptable concentrations based on the DST approach. However, additional studies may show it could be used at higher levels.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/12/19.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, *cis*-3-hexenyl *cis*-3-hexenoate 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 *cis*-3-hexenoate 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 et al., 2009). Based on the lack of absorbance, *cis*-3-Hexenyl *cis*-3-hexenoate 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: 04/03/19.

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 *cis*-3-hexenoate 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 *cis*-3-hexenoate. Based on the Creme RIFM Model, the inhalation exposure is 0.012 mg/day. This exposure is 116.7 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: 04/04/19.

Table 1
Maximum acceptable concentrations for *cis*-3-hexenyl *cis*-3-hexenoate that present no appreciable risk for skin sensitization based on non-reactive DST.

IFRA Category[a]	Description of Product Type	Maximum Acceptable Concentrations in Finished Products Based on Non-reactive DST	Reported 95th Percentile Use Concentrations in Finished Products
1	Products applied to the lips	0.069%	1.0×10^{-5}%
2	Products applied to the axillae	0.021%	0.012%
3	Products applied to the face using fingertips	0.41%	0.0013%
4	Fine fragrance products	0.39%	0.069%
5	Products applied to the face and body using the hands (palms), primarily leave-on	0.10%	0.016%
6	Products with oral and lip exposure	0.23%	NRU[b]
7	Products applied to the hair with some hand contact	0.79%	0.0017%
8	Products with significant ano-genital exposure	0.041%	No Data[c]
9	Products with body and hand exposure, primarily rinse-off	0.75%	0.0063%
10	Household care products with mostly hand contact	2.7%	0.015%
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate	1.5%	No Data[c]
12	Products not intended for direct skin contact, minimal or insignificant transfer to skin	Not Restricted	1.0%

Note:
[a] For a description of the categories, refer to the IFRA/RIFM Information Booklet.
[b] No reported use.
[c] Fragrance exposure from these products is very low. These products are not currently in the Creme RIFM Aggregate Exposure Model.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of *cis*-3-hexenyl *cis*-3-hexenoate 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 *cis*-3-hexenoate was identified as a fragrance material with the potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC > 1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) did not identify *cis*-3-hexenyl *cis*-3-hexenoate as possibly persistent or bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). Data on biodegradation, fate, 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 *cis*-3-hexenoate 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, 2012**:** The ready biodegradability of the test material was determined by the manometric respirometry test according to the OECD 301 F method. Under the test conditions, biodegradation of 74% was observed within 28 days (76% after 35 days).

11.2.1.2.2. Ecotoxicity. RIFM, 2014b**:** The *Daphnia magna* acute immobilization test was performed under semi-static conditions according to the OECD 202 method. The 48-h EC50 and NOEC values based on time-weighted mean measured concentrations were reported to be 6.2 mg/L (95% CI: 5.8–6.4 mg/L) and 2.2 mg/L respectively.

RIFM, 2014c**:** The algae growth inhibition test was performed under static conditions according to the OECD 201 method. The 72-h EC50 and NOEC values based on time-weighted mean measured concentrations were reported to be > 1.8 mg/L (95% CI: 23.8 mg/L) and 0.33 mg/L, respectively.

11.2.1.2.3. Other available data. *cis*-3-Hexenyl *cis*-3-hexenoate has been registered for REACH, with no additional data available at this time.

11.2.2. 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.

	LC_{50} (Fish) (mg/L)	EC_{50} (*Daphnia*) (mg/L)	EC_{50} (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	3.94			1000000	0.00394	
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	1.094	1.766	0.517	10000	0.0517	Esters
ECOSAR Acute Endpoints **(Tier 2)** ***Ver 1.11***	1.217	0.860	1.578			Neutral Organic SAR (Baseline Toxicity)

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	4.1	4.1
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	1–10	1–10
Risk Characterization: PEC/PNEC	**< 1**	**< 1**

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

The RIFM PNEC is 0.0517 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 volumes of use.

Literature Search and Risk Assessment Completed On: 02/21/19.

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
- **TOXNET:** 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 09/30/19.

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.2020.111176.

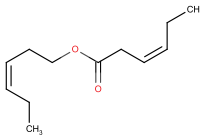
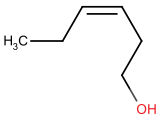
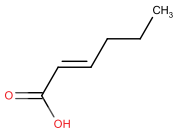
Appendix

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, 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 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 Material	Read-across Material
Principal Name	*cis*-3-Hexenyl *cis*-3-hexenoate	*cis*-3-Hexenol	*trans*-2-Hexenoic acid
CAS No.	61444-38-0	928-96-1	13419-69-7
Structure			
Similarity (Tanimoto Score)		0.33	0.32
Read-across Endpoint		• Genotoxicity	• Genotoxicity
Molecular Formula	$C_{12}H_{20}O_2$	$C_6H_{12}O$	$C_6H_{10}O_2$
Molecular Weight	196.29	100.16	114.14
Melting Point (°C, EPI Suite)	10.62	−38.47	36.5
Boiling Point (°C, EPI Suite)	258.41	165.73	216.5
'Vapor Pressure (Pa @ 25 °C, EPI Suite)	2.31	125	15.3
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	4.36	1.61	1.84
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPI Suite)	8.588	1.6e+004	7069
J_{max} (μg/cm^2/h, SAM)	8.184	446.293	696.627
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.76E+002	1.57E+000	8.08E-002
Genotoxicity			
DNA Binding (OASIS v1.4, QSAR Toolbox v4.2)	• No alert found	• No alert found	• No alert found
DNA Binding (OECD QSAR Toolbox v4.2)	• No alert found	• No alert found	• No alert found
Carcinogenicity (ISS)	• Non-Carcinogen (low reliability)	• Non-Carcinogen (low reliability)	• Non-Carcinogen (low reliability)
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
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 *cis*-3-hexenoate (CAS # 61444-38-0). 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, read-across *cis*-3-hexenol (CAS # 928-96-1) and *trans*-2-hexenoic acid (CAS # 13419-69-7) were identified as read-across analogs with sufficient data for toxicological evaluation.

Conclusions

- Read-across alcohol *cis*-3-hexenol (CAS # 928-96-1) and read-across acid *trans*-2-hexenoic acid (CAS # 13419-69-7) were used as read-across analogs for the target ester *cis*-3-hexenyl *cis*-3-hexenoate (CAS # 61444-38-0) for the genotoxicity endpoint.
  - The resulting alcohol, as well as a geometric isomer of the acid from ester hydrolysis, are used as read-across analogs for the target ester for the endpoints indicated in the table.
  - The read-across materials are major metabolites or analogs of the target.
  - Structural differences between the target material and the read-across analogs are mitigated by the fact that the target could be metabolically hydrolyzed to the read-across analogs. Therefore, the toxicity profile of the target is expected to be similar to that of its metabolites.
  - The target material and the read-across analog have similar physical–chemical properties. Any differences in the physical–chemical properties of the target material and the read-across analogs are toxicologically insignificant.
  - According to the QSAR OECD Toolbox v4.2, structural alerts for the endpoints evaluated are consistent between the target material and the read-across analog.
  - 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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