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## Food and Chemical Toxicology

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## Short Review

## RIFM fragrance ingredient safety assessment, 2-hexenoic acid, 2-methyl-, methyl ester, (2E)-, CAS Registry Number 16493-96-2



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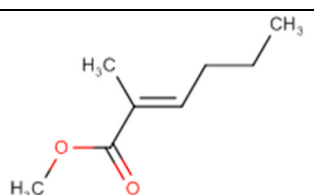
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**2-Box Model** - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration

**AF** - Assessment Factor

**BCF** - Bioconcentration Factor

**CNIH** - Confirmation of No Induction in Humans test. A human repeat insult patch test that is performed to confirm an already determined safe use level for fragrance ingredients (Na et al., 2020)

**Creme RIFM Model** - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic

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estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015a; Safford et al., 2017) compared to a deterministic aggregate approach

**DEREK** - Derek Nexus is an *in silico* tool used to identify structural alerts

**DRF** - Dose Range Finding

**DST** - Dermal Sensitization Threshold

**ECHA** - European Chemicals Agency

**ECOSAR** - Ecological Structure-Activity Relationships Predictive Model

**EU** - Europe/European Union

**GLP** - Good Laboratory Practice

**IFRA** - The International Fragrance Association

**LOEL** - Lowest Observed Effect Level

**MOE** - Margin of Exposure

**MPPD** - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition

**NA** - North America

**NESIL** - No Expected Sensitization Induction Level

**NOAEC** - No Observed Adverse Effect Concentration

**NOAEL** - No Observed Adverse Effect Level

**NOEC** - No Observed Effect Concentration

**NOEL** - No Observed Effect Level

**OECD** - Organisation for Economic Co-operation and Development

**OECD TG** - Organisation for Economic Co-operation and Development Testing Guidelines

**PBT** - Persistent, Bioaccumulative, and Toxic

**PEC/PNEC** - Predicted Environmental Concentration/Predicted No Effect Concentration

**Perfumery** - In this safety assessment, perfumery refers to fragrances made by a perfumer used in consumer products only. The exposures reported in the safety assessment include consumer product use but do not include occupational exposures.

**QRA** - Quantitative Risk Assessment

**QSAR** - Quantitative Structure-Activity Relationship

**REACH** - Registration, Evaluation, Authorisation, and Restriction of Chemicals

**RfD** - Reference Dose

**RIFM** - Research Institute for Fragrance Materials

**RQ** - Risk Quotient

**Statistically Significant** - Statistically significant difference in reported results as compared to controls with a  $p < 0.05$  using appropriate statistical test

**TTC** - Threshold of Toxicological Concern

**UV/Vis spectra** - Ultraviolet/Visible spectra

**VCF** - Volatile Compounds in Food

**VoU** - Volume of Use

**vPvB** - (very) Persistent, (very) Bioaccumulative

**WoE** - Weight of Evidence

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

This safety assessment is based on the RIFM Criteria Document (Api, 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.**

2-Hexenoic acid, 2-methyl-, methyl ester, (2E)- was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- is not genotoxic, provide a calculated Margin of Exposure (MOE)  $> 100$  for the repeated dose toxicity endpoint and a No Expected Sensitization Induction Level (NESIL) of  $1100 \mu\text{g}/\text{cm}^2$  for the skin sensitization endpoint. The reproductive and local respiratory toxicity endpoints were evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material; exposure to 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- is below the TTC (0.03 mg/kg/day and 1.4 mg/day, respectively). The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet (UV) spectra; 2-

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hexenoic acid, 2-methyl-, methyl ester, (2E)- is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- 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  $< 1$ .

**Human Health Safety Assessment**

**Genotoxicity:** Not expected to be genotoxic. (RIFM, 2007c; RIFM, 2010c)

**Repeated Dose Toxicity:** NOAEL = 333 mg/kg/day. RIFM (2010b)

**Reproductive Toxicity:** No NOAEL available. Exposure is below the TTC.

**Skin Sensitization:** NESIL =  $1100 \mu\text{g}/\text{cm}^2$ . RIFM (2010d)

**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: 83% (OECD 310) (RIFM, 2007d)

**Bioaccumulation:** Screening-level: 26.8 L/kg (EPI Suite v4.11; US EPA, 2012a)

**Ecotoxicity:** Screening-level: Fish LC50: 12.58 mg/L (RIFM Framework; Salvito, 2002)

**Conclusion:** Not PBT or vPvB as per IFRA Environmental Standards

**Risk Assessment:**

**Screening-level:** PEC/PNEC (North America and Europe)  $< 1$  (RIFM Framework; Salvito, 2002)

**Critical Ecotoxicity Endpoint:** Fish LC50: 12.58 mg/L (RIFM Framework; Salvito, 2002)

**RIFM PNEC is:** 0.01258  $\mu\text{g}/\text{L}$

• **Revised PEC/PNECs (2015 IFRA VoU):** North America (No VoU) and Europe: Not applicable; cleared at the screening-level

**1. Identification**

- Chemical Name:** 2-Hexenoic acid, 2-methyl-, methyl ester, (2E)-
- CAS Registry Number:** 16493-96-2
- Synonyms:** Davanate; 2-Hexenoic acid, 2-methyl-, methyl ester, (2E)-
- Molecular Formula:** C H O
- Molecular Weight:** 142.19
- RIFM Number:** 7357
- Stereochemistry:** There is no stereoisomer possible.

**2. Physical data**

- Boiling Point:**  $448 \pm 0.5 \text{ K}$  ( $175 \pm 0.5 \text{ }^\circ\text{C}$ ) at 100.97 kPa (RIFM, 2007a)
- Flash Point:**  $63 \pm 2 \text{ }^\circ\text{C}$  (RIFM, 2007a)
- Log K<sub>ow</sub>:** 3.36 (RIFM, 2007b)
- Melting Point:** less than  $253 \pm 0.5 \text{ K}$  (less than  $-20.0 \pm 0.5 \text{ }^\circ\text{C}$ ) (RIFM, 2007a)
- Water Solubility:** 0.486 g/L of solution at  $20.0 \pm 0.5 \text{ }^\circ\text{C}$  (RIFM, 2007a)
- Specific Gravity:** Not Available
- Vapor Pressure:**  $1.7 \times 10^{+1} \text{ Pa}$  at  $25 \text{ }^\circ\text{C}$  (RIFM, 2010a)
- UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark ( $1000 \text{ L mol}^{-1} \cdot \text{cm}^{-1}$ )
- Appearance/Organoleptic:** Not Available

### 3. Volume of use (Worldwide band)

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

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

1. 95th Percentile Concentration in Fine Fragrance: 0.022% (RIFM, 2017)
2. Inhalation Exposure\*: 0.000032 mg/kg/day or 0.0021 mg/day (RIFM, 2017)
3. Total Systemic Exposure\*\*: 0.00028 mg/kg/day (RIFM, 2017)

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

\*\*95th percentile calculated exposure; assumes 100% absorption unless modified by dermal absorption data as reported in Section V. It is derived from concentration survey data in the Creme RIFM Aggregate Exposure Model and includes exposure via dermal, oral, and inhalation routes whenever the fragrance ingredient is used in products that include these routes of exposure (Comiskey, 2015, 2017; Safford, 2015, 2017).

### 5. Derivation of systemic absorption

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

### 6. Computational toxicology evaluation

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

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

#### 2. Analogs Selected:

- a. Genotoxicity: None
  - 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: None

### 7. Metabolism

No relevant data available for inclusion in this safety assessment.

#### 7.1. Additional References

None.

### 8. Natural occurrence

2-Hexenoic acid, 2-methyl-, methyl ester, (2E)- is not reported to occur in foods by the VCF\*.

\*VCF (Volatile Compounds in Food): Database/Nijssen, L.M.; Ingen-Visscher, C.A. van; Donders, J.J.H. (eds). – Version 15.1 – Zeist (The Netherlands): TNO Triskelion, 1963–2014. A continually updated database containing information on published volatile compounds that have been found in natural (processed) food products. Includes FEMA

GRAS and EU-Flavis data.

### 9. REACH dossier

Not pre-registered; no dossier available as of 10/09/20.

### 10. Conclusion

The maximum acceptable concentrations<sup>a</sup> in finished products for 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- are detailed below.

IFRA Category <sup>b</sup>	Description of Product Type	Maximum Acceptable Concentrations <sup>a</sup> in Finished Products (%) <sup>c</sup>
1	Products applied to the lips (lipstick)	0.085
2	Products applied to the axillae	0.025
3	Products applied to the face/body using fingertips	0.51
4	Products related to fine fragrances	0.47
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.12
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.12
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.12
5D	Baby cream, oil, talc	0.040
6	Products with oral and lip exposure	0.28
7	Products applied to the hair with some hand contact	0.96
8	Products with significant anogenital exposure (tampon)	0.040
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.92
10A	Household care products with mostly hand contact (hand dishwashing detergent)	3.3
10B	Aerosol air freshener	3.3
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.040
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	Not restricted

Note: <sup>a</sup>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 2-hexenoic acid, 2-methyl-, methyl ester, (2E)-, the basis was the reference dose of 3.33 mg/kg/day, a predicted skin absorption value of 10%, and a skin sensitization NESIL of 1100 µg/cm<sup>2</sup>.

<sup>b</sup>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>).

<sup>c</sup>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, 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. 2-Hexenoic acid, 2-methyl-, methyl ester, (2E)- 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, 2014). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity

of chemical compounds and mixtures. Additional assays were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

The mutagenic activity of 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- 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 method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- 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 (RIFM, 2007c). Under the conditions of the study, 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- was not mutagenic in the Ames test.

The clastogenicity of 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- 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 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- 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 (RIFM, 2013). Under the conditions of the study, 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the available data, 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- does not present a concern for genotoxic potential.

**Additional References:** RIFM, 1978.

**Literature Search and Risk Assessment Completed On:** 11/03/20.

#### 11.1.2. Repeated dose toxicity

The MOE for 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- is adequate for the repeated dose toxicity endpoint at the current level of use.

**11.1.2.1. Risk assessment.** There are sufficient repeated dose toxicity data on 2-hexenoic acid, 2-methyl-, methyl ester, (2E)-. An OECD 407/GLP repeated dose toxicity study was conducted on Wistar Han:HsdHan:WIST strain rats. Groups of 5 rats/sex/dose were administered via oral gavage test material, 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- at doses of 0 (Arachis Oil BP), 30, 300, or 1000 mg/kg/day for 28 days. There was a statistically significant reduction in bodyweight gain among high-dose males during week 3 of treatment only. In the absence of a convincing effect on the overall bodyweight development, the minimal intergroup difference in isolation was not considered to be of toxicological importance. High-dose animals and mid-dose females showed a statistically significant increase in both the absolute and relative liver weights. No such effects were reported among mid-dose males or low-dose animals. Centrilobular hepatocellular hypertrophy was evident among mid and high-dose animals. The microscopic liver changes together with increased liver weights detected were considered to be an adaptive response to the test material, provided there is a lack of histopathological evidence showing liver cell damage and clinical chemistry alterations (Hall, 2012). Thus, the NOAEL was considered to be 1000 mg/kg/day, the highest dose tested (RIFM, 2010b).

A default safety factor of 3 was used when deriving a NOAEL from an OECD 407 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 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- MOE for the repeated dose toxicity endpoint can be calculated by dividing the 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- NOAEL in mg/kg/day by

the total systemic exposure to 2-hexenoic acid, 2-methyl-, methyl ester, (2E)-, 333/0.00028, or 1189286.

In addition, the total systemic exposure to 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- (0.28 µg/kg bw/day) is below the TTC (30 µg/kg/day; Kroes, 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020) and a reference dose of 3.33 mg/kg/day.

#### Derivation of reference dose (RfD):

Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020) and a reference dose of 3.33 mg/kg/day.

The RIFM Criteria Document (Api, 2015) calls for a default MOE of 100 (10 × 10), based on uncertainty factors applied for interspecies (10 × ) and intraspecies (10 × ) differences. The reference dose for 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- 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:** 10/26/20.

#### 11.1.3. Reproductive toxicity

There are insufficient reproductive toxicity data on 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- or on any read-across materials. The total systemic exposure to 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- 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 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- or on any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure to 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- (0.28 µg/kg/day) is below the TTC (30 µg/kg/day; Kroes, 2007; Laufersweiler, 2012) for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

**Key Studies:** None.

**Additional References:** None.

**Literature Search and Risk Assessment Completed On:** 11/02/20.

#### 11.1.4. Skin sensitization

Based on the existing data, 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- is considered a sensitizer with a defined NESIL of 1100 µg/cm<sup>2</sup>.

**11.1.4.1. Risk assessment.** Based on existing data, 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- is considered a sensitizer with a defined NESIL of 1100 µg/cm<sup>2</sup>. The chemical structure of this material indicates that it would be expected to react with skin proteins directly (Roberts, 2007; Toxtree v3.1.0). In a murine local lymph node assay (LLNA), 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- was found to be sensitizing with an EC3 value of 38.3% (9575 µg/cm<sup>2</sup>) (RIFM, 2007b). Additionally, in a Confirmation of No Induction in Humans test (CNIH) with 1181 µg/cm<sup>2</sup> of 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- in 1:3 ethanol: diethyl phthalate, no reactions indicative of sensitization were observed in any of the 107 volunteers (RIFM, 2010d).



Based on the weight of evidence (WoE) from structural analysis and animal and human studies, 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- is a weak sensitizer with a WoE NESIL of 1100  $\mu\text{g}/\text{cm}^2$  (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, 2020) and a reference dose of 3.33 mg/kg/day.

**Additional References:** None.

**Literature Search and Risk Assessment Completed On:** 10/28/20.

#### 11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- 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 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- in experimental models. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry, 2009). Based on the lack of absorbance, 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- 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  $\text{L mol}^{-1} \cdot \text{cm}^{-1}$  (Henry, 2009).

**Additional References:** None.

**Literature Search and Risk Assessment Completed On:** 11/03/20.

#### 11.1.6. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 2-hexenoic acid, 2-methyl- 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 2-hexenoic acid, 2-methyl-, methyl ester, (2E)-. Based on the Creme RIFM Model, the inhalation exposure is 0.0021 mg/day. This exposure is 667 times lower than the Cramer Class I TTC value of 1.4 mg/day (based on human lung weight of 650 g; Carthew, 2009); therefore, the exposure at the current level of use is deemed safe.

**Key Studies:** None.

**Additional References:** None.

**Literature Search and Risk Assessment Completed On:** 11/05/20.

**Table 1**

Data summary for 2-hexenoic acid, 2-methyl-, methyl ester, (2E)-.

LLNA Weighted Mean EC3 Value $\mu\text{g}/\text{cm}^2$ (No. Studies)	Potency Classification Based on Animal Data <sup>a</sup>	Human Data			WoE NESIL <sup>c</sup>
		NOEL-CNIH (Induction) $\mu\text{g}/\text{cm}^2$	NOEL-HMT (Induction) $\mu\text{g}/\text{cm}^2$	LOEL <sup>b</sup> (Induction) $\mu\text{g}/\text{cm}^2$	
9575	Weak	1181	NA	NA	1100

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.

<sup>a</sup> Based on animal data using classification defined in ECETOC, Technical Report No. 87, 2003.

<sup>b</sup> Data derived from CNIH or HMT.

<sup>c</sup> WoE NESIL limited to 2 significant figures.

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## 11.2. Environmental endpoint summary

### 11.2.1. Screening-level assessment

A screening-level risk assessment of 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- was performed following the RIFM Environmental Framework (Salvito, 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log  $K_{ow}$ , and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity, as discussed in Salvito et al. (2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 2012b), which provides chemical class-specific ecotoxicity estimates. Finally, if necessary, Tier 3 is conducted using measured biodegradation and ecotoxicity data to refine the RQ, thus allowing for lower PNEC uncertainty factors. The data for calculating the PEC and PNEC for this safety assessment are provided in the table below. For the PEC, the range from the most recent IFRA Volume of Use Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- 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 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- as possibly being either persistent or bioaccumulative based on its structure and physical-chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent and bioaccumulative and toxic, or very persistent and very bioaccumulative as defined in the Criteria Document (Api, 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF  $\geq 2000$  L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical-chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). 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), 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- presents a risk to the aquatic compartment in the screening-level assessment.

#### 11.2.2.1. Key Studies. Biodegradation

**RIFM, 2007d:** The ready biodegradability of the test material was evaluated over a period of 28 days using the  $\text{CO}_2$  in sealed vessels (Headspace test) according to the OECD 310 method. Under the conditions of this study, biodegradation of 83% was observed.

**RIFM, 2011d:** The ready biodegradability of the test material was evaluated in a manometric respirometry test according to the OECD 301F method. Under the conditions of this study, biodegradation greater than 50.1% was observed after 28 days.

#### Ecotoxicity

**RIFM, 2011a:** A *Daphnia magna* immobilization test was conducted

according to the OECD 202 method under static conditions in a closed system. The 48-h EC50 value based on the mean measured concentration was determined to be 27 mg/L (95% CI: 22–40 mg/L).

**RIFM, 2011b:** An algae growth inhibition test was conducted according to the OECD 201 method. Under the conditions of this study, the EC50 values based on mean measured concentrations for growth rate and yield were determined to be 25 (95% CI: 23–28 mg/L) and 11 mg/L (95% CI: 9.9–13 mg/L), respectively.

**RIFM, 2011c:** A fish (*Danio rerio*) acute toxicity study was conducted according to the OECD 202 under semi-static conditions. Under the conditions of this study, the 96-h LC50 value based on geometric mean concentrations was reported to be 13.3 mg/L (95% CI: 12–14.7 mg/L).

#### Other available data

2-Hexenoic acid, 2-methyl-, methyl ester, (2E)- has not been pre-registered with REACH, and there are no additional data at this time.

#### 11.2.3. Risk assessment refinement

Since 2-hexenoic acid, 2-methyl-, methyl ester, (2E)- 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.

	LC50 (Fish) (mg/L)	EC50 ( <i>Daphnia</i> ) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (µg/L)	Chemical Class
RIFM Framework Screening-level (Tier 1)	<u>12.58</u>			1,000,000	0.01258	

Exposure information and PEC calculation (following RIFM Environmental Framework: [Salvito, 2002](#)).

Exposure	Europe (EU)	North America (NA)
Log K <sub>ow</sub> Used	3.36	3.36
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	<1	No VoU
<b>Risk Characterization: PEC/PNEC</b>	<b>&lt;1</b>	<b>NA</b>

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

The RIFM PNEC is 0.01258 µg/L. The revised PEC/PNECs for EU and NA (No VoU) are not applicable. The material was cleared at the screening-level; therefore, it does not present a risk to the aquatic environment at the current reported VoU.

**Literature Search and Risk Assessment Completed On:** 11/06/20.

## 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/opthpv/public\\_search\\_publicdetails?submission\\_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User\\_title=DetailQuery%20Results&EndPointRpt=Y#submission](https://ofmpub.epa.gov/opthpv/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](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](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 04/14/21.

## 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.

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