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Short Review

RIFM fragrance ingredient safety assessment, ethyl acetoacetate, CAS Registry Number 141-97-9

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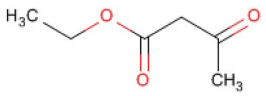

Name: Ethyl acetoacetate

CAS Registry Number: 141-97-9

AAbbreviation/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

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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 acetoacetate was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that ethyl acetoacetate is not genotoxic. Data from ethyl acetoacetate and read-across analog methyl acetoacetate (CAS # 105-45-3) show that there are no safety concerns for ethyl acetoacetate for skin sensitization under the current, declared levels of use. Data provide a calculated MOE > 100 for the repeated dose, developmental, and reproductive toxicity endpoints. The local respiratory toxicity endpoint was evaluated using the TTC for a Cramer Class I material, and the exposure to ethyl acetoacetate is below the TTC (1.4 mg/day). The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; ethyl acetoacetate is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; ethyl acetoacetate 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: Ethyl acetoacetate)
Repeated Dose Toxicity: NOAEL = 333 mg/kg/day.	(ECHA Dossier: Ethyl acetoacetate)
Developmental and Reproductive Toxicity: NOAEL = 225 mg/kg/day	(ECHA Dossier: Ethyl acetoacetate)
Skin Sensitization: No safety concerns at current, declared use levels.	(ECHA dossier)
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic.	(UV Spectra, RIFM DB)

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

Environmental Safety Assessment

Hazard Assessment:

Persistence: Screening-level: 66% (OECD 301D)	(REACH Dossier; accessed 11/2017)
Bioaccumulation: Screening-level: 3.162 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Screening-level: Fish LC50: 6462 mg/L	(RIFM Framework; Salvito et al., 2002)

Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:

Screening-level: PEC/PNEC (North America and Europe) < 1	(RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: Fish LC50: 6462 mg/L	(RIFM Framework; Salvito et al., 2002)

RIFM PNEC is: 6.462 μg/L

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

1. Identification

1. **Chemical Name:** Ethyl acetoacetate
2. **CAS Registry Number:** 141-97-9
3. **Synonyms:** Acetoacetic ester; Butanoic acid, 3-oxo-, ethyl ester; Ethyl acetylacetate; Ethyl β-ketobutyrate; Ethyl 3-oxobutanoate; ｱｾﾄ酢酸ｱﾙｷﾙ(C = 1 ~ 4); Ethyl acetoacetate
4. **Molecular Formula:** $C_6H_{10}O_3$
5. **Molecular Weight:** 130.14
6. **RIFM Number:** 390
7. **Stereochemistry:** Isomer not specified. No stereocenters and no stereoisomers possible.

2. Physical data

1. **Boiling Point:** 181 °C (FMA), 169.06 °C (EPI Suite)
2. **Flash Point:** 74 °C (GHS), 183 °F; CC (FMA Database)
3. **Log K_{ow}:** 0.2 (EPI Suite)
4. **Melting Point**: 19.23 °C (EPI Suite)
5. **Water Solubility:** 56170 mg/L (EPI Suite)
6. **Specific Gravity:** 1.025 (FMA Database)
7. **Vapor Pressure:** 0.645 mm Hg @ 20 °C (EPI Suite v4.0), 0.5 mm Hg 20 °C (FMA), 0.929 mm Hg @ 25 °C (EPI Suite)
8. **UV Spectra:** Minor absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} · cm^{-1})
9. **Appearance/Organoleptic:** Food Chemicals Codex (1972): A colorless liquid that has an ethereal-fruity, sweet and rum-like odor and a sweet, fruity taste (1138)

3. Exposure

1. **Volume of Use (worldwide band):** 100–1000 metric tons per year (IFRA, 2015)
2. **95**th **Percentile Concentration in Hydroalcoholics:** 0.15% (RIFM, 2016)
3. **Inhalation Exposure*:** 0.0016 mg/kg/day or 0.12 mg/day (RIFM, 2016)
4. **Total Systemic Exposure**:** 0.0060 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:** None

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 acetoacetate is reported to occur in the following foods*:

Babaco fruit (*Carica pentagona* Heilborn)
Coffee
Passion fruit (*Passiflora* species)
Strawberry (*Fragaria* species)

*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 12/6/2017.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

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

10.1.1.1. Risk assessment. The mutagenic activity of ethyl 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, TA1538, and *Escherichia coli* strain WP2uvrA were treated with ethyl acetoacetate in water at concentrations up to 10000 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 REACH Dossier). Under the conditions of the study, ethyl acetoacetate was not mutagenic in the Ames test.

The clastogenicity of ethyl acetoacetate was assessed in an *in vitro* chromosome aberration study conducted in compliance with GLP regulations and in accordance with OECD TG 473. Chinese hamster lung fibroblasts (V79) were treated with ethyl acetoacetate in water at concentrations up to 1301.4 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 item, either with or without S9 metabolic activation (ECHA REACH Dossier). Under the conditions of the study, ethyl acetoacetate was considered to be non-clastogenic to mammalian cells.

Based on the data available, ethyl acetoacetate does not present a concern for genotoxic potential.

Additional References: Shimizu et al., 1985; Kusakabe et al., 2002; ECHA Dossier.

Literature Search and Risk Assessment Completed On: 11/29/2017.

10.1.2. Repeated dose toxicity

The margin of exposure for ethyl acetoacetate is adequate for the repeated dose toxicity endpoint at the current level of use.

10.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on ethyl acetoacetate. A GLP 28-day subchronic dietary study was conducted in Sprague Dawley rats. Groups of 16 rats/sex/dose were fed diets containing ethyl acetoacetate at concentrations of 0, 100, 300, or 1000 mg/kg/day for 28 days. The calculated mean food intake was 0, 106, 421, and 1266 mg/kg/day for males and 0, 91, 292, and 1008 mg/kg/day for females, corresponding to the 0, 100, 300, and 1000 mg/kg/day doses. There were no treatment-related adverse effects observed up to the highest dose tested. The NOAEL for repeated dose toxicity was considered to be 1000 mg/kg/day (1266 mg/kg/day for males and 1008 mg/kg/day for females) (RIFM, 1988; data also available at Cook et al., 1992; ECHA Dossier; SIDS Initial Assessment Report for SIAM 12, 2001; EU Risk Assessment Report on Ethyl acetoacetate, 2002). An OECD 407/GLP oral gavage toxicity study was conducted in Sprague Dawley rats. Groups of 5 rats/sex/dose were administered ethyl acetoacetate via oral gavage at doses of 0, 50, 225, or 1000 mg/kg/day for 28 days. Additional groups of 5 rats/sex/dose were assigned to the control and high dose groups to serve as the 14-day treatment-free recovery groups. There were no treatment-related adverse effects observed. Therefore the NOAEL was considered to be 1000 mg/kg/day, the highest dose tested (ECHA Dossier: Ethyl acetoacetate; data also available at SIDS Initial Assessment Report for SIAM 12, 2001; EU Risk Assessment Report on Ethyl acetoacetate, 2002). The NOAEL of 1000 mg/kg/day from the OECD 407 study was considered for this safety assessment. A default safety factor of 3 was used when deriving a NOAEL from an OECD 407 study. The safety factor has been approved by the Expert Panel for Fragrance Safety*. The derived NOAEL for the repeated dose toxicity data is 1000/3 or 333 mg/kg/day.

Therefore, the ethyl acetoacetate MOE for the repeated dose toxicity endpoint can be calculated by dividing the ethyl acetoacetate NOAEL in mg/kg/day by the total systemic exposure for ethyl acetoacetate, 333/0.006 or 55500.

In addition, the total systemic exposure to ethyl acetoacetate (6.0 μg/kg/day) is below the TTC (30 μg/kg bw/day) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

*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: 11/29/17.

10.1.3. Developmental and reproductive toxicity

The margin of exposure for ethyl acetoacetate is adequate for the developmental and reproductive toxicity endpoints at the current level of use.

10.1.3.1. Risk assessment. There are sufficient developmental and reproductive toxicity data on ethyl acetoacetate. An OECD 421/GLP oral gavage reproduction/developmental toxicity screening test was conducted in Sprague Dawley rats. Groups of 10 rats/sex/dose were administered ethyl acetoacetate via oral gavage at doses of 0, 50, 225,

or 1000 mg/kg/day. Males were dosed 2 weeks prior to mating, during the mating period, and approximately 2 weeks post-mating (minimum of 28 days), while females were dosed 2 weeks prior to mating up to day 3 post-partum. In parental animals, there were no treatment-related adverse effects observed for mortality, body weights, food and water consumption, and organ weights (testicle and epididymis). Histopathological examination was limited to the testicle, epididymis, and ovary among animals of the control and high-dose groups, and no treatment-related effects were reported. In high-dose group dams, the number of corpora lutea and implants was reduced slightly when compared to the controls, which led to an increased pre-implantation loss of 5% (control: 2%). The post-implantation loss was increased in the high-dose group (13.2%) as compared to the controls (5.9%). Furthermore, the number of pups at birth and day 4 of lactation was decreased in the high-dose group, correlating to the reduced number of implants. The mean birth index and live birth index (both: 86.8%) was decreased in the high-dose group when compared to controls (both: 94.1%). However, the viability index in the high-dose group was comparable to the control group. These findings were not toxicologically significant when compared to the historical control data. No further treatment-related reproductive alterations were observed. In the F1 generation, no treatment-related adverse effects were reported for mortality, clinical signs, body weights, sexual maturation, organ weights, pathological changes during necropsy, and microscopical evaluation. The NOAEL for reproductive and developmental toxicity was considered to be 225 mg/kg/day, based on increased post-implantation losses among high dose group animals (ECHA Dossier: Ethyl acetoacetate, accessed 11/29/17); data also available at SIDS Initial Assessment Report for SIAM 12, 2001; EU Risk Assessment Report on Ethyl acetoacetate, 2002).

Therefore, the ethyl acetoacetate MOE for the developmental and reproductive toxicity endpoints can be calculated by dividing the ethyl acetoacetate NOAEL in mg/kg/day by the total systemic exposure for ethyl acetoacetate, 250/0.006 or 37500.

In addition, the total systemic exposure to ethyl acetoacetate (6.0 μg/kg/day) is below the TTC (30 μg/kg bw/day) for the developmental and reproductive toxicity endpoints of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 11/29/17.

10.1.4. Skin sensitization

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

10.1.4.1. Risk assessment. Limited data are available for ethyl acetoacetate. Based on the read-across analog methyl acetoacetate (CAS # 105-45-3; see Section V), ethyl acetoacetate 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 be expected to react with skin proteins directly (Toxtree 2.6.13; OECD toolbox v3.4). However, in a guinea pig Freund's complete adjuvant test (FCAT) and an open epicutaneous test (OET), ethyl acetoacetate did not present reactions indicative of sensitization (ECHA dossier; Klecak, 1985). In a murine Local Lymph Node Assay (LLNA), read-across analog methyl acetoacetate was found to be negative up to the maximum tested concentration of 100%, which resulted in a Stimulation Index (SI) of 0.70 (ECHA Dossier: accessed 10/19/17). In a confirmatory human maximization test, no reactions indicative of sensitization were observed with 8% ethyl acetoacetate or read-across analog methyl acetoacetate (5520 μg/cm^2) (RIFM, 1973; RIFM, 1976).

Based on weight of evidence from structural analysis, animal and human studies, and read-across analog methyl acetoacetate, ethyl acetoacetate does not present a safety concern for skin sensitization under the current, declared levels of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 10/19/17.

10.1.5. Phototoxicity/photoallergenicity

Based on UV/Vis absorption spectra, ethyl acetoacetate 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 acetoacetate in experimental models. UV/Vis absorption spectra indicate minor absorbance between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). Based on lack of significant absorbance in the critical range, ethyl acetoacetate does not present a concern for phototoxicity or photoallergenicity.

10.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) for ethyl acetoacetate were obtained. The spectra indicate minor 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: 11/30/17.

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

10.1.6.1. Risk assessment. There is insufficient inhalation data available on ethyl acetoacetate. Based on the Creme RIFM Model, the inhalation exposure is 0.12 mg/day. This exposure is 11.7 times lower than the Cramer Class I TTC value of 1.4 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: Cunzeman and Slotnick, 1984; Smyth et al., 1949; Pinching and Doving, 1974.

Literature Search and Risk Assessment Completed On: 12/13/2016.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of ethyl acetoacetate 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, ethyl acetoacetate 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 v4.1 did not identify ethyl acetoacetate as either being possibly persistent nor 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 v4.1). 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 Volume of Use (2015), ethyl acetoacetate does not present a risk to the aquatic compartment in the screening-level assessment.

Biodegradation: No data available.

Ecotoxicity: No data available.

10.2.2.1. Other available data. Ethyl acetoacetate has been registered under REACH, and the following data is available:

A ready biodegradability study was conducted according to the OECD 301D method. Biodegradation of 66% was observed after 28 days.

A 96-h fish (Zebra fish) acute toxicity study was conducted according to the OECD 203 method under semi-static conditions. The LC50 was reported to be greater than 100 mg/L.

A *Daphnia magna* immobilization test was conducted according to the OECD 202 method under static conditions. The 48-h NOEC was reported to be greater than 100 mg/L (limit concentration).

An algae inhibition test was conducted according to the OECD 201 method, and the 72-h EC50 was reported to be greater than 100 mg/L.

10.2.3. Risk assessment refinement

Since Ethyl acetoacetate has passed the screening criteria, measured data is included for completeness only and has not been used in PNEC calculations.

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

Endpoints used to calculate PNEC are underlined.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	0.2	0.2
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	100–100	100–1000
Risk Characterization: PEC/PNEC	**N/A**	**N/A**

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

The RIFM PNEC is 6.462 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 current reported volumes of use.

Literature Search and Risk Assessment Completed On: 11/30/17.

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/oppthpv/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
- **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 07/30/2018.

Conflicts of interest

The authors declare that they have no conflicts of interest.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	6462			1,000,000	6.462	

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.03.016.

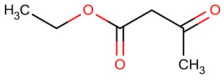
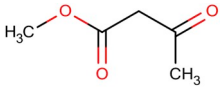
Appendix

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, 2012).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v3.4 (OECD, 2012).
- 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, 2012).
- The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox v3.4 (OECD, 2012).

	Target Material	Read-Across Material
Principal Name	Ethyl acetoacetate	Methyl acetoacetate
CAS No.	141-97-9	105-45-3
Structure		
Similarity (Tanimoto Score)		0.78
Read-Across Endpoint		• Skin Sensitization
Molecular Formula	$C_6H_{10}O_3$	$C_5H_8O_3$
Molecular Weight	130.14	116.12
Melting Point (°C, EPI Suite)	−19.23	−31.21
Boiling Point (°C, EPI Suite)	169.06	147.34
Vapor Pressure (Pa @ 25 °C, EPI Suite)	124	166
Log Kow (KOWWIN v1.68 in EPI Suite)	0.25	−0.69
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPI Suite)	110000	500000
J_{max} (mg/cm^2/h, SAM)	216.632	763.293
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	1.59E-002	1.20E-002
Skin Sensitization		
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)	• Nucleophilic addition	• Nucleophilic addition
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	• No alert found	• No alert found
Metabolism		
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v3.4)	See Supplemental Data 1	See Supplemental Data 2

Summary

There are insufficient toxicity data on ethyl acetoacetate (CAS # 141-97-9). 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, 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 acetoacetate (CAS # 141-97-9) for the skin sensitization endpoint.
  - o The target substance and the read-across analog are structurally similar and belong to the class of beta ketoesters.
  - o The target substance and the read-across analog share an acetoacetate fragment.
  - o The key structural difference between the target substance and the read-across analog is that the target substance is an ethyl ester, whereas the read-across analog is a methyl ester. This structural difference is toxicologically insignificant.
  - o Structural similarity between the target substance and the read-across analog is indicated by the Tanimoto score. The Tanimoto score is mainly

driven by the acetoacetate structure. 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 read-across analog and the target substance have nucleophilic addition alert by OASIS model for protein binding. All other alerts are negative. Data are consistent with *in silico* alerts.
- 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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