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RIFM fragrance ingredient safety assessment, *cis*-3-hexenyl butyrate, CAS Registry Number 16491-36-4

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A R T I C L E I N F O

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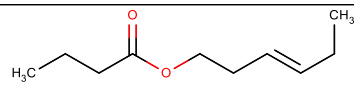

Name: *cis*-3-Hexenyl butyrate
CAS Registry Number: 16491-36-4

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
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., 2017; Safford et al., 2015a; Safford et al., 2017; Comiskey et al., 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DRF - Dose Range Finding
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observed Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
Perfumery - In this safety assessment, perfumery refers to fragrances made by a perfumer used in consumer products only. The exposures reported in the safety assessment include consumer product use but do not include occupational exposures.
QRA - Quantitative Risk Assessment
QSAR - Quantitative Structure-Activity Relationship
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use
vPvB - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

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

This safety assessment is based on the RIFM Criteria Document (Api et al., 2015), which should be referred to for clarifications.

Each endpoint discussed in this safety assessment includes the relevant data that were available at the time of writing (version number in the top box is indicative of the date of approval based on a 2-digit month/day/year), both in the RIFM Database (consisting of publicly available and proprietary data) and through publicly available information sources (e.g., SciFinder and PubMed). Studies selected for this safety assessment were based on appropriate test criteria, such as acceptable guidelines, sample size, study duration, route of exposure, relevant animal species, most relevant testing endpoints, etc. A key study for each endpoint was selected based on the most conservative endpoint value (e.g., PNEC, NOAEL, LOEL, and NESIL).

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*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 butyrate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analog hex-3-enyl acetate (CAS # 1708-82-3) show that *cis*-3-hexenyl butyrate is not expected to be genotoxic. Data on read-across analog *cis*-3-hexen-1-yl acetate (CAS # 3681-71-8) provide a calculated MOE >100 for the repeated dose toxicity and reproductive toxicity endpoints. Data from read-across analog 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 *cis*-3-hexenyl butyrate a NESIL of 1000 μg/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on UV/Vis spectra; *cis*-3-hexenyl butyrate is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was evaluated using the TTC for a Cramer Class I material, and the exposure to *cis*-3-hexenyl butyrate is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; *cis*-3-hexenyl butyrate 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.	(RIFM, 2016a; RIFM, 2014)
Repeated Dose Toxicity: NOAEL = 333 mg/kg/day.	(ECHA REACH Dossier: (Z)-Hex-3-enyl acetate; ECHA, 2013)
Reproductive Toxicity: NOAEL = 1000 mg/kg/day.	(ECHA REACH Dossier: (Z)-Hex-3-enyl acetate; ECHA, 2013)
Skin Sensitization: NESIL = 1000 μg/cm^2.	RIFM (2018)
Phototoxicity/ Photoallergenicity: Not expected to be phototoxic/ photoallergenic.	(UV/Vis Spectra; RIFM Database)

Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:

Persistence:	
Critical Measured Value: 77% (OECD 301F)	RIFM (2011)
Bioaccumulation:	
Screening-level: 109.5 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: 96-h Algae EC50: 1.542 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: 1.542 mg/L	(ECOSAR; US EPA, 2012b)

RIFM PNEC is: 0.1542 μg/L

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

1. Identification

1. **Chemical Name:** *cis*-3-Hexenyl butyrate
2. **CAS Registry Number:** 16491-36-4
3. **Synonyms:** Butanoic acid, 3-hexenyl ester, (Z)-; *cis*-3-Hexenyl butanoate; (Z)-Hex-3-enyl butyrate; Butyric acid, 3-hexenyl ester, (Z)-; *cis*-3-Hexen-1-yl butyrate; *cis*-Butyric acid, 3-hexenyl ester; ｱﾙｶﾝ

酸(C = 1–16)ｱﾙｹﾆﾙ(C = 4–8); Hex-3-en-1-yl butyrate; *cis*-3-Hexenyl butyrate

4. **Molecular Formula:** $C_{10}H_{18}O_2$
5. **Molecular Weight:** 170.25
6. **RIFM Number**: 11
7. **Stereochemistry:** *Cis* isomer specified.

2. Physical data

1. **Boiling Point:** 103 °C at 25 mm Hg (Fragrance Materials Association [FMA]), 192 °C at 760 mm Hg (FEMA [SLR]), 216.64 °C (EPI Suite)
2. **Flash Point:** 77 °C (Globally Harmonized System), 170 °F; CC (FMA)
3. **Log K_{OW}:** 3.6 (EPI Suite)
4. **Melting Point**: 10.31 °C (EPI Suite)
5. **Water Solubility:** 52.1 mg/L (EPI Suite)
6. **Specific Gravity:** 0.89 (FMA), (H_2O = 1) 0.90 (FEMA [SLR])
7. **Vapor Pressure:** 0.103 mm Hg at 20 °C (EPI Suite v4.0), 0.5 mm Hg at 20 °C (FMA), 0.156 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 (1969): Colorless liquid with powerful fruity-winey, green cognac-like or brandy-like, slightly buttery-oily odor. Winey-buttery, sweet-green, and slightly fruity taste in dilutions below 5 ppm.

3. Volume of use (worldwide band)

1. 1–10 metric tons per year (IFRA, 2015)

4. Exposure to fragrance ingredient (Creme RIFM Aggregate Exposure Model v1.0)

1. 95th **Percentile Concentration in Hydroalcoholics:** 0.0042% (RIFM, 2017a)
2. **Inhalation Exposure*:** 0.000059 mg/kg/day or 0.0044 mg/day (RIFM, 2017a)
3. **Total Systemic Exposure**:** 0.00030 mg/kg/day (RIFM, 2017a)

*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM Aggregate Exposure Model (RIFM, 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 (RIFM, 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:** Hex-3-enyl acetate (CAS # 1708-82-3)
   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-hexenyl 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.

7.1. Additional references

None.

8. Natural occurrence

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

Mangifera species	Apricot (*Prunus armeniaca* L.)
Passion Fruit (*Passiflora* species)	Chinese Quince (*Pseudocydonia sinensis* Schneid)
Plum (*Prunus* species)	Citrus fruits
Guava and Feyoa	Mastic (*Pistacia lentiscus*)
Strawberry (*Fragaria* species)	Sea Buckthorn (*Hippophaë rhamnoides* L.)

*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. This is a partial list.

9. REACH dossier

Pre-registered for 2010; no dossier available as of 11/01/21.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for *cis*-3-hexenyl butyrate 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

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(*continued*)

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
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
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 butyrate, 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; December 2019).
[c]Calculations by Creme RIFM Aggregate Exposure Model v3.1.3.

11. Summary

11.1. Human Health Endpoint Summaries

11.1.1. Genotoxicity

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

11.1.1.1. Risk assessment. *cis*-3-Hexenyl butyrate was assessed in the BlueScreen assay and found negative for both cytotoxicity and genotoxicity, with and without metabolic activation (RIFM, 2013). There are no studies assessing the mutagenic activity of *cis*-3-hexenyl butyrate. However, read-across can be made to hex-3-enyl acetate (CAS # 1708-82-3; see Section VI). The mutagenic activity of hex-3-enyl 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/preincubation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with hex-3-enyl 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 dose in the presence or absence of S9 (RIFM, 2016a). Under the conditions of the study, hex-3-enyl acetate was not mutagenic in the Ames test, and this can be extended to *cis*-3-hexenyl butyrate.

The clastogenic activity of *cis*-3-hexenyl butyrate 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-hexenyl butyrate in DMSO at concentrations up to 1703 μg/mL in the presence and absence of metabolic activation (S9) for 4 and 24 h *cis*-3-Hexenyl butyrate did not induce binucleated cells with micronuclei when tested up to cytotoxic levels in either non-activated or S9-activated test systems (RIFM, 2014). Under the conditions of the study, *cis*-3-hexenyl butyrate was considered to be non-clastogenic in the *in vitro* micronucleus test.

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

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

11.1.2.1. Risk assessment. There are insufficient repeated dose toxicity data on *cis*-3-hexenyl butyrate for the repeated dose toxicity endpoint. Read-across material *cis*-3-hexenyl acetate (CAS # 3681-71-8; see Section VI) has an OECD 422/GLP oral gavage combined repeated dose toxicity study with reproduction/developmental screening test conducted in Wistar rats. Groups of 11 rats/sex/dose were administered via gavage with test material *cis*-3-hexenyl acetate 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. There were no dose-responsive, treatment-related adverse effects observed on body weight, hematological and clinical chemistry parameters, and organ weights. Macroscopic and microscopic findings were not attributed to treatment and were within the historical control range among animals of this strain and age. Thus, the NOAEL was considered to be 1000 mg/kg/day, the highest dose tested (ECHA, 2013).

A default safety factor of 3 was used when deriving a NOAEL from an OECD 422 study (ECHA, 2012). The safety factor has been approved by the Expert Panel for Fragrance Safety*.

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

Therefore, the *cis*-3-hexenyl butyrate 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 butyrate, 333/0.0003, or 1110000.

In addition, the total systemic exposure to *cis*-3-hexenyl butyrate (0.3 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, 2020b) 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 butyrate 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 *cis*-3-hexenyl butyrate is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.3.1. Risk assessment. There are insufficient reproductive toxicity data on *cis*-3-hexenyl butyrate for the reproductive toxicity endpoint. Read-across material *cis*-3-hexenyl acetate (CAS # 3681-71-8; see Section VI) has an OECD 422/GLP oral gavage combined repeated dose toxicity study with reproduction/developmental screening test conducted in Wistar rats. Groups of 11 rats/sex/dose were administered via gavage with test material *cis*-3-hexenyl acetate 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 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 the first litter check or on day 4 postpartum. Thus, the NOAEL for maternal toxicity, fertility, and developmental toxicity was considered to be 1000 mg/kg/day, the highest dose tested (ECHA, 2013). **Therefore, the *cis*-3-hexenyl butyrate MOE for the developmental toxicity and fertility endpoint can be calculated by dividing the *cis*-3-hexenyl acetate NOAEL in mg/kg/day by the total systemic exposure to *cis*-3-hexenyl butyrate, 1000/0.0003, or 3333333.**

In addition, the total systemic exposure to *cis*-3-hexenyl butyrate (0.3 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012**) for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.**

Additional references: None.

Literature search and risk assessment completed on: 08/16/20.

11.1.4. Skin sensitization

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

11.1.4.1. Risk assessment. Insufficient skin sensitization studies are available for *cis*-3-hexenyl butyrate. Based on the existing data and read-across material hex-3-enyl acetate (CAS # 1708-82-3; see Section VI) and its additional materials (isomers) *trans*-3-hexenyl acetate and *cis*-3-hexenyl acetate (CAS # 3681-82-1 and CAS # 3681-71-8; see Section VI), *cis*-3-hexenyl butyrate is a skin sensitizer. The chemical structure of these materials indicates that they would not be expected to react with skin proteins (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). The 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, 2017b; RIFM, 2016b). In a murine local lymph node assay (LLNA), read-across material hex-3-enyl acetate was found to be negative up to 100% (RIFM, 2016c). In a guinea pig maximization test, read-across material *cis*-3-hexen-1-yl acetate led to skin sensitization reactions (RIFM, 1996; RIFM, 1997). In a human maximization test, no skin sensitization reactions were observed with additional read-across material *cis*-3-hexen-1-yl acetate (RIFM, 1974). In a human maximization test, no skin sensitization reactions were observed with the target material, 10% *cis*-3-hexenyl butyrate (RIFM, 1978). Additionally, in a Confirmation of No Induction in Humans test (CNIH) with 1102 μg/cm^2 of additional read-across material *cis*-3-hexen-1-yl acetate in 1:3 ethanol:diethyl phthalate (EtOH:DEP), a reaction indicative of sensitization was observed in 1 of the 104 volunteers (RIFM, 2012). However, in 2 separate CNIHs with 969 μg/cm^2 and 1003 μg/cm^2 of additional read-across material *cis*-3-hexen-1-yl acetate in EtOH and 1:3 EtOH:DEP, respectively, no reactions indicative of sensitization were observed in any of the 38 or 110 volunteers, respectively (RIFM, 1965; RIFM, 2018).

Based on weight of evidence (WoE) from structural analysis, human studies, and data on the read-across material hex-3-enyl acetate *cis*-3-hexenyl butyrate is a sensitizer with a Weight of Evidence No Expected Sensitization Induction Level (WoE NESIL) of 1000 μg/cm^2 (see Table 1). Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020b) and a reference dose of 3.33 mg/kg/day.

Additional references: None.

Literature search and risk assessment completed on: 08/01/20.

Table 1
Data summary for hex-3-enyl acetate as read-across material for *cis*-3-hexenyl butyrate.

LLNA Weighted Mean EC3 Value μg/cm^2 [No. Studies]	Potency Classification Based on Animal Data[1]	Human Data			
		NOEL-CNIH (Induction) μg/cm^2	NOEL-HMT (Induction) μg/cm^2	LOEL[2] (Induction) μg/cm^2	WoE NESIL[3] 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.
[1] Based on animal data (guinea pig maximization test) using classification defined in ECETOC, Technical Report No. 87, 2003.
[2] Data derived from CNIH or HMT.
[3] WoE NESIL limited to 2 significant figures.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, *cis*-3-Hexenyl butyrate 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 butyrate 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 butyrate does not present a concern for phototoxicity or photoallergenicity.

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate no significant absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark of concern for phototoxic effects, 1000 L mol^{-1} • cm^{-1} (Henry et al., 2009).

Additional references: None.

Literature search and risk assessment completed on: 08/05/20.

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 butyrate is below the Cramer Class I TTC value for inhalation exposure local effects.

11.1.6.1. Risk assessment. There is insufficient inhalation data available on *cis*-3-hexenyl butyrate. Based on the Creme RIFM Model, the inhalation exposure is 0.0044 mg/day. This exposure is 318 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: Helmig et al., 1999a; Helmig et al., 1999b.

Literature search and risk assessment completed on: 07/29/20.

11.2. Environmental Endpoint Summary

11.2.1. Screening-level assessment

A screening-level risk assessment of *cis*-3-hexenyl butyrate 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 butyrate 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 butyrate 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 persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

11.2.2. Risk assessment

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

11.2.2.1. Key studies

11.2.2.1.1. Biodegradation. RIFM, 2011: The ready biodegradability of the test material was evaluated using the manometric respirometry test according to the OECD 301F method. Under the conditions of the study, biodegradation of 77% was observed after 28 days.

11.2.2.1.2. Ecotoxicity. No data available.

11.2.2.1.3. Other available data. *cis*-3-Hexenyl butyrate has been pre-registered for REACH with no additional data at this time.

11.2.3. Risk assessment refinement

Since *cis*-3-Hexenyl butyrate has passed the screening criteria, measured data is included for completeness only and has not been used in PNEC derivation.

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	North America
Log K_{ow} Used	3.6	3.6
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	1–10	1–10
Risk Characterization: PEC/PNEC	**<1**	**<1**

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

The RIFM PNEC is 0.1542 μg/L. The revised PEC/PNECs for EU and NA are <1; therefore, the material does not present a risk to the aquatic environment at the current reported VoU.

Literature search and risk assessment completed on: 08/18/20.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (µg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	<u>9.312</u>	X	X	1000000	0.009312	X
ECOSAR Acute Endpoints **(Tier 2)** v1.11	2.662	4.665	<u>1.542</u>	10000	0.1542	Esters
ECOSAR Acute Endpoints **(Tier 2)** v1.11	5.158	3.394	4.649			Neutral Organic SAR

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 links listed above were active as of 11/01/21.

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.2021.112758.

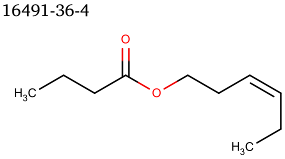
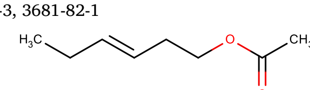
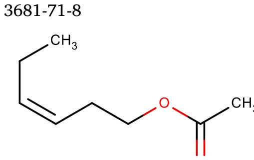
Appendix

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

	Target Material	Read-across Material	Read-across Material
Principal Name	*cis*-3-Hexenyl butyrate	Hex-3-enyl acetate, *trans*-3-hexenyl acetate	*cis*-3-Hexen-1-yl acetate
CAS No.	16491-36-4	1708-82-3, 3681-82-1	3681-71-8
Structure			
Similarity (Tanimoto Score)		0.81	0.81

(*continued on next page*)

(*continued*)

	Target Material	Read-across Material	Read-across Material
Endpoint		• Genotoxicity (CAS # 1708-82-3 only) • Skin sensitization	• Skin sensitization • Repeated dose toxicity • Reproductive toxicity
Molecular Formula	$C_{10}H_{18}O_2$	$C_8H_{14}O_2$	$C_8H_{14}O_2$
Molecular Weight	170.252	142.198	142.198
Melting Point (°C, EPI Suite)	−10.31	−33.28	−33.28
Boiling Point (°C, EPI Suite)	216.64	176.55	176.55
Vapor Pressure (Pa @ 25°C, EPI Suite)	2.08E+01	1.52E+02	1.52E+02
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	5.21E+01	4.81E+02	4.81E+02
Log K_{OW}	3.6	2.61	2.61
J_{max} (μg/cm^2/h, SAM)	5.60	30.25	30.25
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.13E+02	6.44E+01	6.44E+01
Genotoxicity			
DNA Binding (OASIS v1.4, QSAR Toolbox v4.2)	No alert found	AN2|AN2 ≫ 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	
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 domains alerts identified.	No skin sensitization reactivity domains alerts identified.	No skin sensitization reactivity domains 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 butyrate (CAS # 16491-36-4). 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

- Hex-3-enyl acetate (CAS # 1708-82-3) was used as a read-across analog for the target material *cis*-3-hexenyl butyrate (CAS # 16491-36-4) for the genotoxicity endpoint. Hex-3-enyl acetate (CAS # 1708-82-3) and additional material (isomer) *trans*-3-hexenyl acetate (CAS # 3681-82-1) were used as read-across analogs for the target material *cis*-3-hexenyl butyrate (CAS # 16491-36-4), for the skin sensitization endpoint.
  - The target material and the read-across analog are structurally similar and belong to a class of esters.
  - The target material and the read-across analog share a common hexenyl fragment on the alcohol portion of the ester.

- The key difference between the target material and the read-across analog is that the target material has a butyrate fragment on the acid portion, while the read-across analog has an acetate fragment on the acid portion. This structural difference is toxicologically insignificant.
- 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.
- 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 the toxicological endpoints are consistent between the target material and the read-across analog.
- The read-across analog is predicted to have positive DNA binding alerts by the OASIS model for genotoxicity. All the other alerts for genotoxicity were predicted to be negative. According to these predictions, the read-across analog is expected to be more reactive when compared to the target material. Data superseded predictions in this case.
- 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 endpoint 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 butyrate (CAS # 16491-36-4), for the repeated dose toxicity, reproductive toxicity, and skin sensitization endpoints.
  - The target material and the read-across analog are structurally similar and belong to a class of esters.
  - The target material and the read-across analog share a common hexenyl fragment on the alcohol portion of the ester.
  - The key difference between the target material and the read-across analog is that the target material has a butyrate fragment on the acid portion, while the read-across analog has an acetate fragment on the acid portion. This structural difference is toxicologically insignificant.
  - 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.
  - 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.
  - The read-across analog is predicted to be a toxicant by the CAESAR model for developmental toxicity. All the 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 above shows that the read-across analog has an adequate MOE at the current level of use. Therefore, the predictions are superseded by the data.
  - There are no alerts for the skin sensitization endpoint. The *in silico* predictions 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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