



## Short Review

## RIFM fragrance ingredient safety assessment, ethyl levulinate, CAS Registry Number 539-88-8



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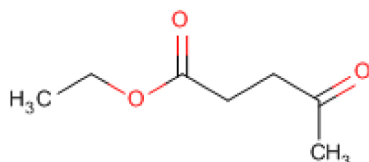
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Version: 090618. This version replaces any previous versions.

Name: Ethyl levulinate

CAS Registry Number: 5-39-88-8



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**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., 2015, 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  
**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  
**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 under the limits 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 use of this material under current conditions is supported by existing information.**

Ethyl levulinate was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analog methyl acetoacetate (CAS # 105-45-3) show that ethyl levulinate is not expected to be genotoxic and is not a safety concern under the current, declared levels of use for the skin sensitization endpoint. The repeated dose, developmental and reproductive, and local respiratory toxicity endpoints were evaluated using the TTC for a Cramer Class I material, and the exposure to ethyl levulinate is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; ethyl levulinate is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; ethyl levulinate 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 genotoxic. (ECHA Dossier: Methyl Acetoacetate; ECHA, 2011; RIFM, 2014)

**Repeated Dose Toxicity:** No data available. Exposure is below the TTC.

**Developmental and Reproductive Toxicity:** No data available. Exposure is below the TTC.

**Skin Sensitization:** No safety concerns at current, declared use levels. (ECHA Dossier: Methyl Acetoacetate; ECHA, 2011)

**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:** Screening-level: (EPI Suite v4.11; US EPA, 2012a) 2.9 (BIOWIN 3)

**Bioaccumulation:** Screening-level: (EPI Suite v4.11; US EPA, 2012a) 3.16 L/kg

**Ecotoxicity:** Screening-level: (RIFM Framework; Salvito et al., 2002) Fish LC50: 5978 mg/L

**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:** (RIFM Framework; Salvito et al., 2002) Fish LC50: 5978 mg/L

**RIFM PNEC is:** 5.978  $\mu\text{g/L}$

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

**1. Identification**

- Chemical Name:** Ethyl levulinate
- CAS Registry Number:** 539-88-8
- Synonyms:** Ethyl acetylpropanoate; Ethyl 4-ketovalerate; Ethyl laevulinate; Ethyl laevulate; Ethyl 4-oxopentanoate; Levulinic acid, ethyl ester; 外脂肪酸(C = 3 ~ 10)アルキル(C = 1 ~ 5); レーリン酸アルキル(C = 1 ~ 2)エステル; Ethyl levulinate
- Molecular Formula:**  $\text{C}_7\text{H}_{12}\text{O}_3$
- Molecular Weight:** 144.17
- RIFM Number:** 1017
- Stereochemistry:** Isomer not specified. No stereocenters and no stereoisomers possible

**2. Physical data**

- Boiling Point:** 93 °C @ 18 mm Hg (FMA Database), 189.88 °C (EPI Suite)
- Flash Point:**  $> 93$  °C (GHS),  $> 200$  °F; CC (FMA Database)
- Log  $K_{ow}$ :** 0.29 (EPI Suite)
- Melting Point:**  $-7.52$  °C (EPI Suite)
- Water Solubility:** 45660 mg/L (EPI Suite)
- Specific Gravity:** 1.016 (FMA Database)
- Vapor Pressure:** 0.181 mm Hg @ 20 °C (EPI Suite v4.0), 0.1 mm Hg @ 20 °C (FMA Database), 0.27 mm Hg @ 25 °C (EPI Suite)
- UV Spectra:** No significant absorbance between 290 and 700 nm; the molar absorption coefficient is below the benchmark ( $1000 \text{ L mol}^{-1} \cdot \text{cm}^{-1}$ )
- Appearance/Organoleptic:** A colorless liquid with an ethereal-fruity, somewhat green and sweet odor; mild-fruity, ethereal-green, unripe-apple-like taste

**3. Exposure**

- Volume of Use (worldwide band):** 1–10 metric tons per year (IFRA, 2015)
- 95th Percentile Concentration in Hydroalcoholics:** 0.025% (RIFM, 2016)

3. **Inhalation Exposure\***: 0.00058 mg/kg/day or 0.041 mg/day (RIFM, 2016)  
 4. **Total Systemic Exposure\*\***: 0.0020 mg/kg/day (RIFM, 2016)

\*95th percentile calculated exposure derived from concentration survey data in the Creme RIFM aggregate exposure model (Comiskey et al., 2015; Safford et al., 2015; Safford et al., 2017; and Comiskey et al., 2017).

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

#### 4. Derivation of systemic absorption

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

#### 5. 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**: methyl acetoacetate (CAS # 105-45-3)
  - b. **Repeated Dose Toxicity**: None
  - c. **Developmental and Reproductive Toxicity**: None
  - d. **Skin Sensitization**: methyl acetoacetate (CAS # 105-45-3)
  - e. **Phototoxicity/Photoallergenicity**: None
  - f. **Local Respiratory Toxicity**: None
  - g. **Environmental Toxicity**: None
3. **Read-across Justification**: See Appendix below

#### 6. Metabolism

Not considered for this risk assessment and therefore not reviewed except where it may pertain in specific endpoint sections as discussed below.

#### 7. Natural occurrence (discrete chemical) or composition (NCS)

Ethyl levulinate is reported to occur in the following foods by the VCF\*:

*Allium* species.  
 Apple brandy (Calvados).  
 Bilberry wine.  
 Cherimoya (*Annona cherimolia* Mill.)  
 Cider (apple wine).  
 Cocoa.  
 Grape brandy.  
 Tequila (*Agave tequilana*).  
 Vanilla.  
 Wheaten bread.  
 Whisky.  
 Wine.

\*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.

#### 8. IFRA standard

None.

#### 9. REACH dossier

Available, accessed 11/06/2018.

#### 10. Summary

##### 10.1. Human health endpoint summaries

##### 10.1.1. Genotoxicity

Based on the current existing data, ethyl levulinate does not present a concern for genetic toxicity.

**10.1.1.1. Risk assessment.** Ethyl levulinate was assessed in the BlueScreen assay and found negative for both cytotoxicity (positive: < 80% relative cell density) and genotoxicity, with and without metabolic activation (RIFM, 2013). BlueScreen is a screening assay that assesses genotoxic stress through human-derived gene expression. Additional assays on a more reactive read-across material were considered to fully assess the potential mutagenic or clastogenic effects on the target material. There are no studies assessing the mutagenic activity of ethyl levulinate; however, read-across can be made to methyl acetoacetate (CAS # 105-45-3; see Section V). The mutagenic activity of methyl acetoacetate 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 TA1538 were treated with methyl acetoacetate in distilled water at concentrations up to 5000 µg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (ECHA, 2011). Under the conditions of the study, methyl acetoacetate was not mutagenic in the Ames test, and this can be extended to ethyl levulinate.

There are no data assessing the clastogenic activity of ethyl levulinate; however, read-across can be made to methyl acetoacetate (CAS # 105-45-3; see Section V). The clastogenic activity of methyl acetoacetate 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 methyl acetoacetate in dimethyl sulfoxide (DMSO) at concentrations up to 1170 µg/mL in the presence and absence of metabolic activation (S9) for 3 h and in the absence of metabolic activation for 24 h. Methyl acetoacetate did not induce binucleated cells with micronuclei when tested up to cytotoxic concentrations or the maximum concentration in either the presence or absence of an S9 activation system (RIFM, 2014). Under the conditions of the study, methyl acetoacetate was considered to be non-clastogenic in the *in vitro* micronucleus test, and this can be extended to ethyl levulinate.

Based on the available data, methyl acetoacetate does not present a concern for genotoxic potential, and this can be extended to ethyl levulinate.

**Additional References:** Shimizu et al., 1985; Kusakabe et al., 2002.  
**Literature Search and Risk Assessment Completed On:** 12/27/2017.

##### 10.1.2. Repeated dose toxicity

There are no repeated dose toxicity data on ethyl levulinate or any of the read-across materials that can be used to support the repeated

dose toxicity endpoint. The total systemic exposure to ethyl levulinate is below the TTC at the current level of use.

**10.1.2.1. Risk assessment.** There are no repeated dose toxicity data on ethyl levulinate or any of the read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to ethyl levulinate (2 µg/kg bw/day) is below the TTC (30 µg/kg bw/day for a Cramer Class I material; Kroes et al., 2007; Laufersweiler et al., 2012) for the repeated dose toxicity endpoint at the current level of use.

**Additional References:** None.

**Literature Search and Risk Assessment Completed On:** 12/20/2017.

#### 10.1.3. Developmental and reproductive toxicity

There are no developmental and reproductive toxicity data on ethyl levulinate or any of the read-across materials that can be used to support the developmental and reproductive toxicity endpoint. The total systemic exposure to ethyl levulinate is below the TTC at the current level of use.

**10.1.3.1. Risk assessment.** There are no developmental and reproductive toxicity data on ethyl levulinate or any of the read-across materials that can be used to support the developmental and reproductive toxicity endpoint. The total systemic exposure to ethyl levulinate (2 µg/kg bw/day) is below the TTC (30 µg/kg bw/day) for a Cramer Class I material; Kroes et al., 2007; Laufersweiler et al., 2012) for the developmental and reproductive toxicity endpoint at the current level of use.

**Additional References:** None.

**Literature Search and Risk Assessment Completed On:** 12/20/2017.

#### 10.1.4. Skin sensitization

Based on existing data and read-across analog methyl acetoacetate (CAS # 105-45-3), ethyl levulinate does not present a safety concern for skin sensitization under the current, declared levels of use.

**10.1.4.1. Risk assessment.** Based on the existing data and read-across to methyl acetoacetate (CAS # 105-45-3; see Section V); ethyl levulinate does not present a safety concern for skin sensitization under the current, declared levels of use. The chemical structures of these materials indicate that they would not be expected to react with skin proteins directly (Toxtree 2.6.13; OECD toolbox v3.4). In a murine Local Lymph Node Assay (LLNA), read-across analog methyl acetoacetate was found to be negative up to 100%, which resulted in a Stimulation Index (SI) of 0.70 (<https://echa.europa.eu/registration-dossier/-/registered-dossier/13864/7/5/2> ECHA, 2011). In human maximization tests, no reactions indicative of sensitization were observed with 4% ethyl levulinate (2760 µg/cm<sup>2</sup>) (RIFM, 1977) or 8% read-across analog methyl acetoacetate (5520 µg/cm<sup>2</sup>) (RIFM, 1976).

Based on weight of evidence from structural analysis, human studies, and read-across analog methyl acetoacetate, ethyl levulinate does not present a concern for skin sensitization.

**Additional References:** None.

**Literature Search and Risk Assessment Completed On:** 12/11/2017.

#### 10.1.5. Phototoxicity/photoallergenicity

Based on UV/Vis absorption spectra, ethyl levulinate would not be expected to present a concern for phototoxicity or photoallergenicity.

**10.1.5.1. Risk assessment.** There are no phototoxicity studies available for ethyl levulinate 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 lack of absorbance, ethyl levulinate does not present a concern for phototoxicity or photoallergenicity.

**10.1.5.2. 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<sup>-1</sup> · cm<sup>-1</sup> (Henry et al., 2009).

**Additional References:** None.

**Literature Search and Risk Assessment Completed On:** 11/07/2017.

#### 10.1.6. Local Respiratory Toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The exposure level for ethyl levulinate is below the Cramer Class I TTC value for inhalation exposure local effects.

**10.1.6.1. Risk assessment.** There are no inhalation data available on ethyl levulinate. Based on the Creme RIFM Model, the inhalation exposure is 0.041 mg/day. This exposure is 34.1 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:** 12/14/2016.

#### 10.2. Environmental endpoint summary

##### 10.2.1. Screening-level assessment

A screening-level risk assessment of ethyl levulinate 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<sub>OW</sub>, 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, ethyl levulinate 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 ethyl levulinate 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.

#### 10.2.2. Risk assessment

Based on the current VoU (2015), ethyl levulinate does not present a risk to the aquatic compartment in the screening-level assessment.

**Biodegradation:** No data available.

**Ecotoxicity:** No data available.

**Other available data** Ethyl levulinate has been pre-registered for REACH with no additional data at this time.

#### 10.2.3. Risk assessment refinement

Ecotoxicological data and PNEC derivation (all endpoints reported in mg/L; PNECs in µg/L).

Endpoints used to calculate PNEC are underlined.

Exposure information and PEC calculation (following RIFM

current reported volumes of use.

**Literature Search and Risk Assessment Completed On:** 12/12/2017.

#### 11. Literature Search\*

- **RIFM Database:** Target, Fragrance Structure Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** <http://echa.europa.eu/>
- **NTP:** <https://ntp.niehs.nih.gov/>
- **OECD Toolbox**
- **SciFinder:** <https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf>
- **PubMed:** <http://www.ncbi.nlm.nih.gov/pubmed>
- **TOXNET:** <http://toxnet.nlm.nih.gov/>
- **IARC:** <http://monographs.iarc.fr>
- **OECD SIDS:** <http://webnet.oecd.org/hpv/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:** <http://www.safe.nite.go.jp/english/db.html>

	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>5978</u>	X	X	1,000,000	5.978	X

Environmental Framework: [Salvito et al., 2002](#)).

Exposure	Europe (EU)	North America (NA)
Log K <sub>ow</sub> Used	0.29	0.29
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	< 1	< 1
<b>Risk Characterization: PEC/PNEC</b>	< 1	< 1

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

The RIFM PNEC is 5.978 µg/L. The revised PEC/PNECs for EU and NA are: not applicable. The material was cleared at screening-level and therefore does not present a risk to the aquatic environment at the

- **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 09/06/2018.

#### Conflicts of interest

The authors declare that they have no conflicts of interest.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fct.2018.12.050>.

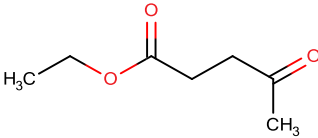
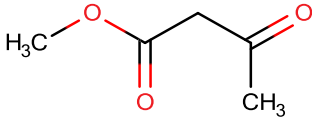
### Appendix

#### Read-across Justification

#### Methods

The read-across analogs were identified following the strategy for structuring and reporting a read-across prediction of toxicity 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 Chemical 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 substance 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 v3.4 (OECD, 2018).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v3.4 (OECD, 2018).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010), and skin sensitization was predicted using Toxtree 2.6.13.
- Protein binding was predicted using OECD QSAR Toolbox v3.4 (OECD, 2018).
- The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox v3.4 (OECD, 2018).

	Target Material	Read-across Material
Principal Name	Ethyl levulinate	Methyl acetoacetate
CAS No.	539-88-8	105-45-3
Structure		
Similarity (Tanimoto Score)		0.553
Read-across Endpoint		<ul style="list-style-type: none"> <li>• Genotoxicity</li> <li>• Skin Sensitization</li> </ul>
Molecular Formula	C <sub>7</sub> H <sub>12</sub> O <sub>3</sub>	C <sub>5</sub> H <sub>8</sub> O <sub>3</sub>
Molecular Weight	144.17	116.12
Melting Point (°C, EPI Suite)	-7.52	-31.21
Boiling Point (°C, EPI Suite)	189.88	147.34
Vapor Pressure (Pa @ 25°C, EPI Suite)	36	166
Log K <sub>ow</sub> (KOWWIN v1.68 in EPI Suite)	0.29	-0.69
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	4.566E+004	5E+005
J <sub>max</sub> (mg/cm <sup>2</sup> /h, SAM)	139.063	763.293
Henry's Law (Pa·m <sup>3</sup> /mol, Bond Method, EPI Suite)	2.11E-002	1.20E-002
<b>Genotoxicity</b>		
DNA Binding (OASIS v1.4, QSAR Toolbox v3.4)	• No alert found	• No alert found
DNA Binding (OECD QSAR Toolbox v3.4)	• No alert found	• No alert found
Carcinogenicity (ISS)	• Non-carcinogen (low reliability)	• Non-carcinogen (low reliability)
DNA Binding (Ames, MN, CA, OASIS v1.1)	• No alert found	• No alert found
<i>In Vitro</i> Mutagenicity (Ames, ISS)	• No alert found	• No alert found
<i>In Vivo</i> Mutagenicity (Micronucleus, ISS)	• No alert found	• No alert found
Oncologic Classification	• Not classified	• Not classified
<b>Skin Sensitization</b>		
Protein Binding (OASIS v1.1)	• Nucleophilic addition	• Nucleophilic addition
Protein Binding (OECD)	• No alert found	• No alert found
Protein Binding Potency	• Not possible to classify	• Not possible to classify
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	• No alert found.	• Nucleophilic addition
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	• No alert found	• No alert found
<b>Metabolism</b>		
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (- OECD QSAR Toolbox v3.4)	See Supplemental Da 1	See Supplemental Data 2

#### Summary

There are insufficient toxicity data on ethyl levulinate (CAS # 539-88-8). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, physical–chemical properties, and expert judgment, methyl acetoacetate (CAS # 105-45-3) was identified as a read-across material with sufficient data for toxicological evaluation.

## Conclusions

- Methyl acetoacetate (CAS # 105-45-3) was used as a read-across analog for the target material ethyl levulinate (CAS # 539-88-8) for genotoxicity and skin sensitization endpoints.
  - o The target substance and the read-across analog are structurally similar and belong to the class of keto esters.
  - o The target substance and the read-across analog share a keto ester functionality.
  - o The key difference between the target substance and the read-across analog is that the read-across analog is a beta keto ester whereas the target substance is not. This structural difference is toxicologically insignificant.
  - o Similarity between the target substance and the read-across analog is indicated by the Tanimoto score. The Tanimoto score is mainly driven by the keto ester functionality. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The physical–chemical properties of the target substance and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - o According to the OECD QSAR Toolbox v3.4, structural alerts for toxicological endpoints are consistent between the target substance and the read-across analog.
  - o The target substance and the read-across analog have a protein binding alert for skin sensitization by OASIS v1.1. As described in the skin sensitization section above, based on existing data, read-across methyl acetoacetate does not present a safety concern for skin sensitization under the current, declared levels of use. Therefore, predictions are superseded by data.
  - o The target substance and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
  - o The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

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