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RIFM fragrance ingredient safety assessment, anethole (isomer unspecified), CAS Registry Number 104-46-1

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Name: Anethole (isomer unspecified)
CAS Registry Number: 104-46-1
Additional CAS Numbers*:
25679-28-1 *cis*-Anethole (no reported use)
4180-23-8 *trans*-Anethole
*Included because the materials are isomers.

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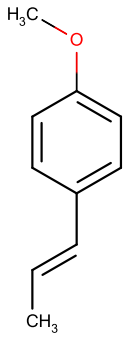

Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
CNIH – Confirmation of No Induction in Humans test. A human repeat insult patch test that is performed to confirm an already determined safe use level for fragrance ingredients (Na et al., 2020)
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015a, 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest Observable Effect Level
MOE - Margin of Exposure
MPPD - Multiple-Path Particle Dosimetry. An *in silico* model for inhaled vapors used to simulate fragrance lung deposition
NA - North America
NESIL - No Expected Sensitization Induction Level
NOAEC - No Observed Adverse Effect Concentration
NOAEL - No Observed Adverse Effect Level
NOEC - No Observed Effect Concentration
NOEL - No Observed Effect Level
OECD - Organisation for Economic Co-operation and Development
OECD TG - Organisation for Economic Co-operation and Development Testing Guidelines
PBT - Persistent, Bioaccumulative, and Toxic
PEC/PNEC - Predicted Environmental Concentration/Predicted No Effect Concentration
QRA - Quantitative Risk Assessment
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use
vPvB - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

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

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

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

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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.

Anethole (isomer unspecified) was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data from the target material and additional material *trans*-anethole (CAS # 4180-23-8) show that anethole (isomer unspecified) is not genotoxic. Data on additional material *trans*-anethole (CAS # 4180-23-8) provide a calculated MOE >100 for the repeated dose toxicity and reproductive toxicity endpoints. Based on the limited existing data and the additional material *trans*-anethole (CAS # 4180-23-8), anethole (isomer unspecified) is considered to be a weak skin sensitizer with a defined NESIL of 5500 μg/cm^2. The phototoxicity/photoallergenicity endpoints were evaluated based on UV/Vis spectra; anethole (isomer unspecified) is not expected to be phototoxic/photoallergenic. The local respiratory toxicity endpoint was evaluated using the TTC for a Cramer Class III material; exposure to anethole (isomer unspecified) is below the TTC (0.47 mg/day). The environmental endpoints were evaluated; anethole (isomer unspecified) was found not to be 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., PEC/PNEC), are <1.

Human Health Safety Assessment

Genotoxicity: Not genotoxic.	(RIFM, 1984; Gorelick, 1995)
Repeated Dose Toxicity: NOAEL = 121 mg/kg/day.	RIFM, (1985)
Developmental and Reproductive Toxicity: NOAEL = 175 mg/kg/day.	RIFM, (1992a)
Skin Sensitization: NESIL = 5500 μg/cm^2.	(RIFM, 2012a; RIFM, 2012b)
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic.	(UV/Vis Spectra, RIFM Database)

Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:	
Persistence: Critical Measured Value: 91% (OECD 301B) for CAS # 104-46-1	RIFM, (1994)
Bioaccumulation: Screening-level: 79.92 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity: Critical Measured Value: 28-Day Fish NOEC: 0.34 mg/L for CAS # 4180-23-8	(ECHA REACH Dossier: (E)-Anethole; ECHA, 2013)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards	
Risk Assessment:	
Screening-level: PEC/PNEC (North America and Europe) > 1	(RIFM Framework; Salvito, 2002)
Critical Ecotoxicity Endpoint: 28-Day Fish NOEC: 0.34 mg/L for CAS # 4180-23-8	(ECHA REACH Dossier: (E)-Anethole; ECHA, 2013)
RIFM PNEC is: 6.8 μg/L	
• **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe <1	

1. Identification

Chemical Name: Anethole (isomer unspecified)	Chemical Name: *cis*-Anethole	Chemical Name: *trans*-Anethole
CAS Registry Number: 104-46-1	**CAS Registry Number:** 25679-28-1	**CAS Registry Number:** 4180-23-8
Synonyms: Benzene, 1-methoxy-4-(1-propenyl)-; 1-Methoxy-4-(1-propenyl) benzene (isomer unspecified); 1-Methoxy-4-propenylbenzene (isomer unspecified); 4-Methoxy-1-propenylbenzene (isomer unspecified); 4-Methoxypropenylbenzene (isomer unspecified); 4-Propenylanisole (isomer unspecified); *p*-Propenylanisole (isomer unspecified); *p*-Propenylphenyl methyl ether	**Synonyms:** Benzene, 1-methoxy-4-(1-propenyl)-, (Z)-; *cis*-1-(p-Methoxyphenyl)-1-propene; *cis*-4-(1-Propenyl)anisole; 1-Methoxy-4-prop-1-en-1-ylbenzene; *cis*-Anethole	**Synonyms:** (E)-Anethole; Benzene, 1-methoxy-4-(1-propenyl)-, (E)-; *p*-Methoxy-α-phenylpropene (E); 1-Methoxy-4-propenylbenzene (E); 4-Methoxy-1-propenylbenzene (E); *p*-Propenylanisole (E); *p*-Propenylphenyl methyl ether (E); 1-ﾒﾄｷｼｰ(1-(又は2-ﾌﾙｵﾍﾞﾆﾙ)ﾍﾟﾝﾃﾍﾟﾝ; 1-

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(isomer unspecified); Anisole, *p*-propenyl-; Anethol; 4-Propenylanisole; 4-Methoxypropenylbenzene; 1-Methoxy-4-propenylbenzene; p-Propenylphenyl methyl ether; Anethole; 1 - ﾒﾄｷｼ - 4 - ﾌﾟﾛﾍﾟﾆﾙﾍﾞﾝｾﾞﾝ; 1-Methoxy-4-prop-1-en-1-ylbenzene; Anethole (isomer unspecified)		ﾒﾄｷｼ-4-ﾌﾟﾛﾍﾟﾆﾙﾍﾞﾝｾﾞﾝ; 1-Methoxy-4-prop-1-en-1-ylbenzene; Anethole Synthetic; *trans*-Anethole
Molecular Formula: $C_{10}H_{12}O$	**Molecular Formula:** $C_{10}H_{12}O$	**Molecular Formula:** $C_{10}H_{12}O$
Molecular Weight: 148.2	**Molecular Weight:** 148.2	**Molecular Weight:** 148.2
RIFM Number: 5137	**RIFM Number:** None	**RIFM Number:** 152
Stereochemistry: No isomer specified. One stereocenter and 2 total stereoisomers possible.	**Stereochemistry:** *Cis* isomer specified. One stereocenter and 2 total stereoisomers possible.	**Stereochemistry:** *Trans* isomer specified. One stereocenter and 2 total stereoisomers possible.

2. Physical data

CAS # 104-46-1	CAS # 25679-28-1	CAS # 4180-23-8
Boiling Point: 217.31 °C (EPI Suite)	**Boiling Point:** 217.31 °C (EPI Suite)	**Boiling Point:** 236 °C (Fragrance Materials Association [FMA]), 217.31 °C (EPI Suite)
Flash Point: 88 °C (Globally Harmonized System [GHS])	**Flash Point:** Not Available	**Flash Point:** 101 °C (GHS), >200 °F; CC (FMA)
Log K_{OW}: 3.39 (EPI Suite)	**Log K_{OW}:** 3.39 (EPI Suite)	**Log K_{OW}:** 3.39 (EPI Suite)
Melting Point: −0.69 °C (EPI Suite)	**Melting Point:** −0.69 °C (EPI Suite)	**Melting Point:** −0.69 °C (EPI Suite)
Water Solubility: 98.68 mg/L (EPI Suite)	**Water Solubility:** 98.68 mg/L (EPI Suite)	**Water Solubility:** 98.68 mg/L (EPI Suite)
Specific Gravity: 0.99 g/mL (RIFM, 1994)	**Specific Gravity:** Not Available	**Specific Gravity:** 0.985 (FMA)
Vapor Pressure: 0.041 mm Hg at 20 °C (EPI Suite v4.0), 0.0634 mm Hg at 25 °C (EPI Suite)	**Vapor Pressure:** 0.0634 mm Hg at 25 °C (EPI Suite)	**Vapor Pressure:** 0.041 mm Hg at 20 °C (EPI Suite v4.0), 0.05 mm Hg 20C (FMA), 0.0634 mm Hg at 25 °C (EPI Suite)
UV Spectra: Minor absorbance between 290 and 700 nm; the molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})	**UV Spectra:** Not Available	**UV Spectra:** Minor absorbance between 290 and 700 nm; the molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})
Appearance/ Organoleptic: Colorless, slightly oily liquid. Very sweet, herbaceous-warm odor	**Appearance/ Organoleptic:** Not Available	**Appearance/ Organoleptic:** Not available

3. Volume of use (worldwide band)

1. **Volume of Use (worldwide band):** 100–1000 metric tons per year (IFRA, 2015)

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

1. **95**th **Percentile Concentration in Hydroalcoholics:** 0.072% (RIFM, 2018)
2. **Inhalation Exposure*:** 0.00024 mg/kg/day or 0.018 mg/day (RIFM, 2018)
3. **Total Systemic Exposure**:** 0.010 mg/kg/day (RIFM, 2019)

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

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

***When a safety assessment includes multiple materials, the highest exposure out of all included materials will be recorded here for the 95th Percentile Concentration in hydroalcoholics, inhalation exposure, and total exposure.

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

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

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

2. Analogs Selected:

a. **Genotoxicity:** None
b. **Repeated Dose Toxicity:** None
c. **Developmental and Reproductive Toxicity:** None
d. **Skin Sensitization:** None
e. **Phototoxicity/Photoallergenicity:** None
f. **Local Respiratory Toxicity:** None
g. **Environmental Toxicity:** None

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

7. Metabolism

The metabolism of *trans*-anethole in rodents has been reviewed by the JECFA Expert Panel (see Fig. 1). The primary pathways for metabolism are O-demethylation, ω-oxidation of the side chain, as well as sidechain epoxidation. In mice, metabolites formed following these pathways accounted for 32%, 28%, and 41%, respectively, whereas in rats, metabolites accounted for 37%, 13%, and 49%, respectively. These initial steps were followed by oxidation and hydration subsequently undergoing extensive conjugation with sulfate, glucuronic acid, glycine, and glutathione. Since O-demethylation is a deactivation pathway, it is considered that the toxicity of *trans*-anethole is mediated through the

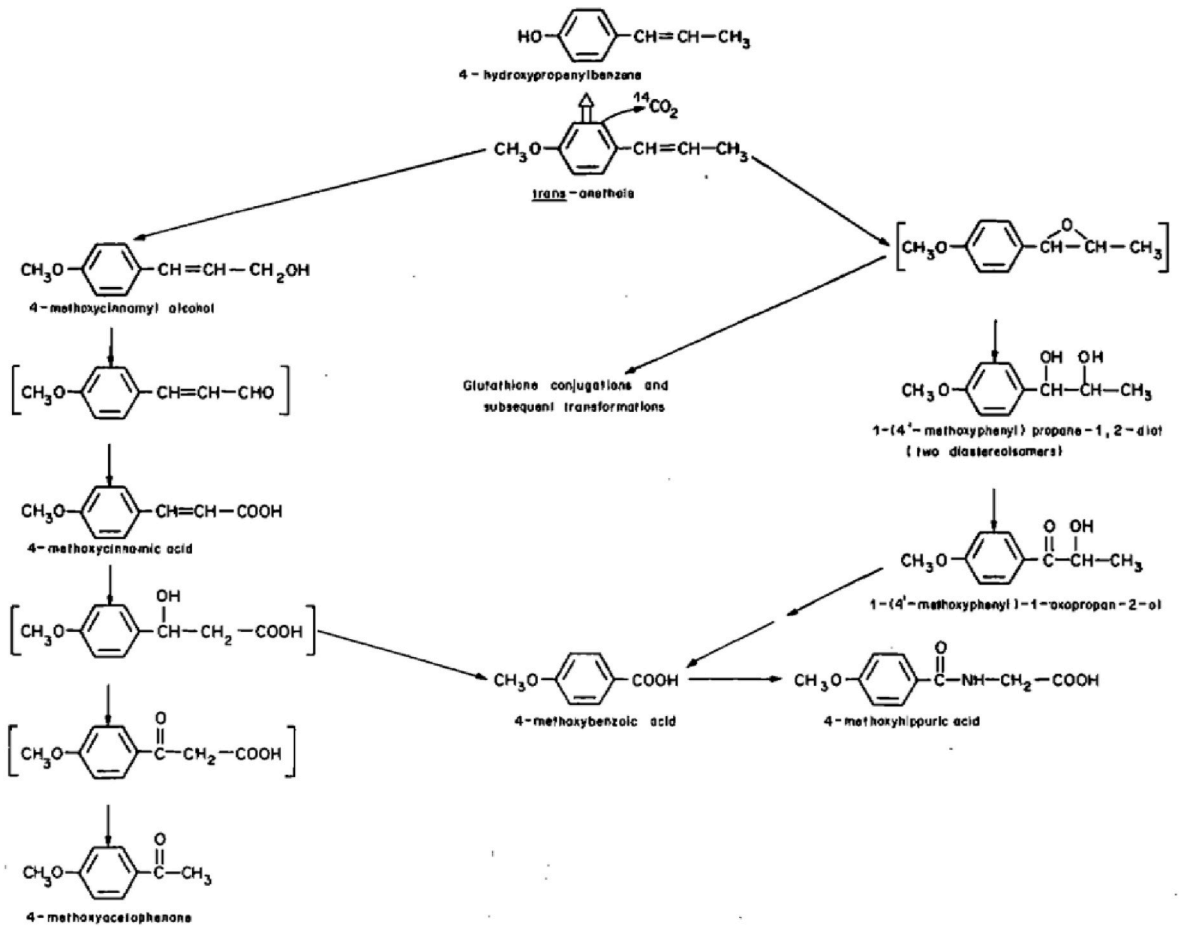

Fig. 1. Metabolism of trans-anethole in rats and mice (Sangster, 1984a).

varying amounts of metabolites resulting from sidechain epoxidation and ω-oxidation pathways. In isolated rat hepatocytes (Bounds, 1996) 82% of *trans*-anethole was metabolized within 6 h, and the 3 major metabolites identified were 4-methoxybenzoic acid (33%), 4-methoxycinnamic acid (7%), and 4-methoxycinnamyl alcohol (WHO, 1999). Metabolites of *trans*-anethole are excreted rapidly and thus unable to accumulate in tissues or transfer to products (EFSA, 2011).

8. Natural occurrence

Anethole (isomer unspecified) is reported to occur in the following foods by the VCF*:

Apple, fresh (*Malus* species)
Buckwheat
Caraway (*Carum carvi* L.)
Cheese, various types
Dill (*Anethum* species)
Elderberry (*Sambucus nigra* L.)
Ginger (*Zingiber* species)
Mustard (*Brassica* species)
Rhubarb
Star anise
Sweetgrass oil (*Hierochloe odorata*)
Sweet marjoram (*Origanum majorana* L.)
Tea

cis-Anethole is reported to occur in the following foods by the VCF*:
Anise (*Pimpinella anisum* L.)
Anise brandy
Fennel (*Foeniculum vulg., ssp. capillaceum*; var.)
Mastic (*Pistacia lentiscus*)
Ocimum species
Star Anise

trans-Anethole is reported to occur in the following foods by the VCF*:
Anise (*Pimpinella anisum* L.)
Apple, fresh (*Malus* species)
Calamus (sweet flag) (*Acorus calamus* L.)
Cinnamomum species
Cloves (*Eugenia caryophyllata* Thunberg)
Fennel (*Foeniculum vulg., ssp. capillaceum*; var.)
Licorice (*Glycrrhiza* species)
Macadamia nut (*Macadamia integrifolia*)
Myrtyle (*Myrtus communis* L.)

Star Anise

*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 for *trans*-Anethole (ECHA, 2013; accessed 07/29/21). Anethole (isomer unspecified) and *cis*-anethole are both pre-registered for 2010; no dossiers for either are available as of 07/29/21.

10. Conclusion

The maximum acceptable concentrations[a] in finished products for anethole (isomer unspecified) 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)	0.42
2	Products applied to the axillae	0.13
3	Products applied to the face/body using fingertips	0.67
4	Products related to fine fragrances	2.4
5A	Body lotion products applied to the face and body using the hands (palms), primarily leave-on	0.60
5B	Face moisturizer products applied to the face and body using the hands (palms), primarily leave-on	0.60
5C	Hand cream products applied to the face and body using the hands (palms), primarily leave-on	0.60
5D	Baby cream, oil, talc	0.20
6	Products with oral and lip exposure	1.4
7	Products applied to the hair with some hand contact	1.3
8	Products with significant ano-genital exposure (tampon)	0.20
9	Products with body and hand exposure, primarily rinse-off (bar soap)	4.6
10A	Household care products with mostly hand contact (hand dishwashing detergent)	4.7
10B	Aerosol air freshener	7.4
11	Products with intended skin contact but minimal transfer of fragrance to skin from inert substrate (feminine hygiene pad)	0.20
12	Other air care products not intended for direct skin contact, minimal or insignificant transfer to skin	No Restriction

Note: [a]Maximum acceptable concentrations for each product category are based on the lowest maximum acceptable concentrations (based on systemic toxicity, skin sensitization, or any other endpoint evaluated in this safety assessment). For anethole (isomer unspecified), the basis was the reference dose of 1.21 mg/kg/day, a predicted skin absorption value of 80%, and a skin sensitization NESIL of 5500 μg/cm^2.

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

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

11. Summary

11.1. Human health endpoint summaries

11.1.1. Genotoxicity

Based on the current existing data and use levels, anethole (isomer unspecified) does not present a concern for genetic toxicity.

11.1.1.1. Risk assessment. The mutagenic activity of anethole (isomer unspecified) has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and equivalent with OECD TG 471 using the standard plate incorporation method. *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 were treated with anethole (isomer unspecified) in dimethyl sulfoxide (DMSO) at concentrations ranging from 0 to 150 μL/plate. Slight increases with TA1538 were observed; however, these increases were not repeatable. No increases in the mean number of revertant colonies were observed with any other tester strain (RIFM, 1984). Under the conditions of the study, anethole (isomer unspecified) was not mutagenic in the Ames test. Additionally, anethole (isomer unspecified) was tested in a mouse lymphoma assay conducted equivalent to OECD TG 476/GLP guidelines and induced a dose-related increase in the mutant frequency in the presence of metabolic activation (RIFM, 1982). However, changes in osmolality and pH fluctuations can be responsible for the increase in mutant frequencies (Scott, 1991; Brusick, 1986) and can lead to false-positive outcomes considering this study was conducted prior to the updated guideline which requires evaluation of chemical associated changes in osmolality and pH. Thus, the positive outcome observed in the *in vitro* MLA study could potentially be a false-positive.

The clastogenicity of anethole was assessed in an *in vitro* chromosome aberration study equivalent to OECD TG 473. Chinese hamster ovary cells were treated with anethole in DMSO at concentrations up to 0.2 μL/mL in the presence and absence of metabolic activation. No statistically significant increases in the frequency of cells with structural chromosomal aberrations or polyploid cells were observed with any concentration of the test material, either with or without S9 metabolic activation (Gorelick, 1995). Under the conditions of the study, anethole was considered to be non-clastogenic in the *in vitro* chromosome aberration assay. Additionally, in 2 separate *in vivo* micronucleus studies, negative results were observed (WHO, 1999). *trans*-Anethole (CAS # 4180-23-8) is mainly metabolized via O-demethylation and epoxidation of the side chain, followed by the formation of diols in rodents and humans (WHO, 1999). Some metabolites of *trans*-anethole have given rise to safety concerns. The epoxide has been shown to be cytotoxic and hepatotoxic and genotoxic in some studies. However, after reviewing the available data, JECFA finally concluded in 2000 that *trans*-anethole was unlikely to be genotoxic *in vivo* (EFSA, 2011).

Based on the data available, anethole (isomer unspecified) does not present a concern for genotoxic potential.

Additional References: RIFM, 1987; RIFM, 1982

Literature Search and Risk Assessment Completed On: 06/01/21

11.1.2. Repeated dose toxicity

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

11.1.2.1. Risk assessment. There are sufficient repeated dose toxicity data on additional material *trans*-anethole (CAS # 4180-23-8). The available data for *trans*-anethole have been reviewed by the EFSA Expert Panel as a flavor additive in food (EFSA, 2011; WHO, 1999) and have been recognized as GRAS by the US FDA (see 21CFR182.60; ECFR,

2020).

In a GLP-compliant study, Sprague Dawley rats were administered t-anethole via diet at concentrations of 0, 0.25, 0.5, and 1% (equivalent to 0, 105, 210, and 420 mg/kg/day in males, and 0, 121, 242, and 484 mg/kg/day in females, as calculated in the study report) for 117 weeks. The control contained 52 Sprague Dawley rats/sex, the low-dose group contained 78 rats/sex, the mid-dose group contained 52 Sprague Dawley rats/sex, and the high-dose group contained 52 Sprague Dawley rats/sex with an additional group of 26 Sprague Dawley rats/sex (78 in total). The additional 26 Sprague Dawley rats/sex were administered the highest dose (1%) through diet for 54 weeks, then 10/26 of each sex were treated as a recovery group, receiving the diet alone (no test material) for the remainder of the study. No treatment-related mortality occurred throughout the study. There were no treatment-related effects on behavior or hematology. Bodyweight gain was reduced at all doses during the first 6 months; this effect was completely attenuated in the low- and mid-dose groups, but a marginal difference remained in the high-dose group. This effect was also seen in the recovery group, and completely reversed after the treatment ceased; thus, it was attributed to an aversion to the taste of the test material. Bodyweight gain decreases were correlated with decreases in food consumption. In all dose groups, there was a dose-dependent increase in female relative liver weight with a correlated increase in hepatocytic hypertrophy at the mid and high doses. Other effects on the liver included hepatocytic vacuolation in males at the high dose; sinusoidal dilatation in mid-dose females and in both sexes at the high dose; nodular hyperplasia in mid-dose males and high-dose males and females. Benign liver cell tumors (hepatocellular adenomas) were seen (1/52 control males, 1/78 low-dose males, 1/52 mid-dose males, 5/78 high-dose males; 3/52 control females, 1/78 low-dose females, 0/52 mid-dose females, 5/78 high-dose females). Malignant liver cell tumors (hepatocellular carcinomas) were seen (2/52 control males, 3/78 low-dose males, 3/52 mid-dose males, 1/78 high-dose males; 0/52 control females, 0/78 low-dose females, 0/52 mid-dose females, 7/78 high-dose females) (RIFM, 1985). Incidences of benign and malignant liver cell tumors were increased in females at the high dose; however, because incidences were low and restricted to a single sex, it was concluded upon further investigation that *trans*-anethole does not present a carcinogenic risk in humans (Newberne, 1989). The data reviewed at the present meeting indicate that *trans*-anethole and its metabolites are unlikely to be genotoxic *in vivo* and suggest that a cytotoxic metabolite, anethole epoxide, is the possible causative agent of the hepatotoxic effects in rats (WHO, 1999). Based on liver effects at the mid and high doses, the NOAEL for this study was considered to be 121 mg/kg/day.

Therefore, the anethole MOE for the repeated dose toxicity endpoint can be calculated by dividing the t-anethole NOAEL in mg/kg/day by the total systemic exposure to anethole, 121/0.010, or 12100. In addition, the ADI established by the EFSA Expert Panel is 2 mg/kg/day (EFSA, 2011).

In addition, the total systemic exposure for anethole (10 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes, 2007) for the repeated dose toxicity endpoint at the current level of use.

11.1.3. Derivation of reference dose (RfD)

Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020) and a reference dose of 1.21 mg/kg/day.

The RIFM Criteria Document (Api, 2015) calls for a default MOE of 100 (10 × 10), based on uncertainty factors applied for interspecies (10 ×) and intraspecies (10 ×) differences. The reference dose for anethole was calculated by dividing the lowest NOAEL (from the Repeated Dose and Reproductive Toxicity sections) of 121 mg/kg/day by the uncertainty factor, 100 = 1.21 mg/kg/day.

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

Additional Studies: Sangster, 1984b; Sangster, 1984a; Caldwell, 1988; RIFM, 1997a; Newberne, 1989; Truhaut, 1989; Sangster, 1983; Marshall, 1992; RIFM, 1997b; RIFM, 1997c; WHO, 1999

Literature Search and Risk Assessment Completed On: 05/20/21

11.1.4. Reproductive toxicity

The MOE for anethole (isomer unspecified) is adequate for the reproductive toxicity endpoint at the current level of use.

11.1.4.1. Risk assessment. There are no developmental toxicity data on anethole (isomer unspecified). However, there are sufficient developmental toxicity data on additional material *trans*-anethole (CAS # 4180-23-8; see Section I). A reproductive and developmental toxicity screening test was conducted in female Sprague Dawley rats. Groups of 10 female rats received daily doses of 0, 35, 175, or 350 mg/kg/day *trans*-anethole in corn oil by gavage for 7 days before cohabitation, during 7 days of mating with untreated male rats through gestation and parturition, up to day 4 of lactation (dams that delivered a litter), or for 25 days after the cohabitation period (dams that did not deliver a litter). All animals were euthanized at day 4 of lactation or at the end of the administration of *trans*-anethole for rats that did not show signs of mating. Gross necropsy was performed on adult animals, and the pups were examined externally. One high-dose dam was found dead on day 20 of gestation; gross necropsy revealed congested lungs, but uterine contents showed 17 normal fetuses and 2 early resorptions. Animals at 350 mg/kg/day appeared to be in poor condition, as indicated by clinical observations of emaciation, pale and ungroomed coat, and stained fur. The body weights of high-dose group dams were significantly lower than the controls during the pre-mating, gestation, and lactation periods. Dams at 175 mg/kg/day also had lower body weights throughout the study, without statistical significance except at several intervals during gestation. Food consumption was significantly reduced during the pre-mating period in animals at 175 and 350 mg/kg/day; significantly lowered food consumption was also seen at 350 mg/kg/day at the end of gestation. The following changes were observed at 350 mg/kg/day: a significantly increased number of dams with stillborn pups and with a total loss of litters by day 4, a significantly increased number of stillborn pups, a significantly decreased number of liveborn pups surviving to day 4 (viability index), and significantly decreased pup body weight sat birth and at day 4 postpartum. Thus, the NOAEL for maternal toxicity was considered to be 35 mg/kg/day, based on clinical signs and reduced body weight and food consumption among the higher-dose group dams. The NOAEL for developmental toxicity was considered to be 175 mg/kg/day, based on increased stillbirths and pup mortality and decreased pup viability and pup body weight among high-dose group pups (RIFM, 1992a). **Therefore, the anethole (isomer unspecified) MOE for the development toxicity endpoint can be calculated by dividing the *trans*-anethole NOAEL in mg/kg/day by the total systemic exposure to anethole (isomer unspecified), 175/0.010 or 17500.**

There are no fertility data on anethole (isomer unspecified). However, there are sufficient reproductive toxicity data on additional material *trans*-anethole (CAS # 4180-23-8; see Section I). In a reproductive and developmental toxicity screening test, groups of 10 female Sprague Dawley rats received daily doses of 0, 35, 175, or 350 mg/kg/day *trans*-anethole in corn oil by gavage for 7 days before cohabitation, during 7 days of mating with untreated male rats through gestation and

parturition, up to day 4 of lactation (dams that delivered a litter), or for 25 days after the cohabitation period (dams that did not deliver a litter). Treatment did not affect female mating performance or fertility up to the dose of 175 mg/kg/day (males were untreated in this study). The NOAEL for female fertility was considered to be 175 mg/kg/day, based on a significantly increased number of dams with stillborn pups at 350 mg/kg/day (RIFM, 1992a).

A 4-generation reproductive toxicity study was conducted in Wistar rats given a single dietary concentration of 1% *trans*-anethole. Groups of 20 4-week-old rats/sex were fed diets containing the test material in concentrations of either 0% (basal diet) or 1% (actual dose varied from 600 to 1500 mg/kg/day) for 70 days prior to mating. The animals were then mated on a 1-to-1 basis for a maximum of 15 days, with 9 pairs of rats fed the control diet (group I), 9 pairs fed the treated diet (group IV), 10 pairs of males fed the control diet and females fed the treated diet (group II), and 10 pairs of males fed the treated diet and females fed the control diet (group III). During the mating period, only animals in group IV were fed the treated diet. After the mating period, the females were housed individually and were fed the control or treated diet as established during the pre-mating period. The dams were allowed to litter and nurse the pups to weaning (3 weeks). After weaning, the offspring received the same dietary treatment as both of their parents (70 days from the time of weaning). At approximately 3 months of age, rats were bred to obtain the next generation. A similar procedure was followed to obtain the third and fourth generations. All groups of rats (F0, F1, F2, and F3 generations) treated with *trans*-anethole had reduced body-weight gain. There was no difference in mating or in the number of dams that brought litters to term (fertility index and gestation index, respectively). There was no treatment-related effect on the number of dams with stillborn pups or on pup viability, survival through lactation, or litter size. Pup body weights per litter were significantly reduced for all pups reared by treated dams, regardless of the diet fed to the males or to the dams during gestation. Since successful mating or the number of dams that brought litters to term (fertility index and gestation index, respectively) was not affected by treatment, the fertility NOAEL was considered to be 1% or 600–1500 mg/kg/day (ECHA, 2013; US EPA, 1973).

The more conservative NOAEL of 175 mg/kg/day from the gavage reproductive and developmental toxicity screening test was considered for the fertility endpoint. **Therefore, the anethole (isomer unspecified) MOE for the fertility endpoint can be calculated by dividing the *trans*-anethole NOAEL in mg/kg/day by the total systemic exposure to anethole (isomer unspecified), 175/0.010 or 17500.**

Additional References: Farook, 1989; Zondek, 1938

Literature Search and Risk Assessment Completed On: 05/31/21

11.1.5. Skin sensitization

Based on the limited existing data and the additional material, *trans*-anethole (CAS # 4180-23-8), anethole (isomer unspecified) is considered to be a weak skin sensitizer with a defined NESIL of 5500 μg/cm^2.

11.1.5.1. Risk assessment. Limited skin sensitization studies are available for anethole (isomer unspecified). Based on the available animal and human data for an additional material, *trans*-anethole (CAS # 4180-23-8), anethole (isomer unspecified) is considered a skin sensitizer.

The chemical structure of the target material and the additional materials indicate that they would be expected to react with skin proteins (Roberts, 2007; Toxtree v3.1.0). In a peptide reactivity assay, anethole (isomer unspecified) showed reactivity with the Cys-peptide (Natsch, 2007). The additional material, *trans*-anethole was found to have minimal reactivity in a DPRA based on the cysteine prediction model and no induction of NRf2-regulated genes in a KeratinoSens assay, but it was found to induce dendritic cell activation in a human cell line activation test (h-CLAT) (RIFM, 2016a; RIFM, 2016b; RIFM, 2017). In a murine local lymph node assay (LLNA), the additional material, *trans*-anethole was found to be sensitizing with an EC3 value of 2.7% (675 μg/cm^2) (RIFM, 2004). In guinea pigs, a maximization test showed reactions indicative of sensitization (ECHA, 2013); Barratt, 1992). In a human maximization test, no skin sensitization reactions were observed (RIFM, 1971). Additionally, in a Confirmation of No Induction in Humans test (CNIH) with 5% (2754 μg/cm^2) of the additional material *trans*-anethole in 1:3 ethanol:diethyl phthalate (EtOH:DEP), no reactions indicative of sensitization were observed in any of the 101 volunteers (RIFM, 2012b). Similarly, in another confirmatory CNIH with 10% (5509 μg/cm^2) of *trans*-anethole, no visible reactions were observed in any of the 105 volunteers (RIFM, 2012a).

Based on the weight of evidence (WoE) from structural analysis and animal and human studies, anethole (isomer unspecified) is a sensitizer with a WoE NESIL of 5500 μg/cm^2 (see Table 1). Section X provides the maximum acceptable concentrations in finished products, which take into account skin sensitization and application of the Quantitative Risk Assessment (QRA2) described by Api et al. (RIFM, 2020) and a reference dose of 1.21 mg/kg/day.

Additional References: Piroird (2015); Gerberick (2005); Natsch (2013); Klecak (1985); Klecak (1979); Ishihara (1986); Natsch (2007).

Literature Search and Risk Assessment Completed On: 05/27/21

Table 1
Data summary for the additional material, *trans*-anethole.

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
675 [1]	Moderate	5509	1386	NA	5500

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

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

[b] Data derived from CNIH or HMT.

[c] WoE NESIL limited to 2 significant figures.

11.1.6. Phototoxicity/photoallergenicity

Based on UV/Vis absorption spectra, anethole (isomer unspecified) would not be expected to present a concern for phototoxicity or photoallergenicity.

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

11.1.7. UV spectra analysis

UV/Vis absorption spectra (OECD TG 101) for anethole (isomer unspecified) were obtained. The spectra indicate minor absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark, 1000 L mol^{-1} • cm^{-1}, of concern for phototoxic effects (Henry, 2009).

Additional References: None

Literature Search and Risk Assessment Completed On: 05/19/21

11.1.8. Local Respiratory Toxicity

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

11.1.8.1. Risk assessment. There is insufficient inhalation data available on anethole (isomer unspecified). Based on the Creme RIFM Model, the inhalation exposure is 0.018 mg/day. This exposure is 26 times lower than the Cramer Class III TTC value of 0.47 mg/day (based on human lung weight of 650 g; Carthew, 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: UGCM, 1997: Regnault-Roger (1995): Buchbauer (1993).

Literature Search and Risk Assessment Completed On: 05/28/21

11.2. Environmental endpoint summary

11.2.1. Screening-level assessment

A screening-level risk assessment of anethole (isomer unspecified) was performed following the RIFM Environmental Framework (Salvito, 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log K_{OW}, and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high uncertainty factor applied is used to predict fish toxicity, as discussed in Salvito et al. (2002). In Tier 2, the RQ is refined by applying a lower uncertainty factor to the PNEC using the ECOSAR model (US EPA, 2012b), which provides chemical class-specific ecotoxicity estimates. Finally, if necessary, Tier 3 is conducted using measured biodegradation and ecotoxicity data to refine the RQ, thus allowing for lower PNEC uncertainty factors. The data for calculating the PEC and PNEC for this safety assessment are provided in the table below. For the PEC, the range from the most recent IFRA Volume of Use Survey is reviewed. The PEC is then calculated using the actual regional tonnage, not the extremes of the range. Following the RIFM Environmental Framework, anethole (isomer unspecified) 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 did not identify anethole (isomer unspecified) as possibly persistent or bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api, 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11). 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 (2015), anethole (isomer unspecified) presents a risk to the aquatic compartment in the screening-level assessment.

11.2.2. Key studies

11.2.2.1. Biodegradation. For CAS # 104-46-1

RIFM, 1994: The ready biodegradability of the test material was evaluated using the sealed vessel test according to the OECD 301B method. Biodegradation of 91% was observed after 28 days.

11.2.2.2. Ecotoxicity. For CAS # 4180-23-8

RIFM, 1992b: A fish (*Brachydanio rerio*) acute toxicity study was conducted according to the EU Method C.1 under semi-static conditions. The geometric mean of LC0/LC100 was reported to be 7 mg/L.

11.2.2.3. Other available data. Additional material *trans*-anethole (CAS # 4180-23-8) has been registered for REACH with the following additional data (ECHA, 2013):

The ready biodegradability of the test material was evaluated using the closed bottle test according to the Method C.4 E. Biodegradation of 79% was observed after 28 days.

A fish (*Danio rerio*) early life stage toxicity test was conducted according to the OECD 210 guidelines under flow-through conditions. The 28-day NOEC value based on measured concentration was reported to be 0.34 mg/L.

A *Daphnia magna* immobilization test was conducted according to the ASTM, 1989 method under flow-through conditions. The 48-h EC50 was reported to be 4.25 mg/L.

A *Daphnia magna* reproduction test was conducted according to the OECD 211 guideline. The 21-day NOEC value based on nominal test concentration was reported to be 1.05 mg/L.

An algae growth inhibition test was conducted according to the ASTM 1988 method. The 96-h IC50 was reported to be 9.57 mg/L.

11.2.2.4. Risk assessment refinement. Ecotoxicological data and PNEC derivation (all endpoints reported in mg/L; PNECs in μg/L)

Endpoints used to calculate PNEC are underlined.

	LC50 (Fish) (mg/L)	EC50 (*Daphnia*) (mg/L)	EC50 (Algae) (mg/L)	AF	PNEC (μg/L)	Chemical Class
RIFM Framework Screening-level **(Tier 1)**	12.08			1000000	0.01208	
ECOSAR Acute Endpoints **(Tier 2)** v1.11	6.895	4.451	5.633	10000	0.445	Neutral Organics
Tier 3: Measured Data including REACH						
	LC50	EC50	NOEC	AF	PNEC	Comments
Fish	7.0		0.34	50	6.8	
Daphnia		4.25	1.05			
Algae		9.57				

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} Used	3.39	3.39
Biodegradation Factor Used	1	1
Dilution Factor	3	3
Regional Volume of Use Tonnage Band*	100–1000	100–1000
Risk Characterization: PEC/PNEC	**<1**	**<1**

*Combined Regional Volume of Use.

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

The RIFM PNEC is 6.8 μg/L. The revised PEC/PNECs for EU and NA are <1; therefore, the material does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed On: 05/25/21

12. Literature Search*

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

Search keywords: CAS number and/or material names

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment. This is not an exhaustive list. The links listed above were active as of 09/07/21.

Declaration of competing interest

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

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