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Short Review

RIFM fragrance ingredient safety assessment, estragole, CAS registry number 140-67-0

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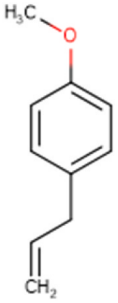
Name: Estragole CAS Registry Number: 140-67-0



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

(*continued on next column*)

(*continued*)

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.

Estragole was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, photoirritation/photoallergenicity, skin sensitization, and environmental safety. Data show that estragole is not expected to be genotoxic and provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose toxicity endpoint and a No Expected Sensitization Induction Level (NESIL) of 1000 μg/cm^2 for the skin sensitization endpoint. Data on estragole and read-across analog eugenyl methyl ether (CAS # 93-15-2) provide a calculated MOE >100 for the reproductive toxicity endpoint. The photoirritation/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; estragole 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; exposure is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; estragole 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.

Human Health Safety Assessment

Genotoxicity: Not expected to be genotoxic at the current level of use in fragrances.	(NTP, 2008; Randerath et al., 1984a; Phillips et al., 1984)
Repeated Dose Toxicity: BMDL10 = 10 mg/kg/day.	(Miller et al., 1983)
Reproductive Toxicity: Developmental toxicity NOAEL = 200 mg/kg/day. Fertility NOAEL = 30 mg/kg/day.	(NTP, 2004; NTP, 2000)
Skin Sensitization: NESIL = 1000 μg/cm^2.	(Gerberick et al., 2001)
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: 84% (OECD 301F) (RIFM, 1997)

Bioaccumulation:
Screening-level: 90.06 L/kg (EPI Suite v4.11; US EPA, 2012a)

Ecotoxicity:
Screening-level: 48-h *Daphnia magna* EC50: 3.81 mg/L (ECOSAR v2.0; US EPA, 2012b)

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: 48-h *Daphnia* magna EC50: 3.81 mg/L	(ECOSAR v2.0; US EPA, 2012b)

RIFM PNEC is: 0.381 μg/L

- Revised PEC/PNECs (2019 IFRA VoU): North America and Europe <1

1. Identification

1. **Chemical Name:** Estragole
2. **CAS Registry Number:** 140-67-0
3. **Synonyms:** p-Allylanisole; Benzene, 1-methoxy-4-(2-propenyl)-; Chavicyl methyl ether; Isoanethole; p-Methoxyallylbenzene; 1-Methoxy-4-(2-propen-1-yl)benzene; Methyl chavicol; 1-ﾒﾄｷｼ-4-ﾌﾟﾛﾍﾟﾆﾙﾍﾞﾝｾﾞﾝ; Methyl Chavicol Coeur; 1-Allyl-4-methoxybenzene; 4-Allylanisole; Estragole
4. **Molecular Formula:** $C_{10}H_{12}O$
5. **Molecular Weight:** 148.2 g/mol
6. **RIFM Number:** 263
7. **Stereochemistry:** Stereoisomer not specified. No stereocenter is present, and no stereoisomer is possible.

2. Physical data

1. **Boiling Point:** 216 °C (Fragrance Materials Association [FMA]), 209.93 °C (EPI Suite)
2. **Flash Point:** 82 °C (Globally Harmonized System), 180 °F; closed cup (FMA)
3. **Log K_{OW}:** 3.4 at 35 °C (RIFM, 1998), 3.47 (EPI Suite)
4. **Melting Point**: -1.19 °C (EPI Suite)
5. **Water Solubility:** 84.55 mg/L (EPI Suite)
6. **Specific Gravity:** 0.962–0.970 (FMA), 0.960–0.968 (FMA)
7. **Vapor Pressure:** 0.109 mm Hg at 20 °C (EPI Suite v4.0), 0.09 mm Hg at 20 °C (FMA), 0.165 mm Hg at 25 °C (EPI Suite)
8. **UV Spectra:** No absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** A colorless, slightly oily liquid that has a sweet-herbaceous Anise-Fennel type odor, and the taste is not nearly as sweet as that of anethole

3. Volume of use (worldwide band)

1. 10–100 metric tons per year (IFRA, 2019)

4. Exposure to fragrance ingredient (Creme RIFM aggregate exposure model v3.1.5)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.039% (RIFM, 2021)
2. **Inhalation Exposure*:** 0.000069 mg/kg/day or 0.0050 mg/day (RIFM, 2021)
3. **Total Systemic Exposure**:** 0.00036 mg/kg/day (RIFM, 2021)

*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; 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; Comiskey et al., 2017).

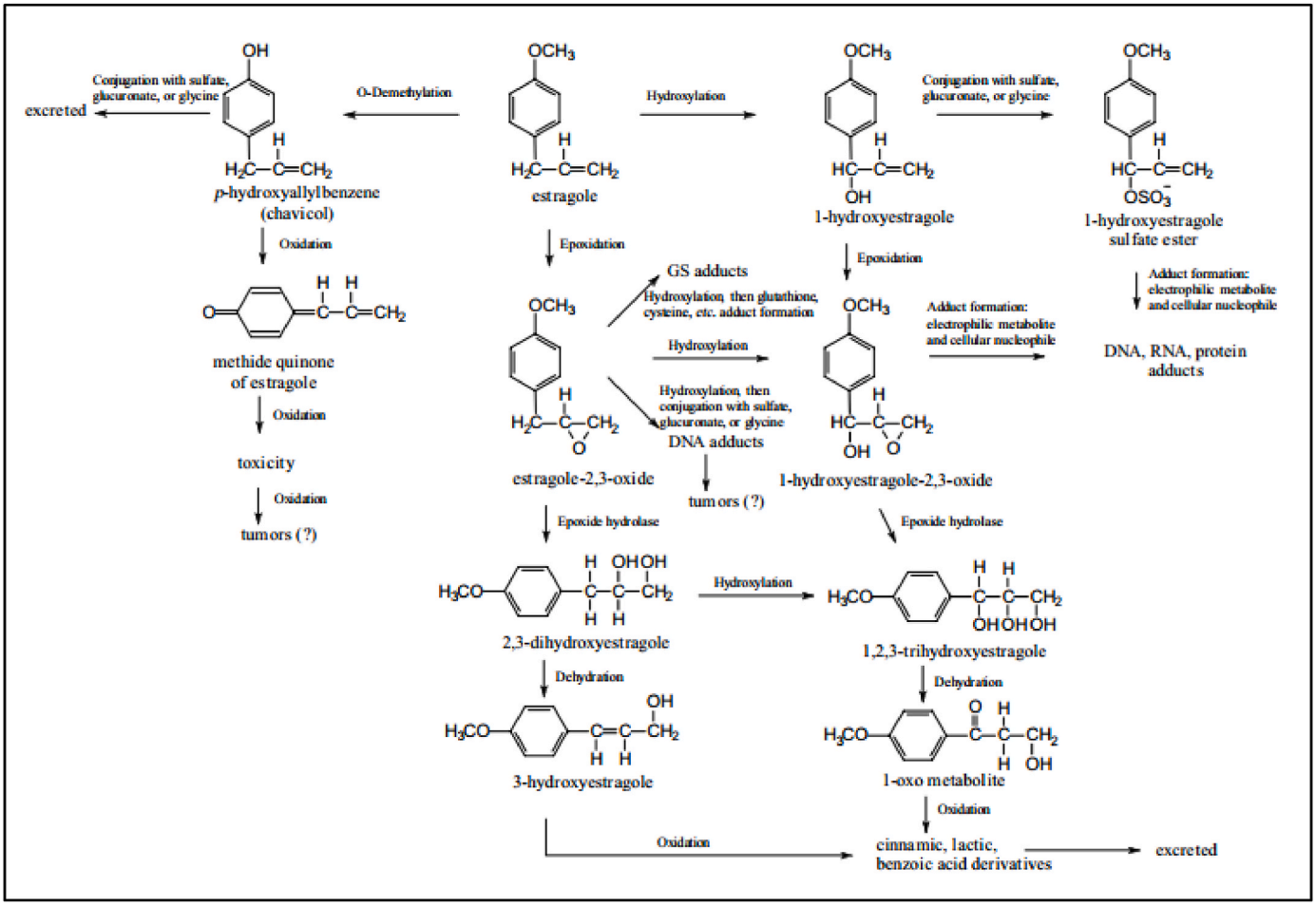


Fig. 1. Metabolism of estragole (cited from NTP, 2011).

5. Derivation of systemic absorption

1. **Dermal:** 22.4%

RIFM, 2001: Skin absorption of estragole was measured in an *in vitro* human skin permeation and distribution study. Under unoccluded conditions, 20 μL/cm^2 [^{14}C]-estragole was applied as 1% (w/v) estragole solution in ethanol (96% v/v). The target dose of 20 μL/cm^2 contained 200 μg/cm^2 of fragrance material. Epidermis from the breast and abdominal skin of 4 female donors was used, and skin integrity was assessed by measuring the permeation of tritiated water (10 μCi/mL^3). Permeation of estragole from a 20 μL/cm^2 dose in 50% v/v ethanol/-water receptor medium was measured at 1, 2, 4, 8, 24, and 48 h after application. Tissue samples were mounted on 14 Franz-type diffusion cells; 12 cells were dosed with estragole, and 2 were assigned as control cells. Samples (200 μL) were diluted in scintillation fluid and analyzed for radioactivity. At the end of 48 h, estragole absorption was assessed after the epidermal membranes were wiped and tape-stripped 10 times. The estragole content of the wipes, strips, and remaining epidermis was determined. Estragole absorption significantly increased up to 8 h, followed by saturation through the end of the study duration. From the applied dose, 16.3% of the estragole permeated into the receptor phase, and only 6.3% was recovered from other compartments. Following a very low recovery, the evaporative loss of estragole was measured by mounting the polytetrafluoroethylene (PTFE) sheets in diffusion cells. After 48 h, the evaporative loss from the PTFE membranes was approximately 93.7%. Hence, a conservative skin absorption value was determined by combining the % permeated as well as % estragole found in the epidermis, tape strips, receptor phase, donor chamber, and surface wipe, which was 44.9 ± 9.1 μg/cm^2, corresponding to 22.4% ± 4.5% of the applied dose.

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

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. **Reproductive Toxicity:** Eugenyl methyl ether (CAS # 93-15-2)
   d. **Skin Sensitization:** None
   e. **Photoirritation/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. Read-across Justification: See Appendix below

7. Metabolism

The metabolism of estragole is well characterized in humans, rats, and mice in both *in vitro* and *in vivo* studies**.** Estragole biotransformation, like methyl eugenol, was reported to be dose dependent. Based on available data, it appears that the O-demethylation pathway becomes saturated at higher doses (>10 mg/kg), which leads to 1′-hydroxylation (formation of 1-hydroxyestragol) and epoxidation of the side chain. CYP450 1A2 and 2A6 are the isoforms responsible for the 1-hydroxylation of estragole. The 1-hydroxyestragol metabolite undergoes sulfate conjugation to form the reactive 1-hydroxyestragole sulfate ester (which binds to DNA and protein to form adducts). Epoxidation of the allyl side chain leading to estragole-2,3-oxide (also may form DNA and protein adducts) is rapidly metabolized by epoxide hydrolase and glutathione transferase to detoxified metabolites. The O-demethylation detoxification pathway, active at lower exposures, results in the formation of *p*-hydroxyallylbenzene and more distal metabolites (and the ultimate formation of CO_2) (NTP, 2008; Smith et al., 2002; EMA, 2015; WHO, 2009a).

Smith et al., 2002: The metabolism and toxicokinetics of allylalkoxybenzene derivatives such as estragole and methyl eugenol have been extensively reviewed by the FEMA Expert Panel. The hazard identified is a mechanistic outcome resulting in the production of the hepatotoxic sulfate conjugate of 1′-hydroxyestragole observed in different species under chronic and subchronic conditions. Both estragole and methyl eugenol are expected to share similar metabolic fates, pharmacokinetics, and toxicological profiles. Both materials are readily absorbed following an oral dose, but the metabolic pathways are dose dependent. At low doses, ring substituents are metabolized, while at higher doses, the allyl side chain undergoes oxidation. The formation of 1'hydroxy moieties is directly proportionate to the dose, whereas the extent of O-demethylation decreases with increasing doses (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 phenolic metabolite, 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 the genotoxicity section). However, the formation of these adducts is dose dependent. Metabolism and toxicokinetic data from an NTP study (NTP, 1996) suggest that, at higher doses, the allylalkoxybenzene metabolism gets saturated and no longer follows the O-demethylation pathway for elimination, leading to a dose-dependent shift toward the CYP450-mediated activation of the 1′-hydroxylation pathway and subsequent sulfation to the sulfate conjugate of the 1′-hydroxylated conjugate, in turn generating the genotoxic metabolite.

Zangouras et al., 1981; NTP, 2008; Smith et al., 2002: Dose-dependent formation of the 1′-hydroxy metabolite was assessed in mice and rats. [^{14}C]-Estragole was administered to female Wistar rats (single oral dose) and male CD-1 mice (single i.p. dose) at dose levels of 0, 0.05, 5, 500, or 1000 mg/kg. Urine and expired air (for $^{14}CO_2$) were collected for 24 h, followed by radioactivity determination and metabolite identification by thin-layer chromatography. A dose-related increase in the glucuronide conjugate of 1′-hydroxyestragole in urine was observed (0.9%–8% in rats and 1.3%–9.5% in mice). The total formation of 1′-hydroxy metabolite increased significantly (1224–255000 nmol/kg/day in rats and 5–279000 nmol/kg/day) with an increase in dose from 5 to 500 mg/kg. This suggests that an increase in dose and a shift in metabolic pathways (i.e., a decrease in O-demethylation) results in a marked increase in the formation of the 1′-hydroxy metabolite (see Fig. 1).

Additional References: Delaforge et al., 1980; Sangster et al., 1987; Drinkwater et al., 1976; Solheim and Scheline, 1973.

8. Natural occurrence

Estragole is reported to occur in the following foods by the VCF*:

Anise (*Pimpinella anisum* L.)	Licorice (*Glycyrrhiza glabra* L.)
Apple, fresh (*Malus* species)	Olive (*Olea europaea*)
Chicken	Sweet grass oil (*Hierochloe odorata*)
Coriander seed (*Coriandrum sativum* L.)	Sweet marjoram (*Origanum majorana* L.)
Elderberry (*Sambucus nigra* 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 (ECHA, 2018); accessed on 12/14/22.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for estragole 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)	0.00031
2	Products applied to the axillae	0.0025
3	Products applied to the face/body using fingertips	0.00063
4	Products related to fine fragrances	0.014
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.0022
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.00063
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.00063
5D	Baby cream, oil, talc	0.00021
6	Products with oral and lip exposure	0.0019
7	Products applied to the hair with some hand contact	0.00063
8	Products with significant ano-genital exposure (tampon)	0.00021
9	Products with body and hand exposure, primarily rinse-off (bar soap)	0.0041
10A	Household care products with mostly hand contact (hand dishwashing detergent)	0.00094
10B	Aerosol air freshener	0.0022
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.00021
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	0.11

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 estragole, the basis was the subchronic reference dose of 0.0010 mg/kg/day, a skin absorption value of 22.40%, and a skin sensitization NESIL of 1000 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, estragole is considered to be potentially genotoxic but safe at the maximum acceptable concentrations outlined in Section X.

11.1.1.1. Risk assessment. Genotoxicity studies have shown that estragole is not mutagenic or very weakly mutagenic in *S. typhimurium*. A study performed by the US NTP assessed estragole for mutagenic potential in a GLP-compliant bacterial reverse mutation assay in accordance with OECD TG 471. *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 were treated with estragole in dimethyl sulfoxide (DMSO) at concentrations up to 220 μg/plate in the presence and absence of metabolic activation (S9). Under the conditions of the study, the test material was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 in the presence or absence of rat or hamster liver activation enzymes (NTP, 2008).

Estragole and its 1′-hydroxy metabolite have been shown to induce unscheduled DNA synthesis in rat hepatocytes both *in vitro* and *in vivo*. The formation of hepatic DNA adducts by estragole and by the 1′-hydroxy metabolite of estragole has also been demonstrated in mice. In 2000, the Committee of Experts on Flavoring Substances (CEFS) of the Council of Europe evaluated estragole as a naturally occurring compound and concluded that it is a genotoxic carcinogen in experimental animals following chronic exposure or after a few repeated doses (WHO, 2009b). It has been reported that estragole and its metabolite 1′-hydroxyestragole induce hepatic tumors in CD-1 or B6C3F1 mice after chronic dietary exposure or after intraperitoneal or subcutaneous injections both prior to and after weaning, with males appearing to be more susceptible than females (Drinkwater et al., 1976; Miller et al., 1983; Wiseman et al., 1987). The available toxicological studies were considered inadequate for evaluation by CEFS, and no allowed daily intake (ADI) has been allocated. The CEFS requested additional long-term studies for evaluation of the carcinogenic potential before an ADI can be established (WHO, 2009b).

At the end of a 3-month study assessing the toxicity of estragole, peripheral blood samples were obtained from male and female B6C3F1 mice treated with estragole in corn oil via oral gavage 5 days per week at concentrations of 37.5, 75, 150, 300, and 600 mg/kg in male mice and 37.5, 75, 150, and 300 mg/kg in female mice and assessed for increased micronuclei formation. No significant increases in the frequencies of micronucleated normochromatic erythrocytes were observed in peripheral blood samples from male and female mice in this study, and no significant changes in percent polychromatic erythrocytes were seen, indicating no treatment-induced toxicity to bone marrow (NTP, 2008). Additionally, The FEMA Expert Panel (Smith et al., 2002; Smith et al., 2005) reviewed the data on estragole and concluded that estragole forms covalent bonds to proteins and DNA following metabolism to 1-hydroxyestragole (Randerath et al., 1984a; Phillips et al., 1984; NTP, 2008). The tumors arise at high dosages and in a non-linear dose-response manner, indicating there is a clear minimum concentration required for carcinogenicity at levels far exceeding the exposure to humans from fragrances (Waddell, 2002).

The lowest oral dosage producing hepatocarcinogenesis in rats was 600 mg/kg/day (NTP, 2008) and 150 mg/kg/day in mice (Miller et al., 1983). Additionally, in a turkey egg DNA adduct study, the administration of estragole lead to the formation of adducts at doses >0.05 mg/egg. However, since no adducts were formed at a dose of 0.025 mg/egg, this dose was considered to be the minimum concentration required for the turkey eggs DNA adduct study. This dose, when adjusted to the fetus's body weights (obtained at 24 days of incubation), leads to a conservative value of 0.68 mg/kg/day. Hence, the minimum concentration required for DNA adduct formation was considered to be 0.68 mg/kg/day (Williams et al., 2018). This minimum concentration required for DNA adduct formation is almost 50 times lower than the DNA adduct dose of 30 mg/kg in an animal study (WHO, 2009a; Paini et al., 2012).

The total fragrance systemic exposure to estragole is 0.00031 mg/

Table 1
Incidences of hepatomas reported in female mice.

Mice		
Doses (mg/kg/day)	Hepatomas	
	Incidence	Total N
0	0	50
176	27	48
352	35	49

kg/day. Considering the DNA adduct formation occurs at much higher doses, estragole does not raise safety concerns at the current level of use in fragrances.

Additional References: Sekizawa, 1982; Dorange et al., 1977; Swanson et al., 1979; To et al., 1982; Zeiger et al., 1987; Zani et al., 1991; RIFM, 2000; Martins et al., 2012; Nesslany et al., 2010; Chan and Caldwell, 1992; Caldwell et al., 1992; Howes et al., 1990; Randerath et al., 1984b; Randerath et al., 1984a; Muller et al., 1994; Phillips et al., 1984.

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

11.1.2. Repeated dose toxicity

The MOE for estragole 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 estragole, has been extensively studied in rodent models. Although a robust epidemiological study is not available to date, several studies have investigated the effects of human consumption of allylalkoxybenzene derivatives containing food products. Dietary human exposure to estragole results from fruits, vegetables, herbs, and spices. Concentrations of estragole vary significantly in spices largely due to variations in plant maturity, harvesting techniques, storage as well as measurement techniques. Several groups, including the FEMA Expert Panel, have reviewed the available rodent carcinogenicity and human exposure data for allylalkoxybenzene. Like methyl eugenol, the primary hazard associated with estragole exposure is dependent on the formation of the 1′-hydroxy metabolite. This metabolite, when conjugated with sulfate, is reactive and forms hepatotoxic DNA and protein adducts (see the genotoxicity section above; Randerath et al., 1984a; Phillips et al., 1984; Gardner et al., 1996). The formation of this active metabolite is dose dependent. At higher doses, the O-demethylation/glucuronidation pathway of estragole metabolism becomes saturated, which triggers a shift toward CYP450-mediated formation of the 1′-hydroxy-metabolite (Smith et al., 2002).

In a subchronic oral toxicity study, 10 F344/N rats/sex/dose were administered estragole through gavage at doses of 0, 37.5, 75, 150, 300, or 600 mg/kg/day, 5 days per week for 90 days. The only clinical observation was staining observed on the ventral surface anterior to the genitalia from week 9 onwards in the 300 mg/kg/day (7/10 males and 3/10 females) and the 600 mg/kg/day groups (4/10 males and 9/10 females). The mean body weight and mean bodyweight gain were significantly reduced at doses ≥300 mg/kg/day in both sexes. Hematological alterations were reported in both sexes, but male rats were more sensitive. The changes were characterized as microcytic, normochromic, and nonresponsive anemia consistent with impaired erythropoiesis. Moreover, serum ALT increased significantly at doses ≥300 mg/kg/day, and sorbitol dehydrogenase activity increased at 600 mg/kg/day. These serum enzyme changes are indicative of hepatotoxicity that was confirmed by liver morphological and histological changes. Dose-dependent increases in serum bile salt concentration (at ≥75 mg/kg/day in males and ≥300 mg/kg/day in females) were observed but without any accompanying changes of serum ALP activity; the bile salt concentration levels probably were not associated with cholestasis. A dose-related significant decrease in serum albumin and, ultimately, total serum protein was observed in all dose groups. Absolute liver weights were increased only in males at 75 and 150 mg/kg/day doses, whereas relative liver weights were significantly increased in males and females receiving doses ≥75 mg/kg/day. Alterations of liver weights were accompanied by gross lesions at ≥300 mg/kg/day, such as mottled discoloration, enlargement (males only), and increased granular appearance. Multiple cholangiocarcinomas (2 animals) and hepatocellular adenoma (1 animal) were observed in males receiving the highest dose. In addition, all males in the 600 mg/kg/day group had cholangiofibrosis. Liver hypertrophy was reported in all males at ≥75 mg/kg/day doses and in all females at doses higher than 150 mg/kg/day. Hepatotoxicity and incidences of hepatomas were accompanied by renal alterations as well. Increases in relative kidney weights, dark focal discolorations, and renal tubule mineralization were reported in treated animals with a dose-dependent increase in incidences. Furthermore, increased incidences of bone marrow hyperplasia in males were reported at 75, 300, and 600 mg/kg/day doses. Incidences of degeneration of the olfactory epithelium were significantly increased in ≥300 mg/kg/day groups. Incidences of atrophy of the gastric glands of the stomach were significantly increased in the ≥150 mg/kg/day groups. In a special study group (rats treated with the same doses for 30 days), serum gastrin concentration and stomach pH were significantly increased at 600 mg/kg/day dose. Therefore, a NOAEL for repeated dose toxicity was considered to be 37.5 mg/kg/day based on the liver, kidney, and bone marrow effects noted at 75 mg/kg/day. However, under the conditions of the study, NTP concluded that estragole showed some carcinogenic activity based on the occurrence of 2 cholangiocarcinomas and 1 hepatocellular adenoma in the liver of male F344/N rats in the high-dose group (NTP, 2008).

In a subchronic oral toxicity study, 10 B6C3F1 mice/sex/dose were administered estragole through gavage at doses of 0, 37.5, 75, 150, 300, or 600 mg/kg/day, 5 days per week for 90 days. Significant incidences of mortalities were reported in the highest dose group (1 male during week 9 and all female mice during week 1). Female deaths were attributed to liver necrosis resulting from estragole exposure. In contrast to the subchronic rat study, female mice were more sensitive to estragole treatment than their male counterparts. Mean body weights and bodyweight gains were lower in males treated with 300 and 600 mg/kg/day doses and in females at doses ≥75 mg/kg/day. In mice that ultimately died, clinical findings noted were lethargy and reduced locomotor and exploratory behavior. Overall, there were no significant changes in hematological parameters. Higher absolute liver weights were reported in males at 75 and 150 mg/kg/day and in females at 300 mg/kg/day. A dose-dependent increase in relative liver weights was reported in animals receiving 75 mg/kg/day or higher doses. Statistically significant increased incidences of hepatocellular hypertrophy and hepatocellular degeneration were observed in males at doses of 300 and 600 mg/kg and at doses of 150 and 300 mg/kg in females. The severity of these lesions increased dose-dependently. Liver necrosis occurred in all treated females (week 1) and 1 male (week 9) at 600 mg/kg/day, along with an increased incidence of diffuse fatty change. Relative thymus weights were significantly increased in all dosed groups of females. There were significantly increased incidences of degeneration of the gastric glands of the glandular stomach, as well as squamous hyperplasia, mineralization, and ulcer in the forestomach in 600 mg/kg/day treated females. Degeneration of the olfactory epithelium occurred in 300 and 600 mg/kg/day treated animals. A LOAEL for systemic toxicity was considered to be 37.5 mg/kg/day based on the reduction in body weight, increased relative liver weights, and histopathological alterations observed at higher doses (NTP, 2008).

In an earlier pioneering study, the carcinogenic potential of alkoxy-substituted allylbenzenes was examined in a multipart bioassay. Groups of CD-1 female mice were maintained on diets containing estragole or 1′-hydroxyestragole in a single-dose dietary study. Survival at 20 months was lower for estragole-fed (68–70%) animals compared with control

Table 2
Summary of data for reproductive toxicity data.

Test Duration	GLP	No. of Animals/ Dose (Species, Strain, Sex)	Route	Limitations	Study Type	Test Material	Doses (in mg/kg/ day; Purity)	NOAEL/LOAEL/ NOEL	Effects Observed	References
3 months	Yes	10 F344/ N rats/ sex/dose	Oral gavage	Effects on the female reproductive cycle or organs not evaluated	Repeated dose toxicity	Estragole	0 (corn oil), 37.5, 75, 150, 300, or 600 mg/kg/day	–	Incidences of bilateral degeneration of the germinal epithelium in the testes and bilateral hypospermia of the epididymis were observed at ≥300 mg/kg/day. Degeneration affected seminiferous tubules, which were devoid of germinal epithelial cells and lined only by Sertoli cells. Epididymal hypospermia was invariably associated with testicular degeneration and consisted of the complete absence or markedly reduced numbers of mature spermatozoa within the tubules.	NTP (2008)
3 months	Yes	10 B6C3F1 mice/sex/ dose	Oral gavage	Effects on the female reproductive cycle or organs not evaluated	Repeated dose toxicity	Estragole	0 (corn oil), 37.5, 75, 150, 300, or 600 mg/kg/day	–	All 600 mg/kg/day females died during week 1, where liver necrosis occurred in all high-dose females, and the incidence of diffuse fatty change was increased in this group.	NTP (2008)
Gestation days 6–19	Yes	25 female Sprague Dawley rats/dose	Oral gavage	The difference in metabolism to estragole	Developmental toxicity	Methyl eugenol	0 (0.5% methylcellulose), 80, 200, or 500 mg/kg/day	Maternal toxicity LOAEL = 80 mg/kg/day Developmental toxicity NOAEL = 200 mg/kg/ day	Maternal toxicity LOAEL is based on increases in maternal liver weights at all dose levels. Developmental toxicity NOAEL is based on decreased fetal body weight and increased incidences of unossified sternebrae at 500 mg/kg/day.	NTP (2004)

animals (78%). The average lifespan of mice administered 1′-hydroxyestragole was 13.6 months compared with 18 months in controls. In all treatment groups, body weights were markedly reduced at 4 and 8 months compared to controls while, at 10 months, the incidence of hepatomas increased significantly in treated animals. Histopathological examinations revealed portal fibrosis, chronic inflammation, and bile duct proliferation in addition to these tumors. Varied numbers of ceroid-laden histiocytes and focal areas of hyperplasia and megalocytosis were also reported. In another part of the study, male (55) and female (49) CD-1 mice were administered 370 mg/kg estragole by gavage twice a week for 10 doses beginning at 4 days of age. The mice were weaned at 35 days of age. Hepatomas in estragole-treated mice were observed as early as 11 months. In males, at 14 months, 3.5 hepatomas/animal were reported in contrast to 0.6 hepatomas/male animal in the control group, whereas hepatoma incidences in treated and control female animals were not statistically different (Miller et al., 1983). 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 NOAEL. The BMD (using BMDS v3.1.2) was derived using the results of the study by vandenBerg et al. (van den Berg et al., 2011). Using dose-response modeling, a BMD lower confidence limit for a benchmark response of 10% ($BMDL_{10}$) was calculated as being 10 mg/kg/day for incidences of hepatoma in female mice (Davidsen et al., 2022) (see Table 1).

In another part of the study, groups of 50 CD-1 mice/sex were administered single intraperitoneal doses of estragole, estragole-2′,3′-oxide, or 1′-hydroxyestragole such that doses were increased with age on postnatal days 1, 8, 15, and 22, and the animals were weaned on

postnatal day 22. A vehicle control group and an untreated group were also included in the study. Increased incidences of hepatocellular carcinomas were observed for mice treated with estragole (30/46, $P < 0.001$) relative to the incidence for the vehicle controls (11/42) (Miller et al., 1983).

Groups of newborn male B6C3F1 mice (50–60 per group) were given single intraperitoneal injections of estragole, 1′-hydroxyestragole, or 1′-hydroxy-2′,3'dehydroestragole. In mice administered 1′-hydroxyestragole and 1′-hydroxy-2′,3′-dehydroestragole, over 50% of the mice died within a week of the first injection. For these compounds, experiments were repeated using lower doses. During the study, a vehicle control group and an untreated group were also included in the study. At 18 months, increased incidences of hepatocellular carcinomas were observed in mice treated with estragole (2.4 hepatomas/animal), 1′-hydroxyestragole (5.6–5.8 hepatomas/animal), and 1′-hydroxy-2′,3′-dehydroestragole (9.4 hepatomas/animal) relative to the incidence for the vehicle controls (0.5 hepatoma/animal). Since these are single-dose studies, a NOAEL could not be determined.

From the available studies, several uncertainties have been identified, the most important being that the rodent studies are conducted at doses significantly higher dose than those encountered by humans from dietary exposure. In addition, metabolism-induced toxicity is dose dependent and is mediated by DNA/protein adduct formation. A NOAEL for adduct formation was determined in the turkey DNA adduct studies, which is 73% lower than the NOAEL derived for the repeated dose endpoint. Like methyl eugenol, herbs and spices also contain estragole, and the presence of other food substances in the matrix will affect its absorption, bioactivation, and detoxification. Moreover, rodent studies demonstrate that doses of 1–10 mg/kg/day (approximately 100–1000 fold higher than human exposure) offer minimal risk for tumor formation. Considering the uncertainties in the available data for estragole, the FEMA Expert Panel is of the opinion that estragole exposure at low levels encountered by humans does not pose a carcinogenic risk (Smith et al., 2002).

Therefore, the estragole MOE for the repeated dose toxicity endpoint can be calculated by dividing the estragole $BMDL_{10}$ in mg/kg/day by the total systemic exposure to estragole, 10/0.00036 = 27778. It should be noted that EFSA Scientific Committee advocates that any genotoxic and carcinogenic material 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).

In addition, the total systemic exposure for estragole (0.36 μg/kg/day) is below the TTC (1.5 μg/kg/day; Kroes et al., 2007) for the repeated dose toxicity endpoint at the current level of use.

11.1.2.1.1. 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 0.0010 mg/kg/day.

For severe irreversible adverse health effects, Gaylor et al. (Gaylor et al., 1999), as well as the Expert Panel for Fragrance Safety*, recommend using a default uncertainty factor of 10000. The RfD for estragole ether was calculated by dividing the BMDL10 (from the Repeated Dose and Developmental and Reproductive Toxicity sections) of 10 mg/kg/day by the uncertainty factor, 10000 = 0.0010 mg/kg/day.

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

Additional References: None.

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

11.1.3. Reproductive toxicity

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

11.1.3.1. Risk assessment. There are limited developmental toxicity data and insufficient fertility data on estragole. The safety of estragole has been thoroughly reviewed by Smith et al. (Smith et al., 2002) as a part of the allylalkoxybenzene derivatives (estragole and methyl eugenol) used as flavoring substances. It was concluded that exposure to methyl eugenol and estragole resulting from the consumption of food does not pose a significant risk to humans.

Data summarized in the table below (Table 2) indicate there are no developmental toxicity concerns from administrating nutmeg oil (containing 10%–16% of allylalkoxybenzenes, including estragole) to mice, rats, or hamsters. However, there are no developmental toxicity data available on estragole as a single component; hence, developmental toxicity data on read-across material eugenyl methyl ether (CAS # 93-15-2; see Section VI) are considered for the safety assessment of estragole. Furthermore, repeated gavage administration of estragole to rats over 3 months duration led to alterations in the male reproductive system. The study did not evaluate the effects on the female reproductive system. Similar effects were observed from the administration of methyl eugenol to rats and mice over a treatment duration of 14 weeks to 2 years. Although there are some differences in enzymes responsible for metabolizing estragole and methyl eugenol, both are metabolized to the 1-hydroxy-derivative, the precursor to the reactive genotoxic metabolite. Thus, developmental toxicity data available on methyl eugenol will be considered for the safety assessment of estragole.

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 methyl eugenol in a 0.5% methylcellulose vehicle from gestation days (GDs) 6–19. Maternal toxicity was manifested by clinical signs (rooting behavior), decreased body weight and 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, and 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 estragole MOE for the developmental toxicity endpoint can be calculated by dividing the eugenyl methyl ether NOAEL in mg/kg/day divided by the total systemic exposure to estragole, 200/0.00036, or 555555.**

There are limited fertility data for estragole. A repeated gavage administration of estragole to rats over 3 months duration led to alterations in the male reproductive system. The study did not evaluate the effects on the female reproductive system. Read-across material eugenyl methyl ether (CAS # 93-15-2; see Section VI; methyl eugenol) has sufficient fertility data that can be used to support the fertility endpoint. A GLP-compliant NTP 14-week subchronic toxicity study was conducted in F344/N rats. Groups of 10 rats/sex/dose were administered methyl eugenol 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 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 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

Table 3
Data summary for estragole.

LLNA Weighted Mean EC3 Value (No. Studies) μg/cm^2	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
4500 [1]	Weak	NA	NA	NA	1000

NOEL = No observed effect level; CNIH = Confirmation of No Induction in Humans; 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 derived from LLNA data as defined in Gerberick et al., 2001.

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 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 methyl eugenol 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, 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, respectfully. Clinical findings of toxicity were manifested as generalized morbidity in the male and female mice, which 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 was 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 significant when compared to the vehicle control group (147% and 145% for 10 and 30 mg/kg/day males, respectively, of 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).

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 estragole MOE for the fertility endpoint can be calculated by dividing the methyl eugenol NOAEL in mg/kg/day by the total systemic exposure to estragole, 30/0.00036, or 83333.**

Data included in the table below provides summaries of developmental toxicity or fertility data on estragole or estragole as a component of nutmeg oil (containing 10%–20% p-allylalkoxybenzene derivatives, including estragole). In addition, the table also includes data on structural analogs considered for the developmental toxicity and fertility endpoints.

When correcting for skin absorption (see Section V), the total systemic exposure to estragole (0.36 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 for 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 available data, estragole is considered to be a skin sensitizer with a WoE NESIL of 1000 μg/cm^2.

11.1.4.1. Risk assessment. Based on the existing data, estragole is considered a skin sensitizer with a NESIL of 1000 μg/cm^2. The chemical structure of this material indicates that it would be expected to react with skin proteins (Toxtree v3.1.0). Estragole was found to be both positive in direct peptide reactivity assays (DPRA) (Natsch, 2013; ECHA, 2018) and U-SENS assay (ECHA, 2018) and negative in the KeratinoSens (Natsch, 2013; Piroird et al., 2015). In a murine local lymph node assay (LLNA), estragole was found to be sensitizing with an EC3 value of 18% (4500 μg/cm^2) (Gerberick et al., 2005).

In a Confirmation of No Induction in Humans (CNIH), no reactions indicative of sensitization were observed when 2.5% (1937 μg/cm^2) estragole in alcohol SDA 39C was used for induction and challenge (RIFM, 1972b). In a human maximization test, no sensitization reactions were observed when 3% (2070 μg/cm^2) estragole in petrolatum was used for induction and challenge (RIFM, 1972a).

Taken together, these data provide WoE to classify estragole as a weak sensitizer. However, no CNIH that conforms to the published protocol is currently available on estragole (Politano and Api, 2008). In the absence of the human data to confirm the quantitative threshold obtained from the LLNA, a default no observed effect level (NOEL) of 1000 μg/cm^2 was used in the QRA as a NESIL, as assigned by Gerberick et al. for weak sensitizers (RIFM, 2008; Gerberick et al., 2001). 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 reference dose of 0.0010 mg/kg/day).

The CNIH conforming to the EC3 value (4500 μg/cm^2) is classified as weak and assigned a conservative default NOEL of 1000 μg/cm^2 for use in the QRA (RIFM, 2008; Gerberick et al., 2001). See Table 3 below for the data summary for estragole.

Additional References: Barratt, 1992.

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

11.1.5. Photoirritation/photoallergenicity

Based on the available UV/Vis absorption spectra, estragole would not be expected to present a concern for photoirritation or

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	12.10			1000000	0.0121	
ECOSAR Acute Endpoints **(Tier 2)** ***v2.0***	5.861	3.810	4.969	10000	0.381	Neutral Organics

photoallergenicity.

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

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate minor absorbance in the range of 290–700 nm. The molar absorption coefficients (183, 187, 218 L mol^{-1} • cm^{-1} under neutral, acidic, and basic conditions, respectively) for wavelengths between 290 and 700 nm are below the benchmark (1000 L mol^{-1} • cm^{-1}) of concern for photoirritating effects (Henry et al., 2009).

Additional References: None.

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

11.1.6. Local Respiratory Toxicity

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

11.1.6.1. Risk assessment. There are insufficient inhalation data available on estragole. Based on the Creme RIFM Model, the inhalation exposure is 0.0050 mg/day. This exposure is 94 times lower than the Cramer Class III TTC value of 0.47 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: Regnault-Roger, 1995; Perrucci et al., 1995.

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 estragole 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 VoU Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, Estragole 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 estragole as possibly being 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), estragole presents a risk to the aquatic compartment in the screening-level assessment.

11.2.1.2. Key studies

11.2.1.2.1. Biodegradation. RIFM, 1997: The ready biodegradability

of the test material was determined by the manometric respirometry test according to the OECD 301F method. The biodegradation rate was 82% at the end of the 10-day window and 84% after 31 days.

11.2.1.2.2. Ecotoxicity. RIFM, 2003b: The acute toxicity of test material to *Daphnia magna* was performed under static-renewal conditions in sealed vials without headspace according to the OECD 202 method. The 48-h EC50 value based on mean measured concertation was reported to be 8.87 mg/L.

RIFM, 2003a: An algae (*Selenastrum capricornutum*) 72-h growth and reproduction toxicity test was performed under static conditions according to the OECD 201 method. The EC50s were reported to be 1.01 mg/L, 1.35 mg/L, and 2.81 mg/L for the area under growth, number of cells, and specific growth rate, respectively. The NOECs of 0.118 mg/L and 0.223 mg/L were reported for the number of cells and growth rate, respectively.

11.2.1.2.3. Other available data. Estragole has been registered for REACH, with the following additional data available at this time (ECHA, 2018):

The acute toxicity of the test material to the *Daphnia magna* was tested according to the OECD 202 method. The 48-h EC50 was reported to be 17.583 mg/L.

The 72-h algae growth inhibition test was performed according to OECD 201 method under static conditions. The EC50 value based on the mean measured concentration for growth rate was reported to be 10.35 mg/L.

11.2.1.3. Risk assessment refinement. Since estragole has passed the screening criteria, measured data are included for completeness 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, 2002).

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	3.4	3.4
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	1–10	1–10
Risk Characterization: PEC/PNEC	**<1**	**<1**

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

The RIFM PNEC is 0.381 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 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:** https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm
- **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 appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 01/31/23.

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.2023.114143.

Appendix

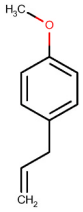
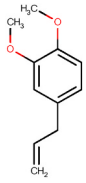
Read-across Justification

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.

Principal Name	Target Material	Read-across Material
	Estragole	Eugenyl methyl ether
CAS No.	140-67-0	93-15-2
Structure		
Similarity (Tanimoto Score)		0.73
Read-across Endpoint		• Reproductive toxicity
Molecular Formula	$C_{10}H_{12}O$	$C_{11}H_{14}O_2$
Molecular Weight (g/mol)	148.20	178.23
Melting Point (°C, EPI Suite)	−1.19	70
Boiling Point (°C, EPI Suite)	215.5	270.5
Vapor Pressure (Pa @ 25°C, EPI Suite)	22	1.60E+000
Log K_{OW} (KOWWIN v1.68 in EPI Suite)	3.47	3.03
Water Solubility (μg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	178	500
J_{max} (μg/cm^2/h, SAM)	16.958	22.564
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	4.68E+001	2.77E+000
Reproductive toxicity		
ER Binding (OECD QSAR Toolbox v4.5)	• Non-binder, without OH or NH_2 group	• Non-binder, without OH or NH_2 group
Developmental Toxicity (CAESAR v2.1.6)	• Non-toxicant (low reliability)	• Non-toxicant (low reliability)
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 estragole (CAS # 140-67-0). 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, eugenyl methyl ether (CAS # 93-15-2) was identified as a read-across analog with sufficient data for toxicological evaluation.

Conclusions

- Eugenyl methyl ether (CAS # 93-15-2) was used as a read-across analog for the target material, estragole (CAS # 140-67-0), for the reproductive toxicity endpoint.
  - o The target material and the read-across analog are structurally similar and belong to a class of phenylpropene ethers.
  - o The target material and the read-across analog share a phenylpropene moiety with a methyl ether group in position 4.
  - o The key difference between the target material and the read-across analog is that the read-across analog has 1 extra methyl ether group in position 3. This structural difference is toxicologically insignificant.
  - o The similarity between the target material and the read-across analog is indicated by the Tanimoto score. Differences between the structures that affect the Tanimoto score are toxicologically insignificant.
  - o The 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 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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