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Short Review

RIFM fragrance ingredient safety assessment, eugenyl methyl ether, CAS Registry Number 93-15-2

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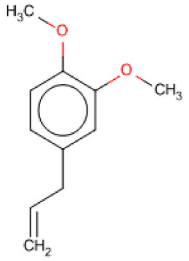
Name: Eugenyl methyl ether CAS Registry Number: 93-15-2



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., 2021)
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015, 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
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 Observed 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

(*continued on next column*)

(*continued*)

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.**

Eugenyl methyl ether was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, photoirritation/photoallergenicity, skin sensitization, and environmental safety. Data show that eugenyl methyl ether is potentially genotoxic and should be used as per the International Fragrance Association (IFRA) Standards. Data on eugenyl methyl ether provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity and reproductive toxicity endpoints. Data from read-across analog isoeugenyl methyl ether (CAS # 93-16-3) provided eugenyl ethyl ether a No Expected Sensitization Induction Level (NESIL) of 9400 μg/cm^2 for the skin sensitization endpoint. The photoirritation/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; eugenyl methyl ether is not expected to be photoirritating/photoallergenic. The local respiratory toxicity endpoint was evaluated using the Threshold of Toxicological Concern (TTC) for a Cramer Class III material, and the exposure to eugenyl methyl ether is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; eugenyl methyl ether was found not to be Persistent, Bioaccumulative, and Toxic (PBT) as per the IFRA Environmental Standards, and its risk quotients, based on its current volume of use in Europe and North America (i.e., Predicted Environmental Concentration/Predicted No Effect Concentration [PEC/PNEC]), are <1.

Human Health Safety Assessment

Genotoxicity: Potentially genotoxic but safe under the current conditions of use.	(NTP, 2000; Witt et al., 2000)
Repeated Dose Toxicity: BMDL10 = 7.7 mg/kg/day.	NTP (2000)
Developmental and Reproductive Toxicity: NOAEL = 200 and 30 mg/kg/day, respectively.	(NTP, 2004; NTP, 2000)
Skin Sensitization: NESIL = 9400 μg/cm^2.	RIFM (2018b)
Photoirritation/Photoallergenicity: Not expected to be photoirritating/photoallergenic.	(UV/Vis Spectra, RIFM Database)
Local Respiratory Toxicity: No NOAEC Available. Exposure is below the TTC.	

Environmental Safety Assessment

Hazard Assessment:

Persistence: Critical Measured Value: 92.1% (OECD 301B)	(ECHA REACH Dossier: 4-Allylveratrole; ECHA, 2018)
Bioaccumulation: Screening-level: 46.44 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Screening-level: Fish LC50: 107.9 mg/L	(RIFM Framework; Salvito et al., 2002)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards	

Risk Assessment:

Screening-level: PEC/PNEC (North America and Europe) < 1	(RIFM Framework; Salvito et al., 2002)
Critical Ecotoxicity Endpoint: Fish LC50: 107.9 mg/L	(RIFM Framework; Salvito et al., 2002)

RIFM PNEC is: 0.1079 μg/L

- **Revised PEC/PNECs (2019 IFRA VoU)**: North America and Europe: Not applicable; cleared at screening-level

1. Identification

1. **Chemical Name**: Eugenyl methyl ether
2. **CAS Registry Number**: 93-15-2
3. **Synonyms**: 4-Allyl-1,2-dimethoxybenzene; 4-Allylveratrole; Benzene, 1,2-dimethoxy-4-(2-propenyl)-; 1,2-Dimethoxy-4-allylbenzene; Eugenol methyl ether; Methyl eugenol ether; Methyl eugenol; Veratrole methyl ether; 1,2-Dimethoxy-4-(2-propenyl)benzene; 3,4-

Dimethoxyallylbenzene; Allylveratrole; o-ﾒﾄｷｼ-p-ﾌﾟﾛﾍﾟﾆﾙﾌｪﾉｰﾙｱﾙｷﾙ (C = 1 ~ 5); Eugenyl methyl ether
4. **Molecular Formula**: $C_{11}H_{14}O_2$
5. **Molecular Weight**: 178.23 g/mol
6. **RIFM Number**: 302
7. **Stereochemistry**: Stereoisomer not specified. No stereocenter is present, and no stereoisomer is possible.

2. Physical data

1. **Boiling Point**: 248 °C (Fragrance Materials Association [FMA]), 254.7 °C (NTP, 2000), 247.71 °C (EPI Suite)
2. **Flash Point**: >200 °F; closed cup (FMA)
3. **Log K_{OW}**: 2.4 (RIFM, 2001b), 3.03 (EPI Suite)
4. **Melting Point**: 32.85 °C (EPI Suite)
5. **Water Solubility**: 144.8 mg/L (EPI Suite)
6. **Specific Gravity**: 1.032–1.035 (FMA), 1.034–1.037 (FMA)
7. **Vapor Pressure**: 0.00191 mm Hg at 20 °C (EPI Suite v4.0), 0.01 mm Hg at 20 °C (FMA), 0.00347 mm Hg at 25 °C (EPI Suite)
8. **UV Spectra**: Minor absorbance in the region 290–700 nm; the molar absorption coefficients (213, 177, 201 L mol^{-1} • cm^{-1} under neutral, acidic, and basic conditions, respectively) are below the benchmark (1000 L mol^{-1} • cm^{-1}).
9. **Appearance/Organoleptic**: Almost colorless oily liquid with a peculiar musty, tea-like, warm and mildly spicy, slightly earthy, and tenacious odor; the taste is somewhat dry tea-like, warm, and mildly spicy

3. Volume of use (worldwide band)

1.0.1–1 metric ton per year (IFRA, 2019)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v2.0)

1. **95th Percentile Concentration in Fine Fragrance**: 0.0015% (RIFM, 2018a)
2. **Inhalation Exposure***: 0.0000030 mg/kg/day or 0.00021 mg/day (RIFM, 2018a)
3. **Total Systemic Exposure****: 0.000019 mg/kg/day (RIFM, 2018a)

*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, 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, 2017).

5. Derivation of systemic absorption

1. **Dermal**: 46.5%

RIFM, 2000: *In vivo* dermal absorption of [^{14}C]-labeled test material eugenyl methyl ether was measured in male Fischer-344 rats. The test material was either applied through a charcoal trap or directly to the skin, and the vehicles used were 100% ethanol or 25% diethyl phthalate (DEP):75% ethanol. The [^{14}C]-labeled test material (5 mg/kg/10 cm^2) in either vehicle was applied topically on the shaved skin of the dorsal region. Following the application, the animals were housed in metabolic cages for the collection of urine and feces. Urine was collected for 24 h prior to dosing and up to 144 h after dosing. Collected feces, urine, and cage rinses were analyzed for the presence of test material. At the end of 144 h, animals were euthanized, and tissues, including blood, were collected. The radioactivity of each tissue was measured, and the study was divided into 3 sections, as shown below (see Table 1).

Table 1
Results from *in vivo* dermal absorption study on eugenyl methyl ether.

Site Measured	Study 1 (n = 6), Charcoal present in an aluminum trap, Vehicle: 100% ethanol		Study 2 (n = 3), No Charcoal in an aluminum trap Vehicle: 100% ethanol		Study 3 (n = 2) Charcoal present in an aluminum trap, Vehicle: DEP-Ethanol	
	Mean	SD	Mean	SD	Mean	SD
Urine of absorbed dose	12.9	3.8	14.6	1.5	34.5	5.1
Cage rinse	2.4	0.8	3.4	1	5.5	1.5
Feces	4.2	1.2	2.3	0.4	6.1	0.1
Skin rinse	11.9	13.7	2.3	1.8	0.1	0.1
Charcoal elution	24.2	7.9	–	–	15.9	3.1
Charcoal burn	19.3	7.2	–	–	14	2.5
Trap wash	9.2	4.4	4.6	0.7	3.5	3.1
Application site	1.1	0.7	0.4	0.6	0.2	0.2
Other tissues	0.3	0.2	0.3	0.1	0.2	0.1
% Absorbed (urine, cage rinse, feces, application site, and tissues)	22.9	5.5	21	1.5	46.5	3.9
Total recovery	84.3	10.9	27.8	1.2	80	4.7
Not recovered	15.7	10.9	72.2	1.2	20	4.7

From the data represented above, the most conservative skin absorption value of 46.5% was considered for the safety assessment of eugenyl methyl ether. Several studies report eugenyl methyl ether skin absorption (see Table 2) but were excluded due to the poor recovery data.

6. Computational toxicology evaluation

1. **Cramer Classification**: Class III, High

Expert Judgment	Toxtree v3.1	OECD QSAR Toolbox v4.5
III	III	III

2. **Analogs Selected**:

a. **Genotoxicity**: None
b. **Repeated Dose Toxicity**: None
c. **Developmental and Reproductive Toxicity**: None
d. **Skin Sensitization**: Isoeugenyl methyl ether (CAS # 93-16-3)
e. **Photoirritation/Photoallergenicity**: None
f. **Local Respiratory Toxicity**: None
g. **Environmental Toxicity**: None

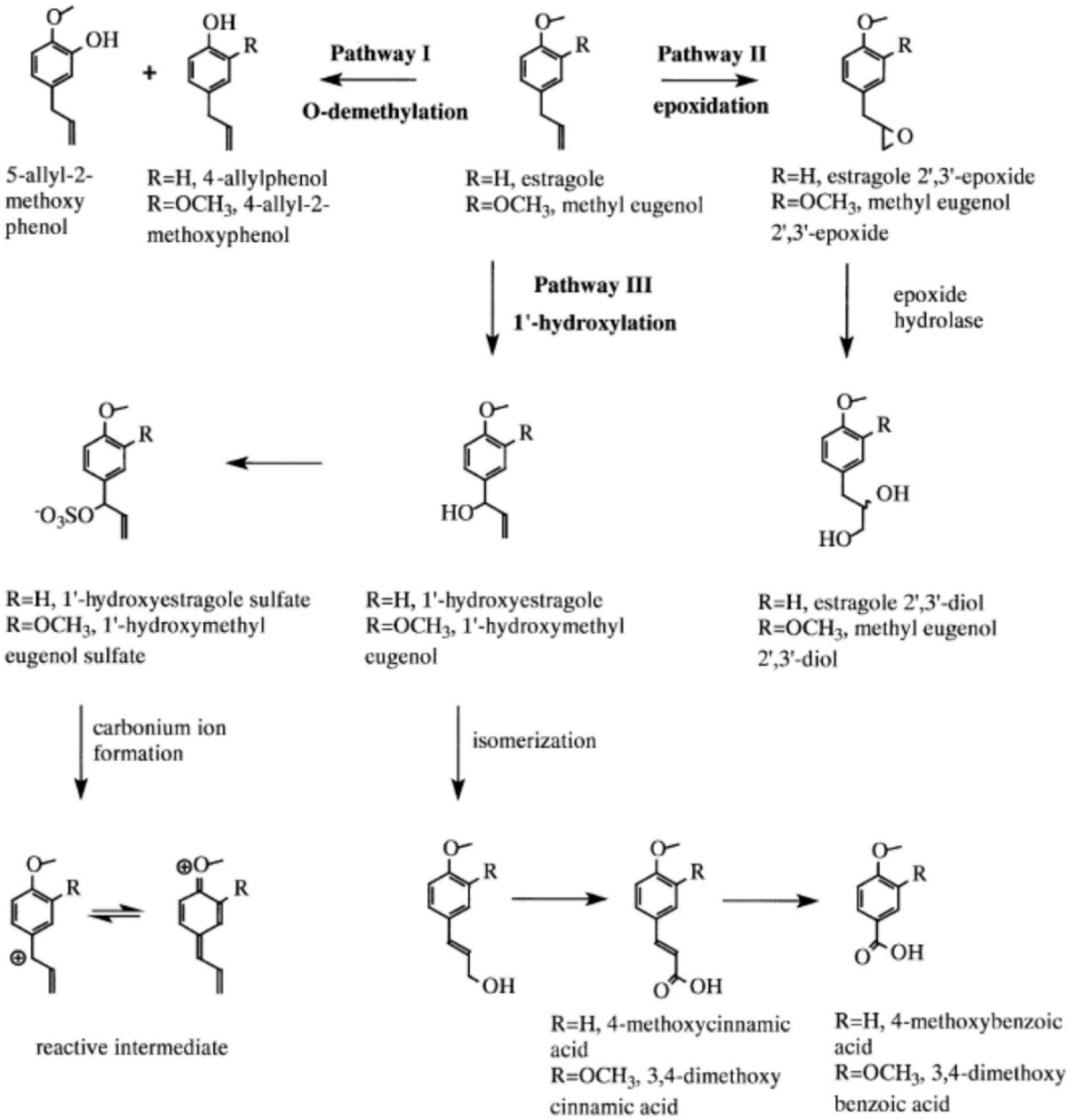
3. **Read-across Justification**: None

7. Metabolism

Smith et al., 2002: The metabolism and toxicokinetics of allylalkoxybenzene derivatives such as estragole and eugenyl methyl ether have been extensively reviewed by the Flavor and Extract Manufacturers Association (FEMA) Expert Panel. The hazard identified is a mechanistic outcome resulting in the production of the hepatotoxic sulfate conjugate of 1′-hydroxymetabolite observed in different species under chronic and subchronic conditions. Both estragole and eugenyl methyl ether are expected to share similar metabolic fates, pharmacokinetics, and toxicological profiles. Overall, both materials are readily absorbed following an oral dose, but the metabolic pathways are dose dependent. At low

Table 2
Other skin absorption studies on eugenyl methyl ether.
2. **Oral**: Assumed 100%
3. **Inhalation**: Assumed 100%

Skin Absorption Data						
Species Tested	Method	Occluded/Non-Occluded	Vehicle	% skin absorption	% Recovery	Reference
Human	*In vitro*	Occluded	Ethanol	49.70%	Not Reported	Yourick and Bronaugh, 2003
Human	*In vitro*	Non-Occluded	Ethanol	2.70%	Not Reported	Yourick and Bronaugh, 2003
Human	*In vitro*	Occluded	Emulsion	18.30%	Not Reported	Yourick and Bronaugh, 2003
Human	*In vitro*	Non-Occluded	Emulsion	0.90%	Not Reported	Yourick and Bronaugh, 2003
Harlan Sprague Dawley	*In vitro*	Occluded	Ethanol	77.30%	Not Reported	Yourick and Bronaugh, 2003
Harlan Sprague Dawley	*In vitro*	Non-Occluded	Ethanol	3.30%	Not Reported	Yourick and Bronaugh, 2003
Harlan Sprague Dawley	*In vitro*	Occluded	Emulsion	36.20%	Not Reported	Yourick and Bronaugh, 2003
Harlan Sprague Dawley	*In vitro*	Non-Occluded	Emulsion	1.10%	Not Reported	Yourick and Bronaugh, 2003
Harlan Sprague Dawley	*In vivo*	Occluded	Ethanol	19.10%	52.90%	Yourick and Bronaugh, 2003
Human	*In vitro*	Non-Occluded	Ethanol	33.60%	35.90%	RIFM (2001a)



Scheme 1. Metabolism of allylalkoxybenzene derivatives in animals.

Fig. 1. Metabolic pathway of eugenyl methyl ether (Smith et al., 2002).

doses, ring substituents are metabolized, while at higher doses, the allyl side chain undergoes oxidation. The formation of 1′-hydroxy moieties is directly proportional to dose, whereas the extent of O-demethylation decreases with increasing dose (0.05–1000 mg/kg/day) in both rats and mice. The extent of toxicity from the epoxidation of the allyl side chain is not as significant as toxicity resulting from 1′-hydroxylation conjugates. Dose-dependent metabolism studies of propenylalkoxybenze derivatives confirm that the O-demethylation pathway is predominant in rodents at doses <10 mg/kg/day. Moreover, this pathway results in the formation of a corresponding phenol, which forms a sulfate or glucuronic acid conjugate. In contrast, the 1′-hydroxylation that is the primary pathway resulting in toxicity produces a reactive hepatotoxic and hepatocarcinogenic moiety in rodents. The unstable sulfate moiety thus formed is anticipated to form a reactive electrophilic intermediate capable of binding to proteins and DNA in the liver to ultimately form DNA adducts (see genotoxicity section). However, the formation of these adducts is dose dependent. The NTP metabolism and toxicokinetic data suggest that, at higher doses, the O-demethylation pathway of eugenyl methyl ether metabolism is saturated, which leads to a dose-dependent shift towards the CYP-1A2-mediated activation of the 1′-hydroxylation pathway (NTP, 2000). The resulting 1′-hydroxy metabolite undergoes sulfation to form the highly reactive sulfate conjugate of the 1′-hydroxylated eugenyl methyl ether, in turn generating the genotoxic metabolite of eugenyl methyl ether. The overall metabolic pathway for eugenyl methyl ether is shown in Fig. 1 below:

NTP, 2000: Single-dose intravenous (I.V.) and oral gavage toxicokinetic studies of eugenyl methyl ether in male and female F344/N rats and B6C3F1 mice were conducted. Groups of 12 rats/sex were administered a single I.V. injection of 37 mg/kg or single oral gavage doses of 37, 75, or 150 mg/kg. In rats, following I.V. administration, blood was collected from 3 animals/sex at 2, 5, 15, 30, 45, 90, 180, and 360 min, while, following the oral dose administration, blood was collected (3 animals/sex) at 5, 15, 30, 60, 90, 120, 240, and 360 min. Groups of 24 mice/sex were administered a single I.V. dose of 25 mg/kg or single doses of 25, 50, or 75 mg/kg by gavage. In mice, following an I.V. dose administration, blood was collected from 2 to 4 mice at 2, 5, 15, 30, 45, 60, 180, and 300 min, while, after oral administration, blood was collected (3 animals/sex) at 5, 15, 30, 45, 60, 90, 120, and 240 min. Each rat was bled twice, and each mouse was bled once; blood was collected from the retroorbital sinus (rats and mice) or by cardiac puncture (mice), followed by plasma concentration determination. The tables below show the reported values of the maximum mean concentration (C_{max}), time of maximum mean concentration (T_{max}), and elimination half-life ($t_{1/2}$) (see Tables 3 and 4) (see Table 5).

In rats, the absorption from oral doses was rapid, with peak plasma levels achieved within the first 5 min at all doses in males and females. In both males and females, eugenyl methyl ether bioavailability was low: ≤6% at 37 mg/kg, increasing to approximately 13% at 75 mg/kg and 15%–20% at 150 mg/kg. These findings suggest a strong but saturable first-pass metabolic effect leading to a nonlinear relationship between dose and parent chemical dosimetry. Elimination of eugenyl methyl ether from the bloodstream was rapid and multiphasic, with initial half-lives in the order of 5 min and terminal half-lives in the order of 1–2 h in males and females.

Table 3
Reported values of C_{max}, T_{max}, and $t_{1/2}$ in the oral gavage study in rats.

Single Gavage Dosing (Rats)						
	Male			Female		
Gavage Dose (mg/kg)	37	75	150	37	75	150
C_{max} (μg/mL)	0.656	1.52	3.84	1.14	3.22	8.25
T_{max} (minutes)	5	5	5	5	5	5
t½ (minutes)	60	75	115	95	80	105
AUC (μg/mL · min)	33.5	155.6	459.5	27.0	133.1	307.9
Absolute Bioavailability (%)	5.8	13.2	19.5	5.5	13.3	15.3

AUC = area under the curve calculated using the trapezoidal rule; absolute bioavailability was calculated as $AUC_{oral}/AUC_{IV} \times Dose_{IV}/Dose_{oral} \times 100$.

Table 4
Reported values of C_{max}, T_{max}, and $t_{1/2}$ in the single IV dose study in rats.

Single IV Dosing (Rats)		
	Male	Female
IV Dose (mg/kg)	37	37
C_{max} (μg/mL)	45.7	49.5
T_{max} (minutes)	2	2
t½ (minutes)	75	75
AUC (μg/mL · min)	581.4	495.4

AUC = area under the curve calculated using the trapezoidal rule.

Table 5
Reported values of C_{max}, T_{max}, and $t_{1/2}$ in the oral gavage study in mice.

Single Gavage Dosing (Mice)						
	Male Mice			Female Mice		
Gavage Dose (mg/kg)	25	50	75	25	50	75
C_{max} (μg/mL)	0.382	1.40	3.10	0.123	1.01	4.39
T_{max} (minutes)	5	5	5	15	5	5
t½ (minutes)	30	30	30	30	30	30
AUC (μg/mL · min)	4.91	27.4	48.4	3.27	25.0	60.5
Absolute Bioavailability (%)	4.2	11.8	13.9	3.1	11.7	18.9

AUC = area under the curve calculated using the trapezoidal rule; absolute bioavailability was calculated as $AUC_{oral}/AUC_{IV} \times Dose_{IV}/Dose_{oral} \times 100$.

In mice, the absorption from oral doses was rapid, with peak plasma levels achieved within 15 min for all doses in males and females. Eugenyl methyl ether bioavailability was low: 3%–5% at 25 mg/kg increasing to 12% at 50 mg/kg and 13%–19% at 75 mg/kg. As observed in rats, mice studies present evidence for a strong but saturable, first-pass metabolic effect, leading to a nonlinear relationship between dose and parent chemical dosimetry. Elimination of eugenyl methyl ether from the bloodstream was rapid and multiphasic, with terminal half-lives ranging from 15 to 30 min.

NTP, 2000: Following oral and I.V. administration of [^{14}C]-eugenyl methyl ether (118 mg/kg, 50 μCi/kg) in corn oil, the absorption, distribution, metabolism, and excretion were measured in male Fischer 344 rats as described above. Urine (6, 12, 24, 48, and 72 h) and feces (24, 48, and 72 h) were collected along with blood and other tissues. Total radioactivity was measured in blood, feces, and tissue samples using a liquid scintillation counter. Urine was also analyzed for the presence of parent and metabolites by high-performance liquid chromatography (HPLC). A single dose of [^{14}C]-eugenyl methyl ether (11.8 mg/kg, 120 μCi/kg) in ethanol:Emulphor:saline (10:10:80, 2 mL/kg) was administered intravenously to 3 male Fischer 344 rats via an indwelling jugular vein cannula. Blood samples were collected at various time points (0, 1, 4, 8, 12, 15, 20, 30, 40, and 50 min and 1, 6, 12, 24, 48, and 72 h) and analyzed for radioactivity or extracted with ethyl acetate for HPLC analysis. Blood and feces were analyzed for total radioactivity by scintillation counting of oxidized samples. Urine was also analyzed for the presence of parent and metabolites by HPLC. Eugenyl methyl ether undergoes rapid metabolism (kinetic parameters described above). The parent compound and its metabolites were preferentially distributed to the liver 72 h after gavage or I.V. administration in males. Tissue:blood ratios of eugenyl methyl ether were 2–3 in the liver, 0.9–1.4 in the kidney, and significantly less than 1 in all other tissues tested after 72 h. Approximately 72% of the orally administered compound was eliminated through urine within 72 h after dosing. Approximately 13% of the orally administered dose was recovered in feces, and less than 0.1% was recovered as expired air. Following an I.V. dose, approximately 85% of the total dose was excreted in the urine within 72 h, approximately 6%

was recovered in feces, and less than 0.1% was recovered as expired air. [^{14}C]-Equivalents determined in tissues accounted for less than 0.3% of the administered dose. However, eugenyl methyl ether as a parent compound was not found in the urine after oral or intravenous administration. The metabolites identified suggest that eugenyl methyl ether can undergo O-demethylation and side chain hydroxylation, followed by sulfation or glucuronidation of the hydroxylated metabolites.

Delaforge et al., 1980: A single intraperitoneal injection of 200 mg/kg of eugenyl methyl ether was given to male Wistar rats, and urine was collected every 2 h for 24 h. Twenty-four hours after treatment, animals were euthanized, and livers were excised. Urinary metabolites included the epoxide of the parent substance and the O-demethylated metabolites of eugenyl methyl ether (allylcatechol epoxide). Liver microsomal preparations show the presence of the epoxide metabolite identified in the urine for eugenyl methyl ether.

Gardner et al., 1997a: Results of studies with rat and human liver microsomes indicate that the 1′-hydroxylation pathway is catalyzed mainly by the CYP2E1 and/or CYP2C6 enzymes. Results of studies that investigated inter-individual variability in hepatic microsomes obtained from 13 humans indicated a 37-fold difference in the rate of 1′-hydroxylation of eugenyl methyl ether among these 13 human liver microsome samples. Autoinduction of the 1′-hydroxylation pathway was reported in hepatic microsomes of rats given 30–300 mg eugenyl methyl ether/kg/day orally for 5 days but not in rats given 10 mg/kg/day for 5 days.

8. Natural occurrence

Eugenyl methyl ether is reported to occur in the following foods by the VCF*:

Agastache species	Nutmeg (*Myristica fragrans* Houttuyn)
Ashanti pepper (*Piper guineense* Schum and Thom)	Ocimum species
Laurel (*Laurus nobilis* L.)	Pimento (Allspice) (*Pimenta dioica* L. Merr.)
Mastic (*Pistacia lentiscus*)	Star anise
Myrtle (*Myrtus communis* L.)	Tarragon (*Artemisia dracunculus* L.)

*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. This is a partial list.

9. REACH dossier

Available; accessed 12/01/22 (ECHA, 2018).

10. Conclusion

The maximum acceptable concentrations[a] in finished products for eugenyl methyl ether 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)	0.00042
2	Products applied to the axillae	0.0015
3	Products applied to the face/body using fingertips	0.00042
4	Products related to fine fragrances	0.011
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.0015
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.00021
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.00042
5D	Baby cream, oil, talc	0.000069
6	Products with oral and lip exposure	0.0010
7	Products applied to the hair with some hand contact	0.00042
8	Products with significant ano-genital exposure (tampon)	0.000069
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.0017
10A	Household care products with mostly hand contact (hand dishwashing detergent)	0.00062
10B	Aerosol air freshener	0.0021
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.000069
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	0.066

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 eugenyl methyl ether, the basis was the reference dose of 0.000770 mg/kg/day, a predicted skin absorption value of 46.5%, and a skin sensitization NESIL of 9400 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 v3.2.9.

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data detailed below, eugenyl methyl ether is considered to be potentially genotoxic but safe at the maximum acceptable concentrations outlined in Section X.

11.1.1.1. Risk assessment. The National Toxicology Program (NTP) reported that eugenyl methyl ether was not mutagenic in the bacteria *S. typhimurium* (NTP, 2000). An Ames test was conducted in accordance with OECD TG 471 using the plate incorporation method in which *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 were treated with eugenyl methyl ether in dimethyl sulfoxide (DMSO) at concentrations ranging from 3 to 333 μg/plate with and without metabolic activation (rat and hamster liver S9 mix) (NTP, 2000). No significant induction of revertant colonies was produced at any of the dose levels with or without S9, and the test material was considered not mutagenic under the conditions of the Ames test. Furthermore, older studies also support the lack of mutagenic potential of eugenyl methyl ether in various strains of *S. typhimurium* and the *E. coli* WP2uvrA strain with and without S9 (Sekizawa and Shibamoto, 1982). It was also reported that eugenyl methyl ether did not induce chromosomal aberrations in CHO cells but did induce sister chromatid exchanges (SCEs) only in the presence of S9 (NTP, 2000).

At the end of a 3-month study conducted by NTP when assessing the toxicity of eugenyl methyl ether, peripheral blood samples were collected from groups of male and female B6C3F1 mice treated with

eugenyl methyl ether at the dose of 1000 mg/kg in 0.5% methylcellulose via oral gavage for 90 days (Witt et al., 2000). Under the conditions of the study, eugenyl methyl ether did not induce the formation of micronuclei. However, other studies have shown that eugenyl methyl ether forms adducts with DNA and proteins in transformed human V79 fibroblasts expressing human sulfotransferase, as well as in mouse livers *in vivo,* resulting in hepatotoxicity and carcinogenicity (Gardner et al., 1996; Randerath et al., 1984a; Phillips et al., 1984). Induction of liver DNA adducts was shown in a 32P-post-labeling study (Randerath et al., 1984a) in which adult CD-1 female mice were administered 100 or 500 mg/kg eugenyl methyl ether by intraperitoneal (i.p.) injection. The DNA-binding activities of eugenyl methyl ether were higher than those of other alkenylbenzene derivatives. In a related study (Phillips et al., 1984), newborn male B6C3F1 mice were treated on postnatal days 1, 8, 15, and 22 via i.p. injection with eugenyl methyl ether (doses: 0.25, 0.5, 1.0, and 3 μmol). The auto-radiographic map by a modified 32P-post--labeling procedure showed liver DNA adducts predominantly on the N2 of guanine and, to a lesser extent, on the N6 of adenine. Eugenyl methyl ether and the metabolite 1′-hydroxyeugenyl methyl ether induced dose-related unscheduled DNS synthesis (UDS) in cultured primary rat hepatocytes (Howes et al., 1990a; Chan and Caldwell, 1992). The metabolite 1′-hydroxyeugenyl methyl ether showed a stronger induction of UDS than the parent substance. In 1999, eugenyl methyl ether was evaluated by the Committee of Experts on Flavouring Substances of the Council of Europe, and it concluded that eugenyl methyl ether is a naturally occurring genotoxic carcinogenic compound with a DNA-binding potency similar to that of safrole. Because eugenyl methyl ether has been demonstrated to have genotoxic and carcinogenic potential, the existence of a minimum concentration required cannot be assumed, and the Committee could not establish a safe exposure limit. Consequently, reductions in exposure and restrictions in use levels were indicated.

Additionally, the FEMA Expert Panel (Smith et al., 2002; Smith et al., 2005; Gooderham et al., 2020) reviewed the data on eugenyl methyl ether and concluded that it forms covalent bonds with proteins and DNA following metabolism to the proximate carcinogen 1′-hydroxyeugenyl methyl ether. Eugenyl methyl ether and several metabolites were found to induce DNA strand breaks in the comet assay (Groh et al., 2012). Eugenyl methyl ether formed liver DNA adducts in a turkey egg DNA adduct study at a dose >0.03 mg/egg (total dose from 3 injections). No adducts were formed at 0.025 mg/egg, and hence, this was considered to be the minimum concentration required for this DNA adduct study. This dose, when adjusted by the weight of the turkey fetus at day 24, leads to a conservative (as compared to rat study) minimum required dose of 0.67 mg/kg (adjusted by fetus weight). Hence, the minimum concentration required for DNA adduct formation was considered to be 0.67 mg/kg/day (Williams et al., 2018). This minimum concentration required for DNA adduct formation is almost 3 times lower than the DNA adduct dose of 2 mg/kg in an animal study (Randerath et al., 1984a). Among all metabolites of eugenyl methyl ether, in terms of DNA adduct formation, 1′-hydroxyeugenyl methyl ether was the most potent and is considered the major proximate mutagen/carcinogen formed from eugenyl methyl ether (Cartus et al., 2012). Evidence points to sulfation by human and murine sulfotransferases (SULTs) of hydroxylated eugenyl methyl ether metabolites as key to the metabolic activation, which results in the formation of DNA adducts (Herrmann et al., 2013; Herrmann et al., 2014). Since DNA adducts have been detected in the rat liver at dose levels as low as 5 mg/kg/day (RIFM, 2007), the hepatic bioactivation of eugenyl methyl ether at lower doses could not be definitively excluded and could be considered indicative of a direct genotoxicity risk upon metabolic activation. In human liver samples, eugenyl methyl ether undergoes bioactivation via 1′-hydroxylation and subsequent sulfation, forming reactive metabolites (Al-Subeihi et al., 2012; Herrmann et al., 2013; Herrmann et al., 2014). The formation of DNA adduct in humans by eugenyl methyl ether has been shown to be directly related to mRNA and protein levels of SULT1A1 (Tremmel et al., 2017). Even though human SULTs are more effective than the murine counterparts, which may lead to the more readily formation of DNA adducts in humans, the formation of the 1′-hydroxy intermediate responsible for forming adducts is less efficient in humans than in rodents (Al-Subeihi et al., 2012). Eugenyl methyl ether-induced DNA adducts (up to 37 per 10^8 nucleosides or 4700 adducts per diploid genome) were detected in the liver of 29 of 30 subjects (median of 13 per 10^8 nucleosides or 1700 adducts per diploid genome) (Herrmann et al., 2013). However, these could be an outcome of excessive intake of eugenyl methyl ether. Hence, new data is required related to a dose-dependent increase in the metabolic rate (leading to the formation of a 1-hydroxy metabolite) and also the detoxification rate, along with DNA repair proficiency in humans, to confirm the effects. Additionally, possible participation of genotoxic mechanisms in a eugenyl methyl ether-induced increase in liver preneoplastic lesions was concluded in a 13-week *gpt delta* transgenic rat (carrying approximately 5 tandem copies of the transgene lambda EG10 per haploid genome) study (Jin et al., 2013; Nohmi et al., 2017).

The total fragrance systemic exposure to eugenyl methyl ether is 0.000019 mg/kg/day. Considering that the DNA adduct formation and genotoxic effect occur at much higher doses, eugenyl methyl ether does not raise safety concerns at the current level of use in fragrances.

Additional References: Dorange et al., 1977; Sekizawa and Shibamoto, 1982; Mortelmans et al., 1986; Schiestl et al., 1989l; Schiestl (1993); Randerath et al., 1984b; Howes et al., 1990b; Marshall and Caldwell, 1996; Brennan et al., 1996; Levy and Weber, 1988; Phillips et al., 1984; Randerath et al., 1984a; Auman et al., 2004; RIFM, 2003a; Burkey et al., 1999a; Lewis-Burkey et al., 2000; Tyrrell et al., 2000; Sipes et al., 1999; Duerksen-Hughes et al., 1999; Iida et al., 2007; Ding et al., 2011; Groh et al., 2012; RIFM, 2012; Riejens et al., 2014

Literature Search and Risk Assessment Completed On: 10/26/22

11.1.2. Repeated dose toxicity

The MOE of eugenyl methyl ether is adequate for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1. Risk assessment. The repeated dose toxicity of allylalkoxybenzene derivatives, including eugenyl methyl ether, has been extensively studied in rodent models. In addition, although a robust epidemiological study is not available to date, several studies have investigated the effects of human consumption of foods containing allylalkoxybenzenes. Dietary human exposure to eugenyl methyl ether results from fruits, vegetables, herbs, and spices. Basil is one of the highest sources of eugenyl methyl ether exposure. Several groups, including the FEMA Expert Panel, have reviewed the available rodent carcinogenicity and human exposure data for eugenyl methyl ether. The primary hazard associated with eugenyl methyl ether exposure is dependent on the formation of the 1′-hydroxy metabolite, which forms a reactive sulfate conjugate that leads to hepatotoxicity and DNA adduct formation. The formation of this active metabolite is dose dependent. At higher doses, the O-demethylation/glucuronidation pathway of eugenyl methyl ether metabolism becomes saturated, which triggers a shift toward CYP450-mediated formation of the 1′-hydroxy-metabolite (Smith et al., 2002). The sulfate ester of 1′-hydroxyeugenyl methyl ether is reactive and readily forms adducts with proteins and DNA, as described above in the genotoxicity section (Randerath et al., 1984a; Phillips et al., 1984; Gardner et al., 1996). Although other reactive metabolites of eugenyl methyl ether are formed (an epoxide on the alkyl side chain), genotoxicity best correlates with the formation of a 1′-hydroxy metabolite. The formation of hepatic tumors occurs in a linear dose response, but the zero percent tumor intercept is several orders of magnitude higher than human consumption. This indicates that the minimum concentration required for carcinogenicity in animals exceeds human exposure, and it does not present a carcinogenicity risk in humans (Waddell, 2002) (see Table 6).

Table 6
Reported values of C_{max}, T_{max}, and $t_{1/2}$ in the single IV dose study in mice.

Single IV Dosing (Mice)		
	Male Mice	Female Mice
IV Dose (mg/kg)	25	25
C_{max} (μg/mL)	18.2	9.34
T_{max} (minutes)	2	2
t½ (minutes)	15	15
AUC (μg/mL · min)	116.4	106.5

AUC = area under the curve calculated using the trapezoidal rule.

Table 7
Incidences of neoplastic lesions reported in rats.

F344/N rats						
Doses (mg/kg/day)	Hepatocellular adenoma		Hepatocellular carcinoma		Hepatocellular carcinoma or adenoma	
	Male	Female	Male	Female	Male	Female
0	5	1	2	0	7	1
37	12	8	3	0	14	8
75	23	11	14	4	28	14
150	38	33	25	8	43	34

A 2-year NTP carcinogenicity study (gavage doses of 37, 75, and 150 mg/kg/day) provided clear evidence of eugenyl methyl ether-induced carcinogenicity in F344 rats of either sex based on the incidences of hepatocellular carcinoma combined with cholangioma and cholangiocarcinoma, neuroendocrine tumors, and malignant, metastatic, and glandular/endocrine tumors of the glandular stomach in rats. Male rats were also reported to have increased incidences of neoplasms, malignant mesothelioma, mammary gland fibroadenoma, and subcutaneous fibroma, or fibrosarcoma following exposure to eugenyl methyl ether. In rats, even the lowest dose of 37 mg/kg/day caused tumors of the liver in either sex (see Table 7). All tumor incidences in rats exhibited a dose-response relationship. Similarly, a 2-year gavage bioassay at the same doses demonstrated clear carcinogenicity evidence in B6CF31 mice of both sexes (see Table 8). The reported effects were increased incidences of hepatocellular adenoma, hepatoblastoma, hepatocholangiocarcinoma, and neuroendocrine tumors of the glandular stomach in male mice. In mice, the hepatic tumors were accompanied by *H. hepaticus* infection in the livers, as well as damaged gastric mucosa. Complicating the interpretation of these results was the fact that male and female mice in the control group were reported to have 63% and 50% tumor rates, respectively, in comparison to the incidences of 80%–100% in the eugenyl methyl ether treated mice. Overall, carcinogenicity was reported at all dose levels in animal toxicity studies, with tumor incidences following a dose response across the tested doses. Since hepatotoxicity is the most sensitive endpoint from the NTP studies, benchmark dosing and MOE calculations were performed based on these data. In the mice studies, tumors were reported in control as well as treatment animals along with *H. hepaticus* infection. Because these effects cloud the interpretation of hepatotoxicity/hepatocarcinogenicity findings in mice, benchmark dose modeling was restricted to rat data only (Smith et al., 2010; NTP, 2000).

Table 8
Incidences of neoplastic lesions reported in mice.

B6C3F1 Mice						
Doses (mg/kg/day)	Hepatocellular adenoma		Hepatocellular carcinoma		Hepatocellular carcinoma or adenoma	
	Male	Female	Male	Female	Male	Female
0	26	20	10	7	31	25
37	43	48	20	37	47	50
75	38	46	19	47	46	49
150	39	41	9	47	40	49

An epidemiological study demonstrated that exposure to eugenyl methyl ether in humans primarily occurs through diet (Al-Malahmeh et al., 2017). Eugenyl methyl ether is generally found in higher concentrations in herbs and spices such as basil and nutmeg. Hence, the consumers of pesto sauce containing fresh basil could be exposed to high levels of eugenyl methyl ether with an estimated daily intake of up to 44.3 μg/kg/day in 10 g of basil, which is an overestimated human consumption value. Al-Malahmeh et al. concluded that consumption of 30 g of basil per day for a short duration does not represent cancer risk. However, the study is unable to support the safe use of eugenyl methyl ether as a constituent of basil for long periods (Al-Malahmeh et al., 2017).

Several uncertainties and a lack of human relevance have been identified in the available data for eugenyl methyl ether-induced carcinogenicity. The data demonstrate that in rodents, eugenyl methyl ether induces hepatocellular carcinomas along with tumors of other sites, but because of the study design, the human relevance of these effects remains questionable. In the NTP studies, the test material was administered as high bolus doses of 99% pure eugenyl methyl ether resulting in greatly exaggerated blood levels of the test compound, and eugenyl methyl ether-induced severe gastric damage would further result in more rapid and extensive absorption of the test material. Thus, bolus dosing of a high dose has the potential to cause effects often not observed at doses encountered through diet. High doses of eugenyl methyl ether can also overwhelm the metabolic pathway, leading to the autoinduction of CYP-mediated metabolic activation and to an imbalance between bioactivation and detoxification (Smith et al., 2010). In addition to dose-dependent metabolic considerations, the relevance of rodent toxicity from single compounds tested by gavage at high doses versus their consumption in the diet as natural spices should also be considered. Bioactivation and/or detoxification of eugenyl methyl ether can also be influenced by other components of natural spices and the food matrix (Al-Malahmeh et al., 2017; Rietjens et al., 2008). These considerations raise serious questions about the human relevance of these carcinogenicity findings in high-dose rodent cancer bioassays in comparison to the low-dose dietary exposures encountered in humans. Typically, in the absence of data to establish a dose response at low human exposure levels, high-dose to low-dose linear extrapolation is used to estimate the carcinogenic risk. The NOAEL thus derived is divided by appropriate safety factors based on the nature of observed effects. For severe irreversible adverse health effects, the Expert Panel for Fragrance Safety* and Gaylor et al. (Gaylor et al., 1999) recommend using an uncertainty factor of 10000.

Since carcinogenic effects were observed even at the lowest dose, the Expert Panel for Fragrance Safety supported the use of the benchmark dose (BMD) approach instead of the NOAEL. The BMD (using BMDS v1.3.2) was derived using the long-term toxicity study from the NTP following the pioneering efforts presented by Smith et al. (Smith et al., 2010). Using dose-response modeling, a BMD lower confidence limit for a benchmark response of 10% ($BMDL_{10}$) was calculated as being 10.76 mg/kg/day** for incidences of rat combined liver adenoma and carcinoma. Adjusted for the dosing schedule of 5/7 days per week, 10.76 mg/kg/day × (5/7) = 7.7 mg/kg/day (Davidsen et al., 2022).

A $BMDL_{10}$ for methyl eugenol has previously been calculated to be 15.3 mg/kg/day based on the incidence of carcinomas alone (Suparmi et al., 2018). Based on the $BMDL_{10}$ using the combined incidences of carcinomas and adenomas as the point of departure, a reference dose (RfD) for humans was established by including a safety factor of 10000.

Therefore, the MOE for repeated dose toxicity is equal to the $BMDL_{10}$ in mg/kg/day divided by the total systemic exposure, 7.7/0.000019, or 405263. It should be noted that EFSA Scientific Committee advocates that, for any genotoxic and carcinogenic material, a MOE of 10000 or greater derived based on $BMDL_{10}$ from animal studies offers minimal human health concern and is considered low priority for risk

management (EFSA, 2005).

11.1.2.2. Derivation of subchronic 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. (2020) and a subchronic RfD of 0.000770 mg/kg/day.

The RIFM Criteria Document (Api et al., 2015) calls for an MOE of 10000. The subchronic RfD for eugenyl methyl ether was calculated by dividing the $BMDL_{10}$ of 7.7 mg/kg/day by the uncertainty factor, 10000 = 0.000770 mg/kg/day.

The RfD for eugenyl methyl ether was calculated by dividing the $BMDL_{10}$ of 7.7 mg/kg/day by the uncertainty factor, 10000 = 0.00077 mg/kg/day.

*The Expert Panel is composed of technical experts in their respective fields. This group provides technical advice and guidance.

**The only difference between the RIFM (2018) and Smith et al. (2010) approaches to derive the $BMDL_{10}$ value was the software versions. Smith et al. (2010) used BMDS v1.3.2.

Additional References: Ellis et al., 2005; Ellis et al., 2006; RIFM, 2007; Caujolle and Meynier, 1960; Snell et al., 2000; RIFM, 1981; Miller et al., 1983; Zondek and Bergmann, 1938; Solheim and Scheline, 1976; Graves and Runyon, 1995; Burkey et al., 1999b; Delaforge et al., 1980; Fujii et al., 1970; Seto and Keup, 1969; Jaffe et al., 1968; Wagstaff, 1971; Ioannides et al., 1985; Gardner et al., 1997a; Rompelberg et al., 1996; Gardner et al., 1997b; Delaforge et al., 1978; Wakazono et al., 1995; Jimbo (1983); RIFM, 2001a; RIFM, 2002; RIFM, 2000.

Literature Search and Risk Assessment Completed On: 10/27/22

*11.1.3. Reproductive toxicity***

The MOE for eugenyl methyl ether is adequate for the reproductive toxicity endpoint at the current level of use.

**IFRA Standard Restricted. Potentially genotoxic and should be used as per IFRA Standard.

11.1.3.1. Risk assessment. There are sufficient developmental toxicity data on eugenyl methyl ether for the developmental toxicity endpoint. A GLP-compliant NTP prenatal developmental toxicity study was conducted in pregnant female Sprague Dawley CD rats. Groups of 25 rats were administered by gavage with 0, 80, 200, or 500 mg/kg/day eugenyl methyl ether in a 0.5% methylcellulose vehicle from gestation days (GDs) 6–19. Maternal toxicity was manifested by clinical signs (rooting behavior), decreased body weight, bodyweight gains, and increased liver weights in all treatment group dams. However, no treatment-related changes were reported for the number of corpora lutea, pregnancy indices, number of resorptions, or dead and live fetuses at any dose level. The average fetal body weight per litter was statistically significantly reduced at 500 mg/kg/day. An increased incidence of unossified sternebra(e), a skeletal variation, was observed at 500 mg/kg/day. Thus, the NOAEL for maternal toxicity could not be determined for this study, based on treatment-related adverse effects reported even at the lowest dose; therefore, the LOAEL for maternal toxicity was considered to be 80 mg/kg/day, based on aversion to treatment and increase in liver weight at all dose levels. The NOAEL for developmental toxicity was considered to be 200 mg/kg/day, based on decreased fetal body weights and increased incidences of a skeletal variation (unossified sternebrae) observed at 500 mg/kg/day (NTP, 2004). **Therefore, the eugenyl methyl ether MOE for the developmental toxicity endpoint can be calculated by dividing the eugenyl methyl ether NOAEL in mg/kg/day by the total systemic exposure to eugenyl methyl ether, 200/0.000019 or 10526316.**

There are sufficient fertility data on eugenyl methyl ether for the reproductive toxicity endpoint. A GLP-compliant NTP 14-week subchronic toxicity study was conducted in F344/N rats. Groups of 10 rats/sex/dose were administered eugenyl methyl ether in 0.5% methylcellulose by gavage at doses of 0, 10, 30, 100, 300, or 1000 mg/kg/day, 5 days per week for 14 weeks. Another group of 10 rats/sex received water alone. In addition to systemic toxicity parameters, reproductive toxicity parameters were assessed. At the end of the study, samples were collected for sperm motility and vaginal cytology (vaginal samples were collected for up to 12 consecutive days prior to the end of the study) on vehicle control, 30, 100, and 300 mg/kg/day rats. At 1000 mg/kg/day, the absolute and relative right testis weights were statistically significantly increased, and males had a statistically significant increase in the incidence of moderate dilatation of the seminiferous tubules and testicular degeneration. However, spermatogonia remaining within the seminiferous and epididymal tubules were morphologically normal at 1000 mg/kg/day. Statistically significantly increased incidences of mild uterine atrophy were reported for 300 and 1000 mg/kg/day females. However, no changes were reported for the uterus during the microscopic examination. There were no significant differences in sperm motility or in vaginal cytology parameters between rats treated up to 300 mg/kg/day and the vehicle control rats. Thus, the NOAEL for male and female reproductive toxicity was considered to be 300 mg/kg/day, based on increased right testis weights and increased incidence of moderate dilatation of the seminiferous tubules and testicular degeneration observed at 1000 mg/kg/day (NTP, 2000).

Simultaneously, a GLP-compliant NTP 14-week subchronic toxicity study was conducted in B6C3F1 mice. Groups of 10 mice/sex/dose were administered eugenyl methyl ether in 0.5% methylcellulose by gavage at doses of 0, 10, 30, 100, 300, or 1000 mg/kg/day, 5 days per week for 14 weeks. Another group of 10 mice/sex received water alone. In addition to systemic toxicity parameters, reproductive toxicity parameters were also assessed. At the end of the study, samples were collected for sperm motility and vaginal cytology (vaginal samples were collected for up to 12 consecutive days prior to the end of the study) on the vehicle control and 10, 30, and 100 mg/kg/day mice. Animal deaths before the end of the study were: 9/10, 1/10, and 1/10 for 1000, 300, and 10 mg/kg/day, respectively. Clinical findings of toxicity were manifested as generalized morbidity in the male and female mice that died at 1000 mg/kg/day. Male mice administered 10 or 30 mg/kg/day had statistically significantly lower left cauda epididymis, left epididymis, and left testis weights than the vehicle controls, which were not dose dependent. At 100 mg/kg/day, males had statistically significantly decreased spermatozoa concentrations (66% of vehicle control). However, the spermatozoa concentrations for 10 and 30 mg/kg/day were increased but not significantly when compared to the vehicle control group (147% and 145% for 10 and 30 mg/kg/day males, respectively, of the vehicle control). Hence, the decrease in spermatozoa concentration attributed to treatment is uncertain. There were no significant differences in vaginal cytology parameters between mice treated up to 100 mg/kg/day and the vehicle control mice. Thus, the NOAEL for male and female reproductive toxicity was considered to be 30 mg/kg/day, based on decreased spermatozoa concentrations at 100 mg/kg/day (NTP, 2000).

Furthermore, male rats at the end of a 2-year NTP-conducted carcinogenicity study were reported to have increased sperm granulomas at 150 mg/kg/day (highest treatment group) and 300 mg/kg/day (stop-exposure group; 52 weeks of treatment followed by vehicle control for the remaining 53 weeks of study) (NTP, 2000; (data also available in NTP, 1989; Abdo et al., 2001).

Thus, the most conservative NOAEL of 30 mg/kg/day from the 14-week mice study was selected for the reproductive toxicity endpoint. **Therefore, the eugenyl methyl ether MOE for the reproductive toxicity endpoint can be calculated by dividing the eugenyl methyl ether NOAEL in mg/kg/day by the total systemic exposure to eugenyl methyl ether, 30/0.000019 or 1578947.**

When correcting for skin absorption (see Section V), the total systemic exposure to eugenyl methyl ether (0.019 μg/kg/day) is below the TTC (1.5 μg/kg/day; Kroes et al., 2007; Laufersweiler et al., 2012) for the reproductive toxicity endpoint of a Cramer Class III material at the current level of use.

Additional References: None

Literature Search and Risk Assessment Completed On: 10/27/22

11.1.4. Skin sensitization

Based on the existing data and read-across material isoeugenyl methyl ether (CAS # 93-16-3), eugenyl methyl ether is considered a skin sensitizer with a defined NESIL of 9400 μg/cm^2.

11.1.4.1. Risk assessment. Limited skin sensitization studies are available for eugenyl methyl ether. Based on the existing data and read-across material isoeugenyl methyl ether (CAS # 93-16-3; see Section VI), eugenyl methyl ether is considered a skin sensitizer. The chemical structures of these materials indicate that they would be expected to react with skin proteins (Roberts et al., 2007; Toxtree v3.1.0). Eugenyl methyl ether was found to be negative in an *in vitro* direct peptide reactivity assay (DPRA) (ECHA, 2018). In a guinea pig maximization test with eugenyl methyl ether, reactions indicative of sensitization were seen at 100% (RIFM, 1982a); with read-across material isoeugenyl methyl ether, reactions indicative of sensitization were seen at 25% (RIFM, 1982b). When eugenyl methyl ether and read-across material isoeugenyl methyl ether were tested in 2 guinea pig open epicutaneous tests and 2 closed epicutaneous tests in guinea pigs, no skin sensitization reactions were observed (Ishihara et al., 1986; Itoh, 1982; Klecak, 1985). In 2 human maximization tests, no skin sensitization reactions were observed with eugenyl methyl ether and read-across material isoeugenyl methyl ether at 8% or 5520 μg/cm^2 (RIFM, 1972). In a Confirmation of No Induction in Humans test (CNIH) with the read-across material, isoeugenyl methyl ether, tested at 25% (29527 μg/cm^2) in 3:1 ethanol:diethyl phthalate (EtOH:DEP), reactions indicative of sensitization were observed in 1/28 volunteers (RIFM, 2003b). In other CNIHs, isoeugenyl methyl ether did not present reactions indicative of sensitization when tested at 25% (29527 μg/cm^2) in 3:1 EtOH:DEP in 28 volunteers (RIFM, 2003c), at 20% (23622 μg/cm^2) in 3:1 EtOH:DEP in 54 volunteers (RIFM, 2005), or at 8% (9448 μg/cm^2) in 27 and 24 volunteers (RIFM, 2004). Another CNIH with 106 volunteer subjects did not present any reactions indicative of skin sensitization when 8% (9448 μg/cm^2) of the read-across material, isoeugenyl methyl ether in 1:3 EtOH:DEP, was used for induction and challenge (RIFM, 2018b).

Based on the weight of evidence (WoE) from structural analysis and animal and human studies, eugenyl methyl ether is a sensitizer with a WoE NESIL of 9400 μg/cm^2 (Table 9). 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. (2020) and a subchronic RfD of 0.000770 mg/kg/day.

Additional References: Natsch and Haupt, 2013; RIFM, 1962.

Table 9
Data Summary for isoeugenyl methyl ether as read-across material for eugenyl methyl ether.

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
NA	Weak	9448	NA	29,527	9400

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.

Literature Search and Risk Assessment Completed On: 10/28/22

11.1.5. Photoirritation/photoallergenicity

Based on available UV/Vis spectra, eugenyl methyl ether would not be expected to present a concern for photoirritation or photoallergenicity.

11.1.5.1. Risk assessment. There are no photoirritation studies available for eugenyl methyl ether in experimental models. UV/Vis absorption spectra indicate minor absorbance between 290 and 700 nm. The corresponding molar absorption coefficients are below the benchmark of concern for photoirritation and photoallergenicity (Henry et al., 2009). Based on the lack of significant absorbance in the critical range, eugenyl methyl ether does not present a concern for photoirritation or photoallergenicity.

11.1.6. UV spectra analysis

UV/Vis absorption spectra (OECD TG 101) for eugenyl methyl ether were obtained. The spectra indicate minor absorbance in the range of 290–700 nm. The molar absorption coefficients ((213, 177, 201 L mol^{-1} • cm^{-1} under neutral, acidic, and basic conditions, respectively) are below the benchmark of concern for photoirritating effects, 1000 L mol^{-1} • cm^{-1} (Henry et al., 2009).

Additional References: None

Literature Search and Risk Assessment Completed On: 10/27/22

11.1.7. Local Respiratory Toxicity

The MOE could not be calculated due to a lack of appropriate data. The exposure level for eugenyl methyl ether is below the Cramer Class III TTC value for inhalation exposure local effects.

11.1.7.1. Risk assessment. There are insufficient inhalation data available on eugenyl methyl ether. Based on the Creme RIFM Model, the inhalation exposure is 0.00021 mg/day. This exposure is 2238 times lower than the Cramer Class III TTC value of 0.47 mg/day (based on a human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: Beroza et al., 1975.

Literature Search and Risk Assessment Completed On: 10/28/22

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of eugenyl methyl ether 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 of 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, eugenyl methyl ether 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 eugenyl methyl 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, 2017a). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

11.2.1.1. Risk assessment. Based on the current VoU (2019), eugenyl methyl ether does not present a risk to the aquatic compartment in the screening-level assessment.

11.2.1.2. Key studies. ***Biodegradation***:

No data available.

Ecotoxicity:

Beroza et al., 1975: A fish (bluegill sunfish and rainbow trout) acute toxicity study was conducted according to the OECD 203 method under static conditions. The 96-h LC50 was reported to be 8.1 mg/L and 6.0 mg/L for bluegill sunfish and rainbow trout, respectively.

11.2.1.3. Other available data. Eugenyl methyl ether has been registered for REACH, and the following data is available (ECHA, 2018):

A ready biodegradability test was conducted using the CO_2 evolution test according to the OECD 301B guideline. After 29 days, mean biodegradation of 92.1% was observed.

An acute fish (*Cyprinus carpio*) toxicity test was conducted according to the OECD 203 guidelines under semi-static conditions. The 96-h No Observed Effect and Lowest Observed Effect loading rates were reported to be 2.0 and 3.8 mg/L, respectively, and the acute median lethal loading rate (LL50) value was reported to be 8.72 mg/L. All the results were based on the nominal test concentration.

A *Daphnia magna* immobilization test was conducted according to the OECD 202 method under static conditions. The 48-h EC50 based on measured concentration was reported to be 38 mg/L.

An algae growth inhibition study was conducted according to the OECD 201 method. The 72-h EC50 and No Observed Effect Concentration (NOEC) values based on nominal test concentration for growth rate were reported to be 22 mg/L and 4.6 mg/L, respectively.

A *Daphnia magna* reproduction test was conducted according to the OECD 211 method. The 21-day NOEC was reported to be 1.1 mg/L.

11.2.2. Risk assessment refinement

Since eugenyl methyl ether has passed the screening criteria, measured data are included for completeness only and have not been used in PNEC derivation.

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	2.4	2.4
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 0.1079 μg/L. The revised PEC/PNECs for the EU and North America 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 VoU.

Literature Search and Risk Assessment Completed On: 10/31/22

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**
- **SciFinder**: https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PubChem**: https://pubchem.ncbi.nlm.nih.gov/
- **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 ChemView**: https://chemview.epa.gov/chemview/
- **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

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*)	EC50 (Algae)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	107.9			1000000	0.1079	

appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 12/01/22.

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.2023.114209.

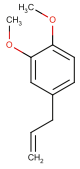
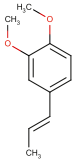
Appendix

Read-across Justification

Methods

The read-across analog was identified using RIFM fragrance chemicals inventory clustering and read-across search criteria (Date et al., 2020). These criteria are in compliance with the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015) and are consistent with the guidance provided by OECD within Integrated Approaches for Testing and Assessment (OECD, 2015) and the European Chemicals Agency read-across assessment framework (ECHA, 2017b).

- 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 (US EPA, 2012a).
- J_{max} values were calculated using RIFM's skin absorption model (SAM). The parameters were calculated using the consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts, and oncologic classification predictions were generated using OECD QSAR Toolbox v4.5 (OECD, 2021).
- ER binding and repeat dose categorization were generated using the OECD QSAR Toolbox v4.5 (OECD, 2021).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010), and skin sensitization was predicted using Toxtree v2.6.13.
- Protein binding was predicted using OECD QSAR Toolbox v4.5 (OECD, 2021).
- The major metabolites for the target material and read-across analogs were determined and evaluated using the OECD QSAR Toolbox v4.5 (OECD, 2021).
- To keep continuity and compatibility with *in silico* alerts, OECD QSAR Toolbox v4.5 was selected as the alert system.

	Target Material	Read-across Material
Principal Name	Eugenyl methyl ether	Isoeugenyl methyl ether
CAS No.	93-15-2	93-16-3
Structure		
Similarity (Tanimoto Score)		0.59
Read-across Endpoint		• Skin Sensitization
Molecular Formula	$C_{11}H_{14}O_2$	$C_{11}H_{14}O_2$
Molecular Weight (g/mol)	178.23	178.23
Melting Point (°C, EPI Suite)	70	18
Boiling Point (°C, EPI Suite)	270.5	270.5
Vapor Pressure (Pa @ 25°C, EPI Suite)	1.6	1.2
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	3.03	2.95
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	500	169.1
J_{max} (μg/cm^2/h, SAM)	22.564	12.359
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	5.67E-001	1.54E+000
Skin Sensitization		
Protein Binding (OASIS v1.1)	• No alert found	• No alert found
Protein Binding (OECD)	• No alert found	• No alert found
Protein Binding Potency	• Not possible to classify according to these rules (GSH)	• Not possible to classify according to these rules (GSH)

(*continued on next page*)

(*continued*)

	Target Material	Read-across Material
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	• No alert found	• No alert found
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	• Alert for Michael acceptor	• Alert for Michael acceptor
Metabolism		
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.5)	•See Supplemental Data 1	• See Supplemental Data 2

Summary

There are insufficient toxicity data on eugenyl methyl ether (CAS # 93-15-2). 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, isoeugenyl methyl ether (CAS # 93-16-3) was identified as a read-across analog with sufficient data for toxicological evaluation.

Conclusions

- Isoeugenyl methyl ether (CAS # 93-16-3) was used as a read-across analog for the target material, eugenyl methyl ether (CAS # 93-15-2), for the skin sensitization endpoint.
  - o The target material and the read-across analog are structurally similar and belong to a class of eugenyls.
  - o The target material and the read-across analog share a benzyl ring with 2 methoxy groups.
  - o The key difference between the target material and the read-across analog is that the target material has 2 possible modes of action related to skin sensitization. These are catechol formation and quinone methide formation. On the other hand, the read-across analog can undergo only catechol formation. The target material is 1 step away from forming quinone methide, as the molecule has to first undergo O-demethylation with respect to the p-propylyne group first. Therefore, the probability of quinone methide is low, and it is a phase II metabolite. The phase I metabolite, which is catechol, will be the same in both the target material and the read-across analog. Therefore the structural differences between the target material and the read-across analog are not significant for the skin sensitization endpoint.
  - 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 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 v4.5, structural alerts for toxicological endpoints are consistent between the target material and the read-across analog.
  - o Both the target and read-across materials have a Skin Sensitization Reactivity Domains by Toxtree v2.6.13 alert for Michael acceptor. This alert is due to the eugenyl moiety in the target material and the unsaturated branch in the isoeugenyl moiety in the read-across analog. The data described in the skin sensitization section confirm that the target material is a skin sensitizer. Therefore, *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.

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