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Short review

RIFM fragrance ingredient safety assessment, 2-methyl-2-pentenoic acid, CAS registry number 16957-70-3

A.M. Api[a], D. Belsito[b], D. Botelho[a], M. Bruze[c], G.A. Burton Jr.[d], J. Buschmann[e], M.L. Dagli[f], M. Date[a], W. Dekant[g], C. Deodhar[a], M. Francis[a], A.D. Fryer[h], L. Jones[a], K. Joshi[a], S. La Cava[a], A. Lapczynski[a], D.C. Liebler[i], D. O'Brien[a], A. Patel[a], T.M. Penning[j], G. Ritacco[a], J. Romine[a], N. Sadekar[a], D. Salvito[a], T.W. Schultz[k], I.G. Sipes[l], G. Sullivan[a,*], Y. Thakkar[a], Y. Tokura[m], S. Tsang[a]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ, 07677, USA*
[b] *Member RIFM Expert Panel, Columbia University Medical Center, Department of Dermatology, 161 Fort Washington Ave., New York, NY, 10032, USA*
[c] *Member RIFM Expert Panel, Malmo University Hospital, Department of Occupational & Environmental Dermatology, Sodra Forstadsgatan 101, Entrance 47, Malmo SE, 20502, Sweden*
[d] *Member RIFM Expert Panel, School of Natural Resources & Environment, University of Michigan, Dana Building G110, 440 Church St., Ann Arbor, MI, 58109, USA*
[e] *Member RIFM Expert Panel, Fraunhofer Institute for Toxicology and Experimental Medicine, Nikolai-Fuchs-Strasse 1, 30625, Hannover, Germany*
[f] *Member RIFM Expert Panel, University of Sao Paulo, School of Veterinary Medicine and Animal Science, Department of Pathology, Av. Prof. dr. Orlando Marques de Paiva, 87, Sao Paulo CEP 05508-900, Brazil*
[g] *Member RIFM Expert Panel, University of Wuerzburg, Department of Toxicology, Versbacher Str. 9, 97078, Würzburg, Germany*
[h] *Member RIFM Expert Panel, Oregon Health Science University, 3181 SW Sam Jackson Park Rd., Portland, OR, 97239 USA;*
[i] *Member RIFM Expert Panel, Vanderbilt University School of Medicine, Department of Biochemistry, Center in Molecular Toxicology, 638 Robinson Research Building, 2200 Pierce Avenue, Nashville, TN, 37232-0146, USA*
[j] *Member of RIFM Expert Panel, University of Pennsylvania, Perelman School of Medicine, Center of Excellence in Environmental Toxicology, 1316 Biomedical Research Building (BRB) II/III, 421 Curie Boulevard, Philadelphia, PA, 19104-3083, USA*
[k] *Member RIFM Expert Panel, The University of Tennessee, College of Veterinary Medicine, Department of Comparative Medicine, 2407 River Dr., Knoxville, TN, 37996-4500, USA*
[l] *Member RIFM Expert Panel, Department of Pharmacology, University of Arizona, College of Medicine, 1501 North Campbell Avenue, P.O. Box 245050, Tucson, AZ, 85724-5050, USA*
[m] *Member RIFM Expert Panel, The Journal of Dermatological Science (JDS), Editor-in-Chief, Professor and Chairman Department of Dermatology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, 431-3192, Japan*

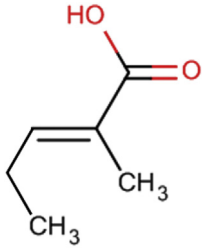
Version: 050818. This version replaces any previous versions

Name: 2-Methyl-2-pentenoic acid

CAS Registry Number: 16957-70-3

Additional CAS Numbers*:
3142-72-1 2-Methylpent-2-en-1-oic acid

*This material was included in this assessment because the materials are isomers.



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

* Corresponding author.
E-mail address: gsullivan@rifm.org (G. Sullivan).

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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.

2-Methyl-2-pentenoic acid was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that 2-methyl-2-pentenoic acid is not genotoxic. Data on read-across analog *trans*-crotonic acid (CAS# 107-93-7) show that 2-methyl-2-pentenoic acid is not a safety concern for skin sensitization at the current, declared levels of use. The repeated dose, reproductive, and local respiratory toxicity endpoints were completed using the TTC for a Cramer Class I material, and the exposure to 2-methyl-2-pentenoic acid is below the TTC (0.03 mg/kg/day, 0.03 mg/kg/day, and 1.4 mg/day, respectively). The phototoxicity/photoallergenicity endpoint was completed based on UV spectra; 2-methyl-2-pentenoic acid is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; 2-methyl-2-pentenoic acid was not found to be PBT as per 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.	(RIFM, 2014a; RIFM, 2014b)
Repeated Dose Toxicity: No NOAEL available. Exposure is below the TTC.	
Reproductive Toxicity: No NOAEL available. Exposure is below the TTC.	
Skin Sensitization: Not a concern for skin sensitization.	(ECHA Dossier: Crotonic acid; RIFM, 2013)
Phototoxicity/Photoallergenicity: Not 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: 3.3 (BIOWIN 3)	(EPI Suite v4.1; US EPA, 2012a)
Bioaccumulation: Screening-level: 3.2 L/kg	(EPI Suite v4.1; US EPA, 2012a)

Ecotoxicity: Screening-level: Fish LC50: 191.9 mg/L (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: 191.9 mg/L	(RIFM Framework; Salvito et al., 2002)

RIFM PNEC is: 0.1919 µg/L

- **Revised PEC/PNECs (2011 IFRA VoU):** North America and Europe: not applicable; cleared at the screening-level

1. Identification

Chemical Name: 2-Methyl-2-pentenoic acid	Chemical Name: 2-Methylpent-2-en-1-oic acid
CAS Registry Number: 16957-70-3	**CAS Registry Number:** 3142-72-1
Synonyms: (E)-2-Methylpent-2-en-1-oic acid; 2-Pentenoic acid, 2-methyl-, (E)-; Strawberiff; ｱﾙｹﾆﾙﾓﾉｶﾙﾎﾞﾝ酸(C = 5 ~ 23); 2-Methylpent-2-enoic acid; 2-Methyl-2-pentenoic acid	**Synonyms:** 2-Methylpent-2-en-1-oic acid; 2-Methylpent-2-enoic acid; 2-Pentenoic acid, 2-methyl-; Fragarone; ｱﾙｹﾆﾙﾓﾉｶﾙﾎﾞﾝ酸(C = 5 ~ 23)
Molecular Formula: $C_6H_{10}O_2$	**Molecular Formula:** $C_6H_{10}O_2$
Molecular Weight: 114.14	**Molecular Weight:** 114.14
RIFM Number: 1329	**RIFM Number:** 5289

2. Physical data**

1. **Boiling Point:** 120 °C @ 20 mm Hg (FMA), 208.44 °C (EPI Suite)
2. **Flash Point:** 107 °C (GHS), 225 °F; CC (FMA)
3. **Log K_{ow}:** 1.89 (EPI Suite)
4. **Melting Point**: 16.91 °C (EPI Suite)
5. **Water Solubility:** 6330 mg/L (EPI Suite)
6. **Specific Gravity:** 0.98 (FMA)
7. **Vapor Pressure:** 0.118 mm Hg @ 20 °C (EPI Suite), 0.178 mm Hg @ 25 °C (EPI Suite)
8. **UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L $mol^{-1} \cdot cm^{-1}$)
9. **Appearance/Organoleptic:** White to yellow crystals with a medium woody, strawberry, fruity odor.*

*http://www.thegoodscentscompany.com/data/rw1468281.html#toorgano, retrieved 5/12/2017.

**Physical data for both materials are identical.

3. Exposure

1. **Volume of Use (worldwide band):** 1–10 metric tons per year (IFRA, 2011)
2. **95th Percentile Concentration in Hydroalcoholics:** 0.073% (RIFM, 2016)
3. **Inhalation Exposure*:** 0.00012 mg/kg/day or 0.0085 mg/day (RIFM, 2016)
4. **Total Systemic Exposure**:** 0.0010 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. **Reproductive Toxicity:** None
   d. **Skin Sensitization:** *trans*-crotonic acid (CAS # 107-93-7)
   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.

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

2-Methyl-2-pentenoic acid is reported to occur in the following foods by the VCF*:

Soursop (*Annona muricata* L.)

Vaccinium species.

2-Methylpent-2-en-1-oic acid is reported to occur in the following foods by the VCF*:

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 on 05/08/2018.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data and use levels, 2-methyl-2-pentenoic acid does not present a concern for genetic toxicity.

10.1.1.1. Risk assessment. 2-Methyl-2-pentenoic acid was assessed in the BlueScreen assay and found positive for both cytotoxicity and genotoxicity in the absence of metabolic activation and negative for both cytotoxicity and genotoxicity in the presence of metabolic activation (RIFM, 2014c). BlueScreen is a screening assay that assesses genotoxic stress through human-derived gene expression. Additional assays were considered to fully assess the potential mutagenic or clastogenic effects on the target material. There are no studies assessing the mutagenicity of 2-methyl-2-pentenoic acid. The mutagenic activity of the additional material in this assessment, 2-methylpent-2-en-1-oic acid (CAS # 3142-72-1), 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-methylpent-2-en-1-oic acid in dimethyl sulfoxide (DMSO) at concentrations up to 5000 μg/plate. No increases in the mean number of revertant colonies were observed at any tested dose in the presence or absence of S9 (RIFM, 2014b). Under the conditions of the study, 2-methylpent-2-en-1-oic acid was not mutagenic in the Ames test.

There are no studies assessing the clastogenicity of 2-methyl-2-pentenoic acid. The clastogenic activity of 2-methylpent-2-en-1-oic acid (CAS # 3142-72-1) 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 2-methylpent-2-en-1-oic acid in DMSO at concentrations up to 1150 μg/mL in the presence and absence of metabolic activation (S9) for 3 and 24 h 2-Methylpent-2-en-1-oic acid did not induce binucleated cells with micronuclei when tested up to cytotoxic levels/the maximum dose in either non-activated or S9-activated test systems (RIFM, 2014a). Under the conditions of the study, 2-methylpent-2-en-1-oic acid was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the available data, 2-methylpent-2-en-1-oic acid does not present a concern for genotoxic potential, and this can be extended to 2-methyl-2-pentenoic acid.

Additional References: None.

Literature Search and Risk Assessment Completed On: 5/6/2017.

10.1.2. Repeated dose toxicity

There are insufficient repeated dose toxicity data on 2-methyl-2-pentenoic acid or any read-across materials. The total systemic exposure to 2-methyl-2-pentenoic acid is below the TTC for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

10.1.2.1. Risk assessment. There are no repeated dose toxicity data on 2-methyl-2-pentenoic acid or any read-across materials that can be used to support the repeated dose toxicity endpoint. The total systemic exposure to 2-methyl-2-pentenoic acid (1.0 μg/kg/day) is below the TTC (30 μg/kg bw/day; Kroes et al., 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 05/02/2017.

10.1.3. Reproductive toxicity

There are insufficient reproductive toxicity data on 2-methyl-2-pentenoic acid or any read-across materials. The total systemic exposure to 2-methyl-2-pentenoic acid is below the TTC for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

10.1.3.1. Risk assessment. There are no developmental toxicity data on 2-methyl-2-pentenoic acid or any read-across materials that can be used to support the developmental toxicity endpoint. The total systemic exposure to 2-methyl-2-pentenoic acid (1.0 μg/kg/day) is below the TTC (30 μg/kg bw/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the developmental toxicity endpoint of a Cramer Class I material at the current level of use.

There are no fertility data on 2-methyl-2-pentenoic acid or any read-across materials that can be used to support the fertility endpoint. The total systemic exposure to 2-methyl-2-pentenoic acid (1.0 μg/kg/day) is below the TTC (30 μg/kg bw/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the fertility endpoint of a Cramer Class I material at the current level of use.

Additional references: None

Literature Search and Risk Assessment Completed On: 05/02/2017.

10.1.4. Skin sensitization

Based on the existing data on the target (2-methyl-2-pentenoic acid) and read-across material *trans*-crotonic acid (CAS # 107-93-7), 2-methyl-2-pentenoic acid does not present a concern for skin sensitization.

10.1.4.1. Risk assessment. Limited skin sensitization studies are available for 2-methyl-2-pentenoic acid. Based on the existing data and read-across material *trans*-crotonic acid (CAS # 107-93-7; see Section V), 2-methyl-2-pentenoic acid does not present a concern for skin sensitization. The chemical structures of these materials indicate that they could possibly react with proteins, although little or no reaction would likely occur under physiological conditions (Toxtree 2.6.13; OECD toolbox v3.4). No predictive skin sensitization studies are available for 2-methyl-2-pentenoic acid. However, in a human maximization test, no skin sensitization reactions were observed 2% or 1380 μg/cm^2 2-methyl-2-pentenoic acid (RIFM, 1980). In a local lymph node assay, read-across analog *trans*-crotonic acid did not induce sensitization up to 50% (ECHA Dossier: Crotonic acid). Additionally, in a confirmatory human repeated insult patch test (HRIPT) with 661 μg/cm^2 or 1.2% of 3,7-dimethyl-2-methylenocta-6-enal in 1:3 ethanol:DEP, no reactions indicative of sensitization were observed in any of the 107 volunteers (RIFM, 2013). Based on weight of evidence from structural analysis, human data, and read-across to *trans*-crotonic acid, 2-methylpent-2-en-1-oic acid does not present a concern for skin sensitization.

Additional References: None.

Literature Search and Risk Assessment Completed On: 5/4/17.

10.1.5. Phototoxicity/Photoallergenicity

Based on UV/Vis absorption spectra, 2-methyl-2-pentenoic acid 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 2-methyl-2-pentenoic acid 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, 2-methyl-2-pentenoic acid 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^{-1} · cm^{-1} (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/20/17.

10.1.6. Local respiratory toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The exposure level for 2-methyl-2-pentenoic acid 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 2-methyl-2-pentenoic acid. Based on the Creme RIFM Model, the inhalation exposure is 0.0085 mg/day. This exposure is 165 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: 5/8/2017.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of 2-methyl-2-pentenoic acid 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, 2-methyl-2-pentenoic acid 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 (US EPA, 2012a) did not identify 2-methyl-2-pentenoic acid as 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, 2016). 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 (2011), 2-methyl-2-pentenoic acid 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. 2-Methyl-2-pentenoic acid 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	Europe (EU)	North America (NA)
Log K_{ow} Used	1.9	1.9
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	< 1	1–10
Risk Characterization: PEC/PNEC	**< 1**	**< 1**

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

The RIFM PNEC is 0.1919 μg/L. The revised PEC/PNECs for EU and NA: not applicable; cleared at the screening-level and therefore the material does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 5/3/17.

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.

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)**	191.9			1,000,000	0.1919	

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

Appendix

Read-across justification

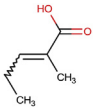
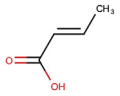
Methods

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, 2012).

- 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 (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	2-Methyl-2-pentenoic acid	*trans*-Crotonic acid
CAS No.	16957-70-3	107-93-7
Structure		
Similarity (Tanimoto score)		0.74
Read-across endpoint		• Skin sensitization
Molecular Formula	$C_6H_{10}O$	$C_4H_6O_2$
Molecular Weight	114.15	86.09
Melting Point (°C, EPI Suite)	16.91	2.39
Boiling Point (°C, EPI Suite)	208.44	173.37
Vapor Pressure (Pa @ 25°C, EPI Suite)	23.7	33
Log Kow (KOWWIN v1.68 in EPI Suite)	1.89	0.72
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	6330	8.6E + 004
J_{max} ($mg/cm^2/h$, SAM)	903.84	1220
Henry's Law ($Pa{\cdot}m^3/mol$, Bond Method, EPI Suite)	9.42E-007	4.59E-002
Skin Sensitization		
Protein Binding (OASIS v1.1)	• AN2, Michael addition	• AN2, Michael 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	No alert found
Skin Sensitization Model (CAESAR v2.1.6)	• Sensitizer (good reliability)	Sensitizer (good reliability)
Metabolism		
OECD QSAR Toolbox (v3.4) Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites	No metabolites	No metabolites

Summary

There are insufficient toxicity data on the 2-methyl-2-pentenoic acid (CAS # 16957-70-3). Hence, *in silico* evaluation was conducted by determining a read-across analog for this material. Based on structural similarity, reactivity, metabolism data, physical–chemical properties, and expert judgment, *trans*-crotonic acid (CAS # 107-93-7) was identified as a read-across analog with sufficient data for toxicological evaluation.

Conclusion

- *trans*-Crotonic acid (CAS # 107-93-7) is used as a read-across analog for target 2-methyl-2-pentenoic acid (CAS # 16957-70-3) for the skin senzitization endpoint.
  - The target substance and the read-across analog are structurally similar and belong to the class of unsaturated carboxylic acids.
  - The target substance and the read-across analog share a carboxylic acid moiety with α,β-unsaturation.
  - The key difference between the target substance and the read-across analog is that the α carbon is substituted in the target substance whereas there is no α carbon substitution in the read-across material. This structural difference is toxicologically insignificant.
  - Similarity between the target substance and the read-across analog is indicated by the Tanimoto score. The Tanimoto score is mainly driven by the carboxylic acid moiety with α,β-unsaturation. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - The physical–chemical properties of the target substance and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - According to the OECD QSAR Toolbox v3.4, structural alerts for toxicological endpoints are consistent between the target substance and the read-across analog.
  - The read-across analog and the target substance are predicted to be sensitizers by the CAESAR model for skin sensitization, and they also have a Michael addition alert by the protein binding model by OASIS. This shows that the target substance and the read-across analog have similar

reactivity. The data described in the skin sensitization section above show that the read-across analog does not pose a concern for the skin sensitization endpoint. Therefore, the alert will be superseded by the availability of the data.

- The target substance and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.
- The structural alerts for the endpoints evaluated are consistent between the metabolites of the read-across analog and the target material.

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