Food and Chemical Toxicology 156 (2021) 112570

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

RIFM fragrance ingredient safety assessment, 2-isopropyl-5-methyl-2--hexenal, CAS Registry Number 35158-25-9

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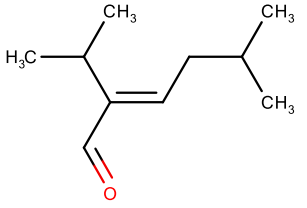
ARTICLE INFO

Handling Editor: Dr. Jose Luis Domingo

Version: 080921. Initial publication. All fragrance materials are evaluated on a five-year rotating basis. Revised safety assessments are published if new relevant data become available. Open access to all RIFM Fragrance Ingredient Safety Assessments is here: fragrance materialsafetyresource.elsevier.com.
Name: 2-Isopropyl-5-methyl-2-hexenal
CAS Registry Number: 35158-25-9



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

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https://doi.org/10.1016/j.fct.2021.112570
Received 5 May 2021; Received in revised form 13 August 2021; Accepted 13 September 2021
Available online 21 September 2021

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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., 2015a, 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DRF - Dose Range Finding
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest 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
Perfumery - In this safety assessment, perfumery refers to fragrances made by a perfumer used in consumer products only. The exposures reported in the safety assessment include consumer product use but do not include occupational exposures.
QRA - Quantitative Risk Assessment
QSAR - Quantitative Structure-Activity Relationship
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use
vPvB - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

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

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

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

*The Expert Panel for Fragrance Safety is an independent body that selects its own members and establishes its own operating procedures. The Expert Panel is comprised of internationally known scientists that provide RIFM with guidance relevant to human health and environmental protection.

Summary: The existing information supports the use of this material as described in this safety assessment.

2-Isopropyl-5-methyl-2-hexenal was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from read-across analog 2-methyl-2-pentenal (CAS # 623-36-9) show that 2-isopropyl-5-methyl-2-hexenal is not expected to be genotoxic. Data on analog hexen-2-al (CAS # 6728-26-3) provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity endpoint. The reproductive and local respiratory toxicity endpoints were evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material; exposure to 2-isopropyl-5-methyl-2-hexenal is below the TTC (0.03 mg/kg/day and 1.4 mg/day, respectively). Data from analog 3,7-dimethyl-2-methylenocta-6-enal (CAS # 22418-66-2) provided 2-isopropyl-5-methyl-2-hexenal a No Expected Sensitization Induction Level (NESIL) of 590 μg/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; 2-isopropyl-5-methyl-2-hexenal is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; 2-isopropyl-5-methyl-2-hexenal was found not to be persistent, bioaccumulative, and toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., PEC/PNEC [Predicted Environmental Concentration/Predicted No Effect Concentration]), are <1.

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Human Health Safety Assessment

Genotoxicity: Not expected to be genotoxic.	(RIFM, 2011; RIFM, 2017c; RIFM, 2016)
Repeated Dose Toxicity: NOAEL = 200 mg/kg/day.	Gaunt (1971)
Reproductive Toxicity: No NOAEL available. Exposure is below TTC.	
Skin Sensitization: NESIL = 590 μg/cm^2.	RIFM (2017b)
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic.	(UV/Vis Spectra; RIFM Database)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.	

Environmental Safety Assessment

Hazard Assessment:

Persistence:	
Screening-level: 2.88 (BIOWIN 3)	(EPI Suite v4.11; US EPA, 2012a)
Bioaccumulation:	
Screening-level: 88.71 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: Fish LC50: 11.17 mg/L	(RIFM Framework; Salvito, 2002)

Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:

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

Critical Ecotoxicity Endpoint: Fish LC50: 11.17 mg/L (RIFM Framework; Salvito, 2002)

RIFM PNEC is: 0.01117 μg/L

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

1. Identification

1. **Chemical Name:** 2-Isopropyl-5-methyl-2-hexenal
2. **CAS Registry Number:** 35158-25-9
3. **Synonyms:** 2-Hexenal, 5-methyl-2-(1-methylethyl)-; Isodihydrolavandulyl aldehyde; 2-Isopropyl-5-methyl-2-hexene-1-al; 2-Isopropyl-5-methylhex-2-enal; 2-Isopropyl-5-methyl-2-hexenal
4. **Molecular Formula:** $C_{10}H_{18}O$
5. **Molecular Weight:** 154.25
6. **RIFM Number:** 408
7. **Stereochemistry:** Isomer not specified. One stereocenter and a total of 2 isomers possible.

2. Physical data

1. **Boiling Point:** 73 °C at 10 mm Hg (Fragrance Materials Association [FMA]), 193.04 °C (EPI Suite)
2. **Flash Point:** 142 °F; CC (FMA)
3. **Log K_{OW}:** 3.46 (EPI Suite)
4. **Melting Point**: −39.34 °C (EPI Suite)
5. **Water Solubility:** 81.08 mg/L (EPI Suite)
6. **Specific Gravity:** 0.84 (FMA)
7. **Vapor Pressure:** 0.348 mm Hg at 20 °C (EPI Suite v4.0), 0.2 mm Hg at 20 °C (FMA), 0.51 mm Hg at 25 °C (EPI Suite)
8. **UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** A colorless oily liquid

3. Volume of use (Worldwide band)

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

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v1.0)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.0% (RIFM, 2017a)
2. **Inhalation Exposure*:** <0.0001 mg/kg/day or <0.0001 mg/day (RIFM, 2017a)
3. **Total Systemic Exposure**:** 0.00054 mg/kg/day (RIFM, 2017a)

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

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

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

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

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

*See the Appendix below for details.

2. Analogs Selected:
   a. **Genotoxicity:** 2-Methyl-2-pentenal (CAS # 623-36-9)
   b. **Repeated Dose Toxicity:** Hexen-2-al (CAS # 6728-26-3)
   c. **Reproductive Toxicity:** None
   d. **Skin Sensitization:** 3,7-Dimethyl-2-methylenocta-6-enal (CAS # 22418-66-2)
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. Read-across Justification: See Appendix below

7. Metabolism

No relevant data available for inclusion in this safety assessment.
Additional References: None.

8. Natural occurrence (Discrete chemical) or composition (NCS)

2-Isopropyl-5-methyl-2-hexenal is reported to occur in the following foods by the VCF*:

Cocoa category.

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

9. REACH dossier

Available; accessed 04/16/21.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for 2-isopropyl-5-methyl-2-hexenal are detailed below.

IFRA Category[b]	Description of Product Type	Maximum Acceptable Concentrations[a] in Finished Products (%)[c]
1	Products applied to the lips (lipstick)	0.045
2	Products applied to the axillae	0.014
3	Products applied to the face/body using fingertips	0.27
4	Products related to fine fragrances	0.25
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.064
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.064
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.064
5D	Baby cream, oil, talc	0.021
6	Products with oral and lip exposure	0.15
7	Products applied to the hair with some hand contact	0.52
8	Products with significant ano-genital exposure (tampon)	0.021
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.49
10A	Household care products with mostly hand contact (hand dishwashing detergent)	1.8
10B	Aerosol air freshener	1.8
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.021
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	No Restriction

Note: [a]Maximum acceptable concentrations for each product category are based on the lowest maximum acceptable concentrations (based on systemic toxicity, skin sensitization, or any other endpoint evaluated in this safety assessment). For 2-isopropyl-5-methyl-2-hexenal, the basis was the reference dose of 2 mg/kg/day, a predicted skin absorption value of 40%, and a skin sensitization NESIL of 590 μg/cm^2.

[b]For a description of the categories, refer to the IFRA RIFM Information Booklet (https://www.rifm.org/downloads/RIFM-IFRA%20Guidance-for-the-use-of-IFRA-Standards.pdf).

[c]Calculations by Creme RIFM Aggregate Exposure Model v3.1.1.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data, 2-isopropyl-5-methyl-2-hexenal does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. There are no data assessing the mutagenic/clastogenic activity of 2-isopropyl-5-methyl-2-hexenal. However, read-across can be made to 2-methyl-2-pentenal (CAS # 623-36-9; see Section VI). The mutagenic activity of 2-methyl-2-pentenal 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 and preincubation methods. *Salmonella*

typhimurium strains TA98, TA100, TA1535, TA1537, and TA102 were treated with 2-methyl-2-pentenal in dimethyl sulfoxide (DMSO) at concentrations up to 5000 μg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (RIFM, 2011). Under the conditions of the study, 2-methyl-2-pentenal was not mutagenic in the Ames test.

The clastogenic activity of 2-methyl-2-pentenal 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-methyl-2-pentenal in DMSO at concentrations up to 981 μg/mL in the dose range finding (DRF) study; micronuclei analysis was conducted at concentrations up to 500 μg/mL in the presence and absence of metabolic activation. 2-Methyl-2-pentenal did induce binucleated cells with micronuclei when tested up to cytotoxic levels in either the presence or absence of an S9 activation system (RIFM, 2017c). Under the conditions of the study, 2-methyl-2--pentenal was considered to be non-clastogenic in the *in vitro* micronucleus test. In order to verify the biological relevance of the outcome in the *in vitro* study, a follow-up *in vivo* study was also conducted. The clastogenic activity of 2-methyl-2-pentenal was evaluated in a combined *in vivo* micronucleus/COMET test conducted in compliance with GLP regulations and in accordance with OECD guidelines. The test material was administered in corn oil via oral gavage to groups of male and female Han Wistar rats. Doses of 350, 700, or 1400 mg/kg were administered. Rats from each dose level were euthanized at 48 h, and the bone marrow was extracted and examined for polychromatic erythrocytes. The liver and duodenum were evaluated for COMET analysis. The test material did not induce a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow (RIFM, 2016). Under the conditions of the study, 2-methyl-2--pentenal was considered to be not clastogenic in the *in vivo* micronucleus test.

Based on the data available, 2-methyl-2-pentenal does not present a concern for genotoxic potential, and this can be extended to 2-isopropyl-5-methyl-2-hexenal.

Additional References: None.

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

11.1.2. Repeated dose toxicity

The MOE for 2-isopropyl-5-methyl-2-hexenal is sufficient for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. There are no repeated dose toxicity data on 2-isopropyl-5-methyl-2-hexenal. Read-across material hexen-2-al (CAS # 6728-26-3; see Section VI) has sufficient data for the repeated dose toxicity endpoint. In a non-GLP and non-guideline subchronic study, 15 CFE rats/sex/dose were fed diets containing 0, 260, 640, 1600, or 4000 ppm of hexen-2-al for 13 weeks (equivalent to 0, 13, 32, 80, or 200 mg/kg/day, respectively). No treatment-related mortality was reported for any dose group. No treatment-related changes in food consumption, body weight parameters, hematology, clinical chemistry, organ weights, and histopathology were reported. There was a slight increase in male urine volume with a concurrent decrease in the specific gravity of urine at the highest dose, but there were no alterations in kidney weight or histopathology. In the high-dose group females, ovary weight was significantly increased but without any correlating histopathological changes. Hence, these effects were not considered to be treatment-related adverse effects. Based on the lack of any treatment-related adverse effects at the highest tested dose, the NOAEL for repeated dose toxicity was considered to be 4000 ppm or 200 mg/kg/day (Gaunt, 1971).

Therefore, the 2-isopropyl-5-methyl-2-hexenal MOE can be calculated by dividing the hexen-2-al NOAEL in mg/kg/day by the total systemic exposure to 2-isopropyl-5-methyl-2-hexenal, 200/0.00054, or 370370.

In addition, the total systemic exposure to 2-isopropyl-5-methyl-2-hexenal (0.54 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes, 2007) for the repeated dose toxicity endpoint of a Cramer Class I material at the current level of use.

11.1.3. Derivation of reference dose (RfD)

Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020) and a reference dose of 2 mg/kg/day.

The RIFM Criteria Document (Api, 2015) calls for a default MOE of 100 (10 × 10), based on uncertainty factors applied for interspecies (10 ×) and intraspecies (10 ×) differences. The reference dose for 2-isopropyl-5-methyl-2-hexenal was calculated by dividing the lowest NOAEL (from the Repeated Dose and Reproductive Toxicity sections) of 200 mg/kg/day by the uncertainty factor, 100 = 2 mg/kg/day.

Additional References: None.

Literature Search and Risk Assessment Completed On: 03/23/20.

11.1.4. Reproductive toxicity

There are insufficient reproductive toxicity data on 2-isopropyl-5-methyl-2-hexenal or any read-across materials. The total systemic exposure to 2-isopropyl-5-methyl-2-hexenal is below the TTC for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

11.1.4.1. Risk assessment. There are no reproductive toxicity data on 2-isopropyl-5-methyl-2-hexenal or any read-across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure (0.54 μg/kg/day) is below the TTC for 2-isopropyl-5-methyl-2-hexenal (30 μg/kg/day; Kroes, 2007; Laufersweiler, 2012) for the reproductive toxicity endpoint of a Cramer Class I material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/14/20.

11.1.5. Skin sensitization

Based on the existing data and read-across material 3,7-dimethyl-2-methylenocta-6-enal (CAS # 22418-66-2), 2-isopropyl-5-methyl-2-hexenal is considered a skin sensitizer with a defined NESIL of 590 μg/cm^2.

11.1.5.1. Risk assessment. Limited skin sensitization studies are available for 2-isopropyl-5-methyl-2-hexenal. Based on the existing data and read-across material 3,7-dimethyl-2-methylenocta-6-enal (CAS # 22418-66-2; see Section VI), 2-isopropyl-5-methyl-2-hexenal is considered a skin sensitizer. The chemical structures of these materials indicate that they would be expected to react with skin proteins directly (Roberts, 2007; Toxtree v3.1.0; OECD Toolbox v4.2). In a murine local lymph node assay (LLNA), read-across material 3,7-dimethyl-2-methylenocta-6-enal was found to be sensitizing, but an EC3 value could not be calculated due to a lack of a dose response (RIFM, 2010). In a human maximization test, no skin sensitization reactions were observed with 2-isopropyl-5-methyl-2-hexenal (RIFM, 1972a). Additionally, in 2 Confirmation of No Induction in Humans tests (CNIHs) with 590 μg/cm^2 and 696 μg/cm^2 of read-across material 3,7-dimethyl-2-methylenocta-6-enal in 1:3 ethanol:diethyl phthalate and ethanol, respectively, no reactions indicative of sensitization were observed in any of the 107 and 37 volunteers, respectively (RIFM, 2017b; RIFM, 1966). However, in another CNIH with an unstated concentration and vehicle of 2-isopropyl-5-methyl-2-hexenal, 1/16 female volunteers had a skin sensitization reaction (RIFM, 1972b).

Based on the weight of evidence (WoE) from structural analysis, human studies, and data on the read-across material 3,7-dimethyl-2-methylenocta-6-enal, 2-isopropyl-5-methyl-2-hexenal is a sensitizer with a WoE NESIL of 590 μg/cm^2 (Table 1). Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020) and a reference dose of 2 mg/kg/day.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/02/20.

11.1.6. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, 2-isopropyl-5-methyl-2-hexenal would not be expected to present a concern for phototoxicity or photoallergenicity.

11.1.6.1. Risk assessment. There are no phototoxicity studies available for 2-isopropyl-5-methyl-2-hexenal in experimental models. UV/Vis absorption spectra indicate no significant absorption between 290 and 700 nm. The corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity (Henry, 2009). Based on the lack of absorbance, 2-isopropyl-5-methyl-2-hexenal does not present a concern for phototoxicity or photoallergenicity.

11.1.7. 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, 2009).

Additional References: None.

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

11.1.8. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for 2-Isopropyl-5-methyl-2-hexenal is below the Cramer Class I TTC value for inhalation exposure local effects.

11.1.8.1. Risk assessment. There are no inhalation data available on 2-isopropyl-5-methyl-2-hexenal. Based on the Creme RIFM Model, the inhalation exposure is < 0.0001 mg/day. This exposure is at least 14000 times lower than the Cramer Class I TTC value of 1.4 mg/day (based on human lung weight of 650 g; Carthew, 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: None.

Literature Search and Risk Assessment Completed On: 04/02/20.

Table 1
Data Summary for 3,7-dimethyl-2-methylenocta-6-enal as read-across material for 2-isopropyl-5-methyl-2-hexenal.

LLNA Weighted Mean EC3 Value μg/cm^2 (No. Studies)	Potency Classification Based on Animal Data[a]	Human Data			
		NOEL-CNIH (Induction) μg/cm^2	NOEL-HMT (Induction) μg/cm^2	LOEL[b] (Induction) μg/cm^2	WoE NESIL[c] μg/cm^2
N/A	**N/A**	590	6900	N/A	590

NOEL = No observed effect level; CNIH = Confirmation of No Induction in Humans test; HMT = Human Maximization Test; LOEL = lowest observed effect level; NA = Not Available.

[a] Based on animal data using classification defined in ECETOC, Technical Report No. 87, 2003.

[b] Data derived from CNIH or HMT.

[c] WoE NESIL limited to 2 significant figures.

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of 2-isopropyl-5-methyl-2-hexenal was performed following the RIFM Environmental Framework (Salvito, 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log K_{OW}, and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity, as discussed in Salvito et al. (2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 2012b), which provides chemical class-specific ecotoxicity estimates. Finally, if necessary, Tier 3 is conducted using measured biodegradation and ecotoxicity data to refine the RQ, thus allowing for lower PNEC uncertainty factors. The data for calculating the PEC and PNEC for this safety assessment are provided in the table below. For the PEC, the range from the most recent IFRA Volume of Use Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, 2-isopropyl-5-methyl-2-hexenal was identified as a fragrance material with no potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC <1).

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) did not identify 2-isopropyl-5-methyl-2-hexenal 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, 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).

11.2.1.1. Risk assessment. Based on the current Volume of Use (2015), 2-isopropyl-5-methyl-2-hexenal presents no risk to the aquatic compartment in the screening-level assessment.

11.2.2. Key studies

11.2.2.1. Biodegradation. No data available.

11.2.2.2. Ecotoxicity. No data available.

11.2.2.3. Other available data. 2-Isopropyl-5-methyl-2-hexenal has been registered for REACH with no additional information available at this time.

11.2.2.4. 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

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	11.17			1000000	0.01117	

Environmental Framework: Salvito, 2002)

Exposure	Europe (EU)	North America (NA)
Log K_{OW} Used	3.46	3.46
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	<1	<1
Risk Characterization: PEC/PNEC	**<1**	**<1**

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

The RIFM PNEC is 0.01117 μg/L. The revised PEC/PNECs for EU and NA are not applicable. The material was cleared at the screening-level; therefore, it does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 03/26/20.

12. Literature Search*

- **RIFM Database:** Target, Fragrance Structure-Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** https://echa.europa.eu/
- **NTP:** https://ntp.niehs.nih.gov/
- **OECD Toolbox:** https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubMed:** https://www.ncbi.nlm.nih.gov/pubmed
- **National Library of Medicine's Toxicology Information Services:** https://toxnet.nlm.nih.gov/
- **IARC:** https://monographs.iarc.fr
- **OECD SIDS:** https://hpvchemicals.oecd.org/ui/Default.aspx
- **EPA ACToR:** https://actor.epa.gov/actor/home.xhtml
- **US EPA HPVIS:** https://ofmpub.epa.gov/oppthpv/public_search.publicdetails?submission_id=24959241&ShowComments=Yes&sqlstr=null&recordcount=0&User_title=DetailQuery%20Results&EndPointRpt=Y#submission
- **Japanese NITE:** https://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop
- **Japan Existing Chemical Data Base (JECDB):** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com
- **ChemIDplus:** https://chem.nlm.nih.gov/chemidplus/

Search keywords: CAS number and/or material names.

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 04/16/21.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. RIFM staff are employees of the Research Institute for Fragrance Materials, Inc. (RIFM). The Expert Panel receives a small honorarium for time spent reviewing the subject work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2021.112570.

Appendix

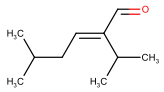
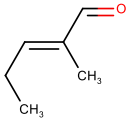
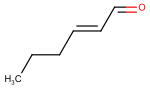
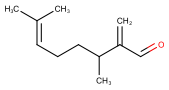
Read-across Justification

Methods

The read-across analogs were identified following the strategy for structuring and reporting a read-across prediction of toxicity as 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, 2017).

- First, materials were clustered based on their structural similarity. Second, data availability and data quality on the selected cluster were examined. Third, appropriate read-across analogs from the cluster were confirmed by expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physical–chemical properties of the target material and the read-across analogs were calculated using EPI Suite v4.11 (US EPA, 2012a).

- J_{max} values were calculated using RIFM's Skin Absorption Model (SAM). The parameters were calculated using the consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts, oncologic classification, ER binding, and repeat dose categorization predictions were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018), and skin sensitization was predicted using Toxtree.
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- To keep continuity and compatibility with *in silico* alerts, OECD QSAR Toolbox v4.2 was selected as the alert system.

	Target Material	Read-across Material	Read-across Material	Read-across Material
Principal Name	2-Isopropyl-5-methyl-2-hexenal	2-Methyl-2-pentenal	Hexen-2-al	3,7-Dimethyl-2-methylenocta-6-enal
CAS No.	35158-25-9	623-36-9	6728-26-3	22418-66-2
Structure				
Similarity (Tanimoto Score)		0.49	0.49	0.52
Read-across Endpoint		• Genotoxicity	• Repeated dose toxicity	• Skin sensitization
Molecular Formula	$C_{10}H_{18}O$	$C_6H_{10}O$	$C_6H_{10}O$	$C_{11}H_{18}O$
Molecular Weight	154.250	98.140	98.140	166.264
Melting Point (°C, EPI Suite)	−39.34	−64.41	−55.63	−26.86
Boiling Point (°C, EPI Suite)	193.04	136.50	146.50	217.71
Vapor Pressure (Pa @ 25°C, EPI Suite)	67.99422	994.58212	629.27984	1.96E+01
Log Kow (KOWWIN v1.68 in EPI Suite)	3.46	1.64	1.58	2.76E+01
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	81.08	4710.00	5261.00	3.94
J_{max} (mcg/cm^2/h, SAM)	9.74	210.24	215.10	3.89
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	36.67965	11.85503	4.95479	4.29E+01
Genotoxicity				
DNA Binding (OASIS v1.4, QSAR Toolbox v4.2)	AN2|AN2 ≫ Nucleophilic addition to α,β-unsaturated carbonyl compounds|AN2 ≫ Nucleophilic addition to α,β-unsaturated carbonyl compounds ≫ α,β-Unsaturated Aldehydes|AN2 ≫ Schiff base formation|AN2 ≫ Schiff base formation ≫ α,β-Unsaturated Aldehydes	AN2|AN2 ≫ Nucleophilic addition to α,β-unsaturated carbonyl compounds|AN2 ≫ Nucleophilic addition to α,β-unsaturated carbonyl compounds ≫ α,β-Unsaturated Aldehydes|AN2 ≫ Schiff base formation|AN2 ≫ Schiff base formation ≫ α,β-Unsaturated Aldehydes		
DNA Binding (OECD QSAR Toolbox v4.2)	Michael addition|Michael addition ≫ Polarized Alkenes-Michael addition|Michael addition ≫ Polarized Alkenes-Michael addition ≫ α,β-unsaturated aldehydes	Michael addition|Michael addition ≫ Polarized Alkenes-Michael addition|Michael addition ≫ Polarized Alkenes-Michael addition ≫ α,β- unsaturated aldehydes		
Carcinogenicity (ISS)	α,β-unsaturated carbonyls (Genotox)|Structural alert for genotoxic carcinogenicity	α,β-unsaturated carbonyls (Genotox)|Structural alert for genotoxic carcinogenicity		
DNA Binding (Ames, MN, CA, OASIS v1.1)	No alert found	AN2|AN2 ≫ Nucleophilic addition to α,β-unsaturated carbonyl compounds|AN2 ≫ Nucleophilic addition to α,β-unsaturated carbonyl compounds ≫ α,β-unsaturated aldehydes|AN2 ≫ Schiff base formation|AN2 ≫ Schiff base formation ≫ α,β-unsaturated aldehydes		
***In Vitro* Mutagenicity (Ames, ISS)**	α,β-unsaturated carbonyls	α,β-unsaturated carbonyls		
***In Vivo* Mutagenicity (Micronucleus, ISS)**	α,β-unsaturated carbonyls	α,β-unsaturated carbonyls		
Oncologic Classification	Aldehyde-type Compounds	Aldehyde-type Compounds		
Repeated Dose Toxicity				
Repeated dose (HESS)	Not categorized		Not categorized	

(*continued on next page*)

(*continued*)

	Target Material	Read-across Material	Read-across Material	Read-across Material
Skin Sensitization				
Protein Binding (OASIS v1.1)	Michael addition|Michael addition ≫ Michael addition on α,β-Unsaturated carbonyl compounds|Michael addition ≫ Michael addition on α,β-Unsaturated carbonyl compounds ≫ α,β-Aldehydes | Schiff base formation|Schiff base formation ≫ Schiff base formation with carbonyl compounds|Schiff base formation ≫ Schiff base formation with carbonyl compounds ≫ Aldehydes			Michael addition|Michael addition ≫ Michael addition on α,β-Unsaturated carbonyl compounds|Michael addition ≫ Michael addition on α,β-Unsaturated carbonyl compounds ≫ α,β-Aldehydes | Schiff base formation|Schiff base formation ≫ Schiff base formation with carbonyl compounds|Schiff base formation ≫ Schiff base formation with carbonyl compounds ≫ Aldehydes
Protein binding (OECD)	Michael addition|Michael addition ≫ Polarized Alkenes|Michael addition ≫ Polarized Alkenes ≫ Polarized alkene - aldehydes|Schiff Base Formers|Schiff Base Formers ≫ Direct Acting Schiff Base Formers|Schiff Base Formers ≫ Direct Acting Schiff Base Formers ≫ Mono-carbonyls			Michael addition|Michael addition ≫ Polarized Alkenes|Michael addition ≫ Polarized Alkenes ≫ Polarized alkene - aldehydes|Schiff Base Formers|Schiff Base Formers ≫ Direct Acting Schiff Base Formers|Schiff Base Formers ≫ Direct Acting Schiff Base Formers ≫ Mono-carbonyls
Protein Binding Potency	Moderately reactive (GSH)|Moderately reactive (GSH) ≫ Substituted 2-Alken-1-als (MA)			Moderately reactive (GSH)|Moderately reactive (GSH) ≫ Substituted 2-Alken-1-als (MA)
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	Michael Addition|Michael Addition ≫ Michael addition on α,β-Unsaturated carbonyl compounds|Michael Addition ≫ Michael addition on α,β-Unsaturated carbonyl compounds ≫ α,β-Aldehydes			Michael Addition|Michael Addition ≫ Michael addition on α,β-Unsaturated carbonyl compounds|Michael Addition ≫ Michael addition on α,β-Unsaturated carbonyl compounds ≫ α,β-Aldehydes
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	Alert for Schiff base formation identified.			Alert for Michael Acceptor identified.
Metabolism				
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	See Supplemental Data 1	See Supplemental Data 2	See Supplemental Data 3	See Supplemental Data 4

Summary

There are insufficient toxicity data on 2-isopropyl-5-methyl-2-hexenal (CAS # 35158-25-9). 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, 2-methyl-2-pentenal CAS # 623-36-9), hexen-2-al (CAS # 6728-26-3), and 3,7-dimethyl-2-methylenocta-6-enal (CAS # 22418-66-2) were identified as read-across analogs with sufficient data for toxicological evaluation.

Conclusions

- 2-Methyl-2-pentenal CAS # 623-36-9) was used as a read-across analog for the target material 2-methyl-2-pentenal (CAS # 623-36-9) for the genotoxicity endpoint.
  - o The target material and the read-across analog are structurally similar and belong to a class of α,β-unsaturated aldehydes.
  - o The key difference between the target material and the read-across analog is that the target material has a methyl position on the α-carbon, which will sterically hinder the Michael reaction, while the target material has α-as well as a β-carbon unsubstitutions, which makes it more reactive compared to the target material.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The difference of log Kow ~1.5 will not affect the reactivity/toxicity of the read-across analog compared to the target material. With the lower log Kow (higher solubility), the read-across analog is expected to have higher bioavailability than the target material. The reactive sub-structural fractures, activated (α,β-unsaturated) aldehydes, are the same between the read-across analog and the target material. Therefore, the read-across analog will be more reactive/toxic via the same MOA compared to the target material. Thus, the physical-chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - o According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - o The target material and the read-across analog do not have alerts for nucleophilic addition to the carbonyl carbon, Michael addition, or Schiff base formation. The data on the read-across analog confirm that the material does not pose a concern for genetic toxicity. Therefore, based on the structural similarity between the target chemical and the read-across analog, and the data for the read-across analog, the *in silico* alerts are consistent.
  - o The target material 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.

- Hexen-2-al (CAS # 6728-26-3) was used as a read-across analog for the target material 2-methyl-2-pentenal (CAS # 623-36-9) for the repeated dose toxicity endpoint.
  - o The target material and the read-across analog are structurally similar and belong to a class of α,β-unsaturated aldehydes.
  - o The target material and the read-across analog share an α,β-unsaturated aldehyde with an α-substitution as a common feature.
  - o The key difference between the target material and the read-across analog is in the chain length of the aliphatic hydrocarbon fragment attached to the aldehyde as well as the substitution on the α-carbon. In the target material, both of these fragments are branched while in the read-across analog they are a straight chain. This structural difference is toxicologically insignificant.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The difference of log K_{ow} ~1.5 will not affect the reactivity/toxicity of the read-across analog compared to the target material. With the lower log Kow (higher solubility), the read-across analog is expected to have higher bioavailability than the target material. The reactive sub-structural fractures, activated (α,β-unsaturated) aldehydes, are the same between the read-across analog and the target material. Therefore, the read-across analog will be more reactive/toxic via the same MOA compared to the target material. Thus, the physical-chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - o According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - o The target material and the read-across analog do not have alerts for repeated dose toxicity. The MOE of the read-across analog is adequate at the current level of use. Therefore, the *in silico* alerts are consistent with the data.
  - o The target material 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.
- 3,7-Dimethyl-2-methylenocta-6-enal (CAS # 22418-66-2) was used as a read-across analog for the target material 2-methyl-2-pentenal (CAS # 623-36-9) for the skin sensitization endpoint.
  - o The target material and the read-across analog are structurally similar and belong to a class of α,β-unsaturated aldehydes.
  - o The target material and the read-across analog share an α,β-unsaturated aldehyde with an α-substitution as a common feature.
  - o The key difference between the target material and the read-across analog is that the α,β unsaturation in the target material is vinylene while that in the read-across analog is a vinyl group. With this difference, the read-across analog is predicted to be more reactive compared to the target material.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The difference of log K_{ow} ~1.5 will not affect the reactivity/toxicity of the read-across analog compared to the target material. With the lower log Kow (higher solubility), the read-across analog is expected to have higher bioavailability than the target material. The reactive sub-structural fractures, activated (α,β-unsaturated) aldehydes, are the same between the read-across analog and the target material. Therefore, the read-across analog will be more reactive/toxic via the same MOA compared to the target material. Thus, the physical-chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
  - o According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - o The target material and the read-across analog have alerts for a Michael addition reaction and Schiff base formation. The limited data for the target material and the sufficient data for the read-across analog confirm that the target material is a skin sensitizer. Therefore, the *in silico* alerts are consistent with the data.
  - o The target material 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.
- Explanation of Cramer Classification:

Due to potential discrepancies with the current *in silico* tools (Bhatia et al., 2015), the Cramer class of the target material was determined using expert judgment based on the Cramer decision tree (Cramer et al., 1978).

1N,2N,3N,43N,5N,6N,42N,7N,16Y.

Q1. A normal constituent of the body? No.
Q2. Contains functional groups associated with enhanced toxicity? No.
Q3. Contains elements other than C, H, O, N, and divalent S? No.
Q43. Possibly harmful divalent sulfur? No.
Q5. Simply branched aliphatic hydrocarbon or a common carbohydrate? No.
Q6. Benzene derivative with certain substituents? No.
Q42. Possibly harmful analogue of benzene? No.
Q7. Heterocyclic? No.
Q16. Common terpene (See Cramer et al., 1978 for detailed explanation) Yes. Class I (Class Low)

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