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RIFM fragrance ingredient safety assessment, ethyl anthranilate, CAS registry number 87-25-2

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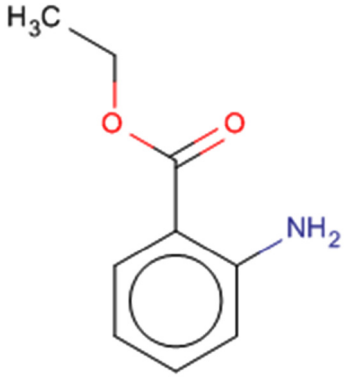

Name: Ethyl anthranilate

CAS Registry Number: 87-25-2

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Abbreviation/Definition list:
2-Box Model – a RIFM, Inc. proprietary *in silico* tool used to calculate fragrance air exposure concentration
97.5th percentile – The concentration of the fragrance ingredient is obtained from examination of several thousand commercial fine fragrance formulations. The upper 97.5 percentile concentration is calculated from these data and is then used to estimate the dermal systemic exposure in ten types of the most frequently used personal care and cosmetic products. The dermal route is the major route in assessing the safety of fragrance ingredients. Further explanation of how the data were obtained and of how exposures were determined has been previously reported by Cadby et al. (2002) and Ford et al. (2000).
AF – Assessment Factor
DEREK – Derek nexus is an *in silico* tool to predict whether a chemical will be toxic
DST – Dermal Sensitization Threshold
ECHA – European Chemicals Agency
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
NESIL – No Expected Sensitization Induction Level
NOAEC – No Observed Adverse Effect Concentration
NOAEL – No Observed Adverse Effect Level
NOEC – No Observed Effect Concentration
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
RIFM – Research Institute for Fragrance Materials
RQ – Risk Quotient
TTC – Threshold of Toxicological Concern
UV/Vis Spectra – Ultra Violet/Visible spectra
VCF – Volatile Compounds in Food
VoU – Volume of Use
vPvB – (very) Persistent, (very) Bioaccumulative
RIFM's Expert Panel* concludes that this material is safe under the limits described in this safety assessment.
This safety assessment is based on RIFM's Criteria Document (Api et al., 2015) and should be referred to for clarifications.
Each endpoint discussed in this safety assessment reviews 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 two digit month/day/year), both in the RIFM database (consisting of publicly available and proprietary data) and through publicly available information sources (i.e., 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 end-point value (e.g., PNEC, NOAEL, LOEL, and NESIL).
* RIFM's Expert Panel 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 guidance relevant to human health and environmental protection.

Summary: The use of this material under current use conditions is supported by the existing information.
This material was evaluated for Genotoxicity, Repeated Dose Toxicity, Developmental Toxicity, Reproductive Toxicity, Local Respiratory Toxicity, Phototoxicity, Skin Sensitization potential as well as Environmental assessment. Reproductive toxicity was based on the Threshold of Toxicological Concern (TTC) of 0.009 mg/kg/day for a Cramer Class II material. The estimated systemic exposure is determined to be below this value assuming 100% absorption from skin contact and inhalation. A systemic exposure below this TTC value is acceptable.

Human Health Safety Assessment
Genotoxicity: Not genotoxic. (Mortelmans et al., 1986; ECHA REACH Dossier: Anthranilic acid)
Repeated Dose Toxicity: NOAEL = 75 mg/kg/day (NCI, 1978)
Developmental and Reproductive Toxicity: Developmental NOAEL = 768.4 mg/kg/day. No reproductive NOAEL. Exposure is below the TTC. (RIFM, 2012)
Skin Sensitization: Not sensitizing (Klecak, 1979; Klecak, 1985; RIFM, 1964, 1973, 1974b, 1975, 2007)
Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic (RIFM, 1976a, 1976)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment
Hazard Assessment:
Persistence: Screening Level: BIOWIN 3: 2.84 (EPISUITE ver 4.1)
Bioaccumulation: Screening Level: 23.1 L/kg (EPISUITE ver 4.1)
Ecotoxicity: Screening Level: LC50: 48.61 mg/L (Salvito et al., 2002)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards

Risk Assessment:
Screening-Level: PEC/PNEC (North America and Europe) <1 (Salvito et al., 2002)
Critical Ecotoxicity Endpoint: LC50: 48.61 mg/L (Salvito et al., 2002)
RIFM PNEC is: 0.048 μg/L
• Revised PEC/PNECs (2011 IFRA VoU): North America and Europe Not Applicable: Cleared at Screening Level

1. Identification

1. **Chemical Name:** Ethyl anthranilate
2. **CAS Registry Number:** 87-25-2
3. **Synonyms:** Benzoic acid, 2-amino-, ethyl ester, Ethyl o-aminobenzoate, Ethyl 2-aminobenzoate, Ethyl anthranilate, アミノ安息香酸　アルキル（Ｃ＝１～１０）
4. **Molecular Formula:** $C_9H_{11}NO_2$
5. **Molecular Weight:** 165.19
6. **RIFM Number:** 666

2. Physical data

1. **Boiling Point:** 260 °C [FMA], 279.9 °C [EPI Suite]
2. **Flash Point:** >200 °F; CC [FMA]
3. **Log K_{ow}:** 2.76 [EPI Suite]

4. **Melting Point**: 13 °C [FMA], 66.17 °C [EPI Suite]
5. **Water Solubility:** 413.6 mg/L [EPI Suite]
6. **Specific Gravity:** 1.118 [FMA]
7. **Vapor Pressure:** 0.002 mm Hg 20 °C [FMA], 0.00635 mm Hg @ 20 °C [EPI Suite 4.0], 0.0103 mm Hg @ 25 °C [EPI Suite]
8. **UV Spectra:** Not available
9. **Appearance/Organoleptic:** A colorless liquid which has a sweet-fruity, grape like odor, milder and less harsh than the Methyl ester.

3. Exposure

1. **Volume of Use (worldwide band):** <1 metric tons per year (IFRA, 2011)
2. **Average Maximum Concentration in Hydroalcoholics:** 0.009% (IFRA, 2008)
3. **97.5th Percentile:** 0.047% (IFRA, 2008)
4. **Dermal Exposure*:** 0.0012 mg/kg/day (IFRA, 2008)
5. **Oral Exposure:** Not available
6. **Inhalation Exposures**:** 0.000073 mg/kg/day (IFRA, 2008)
7. **Total Systemic Exposure (Dermal + Inhalation):** 0.0013 mg/kg/day

* Calculated using the reported 97.5th percentile concentration based on the levels of the same fragrance ingredient in ten of the most frequently used personal care and cosmetic products (i.e., anti-perspirant, bath products, body lotion, eau de toilette, face cream, fragrance cream, hair spray, shampoo, shower gel, and toilet soap) (Cadby et al. 2002; Ford et al. 2000).

** Combined (fine fragrances, hair sprays, antiperspirants/deodorants, candles, aerosol air fresheners, and reed diffusers/heated oil plug-ins) result calculated using RIFM's 2-Box/MPPD *in silico* models, based on the IFRA survey results for the 97.5th percentile use in hydroalcoholics for a 60 kg individual.

4. Derivation of systemic absorption

1. **Dermal:** Assumed 100%
2. **Oral:** Data not available – not considered.
3. **Inhalation:** Assumed 100%
4. **Total:** Since data not available, assume Dermal + Inhalation exposure is 100% absorbed = 0.0013 mg/kg/day

5. Computational toxicology evaluation

1. **Cramer Classification:** Class II, Intermediate (Expert Judgment)

Expert Judgment	Toxtree (v 2.6.0)	OECD QSAR Toolbox (v. 3.2)
II*	III	II

* See Appendix below for explanation.

2. **Analogs Selected:**
   a. **Genotoxicity:** Benzoic acid, 2-amino- (CAS # 118-92-3)
   b. **Repeated Dose Toxicity:** Benzoic acid, 2-amino- (CAS # 118-92-3)
   c. **Developmental and Reproductive Toxicity:** Methyl anthranilate (CAS # 134-20-3)
   d. **Skin Sensitization:** Methyl anthranilate (CAS # 134-20-3)
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. **Read across justifications: See Appendix below**

6. Natural occurrence (discrete chemical) or composition (NCS)

Ethyl anthranilate is reported to occur in the following foods*:

Citrus fruits
Grape (Vitis species)
Starfruit (Averrhoa carambola 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, contains information on published volatile compounds which have been found in natural (processed) food products. Includes FEMA GRAS and EU-Flavis data.

7. IFRA standard

None.

8. REACH dossier

Pre-Registered for 2010; No dossier available as of 01/22/15.

9. Summary

9.1. Human health endpoint summaries

9.1.1. Genotoxicity

Based on the current existing data and use levels, Ethyl anthranilate does not present a concern for genetic toxicity.

9.1.1.1. Risk assessment. The genotoxic potential of ethyl anthranilate was evaluated in an Ames study sponsored by the NTP (National Toxicology Program) and performed in accordance with OECD TG 471 using the standard plate incorporation method. Four *Salmonella typhimurium* strains were dosed up to 1.6 mg both with and without metabolic activation. The test material was unable to induce an increase in the amount of revertant colonies in any of the strains at the concentrations tested with or without metabolic activation (Mortelmans et al., 1986). Under the conditions of the study, the test material was unable to induce an increase in the amount of revertant colonies in any of the test strains and was considered not mutagenic.

There are no data assessing the clastogenic activity of ethyl anthranilate. The material anthranilate (CAS # 118-92-3; see Section 5) was identified as a read across analog. The aneugenic/clastogenic potential of anthranilate was assessed in an *in vivo* micronucleus assay conducted in compliance with GLP regulations and in accordance to OECD TG 474 (ECHA REACH Dossier: Anthranilic acid Exp Key Genetic toxicity in vivo.001). Under the conditions of the study, anthranilate was considered not clastogenic and this can be extended to ethyl anthranilate.

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

Additional References: Kawachi et al., 1980; Foltínová & Grones, 1997; Miyawaga et al., 1995; Hughes et al., 2012; Fowler et al., 2012.

Literature Search and Risk Assessment Completed on: 01/17/14.

9.1.2. Repeated dose toxicity

The margin of exposure for ethyl anthranilate is adequate for the repeated dose toxicity endpoint at the current level of use.

9.1.2.1. Risk assessment. There are no repeated dose toxicity data on ethyl anthranilate. Read across material benzoic acid, 2-amino- (CAS # 118-92-3; see Section 5) has dietary subchronic and chronic toxicity data in rats and mice. The LOAEL was determined to be 750 mg/kg/day in rats, based on reduced body weights (NCI, 1978). The NOAEL was derived by dividing the LOAEL by a safety factor of 10, which is equal to 75 mg/kg/day. **Therefore, the MOE is equal to the benzoic acid, 2-amino- NOAEL in mg/kg/day divided by the total systemic exposure, 75/0.0013 or 57692.**

Additional References: Hagan et al., 1967; Bär & Griepentrog, 1967; Stoner et al., 1973; Schafer et al., 1985; Clark et al., 1980; Cutting et al., 1966; Verrett et al., 1980; RIFM, 1974a; Grundschober, 1977; Yamaori et al., 2005; Ekman et al., 1949.

Literature Search and Risk Assessment Completed on: 01/17/14.

9.1.3. Developmental and reproductive toxicity

The margin of exposure for ethyl anthranilate is adequate for the developmental toxicity endpoint at the current level of use.

There are insufficient reproductive toxicity data on ethyl anthranilate or any read across materials. However, the exposure is below the Threshold of Toxicological Concern (TTC).

9.1.3.1. Risk assessment. There are no developmental toxicity data on ethyl anthranilate. Read across material methyl anthranilate (CAS # 134-20-3; see Section 5) has an OECD 414 dietary developmental toxicity study that was conducted in rats (RIFM, 2012). The NOAEL for developmental toxicity was set to be 768.4 mg/kg/day, based on the highest dosage tested. **Therefore, the MOE for developmental toxicity is equal to the methyl anthranilate NOAEL in mg/kg/day divided by the total systemic exposure, 768.4/0.0013 or 591077.**

There are no reproductive toxicity data on ethyl anthranilate. Read across material methyl anthranilate (CAS # 134-20-3) has an OECD 414 dietary developmental toxicity study conducted in rats which determined the NOAEL for maternal toxicity to be 80.4 mg/kg/day, based on body weights and food consumption (RIFM, 2012). There are no male reproductive data on ethyl anthranilate or any read across materials that can be used to support the reproductive toxicity endpoint. The total systemic exposure (1.3 μg/kg/day) is below the TTC for ethyl anthranilate (9 μg/kg bw/day).

Additional References: Hagan et al., 1967; Bär & Griepentrog, 1967; Stoner et al., 1973; Schafer et al., 1985; Clark et al., 1980; Cutting et al., 1966; Verrett et al., 1980; RIFM, 1974a; Grundschober, 1977; Yamaori et al., 2005; Ekman et al., 1949.

Literature Search and Risk Assessment Completed on: 01/17/14.

9.1.4. Skin sensitization

Based on the available data for the read across material methyl anthranilate and material specific data, ethyl anthranilate does not present a concern for skin sensitization.

9.1.4.1. Risk assessment. Based on the available data for the read across material (methyl anthranilate, CAS # 134-20-3; see Section 5) and material specific data, ethyl anthranilate does not present a concern for skin sensitization. The chemical structure of these materials indicates that they would not be expected to react directly with skin proteins (Roberts et al., 2007; Toxtree 2.5.0; OECD toolbox v3.1). In guinea pig test methods and the local lymph node assay no results indicative of sensitization were observed to methyl anthranilate (Klecak, 1979; Klecak, 1985; RIFM, 2007). Additionally, no reactions indicative of skin sensitization were observed in the human maximization test and/or repeated insult patch test to either material (RIFM, 1964, 1973, 1974b, 1975).

Additional References: None.

Literature Search and Risk Assessment Completed on: 01/17/14.

9.1.5. Phototoxicity/photoallergenicity

Based on the available data, ethyl anthranilate is not considered to present a concern for phototoxicity or photoallergenicity.

9.1.5.1. Risk assessment. There are no suitable UV absorption spectra available for ethyl anthranilate. However, no phototoxic or photoallergic responses were reported in experimental animal studies (RIFM, 1976a, 1976). Based on the available data, ethyl anthranilate is not considered to present a concern for phototoxicity or photoallergenicity.

Additional References: None.

Literature Search and Risk Assessment Completed on: 01/17/14.

9.1.6. Local respiratory toxicity

The ethyl anthranilate exposure level is below the inhalation TTC Cramer Class III limit for local effects.

9.1.6.1. Risk assessment. There are no inhalation data available on ethyl anthranilate. Based on the IFRA survey results for hydroalcoholics, the 97.5th percentile was reported to be 0.047%. Assuming the same amount is used in all product types (fine fragrances, hair sprays, antiperspirants/deodorants, candles, aerosol air fresheners, and reed diffusers/heated oil plug-ins), the inhalation combined exposure would be 0.0044 mg/day as calculated by the RIFM 2 Box Model and further refined using Multiple Path Particle Deposition Model using the 97.5th percentile. This exposure level would be below the recommended Cramer Class III TTC level (Respiratory Cramer Class II defaults to the Cramer Class III value) of 0.47 mg/day (based on human lung weight of 650 g; Carthew et al., 2009) and is deemed safe for use at the reported use level.

Additional References: None.

Literature Search and Risk Assessment Completed on: 01/17/14.

9.2. Environmental endpoint summary

9.2.1. Screening-level assessment

A screening level risk assessment of ethyl anthranilate performed following the RIFM Environmental Framework (Salvito et al., 2002) which provides for 3 levels of screening for aquatic risk. In Tier 1, only the material's volume of use in a region, its log K_{ow} and molecular weight are needed to estimate a conservative risk quotient (RQ; Predicted Environmental Concentration/Predicted No Effect Concentration or PEC/PNEC). In Tier 1, a general QSAR for fish toxicity is used with a high uncertainty factor as discussed in Salvito et al (2002). At Tier 2, the model ECOSAR (providing chemical class specific ecotoxicity estimates) is used and a lower uncertainty factor is applied. Finally, if needed, at Tier 3, measured biodegradation and ecotoxicity data are used to refine the RQ (again, with lower uncertainty factors applied to calculate the PNEC). Following the RIFM Environmental Framework, ethyl anthranilate was identified as a fragrance material with no potential to present a possible risk to the aquatic environment (i.e., its screening level PEC/PNEC <1).

A screening-level hazard assessment using EPISUITE ver.4.1 identify ethyl anthranilate as not persistent and not bioaccumulative based on its structure and physical–chemical properties. This screening level hazard assessment is a weight of evidence review of a material's physical–chemical properties, available data on environmental fate (e.g., OECD Guideline biodegradation studies or die-away studies) and fish bioaccumulation, and review of model outputs (e.g., USEPA's BIOWIN and BCFBAF found in EPISUITE ver.4.1). Specific key data on biodegradation and fate and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

9.2.2. Risk assessment

Based on the most current VoU survey (2011), ethyl anthranilate presents a risk to the aquatic compartment in the screening level assessment.

9.2.2.1. Biodegradation. Not Available.

9.2.2.2. Ecotoxicity. Not Available.

9.2.3. Other available data

Ethyl anthranilate has been pre-registered for REACH with no additional data at this time.

9.2.4. 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)**	84.61 mg/L			1,000,000	0.04861 µg/L	

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} used	2.76	
Biodegradation Factor Used	0	
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	<1	<1
Risk Characterization: PEC/PNEC	**<1**	**<1**

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

The RIFM PNEC is 0.048 µg/L. The revised PEC/PNECs for EU and NA are not applicable: cleared at screening level at the current reported volumes of use.

Literature Search and Risk Assessment Completed on: 01/17/14.

10. Literature search*

- **RIFM database:** target, Fragrance Structure Activity Group materials, other references, JECFA, CIR, SIDS
- **ECHA:** http://echa.europa.eu/
- **NTP:** http://tools.niehs.nih.gov/ntp_tox/index.cfm
- **OECD Toolbox**
- **SciFinder:** https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf
- **PUBMED:** http://www.ncbi.nlm.nih.gov/pubmed
- **TOXNET:** http://toxnet.nlm.nih.gov/
- **IARC:** (http://monographs.iarc.fr)
- **OECD SIDS:** http://www.chem.unep.ch/irptc/sids/oecdsids/sidspub.html
- **EPA Actor:** http://actor.epa.gov/actor/faces/ACToRHome.jsp;jsessionid=0EF5C212B7906229F477472A9A4D05B7
- **US EPA HPVIS:** http://www.epa.gov/hpv/hpvis/index.html
- **US EPA Robust Summary:** http://cfpub.epa.gov/hpv-s/
- **Japanese NITE:** http://www.safe.nite.go.jp/english/db.html
- **Japan Existing Chemical Data Base:** http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp
- **Google:** https://www.google.com/webhp?tab=ww&ei=KMSoUpiQK-arsQS324GwBg&ved=0CBQQ1S4

*Information sources outside of RIFM's database are noted as appropriate in the safety assessment.

This is not an exhaustive list.

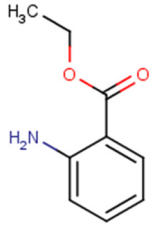
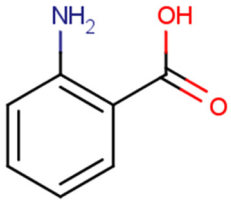
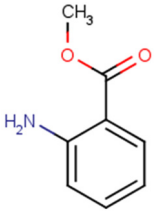
Conflict of interest

A.M. Api, S. Bhatia, L. Kromidas, S. La Cava, J.F. Lalko, A. Lapczynski, V.T. Politano, G. Ritacco, D. Salvito, J. Shen, B. Wall, D.K. Wilcox are employees of the Research Institute for Fragrance Materials, Inc. (RIFM); D. Belsito, M. Bruze, P. Calow, M.L. Dagli, W. Dekant, A.D. Fryer, D.C. Liebler, Y. Miyachi, T.W. Schultz, I.G. Sipes are members of the RIFM Expert Panel.

Transparency document

The Transparency document associated with this article can be found in the online version.

Appendix

	Target Material	Read across Material	
Principal Name	Ethyl anthranilate	Benzoic acid, 2-amino-	Methyl anthranilate
CAS No.	87-25-2	118-92-3	134-20-3
Structure			
3D Structure	http://www.thegoodscentscompany.com/opl/87-25-2.html	http://www.thegoodscentscompany.com/opl/118-92-3.html	http://www.thegoodscentscompany.com/opl/134-20-3.html
Read-across endpoint		Genotoxicity Repeated Dose	Devel/Repro Skin sensitization
Molecular Formula	C9H11NO2	C7H7NO2	C8H9NO2
Molecular Weight	165.19	137.14	151.17

(*continued on next page*)

Appendix (*continued*)

	Target Material	Read across Material	
Melting Point (°C, EPISUITE)	66.17	94.08	55.76
Boiling Point (°C, EPISUITE)	279.90	307.70	263.57
Vapor Pressure (Pa @ 25 °C, EPISUITE)	1.373	0.01049	2.626
Log Kow (KOWWIN v1.68 in EPISUITE)	2.76	1.36	2.26
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPISUITE)	413.6	7970	1860
J_{max} (mg/cm^2/h, SAM)	57.9330157	243.2199995	80.540791
Henry's Law (Pa·m^3/mol, Bond Method, EPISUITE)	0.00165	0.000004	0.001243
Similarity (Tanimoto score)[a]		NA[b]	78%
Genotoxicity			
DNA binding (OASIS v1.1)	• Radical • Radical >> ROS formation after GSH depletion • Radical >> ROS formation after GSH depletion >> Aromatic and Heterocyclic Primary Amines • SN1 • SN1 >> Nitrenium ion formation • SN1 >> Nitrenium ion formation >> Aromatic and Heterocyclic Primary Amines	• Radical • Radical >> ROS formation after GSH depletion • Radical >> ROS formation after GSH depletion >> Aromatic and Heterocyclic Primary Amines • SN1 • SN1 >> Nitrenium ion formation • SN1 >> Nitrenium ion formation >> Aromatic and Heterocyclic Primary Amines	
DNA binding (OECD)	• SN1 • SN1 >> Nitrenium Ion formation • SN1 >> Nitrenium Ion formation >> Primary aromatic amine	• No alert found	
Carcinogenicity (genotox and non-genotox) alerts (ISS)	• Primary aromatic amine, hydroxyl amine and its derived esters (Genotox) • Structural alert for genotoxic carcinogenicity	• No alert found	
DNA alerts for Ames, MN, CA (OASIS v1.1)	• No alert found	• No alert found	
In vitro mutagenicity (Ames test) alerts (ISS)	• Primary aromatic amine, hydroxyl amine and its derived esters	• No alert found	
In vivo mutagenicity (Micronucleus) alerts (ISS)	• H-acceptor-path3-H-acceptor • Primary aromatic amine, hydroxyl amine and its derived esters	• H-acceptor-path3-H-acceptor	
Oncologic classification (OECD)	• Aromatic Amine Type Compounds	• Aromatic Amine Type Compounds	
Repeated Dose Toxicity			
Repeated dose (HESS)	Not categorized	Not categorized	
Developmental and Reproductive Toxicity			
ER binding (OECD)	Weak binder, NH2 group		Weak binder, NH2 group
Developmental toxicity model (CAESAR v2.1.6)	Toxicant (low reliability)		Toxicant (low reliability)
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 (OECD)	• 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 model (CAESAR v2.1.5)	NON-Sensitizer (good reliability)		NON-Sensitizer (good reliability)
Metabolism			
Rat liver S9 metabolism simulator (OECD)	See supplemental data 1	No metabolites	See supplemental data 2

[a] Values calculated using JChem with FCFP4 1024 bits fingerprint (Rogers and Hahn, 2010).
[b] N/A, Not Applicable, The major metabolite of the target.

Summary

There are insufficient toxicity data on Ethyl anthranilate (RIFM # 666, CAS # 87-25-2). Hence, *in silico* evaluation was conducted to determine suitable read-across material. Based on structural similarity, reactivity, metabolism data, physicochemical properties and expert judgment, the above shown read-across materials were identified as proper read across for their respective toxicity endpoints.

Methods

- The identified read-across analogs were confirmed by using expert judgment
- The physicochemical properties of target and analogs were calculated using EPI Suite™ v4.11 developed by US EPA (USEPA, 2012)
- The J_{max} were calculated using RIFM skin absorption model (SAM), the parameters were calculated using consensus model (Shen et al., 2014)
- DNA binding, mutagenicity, genotoxicity alerts and oncologic classification were estimated using OECD QSAR Toolbox (v3.1) (OECD, 2012)
- ER binding and repeat dose categorization were estimated using OECD QSAR Toolbox (v3.1) (OECD, 2012)
- Developmental toxicity and skin sensitization were estimated using CAESAR (v.2.1.6) (Cassano et al., 2010)
- Protein binding were estimated using OECD QSAR Toolbox (v3.1) (OECD, 2012)

Conclusion/Rationale

- Benzoic acid, 2-amino- (analog) was used as a read-across for Ethyl anthranilate (target) based on:
  - The target and analog both belong to the generic class of amines.
  - The analog is the major metabolite of the target.
  - The only difference is that the target is an ester and has an additional ethanol part, while the analog is the 2-aminobenzoic acid. Such difference could be mitigated since the target is predicted to be hydrolyzed to the methanol and the analog. Besides, the differences between structures do not essentially change the physicochemical properties nor raise any additional structural alerts and therefore, the genotoxicity profiles are expected to be similar.
  - Both the target and the analog show similar alerts for DNA binding, mutagenicity, genotoxicity and oncologic classification.
  - As per the OECD Toolbox the target is predicted to be metabolized to the analog (metabolites #1).
- Methyl anthranilate (analog) was used as a read-across for Ethyl anthranilate (target) based on:
  - The target and analog both belong to the generic class of amines, specifically, esters/anthranilates/amino.
  - Both have the common structure of anthranilate and ester function groups.
  - The only difference is that the target is the ethanol ester, while the analog is the methanol ester. The differences between structures do not essentially change the physicochemical properties nor raise any additional structural alerts and therefore, the developmental and reproductive toxicity profiles are expected to be similar.
  - They both show similar alerts for Repeated Dose (HESS) Categorization and ER Binding. ER Binding is molecular initiating event analogous to protein binding. ER binding is not necessarily predictive of endocrine disruption given the complex pre- and post-receptor events that determine activity.
  - Both materials are expected to be metabolized similarly. As per the OECD Toolbox both materials are predicted to have similar metabolites.

Explanation of Cramer class

Due to potential discrepancies with the current *in silico* tools (Bhatia et al., 2015), the Cramer class of the target material was determined using expert judgment based on the Cramer decision tree (Cramer et al., 1978).

Q1. Normal constituent of the body **No**
Q2. Contains functional groups associated with enhanced toxicity **No**
Q3. Contains elements other than C, H, O, N, divalent S **No**
Q5. Simply branched aliphatic hydrocarbon or a common carbohydrate **No**
Q6. Benzene derivative with certain substituents **No**
Q7. Heterocyclic **No**
Q16. Common terpene **No**
Q17. Readily hydrolyzed to a common terpene **No**
Q19. Open chain **No**
Q23. Aromatic **Yes**
Q27. Rings with substituents **Yes**
Q28. More than one aromatic ring **No**
Q30. Aromatic Ring with complex substituents **Yes**
Q31. Is the substance an acyclic acetal or ester of substances defined in Q30? **No**
Q32. Contains only the functional groups listed in Q30 or Q31? **No**
Q22. Common component of food or structurally closely related to a common component of food **Yes**
Class Intermediate (Class II)

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.fct.2015.03.017.

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