Food and Chemical Toxicology 163 (2022) 112974

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

RIFM fragrance ingredient safety assessment, isoeugenyl acetate, CAS Registry Number 93-29-8

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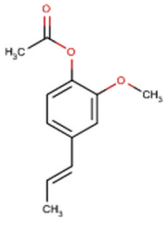
ARTICLE INFO

Handling Editor: Dr. Jose Luis Domingo

Version: 092021. 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: fragrancematerialsafetyresource.elsevier.com.

Name: Isoeugenyl acetate CAS Registry Number: 93-29-8



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Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
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.2022.112974
Received 30 September 2021; Received in revised form 29 November 2021; Accepted 27 March 2022
Available online 31 March 2022
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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; Safford et al., 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DRF - Dose Range Finding
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest 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 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 existing information supports the use of this material as described in this safety assessment.

Isoeugenyl acetate was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that isoeugenyl acetate is not genotoxic. Data on read-across materials isoeugenol (CAS # 97-54-1) and acetic acid (CAS # 64-19-7) provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose and reproductive toxicity endpoints. Data provide isoeugenyl acetate a defined No Expected Sensitization Induction Level (NESIL) of 2300 μg/cm^2 for the skin sensitization endpoint. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet/visible spectra (UV/Vis) spectra; isoeugenyl acetate is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class I material, and the exposure to isoeugenyl acetate is below the TTC (1.4 mg/day). The environmental endpoints were evaluated; isoeugenyl acetate was found not to be Persistent, Bioaccumulative, and Toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

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Human Health Safety Assessment	
Genotoxicity: Not genotoxic.	(RIFM, 2002a; RIFM, 2015a)
Repeated Dose Toxicity: NOAEL = 7.5 mg/kg/day.	NTP (2010)
Reproductive Toxicity: Developmental toxicity: NOAEL = 500 mg/kg/day. Fertility: NOAEL = 230 mg/kg/day	(NTP, 1999; NTP, 2002)
Skin Sensitization: NESIL = 2300 μg/cm^2.	(RIFM, 2000a; RIFM, 2000b)
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: 2.8 (BIOWIN 3)	(EPI Suite v4.11; US EPA, 2012a)
Bioaccumulation: Screening-level: 43.31 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Screening-level: 96-h algae EC50: 4.99 mg/L	(ECOSAR; US ECHA, 2012b)
Conclusion: Not PBT 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: 96-h Algae EC50: 4.99 mg/L	(ECOSAR; US ECHA, 2012b)
RIFM PNEC is: 0.499 μg/L	
• **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe <1	

1. Identification

1. **Chemical Name:** Isoeugenyl acetate
2. **CAS Registry Number:** 93-29-8
3. **Synonyms:** Acetisoeugenol; 4-Acetoxy-3-methoxy-1-(1-propen-1-yl) benzene; Acetyl iso-eugenol; Acetyl isoeugenol; Isoeugenol acetate; 2-Methoxy-4-propenylphenyl acetate; Phenol, 2-methoxy-4-(1-propenyl)-, acetate; 3-ﾒﾄｷｼｺｰ4-ｱｾﾄｷｼﾌﾞﾛﾍﾞﾝｾﾞﾝ; 2-Methoxy-4-prop-1-en-1-ylphenyl acetate; Acetyl Isoeugenol Crystals; Isoeugenyl acetate
4. **Molecular Formula:** $C_{12}H_{14}O_3$
5. **Molecular Weight:** 206.24
6. **RIFM Number:** 455
7. **Stereochemistry:** Isomer not specified. One geometric center and 2 total geometric isomers possible.

2. Physical data

1. **Boiling Point:** 282 °C (Fragrance Materials Association [FMA]), 290.44 °C (EPI Suite)
2. **Flash Point:** >212 °F; CC (FMA)
3. **Log K_{OW}:** 2.99 (EPI Suite)
4. **Melting Point**: 59.35 °C (EPI Suite)
5. **Water Solubility:** 114.7 mg/L (EPI Suite)
6. **Specific Gravity:** 1.087 (SLR)
7. **Vapor Pressure:** 0.000839 mm Hg at 20 °C (EPI Suite v4.0), 0.00155 mm Hg at 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:** White crystals or powder having a weak rose-carnation odor

3. Volume of use (worldwide band)

1. **Volume of Use (worldwide band):** 10–100 metric tons per year (IFRA, 2015)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v3.0.4)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.11% (RIFM, 2019)
2. **Inhalation Exposure*:** 0.00019 mg/kg/day or 0.014 mg/day (RIFM, 2019)
3. **Total Systemic Exposure**:** 0.0021 mg/kg/day (RIFM, 2019)

*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 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 et al., 2015; Safford et al., 2015; Safford et al., 2017; and Comiskey et al., 2017).

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

6.1. *Cramer Classification: Class I, Low*

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

6.2. *Analogs Selected*

a. **Genotoxicity:** None
b. **Repeated Dose Toxicity:** Isoeugenol (CAS # 97-54-1); acetic acid (CAS # 64-19-7)
c. **Reproductive Toxicity:** Isoeugenol (CAS # 97-54-1); acetic acid (CAS # 64-19-7)
d. **Skin Sensitization:** None
e. **Phototoxicity/Photoallergenicity:** None
f. **Local Respiratory Toxicity:** None
g. **Environmental Toxicity:** None

6.3. *Read-across justification: See Appendix below*

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7. Metabolism

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

8. Natural occurrence

Isoeugenyl acetate has not been reported to occur in food by the VCF*.

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

9. REACH dossier

Pre-registered for 2010; no dossier available as of 09/20/21.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for isoeugenyl acetate 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)	0.18
2	Products applied to the axillae	0.053
3	Products applied to the face/body using fingertips	0.061
4	Products related to fine fragrances	0.99
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.25
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.061
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.082
5D	Baby cream, oil, talc	0.020
6	Products with oral and lip exposure	0.020
7	Products applied to the hair with some hand contact	0.061
8	Products with significant ano-genital exposure (tampon)	0.020
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.20
10A	Household care products with mostly hand contact (hand dishwashing detergent)	0.061
10B	Aerosol air freshener	0.45
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.020
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	16

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 isoeugenyl acetate, the basis was the reference dose of 0.075 mg/kg/day, a predicted skin absorption value of 40%, and a skin sensitization NESIL of 2300 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; December 2019).
[c]Calculations by Creme RIFM Aggregate Exposure Model v3.0.5.

Summary

Human Health Endpoint Summaries

Genotoxicity

Based on the current existing data and use levels isoeugenyl acetate does not present a concern for genotoxic potential.

Risk assessment. Isoeugenyl acetate was assessed in the BlueScreen assay and found positive for both cytotoxicity (positive: <80% relative cell density) and genotoxicity, with and without metabolic activation (RIFM, 2015b). BlueScreen is a human cell-based assay for measuring the genotoxicity and cytotoxicity of chemical compounds and mixtures. Additional assays were considered to fully assess the potential mutagenic or clastogenic effects of the target material.

The mutagenic activity of isoeugenyl acetate 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 method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102 were treated with isoeugenyl acetate in ethanol 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, 2002a). Under the conditions of the study, isoeugenyl acetate was not mutagenic in the Ames test.

The clastogenicity of isoeugenyl acetate was assessed 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 isoeugenyl acetate in dimethyl sulfoxide (DMSO) at concentrations up to 2060 μg/mL in the dose range finding (DRF) study. Micronuclei analysis was conducted at 700 μg/mL in the presence and absence of S9 metabolic activation for 4 h and in the absence of metabolic activation for 24 h. Isoeugenyl acetate did not induce binucleated cells with micronuclei when tested up to cytotoxic levels in either the presence or absence of an S9 activation system (RIFM, 2015a). Under the conditions of the study, isoeugenyl acetate was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the available data, isoeugenyl acetate does not present a concern for genotoxic potential.

Additional References: RIFM, 2002b.

Literature Search and Risk Assessment Completed On: 06/09/21.

Repeated dose toxicity

The MOE for isoeugenyl acetate is adequate for the repeated dose toxicity endpoint at the current level of use.

Risk assessment. There are no repeated dose toxicity data on isoeugenyl acetate. The target material, isoeugenyl acetate, is expected to hydrolyze to isoeugenol (CAS # 97-54-1; see Section VI) and acetic acid (CAS # 64-19-7; see Section VI). The hydrolysis product, acetic acid, has been reviewed by several agencies including the US Food and Drug Administration (US FDA, 2018), and has been granted a generally recognized as safe (GRAS) status. In 2004, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated acetic acid and suggested that establishing acceptable daily human intake limits for acetic acid is not necessary (WHO, 2004; accessed 12/18/18). The European food safety authority (EFSA) reviewed the data on acetic acid in their "Scientific Opinion on the safety and efficacy of acetic acid, sodium diacetate, and calcium acetate as preservatives for feed for all animal species" (EFSA, 2012) and declared that there is an application for reauthorizing acetic acid and these salts as preservatives in feed as well as a preservative (acetic acid) in drinking water. These salts may be used alone or in combination with other organic acids typically at a concentration of 200–2500 mg acetate/kg. The Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS) provides a comprehensive review of the toxicity data on acetic acid as a part of their Human health Tier II assessment for acetic acid (NICNAS, 2018). The review establishes that acetates are normal components in human and animal diets and are produced daily in relatively small (molar) quantities in the gastrointestinal tract, where they are rapidly and completely metabolized. Acetate is produced as a major intermediate in normal metabolic processes. Various isotope experiments have shown that the different carbon atoms of acetic acid are used in glycogen formation as intermediates of carbohydrates and fatty acid synthesis as well as in cholesterol synthesis. In addition, acetic acid also participates in the acetylation of amines and the formation of proteins of plasma, liver, kidney, gut mucosa, muscle, and brain. Acetic acid is absorbed from the lungs and gastrointestinal tract. Following absorption, acetic acid is almost completely metabolized by most tissues and produces ketone bodies as intermediates. The level of the acetate ion in humans has been estimated at about 50–60 μmol/L (3.0–3.6 mg/L) in plasma and 116 μmol/L (7 mg/L) in cerebrospinal fluid. The daily turnover of the acetate ion in humans is estimated at about 7.5 μmol/kg/min representing about 45 g/day. Based on the treatment-related effects reported in limited repeated dose toxicity studies, acetic acid is not considered to cause any serious damage to health from repeated oral exposure. The effects observed in some cases are predominantly due to the corrosive activity of acetic acid. Results from repeated human (oral, inhalation, and dermal) exposure to acetic acid have been reported with gastrointestinal tract effects, digestive disorders including heartburn and constipation, chronic inflammation of the respiratory tract, pharyngitis, catarrhal bronchitis, darkening of the skin, skin dermatitis, and erosion of the exposed front teeth enamel. In addition, the skin on the palms of the hands can become dry, cracked, and hyperkeratotic. These observed effects were not associated with any systemic findings, further suggesting the effects observed are due to its corrosive activity. Based on the limited data available, acetic acid does not pose a carcinogenic risk. Thus, acetic acid does not pose systemic toxicity to human health when used in fragrances.

Read-across material isoeugenol (CAS # 97-54-1; see Section VI) has sufficient repeated dose toxicity data. In a DRF study, 10 F344N rats/sex/dose were administered isoeugenol by oral gavage at doses of 0, 37.5, 75, 150, 300, or 600 mg/kg 5 days per week for 14 weeks. All animals survived the study duration except 1 animal each in the 37.5 mg/kg (female) and 600 mg/kg (male) groups that reportedly died due to gavage errors. In males, mean body weight was significantly reduced in all treatment groups. At doses of 150 mg/kg/day and higher, alterations of the liver and nasal epithelium were reported. Based on the effects observed at doses of 150 mg/kg/day or higher, a conservative NOAEL of 75 mg/kg/day was established for this study (NTP, 2010).

In a 2-year NTP study, 50 F344N rats/sex/dose were orally administered isoeugenol in corn oil by gavage at doses of 0, 75, 150, and 300 mg/kg/day 5 days per week for 105 weeks. No treatment-related deaths were reported during the study in either sex. Contrary to the DRF study, male mean body weights of animals in the 300 mg/kg/day group were significantly higher than control animals. In the highest-dose group, 2 male rats were reported to have thymomas along with the other 2 males demonstrating rare mammary gland carcinomas. In animals of the 150 and 300 mg/kg/day dose groups, minimal atrophy and minimal to mild respiratory metaplasia of the olfactory epithelium were reported in either sex. In males, increased incidences of minimal to mild olfactory epithelial degeneration at 300 mg/kg/day dose were also reported. Incidences of minimal to mild atrophy as well as respiratory metaplasia of the olfactory epithelium were higher at 75 mg/kg (males) and significantly increased at 150 mg/kg (males) and 300 mg/kg (both sexes). Incidences of basophilic focus were significantly decreased in all groups (males only), while eosinophilic focus incidences were significantly decreased in males receiving the 75 and 150 mg/kg doses. In 300 mg/kg males, incidences of clear cell focus and bile duct hyperplasia also decreased significantly. However, these decreases were not observed in

female rats. Under the study conditions, the NTP considers there is equivocal evidence of carcinogenicity in male rats. Since significant adverse effects are observed at the lowest dose, a LOAEL for repeated dose toxicity was determined to be 75 mg/kg/day (NTP, 2010). A default safety factor of 10 is used to convert LOAEL to NOAEL. **Thus, the NOAEL for repeated dose toxicity endpoint is 7.5 mg/kg/day.**

Therefore, the MOE is equal to the isoeugenol NOAEL in mg/kg/day divided by the total systemic exposure of isoeugenyl acetate, 7.5/0.0021 or 3571.

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 0.075 mg/kg/day.

The RIFM Criteria Document (Api et al., 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 isoeugenol acetate was calculated by dividing the lowest NOAEL (from the Repeated Dose and Reproductive Toxicity sections) of 7.5 mg/kg/day by the uncertainty factor, 100 = 0.075 mg/kg/day.

In addition, the total systemic exposure to isoeugenyl acetate (2.1 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007) of a Cramer Class I material for the repeated dose toxicity endpoint at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed On: 06/03/21.

Reproductive toxicity

The MOE for isoeugenyl acetate is adequate for the reproductive toxicity endpoint at the current level of use.

Risk assessment. There are no developmental or reproductive toxicity data on isoeugenyl acetate. Isoeugenyl acetate is expected to hydrolyze to isoeugenol (CAS # 97-54-1; see Section VI) and acetic acid (CAS # 64-19-7; see Section VI). Based on the available data on acetic acid (EFSA, 2012; NICNAS, 2018; US FDA, 2018; WHO, 2004), acetic acid does not show specific developmental and reproductive toxicity. Thus, acetic acid does not pose any systemic (repeated dose), developmental, or reproductive toxicity to human health when used in fragrances.

Read-across material isoeugenol (CAS # 97-54-1; see Section VI) has sufficient developmental and reproductive toxicity data. In a GLP-compliant NTP developmental toxicity study, isoeugenol was administered via oral gavage at doses of 0, 250, 500, or 1000 mg/kg/day in corn oil to pregnant female Sprague Dawley rats (25 dams/group) on gestation days (GDs) 6–19. High incidences of aversion to treatment (i.e., rooting behavior) were noted in all treatment group dams. A dose-related statistically significant decrease in maternal bodyweight gain and gestational weight gain was reported at all dose levels. A statistically significant decrease in food consumption was reported at 1000 mg/kg/day. The gravid uterine weight was significantly decreased among 500 and 1000 mg/kg/day dose group dams. A statistically significant decrease in body weight and a statistically significant increase in the incidence of non-ossified sternebrae were reported in 1000 mg/kg/day dose group pups. The LOAEL for maternal toxicity was considered to be 250 mg/kg/day, based on reduced body weight, gestational weight gain, and aversion to treatment. The NOAEL for developmental toxicity was considered to be 500 mg/kg/day, based on decreased pup body weight and increased incidences of non-ossified sternebrae among high-dose group pups (NTP, 1999; George et al., 2001).

Therefore, the isoeugenyl acetate MOE for the developmental toxicity endpoint can be calculated by dividing the isoeugenol NOAEL in mg/kg/day by the total systemic exposure to isoeugenyl acetate, 500/0.0021 or 238095.

In a GLP-compliant NTP multigenerational continuous breeding study, isoeugenol was administered via oral gavage to Sprague Dawley rats (20 animals/sex/group) (F0) at doses of 0, 70, 230, or 700 mg/kg/day in corn oil from 1 week prior to mating to study day 179. One of 3 litters (F1) from each dose group was dosed starting on postnatal day (PND) 21 until necropsy on PND 186. This litter was assigned to mating at approximately PND 80 and produced F2 litters. Mortality in F0 was as follows: 2 males at 70 mg/kg/day; 1 male and 2 females at 230 mg/kg/day; 1 male and 8 females at 700 mg/kg/day. Under the conditions of this study, isoeugenol produced evidence of non-reproductive toxicity at all dose levels as reported by the presence of hyperkeratosis and hyperplasia in the non-glandular stomachs and decreased body weights of F0 and F1 animals (230 mg/kg/day males and 700 mg/kg/day, both sexes). Sperm parameters and vaginal cytology were unaffected in the F0 and F1 generations. A statistically significant decrease in live male pups of the F1 generation and a statistically significant decrease in F1 pup weight were seen at 700 mg/kg/day. An outbreeding study showed that the decrease in live male pups was potentially due to reproductive toxicity in females. Gross necropsy showed no significant changes. Therefore, the NOAEL for developmental toxicity and fertility was considered to be 230 mg/kg/day, based on a decreased number of male pups per litter during the F0 cohabitation and decreased male and female pup weights during the F1 cohabitation among high-dose group animals (NTP, 2002; Layton et al., 2001).

Based on the toxic effects reported at the highest dose levels in both reproductive toxicity studies, a NOAEL of 230 mg/kg/day was selected from the multi-generation study for the fertility endpoint. **Therefore, the isoeugenyl acetate MOE for the reproductive toxicity endpoint can be calculated by dividing the isoeugenol NOAEL in mg/kg/day by the total systemic exposure to isoeugenyl acetate, 230/0.0021 or 109523.**

In addition, the total systemic exposure to isoeugenyl acetate (2.1 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 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: 06/24/21.

Skin sensitization

Based on the available data, isoeugenyl acetate is considered to be a skin sensitizer with a defined NESIL of 2300 μg/cm^2.

Risk assessment. Based on the existing data, isoeugenyl acetate is considered a sensitizer with a NESIL of 2300 μg/cm^2. The chemical structure of this material indicates that it would be expected to react with skin proteins (Roberts et al., 2007; Toxtree v3.1.0). In a murine local lymph node assay (LLNA), isoeugenyl acetate was not found to be sensitizing up to 25% (6250 μg/cm^2) (RIFM, 2005). In a guinea pig closed epicutaneous test (CET) isoeugenyl acetate showed reactions indicative of sensitization (Itoh, 1982; Ishihara et al., 1986). However, in a guinea pig open epicutaneous test (OET), isoeugenyl acetate did not present reactions indicative of sensitization (Klecak, 1985). In human maximization tests, no skin sensitization reactions were observed when isoeugenyl acetate in petrolatum was tested at 10% (6900 μg/cm^2) and 15% (10350 μg/cm^2) (RIFM, 1974; RIFM, 1973). Additionally, in 2 Confirmation of No Induction in Humans tests (CNIHs) with 2362 μg/cm^2 (2%) of isoeugenyl acetate in 1:3 diethyl phthalate:ethanol, no reactions indicative of sensitization were observed in any of the 54 and 49 volunteers, respectively (RIFM, 2000a).

Based on weight of evidence (WoE) from structural analysis and animal and human studies, isoeugenyl acetate is a sensitizer with a WoE NESIL of 2300 μg/cm^2 (see Table 1). Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment

Table 1
Data summary for isoeugenyl acetate.

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
>6250 [1]	NA	2362	10350	NA	2300

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.

(QRA2) described by Api et al. (RIFM, 2020) and a reference dose of 0.075 mg/kg/day.

Additional References: Opdyke (1975).

Literature Search and Risk Assessment Completed On: 06/24/21.

Phototoxicity/photoallergenicity

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

Risk assessment. There are no phototoxicity studies available for isoeugenyl acetate 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 the lack of significant absorbance in the critical range, isoeugenyl acetate does not present a concern for phototoxicity or photoallergenicity.

UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) for isoeugenyl acetate 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: 06/03/21.

Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for isoeugenyl acetate is below the Cramer Class I TTC value for inhalation exposure local effects.

Risk assessment. There are insufficient inhalation data available on isoeugenyl acetate. Based on the Creme RIFM Model, the inhalation exposure is 0.014 mg/day. This exposure is 100 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: Buchbauer et al., 1993; Schnuch et al., 2010; RIFM, 2013.

Literature Search and Risk Assessment Completed On: 06/24/21.

Environmental Endpoint Summary

Screening-level assessment

A screening-level risk assessment of isoeugenyl acetate 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, isoeugenyl acetate was identified as a fragrance material with the 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 isoeugenyl acetate as possibly persistent or bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11).

Risk assessment

Based on current VoU (2015), isoeugenyl acetate presents a risk to the aquatic compartment in the screening-level assessment.

Key studies

Biodegradation. No data available.

Ecotoxicity. No data available.

Other available data. Isoeugenyl acetate has been pre-registered for REACH with no additional data at this time.

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 Framework: Salvito et al., 2002).

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	2.99	2.99
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	10–100	10–100
Risk Characterization: PEC/PNEC	**<1**	**<1**

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	38.28			1000000	0.03828	
ECOSAR Acute Endpoints **(Tier 2)** v1.11	7.330	13.71	4.993	10000	0.4993	Neutral Organics
ECOSAR Acute Endpoints **(Tier 2)** v1.11	22.09	13.73	14.90			Neutral Organics SAR (Baseline Toxicity)

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

The RIFM PNEC is 0.499 μg/L. The revised PEC/PNECs for EU and NA are <1; 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: 06/21/21.

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 09/20/21.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2022.112974.

Appendix

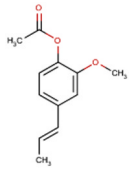
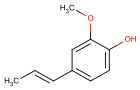
Read-across Justification

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 substance and the read-across analogs were calculated using EPI Suite v4.11 (USEPA, 2012).
- 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 v4.2 (OECD, 2018).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010), and skin sensitization was predicted using Toxtree v3.1.0.
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018).
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).

	Target Material	Read-across Material	Read-across Material
Principal Name	Isoeugenyl acetate	Isoeugenol	Acetic acid
CAS No.	93-29-8	97-54-1	64-19-7
Structure			
Similarity (Tanimoto Score)		N/A	N/A
Read-across Endpoint		• Reproductive toxicity • Repeated dose toxicity	• Reproductive toxicity • Repeated dose toxicity
Molecular Formula	$C_{12}H_{14}O_3$	$C_{10}H_{12}O_2$	$C_2H_4O_2$
Molecular Weight	206.24	164.21	60.05
Melting Point (°C, EPI Suite)	59.35	61.93	16.635
Boiling Point (°C, EPI Suite)	290.44	270.60	117.9
Vapor Pressure (Pa @ 25°C, EPI Suite)	0.207	0.508	2.09E+003
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	2.99	3.04	−0.17
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	114.7	165.9	1e+006
J_{max} (μg/cm^2/h, SAM)	4.994	79.642	6283.044
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	3.12E-001	2.70E-003	1.45E-002
Repeated Dose Toxicity			
Repeated Dose (HESS)	• Not categorized	• Curcumin (renal toxicity alert)	• Acetamide (Renal Toxicity) Alert|Carboxylic acids (Hepatotoxicity) No rank
Developmental and Reproductive Toxicity			
ER Binding (OECD QSAR Toolbox v4.2)	• Non-binder, without OH or NH2 group	• Weak binder, OH group	• Non-binder, non-cyclic structure
Developmental Toxicity (CAESAR v2.1.7)	• Non-toxicant (moderate reliability)	• Non-toxicant (low reliability)	• Toxicant (low reliability)
Metabolism			
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	• See Supplemental Data 1	• See Supplemental Data 2	• No metabolites

Summary

There are insufficient toxicity data on isoeugenyl acetate, CAS # 93-29-8. Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, physical–chemical properties, and expert judgment, isoeugenol (CAS # 97-54-1) and acetic acid (CAS # 64-19-7) were identified as read-across materials with sufficient data for toxicological evaluation.

Metabolism

The metabolism of the target material isoeugenyl acetate (CAS # 93-29-8) was predicted using the Rat Liver S9 Metabolism Simulator (OECD QSAR Toolbox v4.2). The target material is predicted to be metabolized to isoeugenol in the first step with 0.95 probability. Hence, isoeugenol can be used as a read-across analog for the target material. Read-across material isoeugenol was in domain for the *in vivo* rat and in domain for the *in vitro* rat S9 simulator (OASIS TIMES v2.27.19).

Conclusions

- Isoeugenol (CAS # 97-54-1) and acetic acid (CAS # 64-19-7) were used as read-across analogs for the target material isoeugenyl acetate (CAS # 93-29-8) for the repeated dose and reproductive toxicity endpoints.
  - The read-across analog is a direct metabolite of the target material.
  - The target material and the read-across analog share a 4-propenyl anisole substructure.
  - The key structural difference between the target material and the read-across analog is that the read-across analog is the alcohol hydrolysis metabolite of the target material.

- The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable a comparison of their toxicological properties.
- According to the OECD QSAR Toolbox v4.2, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
- The target material does not show any reactivity or toxicity alerts while the read-across analog has a curcumin renal toxicity alert and a weak ER binding alert. According to these predictions, the read-across analog is expected to be more reactive compared to the target material. The data described in the repeated dose toxicity and the reproductive toxicity sections show that the MOE is adequate at the current level of use. Data supersedes predictions in this case.
- The read-across material acetic acid (CAS # 64-19-7) has an acetamide precursor renal toxicity alert and a carboxylic acid hepatotoxicity alert with no rank under the HESS categorization. The wealth of data in the literature suggests fast rates of clearance for acetic acid. Also, acetic acid is one of the natural constituents of the human metabolome according to the Human Metabolome Database (www.hmdb.ca). Therefore, the alerts for acetic acid are superseded by the data.
- The target material 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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