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RIFM fragrance ingredient safety assessment, anisole, CAS Registry Number 100-66-3

A.M. Api [a], D. Belsito [b], D. Botelho [a], M. Bruze [c], G.A. Burton Jr. [d], J. Buschmann [e], M. A. Cancellieri [a], M.L. Dagli [f], M. Date [a], W. Dekant [g], C. Deodhar [a], A.D. Fryer [h], L. Jones [a], K. Joshi [a], M. Kumar [a], A. Lapczynski [a], M. Lavelle [a], I. Lee [a], D.C. Liebler [i], H. Moustakas [a], M. Na [a], T.M. Penning [j], G. Ritacco [a], J. Romine [a], N. Sadekar [a], T.W. Schultz [k], D. Selechnik [a], F. Siddiqi [a], I.G. Sipes [l], G. Sullivan [a,*], Y. Thakkar [a], Y. Tokura [m]

[a] *Research Institute for Fragrance Materials, Inc., 50 Tice Boulevard, Woodcliff Lake, NJ, 07677, USA*
[b] *Member Expert Panel for Fragrance Safety, Columbia University Medical Center, Department of Dermatology, 161 Fort Washington Ave., New York, NY, 10032, USA*
[c] *Member Expert Panel for Fragrance Safety, Malmo University Hospital, Department of Occupational & Environmental Dermatology, Sodra Forstadsgatan 101, Entrance 47, Malmo, SE, 20502, Sweden*
[d] *Member Expert Panel for Fragrance Safety, School of Natural Resources & Environment, University of Michigan, Dana Building G110, 440 Church St., Ann Arbor, MI, 58109, USA*
[e] *Member Expert Panel for Fragrance Safety, Fraunhofer Institute for Toxicology and Experimental Medicine, Nikolai-Fuchs-Strasse 1, 30625, Hannover, Germany*
[f] *Member Expert Panel for Fragrance Safety, University of Sao Paulo, School of Veterinary Medicine and Animal Science, Department of Pathology, Av. Prof. dr. Orlando Marques de Paiva, 87, Sao Paulo, CEP 05508-900, Brazil*
[g] *Member Expert Panel for Fragrance Safety, University of Wuerzburg, Department of Toxicology, Versbacher Str. 9, 97078, Würzburg, Germany*
[h] *Member Expert Panel for Fragrance Safety, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd., Portland, OR, 97239, USA*
[i] *Member Expert Panel for Fragrance Safety, Vanderbilt University School of Medicine, Department of Biochemistry, Center in Molecular Toxicology, 638 Robinson Research Building, 2200 Pierce Avenue, Nashville, TN, 37232-0146, USA*
[j] *Member of Expert Panel for Fragrance Safety, University of Pennsylvania, Perelman School of Medicine, Center of Excellence in Environmental Toxicology, 1316 Biomedical Research Building (BRB) II/III, 421 Curie Boulevard, Philadelphia, PA, 19104-3083, USA*
[k] *Member Expert Panel for Fragrance Safety, The University of Tennessee, College of Veterinary Medicine, Department of Comparative Medicine, 2407 River Dr., Knoxville, TN, 37996- 4500, USA*
[l] *Member Expert Panel for Fragrance Safety, Department of Pharmacology, University of Arizona, College of Medicine, 1501 North Campbell Avenue, P.O. Box 245050, Tucson, AZ, 85724-5050, USA*
[m] *Member Expert Panel for Fragrance Safety, The Journal of Dermatological Science (JDS), Department of Dermatology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, 431-3192, Japan*

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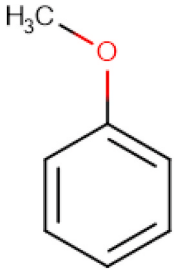
Name: Anisole CAS Registry Number: 100-66-3



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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

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* Corresponding author.
E-mail address: gsullivan@rifm.org (G. Sullivan).

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estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015; Safford et al., 2015a; Safford et al., 2017; Comiskey et al., 2017) compared to a deterministic aggregate approach

DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
DRF - Dose Range Finding
DST - Dermal Sensitization Threshold
ECHA - European Chemicals Agency
ECOSAR - Ecological Structure-Activity Relationships Predictive Model
EU - Europe/European Union
GLP - Good Laboratory Practice
IFRA - The International Fragrance Association
LOEL - Lowest 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/- Predicted Environmental Concentration/Predicted No Effect Concentration
Perfumery - In this safety assessment, perfumery refers to fragrances made by a perfumer used in consumer products only. The exposures reported in the safety assessment include consumer product use but do not include occupational exposures.
QRA - Quantitative Risk Assessment
QSAR - Quantitative Structure-Activity Relationship
REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals
RfD - Reference Dose
RIFM - Research Institute for Fragrance Materials
RQ - Risk Quotient
Statistically Significant - Statistically significant difference in reported results as compared to controls with a $p < 0.05$ using appropriate statistical test
TTC - Threshold of Toxicological Concern
UV/Vis spectra - Ultraviolet/Visible spectra
VCF - Volatile Compounds in Food
VoU - Volume of Use
vPvB - (very) Persistent, (very) Bioaccumulative
WoE - Weight of Evidence

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

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

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

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

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

Anisole was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that anisole is not genotoxic. Data on anisole provide a calculated Margin of Exposure (MOE) > 100 for the repeated dose, reproductive, and local respiratory toxicity endpoints. Data from read-across analog *p*-methylanisole (CAS # 104-93-8) show that there are no safety concerns for anisole for skin sensitization under the current declared levels of use. The phototoxicity/photoallergenicity endpoints were evaluated based on ultraviolet/visible (UV/Vis) spectra; anisole is not expected to be phototoxic/photoallergenic. For the hazard assessment based on the screening data, anisole is not Persistent, Bioaccumulative, and Toxic (PBT) as per the International Fragrance Association (IFRA) Environmental Standards. For the risk assessment, anisole was not able to be risk screened as there were no reported volumes of use for either North America or Europe in the 2015 IFRA Survey.

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Human Health Safety Assessment	
Genotoxicity: Not genotoxic.	(ECHA REACH Dossier: Anisole; ECHA, 2011)
Repeated Dose Toxicity: NOAEL = 269 mg/kg/day.	(ECHA REACH Dossier: Anisole; ECHA, 2011)
Reproductive Toxicity: Developmental toxicity: 200 mg/kg/day. Fertility: No NOAEL available. Exposure is below TTC.	(ECHA REACH Dossier: Anisole; ECHA, 2011)
Skin Sensitization: Not a concern for skin sensitization under the current, declared use levels.	(ECHA REACH Dossier: 4-Methyl-anisole; ECHA, 2015)
Phototoxicity/Photoallergenicity: Not expected to be phototoxic/photoallergenic	(UV/Vis Spectra; RIFM Database)
Local Respiratory Toxicity: NOAEC = 3000 mg/m^3.	(ECHA REACH Dossier: Anisole; ECHA, 2011)
Environmental Safety Assessment	
Hazard Assessment:	
Persistence:	
Screening-level: 2.9 (BIOWIN 3)	(EPI Suite v4.11; US EPA, 2012a)
Bioaccumulation:	
Screening-level: 11.46 L/kg	(EPI Suite v4.11; US EPA, 2012a)
Ecotoxicity:	
Screening-level: Not applicable	
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards	
Risk Assessment:	
• **Revised PEC/PNECs (2015 IFRA VoU):** North America and Europe: Not applicable; no Volume of Use in 2015 reported for Europe and North America	

1. Identification

1. **Chemical Name:** Anisole
2. **CAS Registry Number:** 100-66-3
3. **Synonyms:** Benzene, methoxy; Methoxybenzene; Methyl phenyl ether; Phenyl methyl ether; Anisole
4. **Molecular Formula:** C_7H_8O
5. **Molecular Weight:** 108.14
6. **RIFM Number:** 733
7. **Stereochemistry:** No stereoisomer possible.

2. Physical data

1. **Boiling Point:** 154 °C (Fragrance Materials Association [FMA]), 149.16 °C (EPI Suite)
2. **Flash Point:** 110 °F; CC (FMA)
3. **Log K_{OW}:** 2.11 (Abraham and Rafols, 1995; Patel et al., 2002), 2.07 (EPI Suite)
4. **Melting Point**: -41.21 °C (EPI Suite)
5. **Water Solubility:** 1741 mg/L (EPI Suite)
6. **Specific Gravity:** 0.991 (FMA)
7. **Vapor Pressure:** 2.42 mm Hg at 20 °C (EPI Suite v4.0), 2.4 mm Hg at 20 °C (FMA), 3.38 mm Hg at 25 °C (EPI Suite)
8. **UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L mol^{-1} • cm^{-1})
9. **Appearance/Organoleptic:** Colorless or yellow, straw-colored, mobile liquid with a phenolic anise-like, agreeable aromatic odor * (Arctander, Volume II, 1969)

3. Volume of use (worldwide band)

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

4. Exposure to fragrance ingredient (Creme RIFM Aggregate Exposure Model v3.1)

1. **95**th **Percentile Concentration in Fine Fragrance:** 0.24% (RIFM, 2020b)
2. **Inhalation Exposure*:** 0.0013 mg/kg/day or 0.079 mg/day (RIFM, 2020b)
3. **Total Systemic Exposure**:** 0.0095 mg/kg/day (RIFM, 2020b)

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

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

5. Derivation of systemic absorption

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

6. Computational toxicology evaluation

6.1. Cramer Classification

Class I, Low

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

6.2. Analogs Selected

a. **Genotoxicity:** None
b. **Repeated Dose Toxicity:** None
c. **Reproductive Toxicity:** None
d. **Skin Sensitization:** 4-Methylanisole (CAS # 104-93-8)
e. **Phototoxicity/Photoallergenicity:** None
f. **Local Respiratory Toxicity:** None
g. **Environmental Toxicity:** None

6.3. Read-across Justification

See Appendix below

7. Metabolism

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

8. Natural occurrence

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

Apple fresh (*Malus* species)	Milk and milk products
Apple processed (*Malus* species)	Olive (*Olea europaea*)
Artichoke	Sapodilla fruit (*Achras sapota* L.)
Beef	Truffle
Cheese, various types	Vanilla
Litchi (*Litchi chinensis* Sonn.)	

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

9. REACH dossier

Available; accessed on 09/17/21.

10. Conclusion

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

11. Summary

11.1. Human Health Endpoint Summaries

11.1.1. Genotoxicity

Based on the current existing data, anisole does not present a concern for genotoxicity.

11.1.1.1. Risk assessment. The mutagenic activity of anisole has been evaluated in a bacterial reverse mutation assay conducted in compliance with GLP regulations and in accordance with OECD TG 471 using the standard plate incorporation and preincubation method. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA were treated with anisole in dimethyl sulfoxide (DMSO) at concentrations up to 5000 μg/plate. No increases in the mean number of revertant colonies were observed at any tested concentration in the presence or absence of S9 (ECHA, 2011). Under the conditions of the study, anisole was not mutagenic in the Ames test.

The clastogenicity of anisole was assessed in an *in vitro* chromosome aberration study conducted in compliance with GLP regulations and in accordance with OECD TG 473. Human peripheral blood lymphocytes/Chinese hamster ovary or lung cells were treated with anisole in DMSO at concentrations up to 5000 μg/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 (ECHA, 2011). Under the conditions of the study, anisole was considered to be non-clastogenic in the *in vitro* chromosome aberration assay.

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

Additional references: None.

Literature search and risk assessment completed on: 02/10/21.

11.1.2. Repeated dose toxicity

The MOE for anisole 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 anisole. In a GLP and OECD 412-compliant study, 5 Wistar rats/sex/dose were exposed to anisole via inhalation at concentrations of 0, 120, 600, and 3000 mg/m^3 (calculated to be equivalent to received doses of 0, 32, 161, and 808 mg/kg/day) for 29 days. An additional 5 Wistar rats/sex/dose at 0 and 3 g/m^3 were maintained as recovery groups for 18 days after the treatment period. No mortality occurred throughout the study. No treatment-related effects were seen in clinical signs, body weights, bodyweight gains, food consumption, behavior, organ weights, gross pathology, or histopathology. Prothrombin time was reduced in females at the high dose but was reversed during the recovery period. No other hematological effects were observed. Plasma glucose concentration was increased in females at the high dose, but this effect was reversed during the recovery group. Plasma concentrations of cholesterol and phospholipids were significantly increased in males,

while plasma triglycerides were significantly increased in females. These effects were reversed during the recovery group. Total protein in plasma was decreased in males of the recovery group at the high dose. Because the effects were reversed during the recovered period, the hematological and clinical chemistry effects were not considered adverse. Based on the absence of adverse effects seen up to the highest dose, the NOAEL for this study was considered to be 808 mg/kg/day (3000 mg/m^3) (ECHA, 2011).

A default safety factor of 3 was used when deriving a NOAEL from the OECD 412 repeated dose study (ECHA, 2012). The safety factor has been approved by the Expert Panel for Fragrance Safety*.

Thus, the derived NOAEL for the repeated dose toxicity data is 808/3 or 269 mg/kg/day.

Therefore, the anisole MOE for the repeated dose toxicity endpoint can be calculated by dividing the anisole NOAEL by the total systemic exposure for anisole, 269/0.0095, or 28316.

In addition, the total systemic exposure to anisole (9.5 μg/kg/day) is below the TTC (30 μg/kg/day; Kroes et al., 2007) for the repeated dose endpoint for a Cramer Class I material at the current level of use.

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

Additional references: None.

Literature search and risk assessment completed on: 01/06/21.

11.1.3. Reproductive toxicity

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

11.1.3.1. Risk assessment. There are sufficient developmental toxicity data on anisole. An OECD 414 prenatal developmental toxicity study was conducted with anisole administered to 24 female pregnant Sprague Dawley rats per dose by gavage in corn oil at doses of 50, 200, and 800 mg/kg/day from gestation days 6–20. No mortality was observed at any dose groups. A decrease in bodyweight gains in correlation to a statistically significant decrease in food consumption was observed at 800 mg/kg/day. At the highest dose, there was a lower mean gravid uterus weight resulting in a lower mean total weight change, which was not significant. These findings were considered to be treatment-related. In addition, at 800 mg/kg/day, there was a lower mean number of live fetuses, and mean post-implantation loss was recorded at a higher incidence (than the upper limit of the historical control data). Statistically significant lower mean fetal body weights were observed at 800 mg/kg/day. Furthermore, all fetuses from all litters had discolored skin and moderate subcutaneous edema at the highest dose, effects which were considered to be treatment-related. No skeletal variations were observed in the fetuses at any dose group. Based on lower fetal body weights and increased external fetal variations in pups, the NOAEL for developmental toxicity was considered to be 200 mg/kg/day (ECHA, 2011). **Therefore, the anisole MOE for the developmental toxicity endpoint can be calculated by dividing the anisole NOAEL by the total systemic exposure for anisole, 200/0.0095, or 21053.**

There are insufficient fertility data on anisole. In a GLP and OECD 412-compliant study, 5 Wistar rat/sex/dose were administered anisole via inhalation at concentrations of 0, 120, 600 and 3000 mg/m^3 (calculated to be equivalent to 0, 32, 161, and 808 mg/kg/day) for 29 days. An additional 5 Wistar rat/sex/dose at 0 and 3 g/m^3 were maintained as recovery groups for 18 days after the treatment period. No mortality occurred throughout the study. No treatment-related abnormalities were observed in any reproductive parameters with respect to estrus cyclicity, sperm numbers, motility, or sperm morphology. Based on the absence of adverse effects seen up to the highest dose, the NOAEL for fertility was considered to be 808 mg/kg/day (3000 mg/m^3) (ECHA, 2011). However, 5 rat/sex/dose may not be sufficient to determine the fertility parameters in addition to the exposure being inhalation route. Thus, fertility was cleared using TTC.

There are insufficient fertility data on anisole or any read-across materials that can be used to support the fertility endpoint. The total systemic exposure to anisole (9.5 μg/kg/day) is below the TTC (30 μg/kg bw/day; Kroes et al., 2007) for the fertility endpoint of a Cramer Class I material at the current level of use.

Additional references: None.

Literature search and risk assessment completed on: 02/12/21.

11.1.4. Skin sensitization

Based on the existing data and the read-across *p*-methylanisole (CAS # 104-93-8), anisole does not present a concern for skin sensitization under the current, declared use levels.

11.1.4.1. Risk assessment. Insufficient skin sensitization studies are available for anisole. Based on the existing data and read-across *p*-methylanisole (CAS # 104-93-8; see Section VI), anisole is not a skin sensitizer. The chemical structures of the target material and the read-across material indicate that they would not be expected to react with skin proteins directly (Roberts et al., 2007; Toxtree v3.1.0; OECD Toolbox v4.2). In a guinea pig maximization study and an open epicutaneous test (OET) in guinea pigs, no skin sensitization was observed with anisole (ECHA, 2011; Klecak, 1985). In a murine local lymph node assay (LLNA), the read-across material *p*-methylanisole did not induce sensitization when tested up to 50% (ECHA, 2015). No sensitization reactions were observed with the read-across in an OET (Klecak, 1979, 1985). In addition, in 2 human maximization tests, both the target and the read-across materials did not induce skin sensitization when tested at 4% and 2%, respectively.

Based on the weight of evidence (WoE) from structural analysis, as well as animal and human studies on the target material and the read-across material, anisole does not present a concern for skin sensitization under the current, declared levels of use.

Additional references: RIFM, 2017; ECETOC, 2003; RIFM, 1976; RIFM, 1971None.

Literature search and risk assessment completed on: 02/11/21.

11.1.5. Phototoxicity/photoallergenicity

Based on the available UV/Vis spectra, anisole would not be expected to present a concern for phototoxicity or photoallergenicity.

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

11.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) were obtained. The spectra indicate no significant absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark of concern for phototoxic effects, 1000 L mol^{-1} • cm^{-1} (Henry et al., 2009).

Additional references: None.

Literature search and risk assessment completed on: 01/11/21.

11.1.6. Local respiratory toxicity

The MOE for anisole is adequate for the local respiratory toxicity endpoint at the current level of use.

11.1.6.1. Risk assessment. The inhalation exposure estimated for combined exposure was considered along with toxicological data observed in the scientific literature to calculate the MOE from inhalation exposure when used in perfumery. In a 4 week, OECD 412, GLP-compliant study,

5 male and 5 female Wistar rats were exposed to 0, 120, 600, and 3000 mg/m^3 of anisole vapors via nose-only inhalation exposure for 6 h, 5 days per week (ECHA, 2011). Standard endpoints evaluated included clinical observations, body weight, food consumption, hematology, clinical chemistry, neurobehavioral examinations, gross pathology, and histopathology. Additional evaluations made included estrus cycle evaluations and sperm analysis. Macroscopic and microscopic examinations of all the tissues, including the entire respiratory tract, did not show any treatment-related effects. Therefore, the local effects NOAEC was identified at the highest exposure concentration of 3000 mg/m^3.

This NOAEC expressed in mg/kg lung weight/day is:

- (3000 mg/m^3) × (1 m^3/1000 L) = 3 mg/L
- MV of 0.21 L/min for a Wistar rat × duration of exposure of 360 min per day (min/day) (according to GLP study guidelines) = 75.6 L/day
- (3 mg/L) × (75.6 L/d) = 226.8 mg/day
- (226.8 mg/day)/(0.0016 kg lung weight of rat*) = 141750 mg/kg lung weight/day

The 95th percentile calculated exposure was reported to be 0.079 mg/day—this value was derived from the concentration survey data in the Creme RIFM exposure model (RIFM, 2015; and Safford et al., 2015). To compare this estimated exposure with the NOAEC expressed in mg/kg lung weight/day, this value is divided by 0.65 kg human lung weight (Carthew et al., 2009) to give 0.0514 mg/kg lung weight/day resulting in a MOE of 2757782 (i.e., [141750 mg/kg lung weight of rat/day]/[0.0514 mg/kg lung weight of human/day]).

The MOE is greater than 100. Without adjustment for specific UFs related to inter-species and intra-species variation, the material exposure by inhalation at 0.079 mg/day is deemed to be safe under the most conservative consumer exposure scenario.

*Phalen, R.F. Inhalation Studies. Foundations and Techniques, 2 nd Ed 2009. Published by Informa Healthcare USA, Inc., New York, NY. Chapter 9, Animal Models, in section: "Comparative Physiology and Anatomy," subsection, "Comparative Airway Anatomy."

Additional references: Helmig et al., 1999a; Helmig et al., 1999b; Leclerc et al., 2002; Leclerc et al., 2002.

11.2. Literature search and risk assessment completed on: 02/07/21

2. Environmental Endpoint Summary:

11.2.1. Screening-level assessment

A screening-level risk assessment of anisole was performed following the RIFM Environmental Framework (Salvito et al., 2002), which provides 3 tiered levels of screening for aquatic risk. In Tier 1, only the material's regional VoU, its log K_{OW}, and its molecular weight are needed to estimate a conservative risk quotient (RQ), expressed as the ratio Predicted Environmental Concentration/Predicted No Effect Concentration (PEC/PNEC). A general QSAR with a high UF 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 UF 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 UFs. 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, anisole was not able to be risk screened as there were no reported volumes of use for either North America or Europe in the 2015 IFRA Survey.

A screening-level hazard assessment using EPI Suite v4.11 (US EPA, 2012a) did not identify anisole as possibly persistent or bioaccumulative based on its structure and physical–chemical properties. This screening-level hazard assessment considers the potential for a material to be persistent *and* bioaccumulative *and* toxic, or very persistent *and* very bioaccumulative as defined in the Criteria Document (Api et al., 2015). As noted in the Criteria Document, the screening criteria applied are the same as those used in the EU for REACH (ECHA, 2012). For persistence, if the EPI Suite model BIOWIN 3 predicts a value < 2.2 and either BIOWIN 2 or BIOWIN 6 predicts a value < 0.5, then the material is considered potentially persistent. A material would be considered potentially bioaccumulative if the EPI Suite model BCFBAF predicts a fish BCF ≥2000 L/kg. Ecotoxicity is determined in the above screening-level risk assessment. If, based on these model outputs (Step 1), additional assessment is required, a WoE-based review is then performed (Step 2). This review considers available data on the material's physical–chemical properties, environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies), fish bioaccumulation, and higher-tier model outputs (e.g., US EPA's BIOWIN and BCFBAF found in EPI Suite v4.11).

11.2.2. Risk assessment

Not applicable.

11.2.2.1. Key studies. Biodegradation:No data available.

Ecotoxicity:No data available.

Other available data:Anisole has been registered for REACH with the following additional data available at this time (ECHA, 2011):

The ready biodegradability of the test material was evaluated using the modified MITI test according to the OECD 301 C guideline. Biodegradation of 56% (BOD) was observed after 2 weeks.

The *Daphnia magna* acute immobilization test was conducted according to the OECD 202 guidelines under static conditions. The 48-h EC50 value based on mean measured concentration was reported to be 27 mg/L (95% CI: 18–38 mg/L).

The algae growth inhibition test was conducted according to the OECD 201 guidelines under static conditions. The 72-h EC50 values based on time-weighted average concentration for biomass and growth rate were reported to be 30 mg/L and 47 mg/L, respectively.

11.2.3. Risk assessment refinement

Not applicable.

Literature search and risk assessment completed on: 01/11/21.

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 09/31/21.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2021.112680.

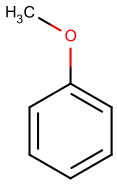
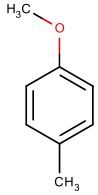
Appendix

Read-across Justification

Methods

The read-across analog was identified using RIFM fragrance materials chemical inventory clustering and read-across search criteria (RIFM, 2020a). These criteria follow 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 Chemical Agency read-across assessment framework (ECHA, 2017).

- First, materials were clustered based on their structural similarity. Second, data availability and data quality on the selected cluster were examined. Third, appropriate read-across analogs from the cluster were confirmed by expert judgment.
- Tanimoto structure similarity scores were calculated using FCFC4 fingerprints (Rogers and Hahn, 2010).
- The physical–chemical properties of the target material and the read-across analogs were calculated using EPI Suite v4.11 (US EPA, 2012a).
- J_{max} values were calculated using RIFM's Skin Absorption Model (SAM). The parameters were calculated using the consensus model (Shen et al., 2014).
- DNA binding, mutagenicity, genotoxicity alerts, oncologic classification, ER binding, and repeat dose categorization predictions were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010).
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018), and skin sensitization was predicted using Toxtree.
- The major metabolites for the target material and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- To keep continuity and compatibility with *in silico* alerts, OECD QSAR Toolbox v4.2 was selected as the alert system.

	Target Material	Read-across Material
Principal Name	Anisole	*p*-Methylanisole
CAS No.	100-66-3	104-93-8
Structure		
Similarity (Tanimoto Score)		0.63
Endpoint		Skin sensitization
Molecular Formula	C_7H_8O	$C_8H_{10}O$
Molecular Weight	108.14	122.17
Melting Point (°C, EPI Suite)	–42.21	−32.00
Boiling Point (°C, EPI Suite)	153.70	175.50
Vapor Pressure (Pa @ 25°C, EPI Suite)	471.96	159.99
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	1040.00	527.10
Log K_{OW}	2.11	2.66
J_{max} (μg/cm^2/h, SAM)	70.61	51.39
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	49.04	35.67

(*continued on next page*)

(*continued*)

	Target Material	Read-across Material
Skin Sensitization		
Protein Binding (OASIS v1.1)	No alert found	No alert found
Protein Binding (OECD)	No alert found	No alert found
Protein Binding Potency	Not possible to classify according to these rules (GSH)	Not possible to classify according to these rules (GSH)
Protein Binding Alerts for Skin Sensitization (OASIS v1.1)	No alert found	No alert found
Skin Sensitization Reactivity Domains (Toxtree v2.6.13)	No skin sensitization reactivity domains alerts identified.	No skin sensitization reactivity domains alerts identified.
Metabolism		
Rat Liver S9 Metabolism Simulator and Structural Alerts for Metabolites (OECD QSAR Toolbox v4.2)	See Supplemental Data 1	See Supplemental Data 2

Summary

There are insufficient toxicity data on anisole (CAS # 100-66-3). Hence, *in silico* evaluation was conducted to determine read-across analogs for this material. Based on structural similarity, reactivity, metabolism data, physical–chemical properties, and expert judgment, *p*-methylanisole (CAS # 104-3-8) was identified as a read-across material with sufficient data for toxicological evaluation.

Conclusion

- *p*-Methylanisole (CAS # 104-3-8) was used as a read-across analog for the target material anisole (CAS # 100-66-3) for the skin sensitization endpoint.
  - The target material and the read-across analog belong to the structural class of aromatic ethers.
  - The key difference between the target material and the read-across analog is that the read-across analog has a para methyl substituent on the benzene ring. This structural difference between the target material and the read-across analog does not affect consideration of the toxic endpoint.
  - The similarity between the target material and the read-across analog is indicated by the Tanimoto score in the above table. Differences between the structures that affect the Tanimoto score do not affect consideration of the toxicity endpoint.
  - The physical–chemical properties of the target material and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - According to the QSAR OECD Toolbox (v4.2), structural alerts for toxicity endpoints are consistent between the target material and the read-across analog.
  - The target material and the read-across analog are expected to be metabolized similarly, as shown by the metabolism simulator.

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