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Short Review

RIFM Fragrance ingredient safety assessment, *cis*-3-hexenyl anthranilate, CAS Registry Number 65405-76-7

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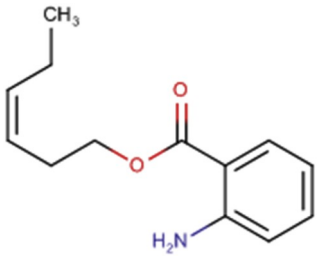


Abbreviation/Definition List:

2-Box Model - A RIFM, Inc. Proprietary *in silico* tool used to calculate fragrance air exposure concentration
AF - Assessment Factor
BCF - Bioconcentration Factor
Creme RIFM Model - The Creme RIFM Model uses probabilistic (Monte Carlo) simulations to allow full distributions of data sets, providing a more realistic estimate of aggregate exposure to individuals across a population (Comiskey et al., 2015, 2017; Safford et al., 2015, 2017) compared to a deterministic aggregate approach
DEREK - Derek Nexus is an *in silico* tool used to identify structural alerts
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 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.

cis-3-Hexenyl anthranilate was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, and environmental safety. Data show that *cis*-3-hexenyl anthranilate is not genotoxic. The skin sensitization endpoint was completed using the DST for non-reactive materials (900 μg/cm^2); exposure is below the DST. The reproductive and local respiratory toxicity endpoints were completed using the TTC for a Cramer Class II material, and the exposure *cis*-3-hexenyl anthranilate is below the TTC (0.009 mg/kg/day and 0.47 mg/day, respectively). Data on read-across analog methyl anthranilate (CAS # 134-20-3) provide a calculated MOE > 100 for the repeated dose and developmental toxicity endpoints. The phototoxicity/photoallergenicity endpoints were evaluated based on UV spectra; *cis*-3-hexenyl anthranilate is not expected to be phototoxic/photoallergenic. The environmental endpoints were evaluated; *cis*-3-hexenyl anthranilate 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, 2016a; RIFM, 2016b)
Repeated Dose Toxicity: NOAEL = 500 mg/kg/day. (Hagan et al., 1967; data also available in Bar, 1967)
Developmental and Reproductive Toxicity: Developmental NOAEL = 768.4 mg/kg/day. No reproductive NOAEL. Exposure is below the TTC. RIFM (2012)
Skin Sensitization: Not a sensitization concern. Exposure is below the DST.
Phototoxicity/Photoallergenicity: Not phototoxic/photoallergenic. (UV Spectra, RIFM DB)
Local Respiratory Toxicity: No NOAEC available. Exposure is below the TTC.

Environmental Safety Assessment

Hazard Assessment:

Persistence: Screening-level: 3.02 (BIOWIN 3) (EPI Suite v4.1; US EPA, 2012a)
Bioaccumulation: Screening-level: 436 L/kg (EPI Suite v4.1; US EPA, 2012a)
Ecotoxicity: Screening-level: Fish LC50: 1.97 mg/L (RIFM Framework; Salvito et al., 2002)
Conclusion: Not PBT or vPvB as per IFRA Environmental Standards
Risk Assessment:
Screening-level: PEC/PNEC (North America and Europe) < 1 (RIFM Framework; Salvito et al., 2002; #40315)
Critical Ecotoxicity Endpoint: Fish LC50: 1.97 mg/L (RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.0019 μg/L
- **Revised PEC/PNECs (2011 IFRA VoU):** North America and Europe: Not Applicable; cleared at screening-level

1. Identification

1. **Chemical Name:** *cis*-3-Hexenyl anthranilate
2. **CAS Registry Number:** 65405-76-7
3. **Synonyms:** 3-Hexen-ol, 2-aminobenzoate, (z)-; 3-Hexenyl 2-aminobenzoate; (Z)-Hex-3-enyl anthranilate; (Z)-3-Hexenyl anthranilate; Hex-3-en-1-yl 2-aminobenzoate; *cis*-3-Hexenyl anthranilate
4. **Molecular Formula:** $C_{13}H_{17}NO_2$
5. **Molecular Weight:** 219.29
6. **RIFM Number:** 10

2. Physical data

1. **Boiling Point:** 160 °C @ 5 mm Hg (FMA), 339.9 °C (EPI Suite)
2. **Flash Point:** 220 °F; CC (FMA)
3. **Log K_{OW}:** 4.51 (EPI Suite)
4. **Melting Point**: 103.68 °C (EPI Suite)
5. **Water Solubility:** 4.933 mg/L (EPI Suite)
6. **Specific Gravity:** 1.05 (FMA)
7. **Vapor Pressure:** 0.0000194 mm Hg @ 20 °C (EPI Suite v4.0), 3.9e-005 mm Hg @ 25 °C (EPI Suite)
8. **UV Spectra:** No significant absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark (1000 L $mol^{-1} \cdot cm^{-1}$)
9. **Appearance/Organoleptic:** Pale straw-colored or pale amber-colored somewhat viscous liquid. Very sweet and tenacious, fruity, American concord-grape-wine-like note (Arctander, 1969).

3. Exposure

1. **Volume of Use (worldwide band):** < 1 metric tons per year (IFRA, 2011)
2. **95**th **Percentile Concentration in Hydroalcoholics:** 0.006% (RIFM, 2016c)
3. **Inhalation Exposure*:** 0.000052 mg/kg/day or 0.0041 mg/day (RIFM, 2016c)
4. **Total Systemic Exposure**:** 0.00034 mg/kg/day (RIFM, 2016c)

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

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

4. Derivation of systemic absorption

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

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:** None
   b. **Repeated Dose Toxicity:** Methyl anthranilate (CAS # 134-20-3)
   c. **Developmental and Reproductive Toxicity:** Methyl anthranilate (CAS # 134-20-3)
   d. **Skin Sensitization:** None
   e. **Phototoxicity/Photoallergenicity:** None
   f. **Local Respiratory Toxicity:** None
   g. **Environmental Toxicity:** None
3. Read-across justifications: See Appendix below

6. Metabolism

Not considered for this risk assessment and therefore not reviewed except where it may pertain in specific endpoint sections as discussed below.

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

Cis-3-Hexenyl anthranilate is not reported to occur in foods by the VCF*.

*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 which have been found in natural (processed) food products. Includes FEMA GRAS and EU-Flavis data.

8. IFRA standard

None.

9. REACH dossier

Pre-registered for 2010; no dossier available as of 4/30/18.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data and use levels, *cis*-3-hexenyl anthranilate does not present a concern for genetic toxicity.

10.1.1.1. Risk assessment. *Cis*-3-hexenyl anthranilate was found negative for genotoxicity in the BlueScreen assay indicating a lack for genotoxic concern (RIFM, 2013). The mutagenic activity of *cis*-3-hexenyl anthranilate 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 method. *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, and *Escherichia coli* strain WP2uvrA were treated with *cis*-3-hexenyl anthranilate 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 dose in the presence or absence of S9 (RIFM, 2016a). Under the conditions of the study, *cis*-3-hexenyl anthranilate was not mutagenic in the Ames test.

The clastogenic activity of *cis*-3-hexenyl anthranilate was evaluated in an *in vitro* micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 487. Human peripheral blood lymphocytes were treated with *cis*-3-hexenyl anthranilate in DMSO at concentrations up to 175 μg/mL in the presence and absence of metabolic activation (S9) at the 3-h and 24-h time points. *cis*-3-Hexenyl anthranilate did not induce binucleated cells with micronuclei when tested up to cytotoxic levels in either non-activated or S9-activated test systems (RIFM, 2016b). Under the conditions of the study, *cis*-3-hexenyl anthranilate was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the available data, *cis*-3-hexenyl anthranilate does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed On: 07/01/16.

10.1.2. Repeated dose toxicity

The margin of exposure for *cis*-3-hexenyl anthranilate is adequate for the repeated dose toxicity endpoint at the current level of use.

10.1.2.1. Risk assessment. There are no repeated dose toxicity data on *cis*-3-hexenyl anthranilate. There are sufficient repeated dose toxicity data on read-across material methyl anthranilate (CAS # 134-20-3; see Section V). A dietary 90-day subchronic toxicity study was conducted in rats. Groups of 10 weanling Osborne-Mendel rats per sex were administered methyl anthranilate in the diet for 13 weeks at dose levels of 0, 1000, or 10000 ppm (equivalent to 0, 50, or 500 mg/kg/day). There were no treatment-related adverse effects reported up to the highest dose tested. Thus, the NOAEL for the repeated dose toxicity endpoint was determined to be 10000 ppm or 500 mg/kg/day (Hagan et al., 1967; data also available in Bar, 1967).

Therefore, the *cis*-3-hexenyl anthranilate MOE for the repeated dose toxicity endpoint can be calculated by dividing the methyl anthranilate NOAEL in mg/kg/day by the total systemic exposure to *cis*-3-hexenyl anthranilate, 500/0.00034 or 1470588.

In addition, the total systemic exposure to *cis*-3-hexenyl anthranilate (0.34 μg/kg/day) is below the TTC (9 μg/kg bw/day) for the repeated dose toxicity endpoint of a Cramer Class II material at the current level of use.

Additional References: Hagan et al., 1967; Bar, 1967; Stoner et al., 1973; Schafer, 1985; Clark et al., 1980; Cutting et al., 1966; Verrett et al., 1980; RIFM, 1974; Grundschober (1977); Yamaori et al., 2005; Ekman, 1949.

Literature Search and Risk Assessment Completed On: 09/15/16.

10.1.3. Developmental and Reproductive Toxicity

The margin of exposure for *cis*-3-hexenyl anthranilate is adequate for the developmental toxicity endpoint at the current level of use.

There are insufficient reproductive toxicity data on *cis*-3-hexenyl anthranilate or any read-across materials. The total systemic exposure to *cis*-3-hexenyl anthranilate is below the TTC for the reproductive toxicity endpoint of a Cramer Class II material at the current level of use.

10.1.3.1. Risk assessment. There are no developmental toxicity data on *cis*-3-hexenyl anthranilate. Read-across material methyl anthranilate (CAS # 134-20-3; see Section V) has sufficient developmental toxicity data. An OECD 414 dietary developmental toxicity study was conducted in rats (RIFM, 2012). Presumed pregnant rats (25/dose) were fed methyl anthranilate in the diet at dose levels of 0, 1000, 5000, or 10000 ppm (average daily consumption of 0, 80.4, 389.9, or 768.4 mg/kg/day) on days 6 through 20 of presumed gestation. The adult animals among the 1000, 5000, and 10000 ppm dose groups had reduced bodyweight gains, and animals among the 5000 and 10000 ppm dose groups had reduced food consumption. There were no developmental toxicity findings reported among the pups up to the highest dose tested. The NOAEL for maternal toxicity was determined to be 1000 ppm or 80.4 mg/kg/day, and the NOAEL for developmental toxicity was determined to be 10000 ppm or 786.4 mg/kg/day, the highest dosage tested.

Therefore, the *cis*-3-hexenyl anthranilate MOE for the developmental toxicity endpoint can be calculated by dividing the methyl anthranilate NOAEL in mg/kg/day by the total systemic exposure to *cis*-3-hexenyl anthranilate, 768.4/0.00034 or 2260000.

In addition, the total systemic exposure to *cis*-3-hexenyl anthranilate (0.34 μg/kg/day) is below the TTC (9 μg/kg bw/day) for the developmental toxicity endpoint of a Cramer Class II material at the current level of use.

There are no reproductive toxicity data on *cis*-3-hexenyl anthranilate or any of the read-across materials. The total systemic exposure to *cis*-3-hexenyl anthranilate (0.34 μg/kg/day) is below the TTC (9 μg/kg bw/day) for the reproductive toxicity endpoint of a Cramer Class II material at the current level of use.

Additional References: Hagan et al., 1967; Bar, 1967; OECD QSAR Toolbox (Dow Chemical, 1967 from MUNRO database); Stoner et al., 1973; Schafer, 1985; Clark et al., 1980; Cutting et al., 1966; Verrett et al., 1980; RIFM, 1974; Grundschober (1977); Yamaori et al., 2005; Ekman, 1949.

Literature Search and Risk Assessment Completed On: 09/14/16.

10.1.4. Skin sensitization

Based on the available data and application of the DST, *cis*-3-hexenyl anthranilate does not present a concern for skin sensitization.

10.1.4.1. Risk assessment. Based on the available data and application of the DST, *cis*-3-hexenyl anthranilate does not present a concern for skin sensitization. The chemical structure of this material indicates that it would not be expected to react with skin proteins (Roberts et al., 2007; Toxtree 2.5.0; OECD toolbox v3.1). In a human maximization test, no reactions to *cis*-3-hexenyl anthranilate were observed (RIFM, 1978). The reported exposure was benchmarked utilizing the non-reactive DST 900 μg/cm^2. The current 95th percentile dermal exposure is below the DST for non-reactive materials when evaluated in all QRA categories. *cis*-3-Hexenyl anthranilate does not present a concern for skin sensitization (Table 1).

Table 1
Acceptable exposure limits for *cis*-3-hexenyl anthranilate based on non-reactive DST.

IFRA Category[a]	Examples of Product Type	Calculated QRA
1	Lip Products	0.026%
2	Deodorant/Antiperspirant	0.033%
3	Hydroalc., Shaved Skin	0.136%
4	Hydroalc., Unshaved Skin	0.407%
5	Women Facial Cream	0.214%
6	Mouthwash	0.652%
7	Intimate Wipes	0.068%
8	Hair Styling Aids Non-Spray	0.91%
9	Conditioners, Rinse-off	4.50%
10	Hard Surface Cleaners	2.5%
11	Candle (Non-Skin/Incidental Skin)	Not Restricted

Note: [a] For a description of the categories, refer to the QRA Informational Booklet (www.rifm.org/doc/QRAInfoJuly2011.pdf).

Additional References: None.

Literature Search and Risk Assessment Completed On: 09/27/16.

10.1.5. Phototoxicity/photoallergenicity

Based on available UV/Vis spectra, *cis*-3-hexenyl anthranilate would not be expected to present a concern for phototoxicity or photoallergenicity.

10.1.5.1. Risk assessment. There are no phototoxicity studies available for *cis*-3-hexenyl anthranilate in experimental models. UV/Vis absorption spectra indicate no significant absorbance between 290 and 700 nm. Corresponding molar absorption coefficient is well below the benchmark of concern for phototoxicity and photoallergenicity (Henry et al., 2009). Based on lack of significant absorbance in the critical range, *cis*-3-hexenyl anthranilate does not present a concern for phototoxicity or photoallergenicity.

There are no studies available on *cis*-3-hexenyl anthranilate in experimental models.

10.1.5.2. UV spectra analysis. UV/Vis absorption spectra (OECD TG 101) for *cis*-3-hexenyl anthranilate were obtained. The spectra indicate no significant absorbance in the range of 290–700 nm. The molar absorption coefficient is below the benchmark, 1000 $L\,mol^{-1} \cdot cm^{-1}$, of concern for phototoxic effects (Henry et al., 2009).

Additional References: None.

Literature Search and Risk Assessment Completed On: 09/13/16.

10.1.6. Local Respiratory Toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The exposure level of *cis*-3-hexenyl anthranilate is below the Cramer Class III* TTC value for inhalation exposure local effects.

10.1.6.1. Risk assessment. There are no inhalation data available on *cis*-3-hexenyl anthranilate. Based on the Creme RIFM model, the inhalation exposure is 0.0041 mg/day. This exposure is 114.6 times lower than the Cramer Class III* TTC value of 0.47 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

*As per Carthew et al., 2009, Cramer Class II materials default to Cramer Class III.

Additional References: None.

Literature Search and Risk Assessment Completed On: 07/20/16.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening-level risk assessment of *cis*-3-hexenyl anthranilate 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 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, *cis*-3-hexenyl anthranilate identified as a fragrance material with no potential to present a possible risk to the aquatic environment (i.e., its screening-level PEC/PNEC < 1).

A screening-level hazard assessment using EPI Suite v4.1 (US EPA, 2012a) did not identify *cis*-3-hexenyl anthranilate as 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 v4.1, US EPA, 2012a). Data on persistence and bioaccumulation are reported below and summarized in the Environmental Safety Assessment section prior to Section 1.

10.2.2. Risk assessment

Based on current Volume of Use (2011), *cis*-3-hexenyl anthranilate does not present a risk to the aquatic compartment in the screening-level assessment.

10.2.2.1. Biodegradation. Not Available.

10.2.2.2. Ecotoxicity. Not Available.

10.2.2.3. Other available data. *cis*-3-Hexenyl anthranilate has been pre-registered for REACH with no additional data at this time.

10.2.3. 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)**	1.97			1,000,000	0.0019	

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} used	4.5	4.5
Biodegradation Factor Used	0	0
Dilution Factor	3	3
Regional Volume of Use Tonnage Band	< 1	< 1
Risk Characterization: PEC/PNEC	< **1**	< **1**

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

The RIFM PNEC is 0.0019 μg/L. The revised PEC/PNECs for EU and NA: Not Applicable: Cleared at Screening-level and therefore does not present a risk to the aquatic environment at the current reported volumes of use.

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

11. 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
- 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://webnet.oecd.org/hpv/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:** http://www.safe.nite.go.jp/english/db.html
- **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.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.110611.

Appendix

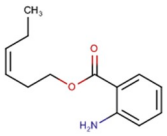
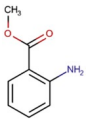
Read-across Justification

Methods

The read-across analogs were identified following the strategy for structuring and reporting a read-across prediction of toxicity as described in Schultz et al. (2015). The strategy is also 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, 2012).

- 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 substance 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, and oncologic classification predictions were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- ER binding and repeat dose categorization were generated using OECD QSAR Toolbox v4.2 (OECD, 2018).
- Developmental toxicity was predicted using CAESAR v2.1.7 (Cassano et al., 2010) and skin sensitization was predicted using Toxtree 2.6.13.
- Protein binding was predicted using OECD QSAR Toolbox v4.2 (OECD, 2018).

- The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox v4.2 (OECD, 2018).

	Target material	Read-across material
Principal Name	*cis*-3-Hexenyl anthranilate	Methyl anthranilate
CAS No.	65405-76-7	134-20-3
Structure		
Similarity (Tanimoto Score)		0.636
Read-across Endpoint		• Repeated dose • Developmental and Reproductive
Molecular Formula	$C_{13}H_{17}NO_2$	$C_8H_9NO_2$
Molecular Weight	219.29	151.16
Melting Point (°C, EPI Suite)	103.68	55.76
Boiling Point (°C, EPI Suite)	339.90	263.57
Vapor Pressure (Pa @ 25°C, EPI Suite)	0.0052	2.63
Log Kow(KOWWIN v1.68 in EPI Suite)	4.51	1.88
Water Solubility (mg/L, @ 25°C, WSKOW v1.42 in EPI Suite)	4.93	2850
J_{max} (μg/cm^2/h, SAM)	6.177	50.57
Henry's Law (Pa·m^3/mol, Bond Method, EPI Suite)	4.45E-008	1.23E-008
Repeated dose toxicity		
Repeated Dose (HESS)	• Not categorized	• Not categorized
Reproductive and developmental toxicity		
ER Binding by OECD QSAR Tool Box (3.4)	• Strong binder NH_2 group	• Weak binder NH_2 group
Developmental Toxicity Model by CAESAR v2.1.6	• Non-toxicant (low reliability)	• Toxicant (low reliability)
Metabolism		
OECD QSAR Toolbox (3.4) Rat Liver S9 Metabolism Simulator	See Supplemental Data 1	See Supplemental Data 2

Summary

There are insufficient toxicity data on *cis*-3-hexenyl anthranilate (CAS # 65405-76-7). Hence *in silico* evaluation was conducted by determining suitable read-across analogs for this material. Based on structural similarity, reactivity, metabolism data, physical–chemical properties, and expert judgment, methyl anthranilate (CAS # 134-20-3) was identified as a read-across material with sufficient data for toxicological evaluation.

Conclusions

- Read-across material methyl anthranilate (CAS # 134-20-3) could be used as a structurally similar read-across analog for the target material (CAS # 65405-76-7) for the reproductive and developmental toxicity and repeated dose toxicity endpoints.
  - o The target substance and the read-across analog are structurally similar and belong to the structural class of anthranilates.
  - o The target substance and the read-across analog have the methyl anthranilate fragment common among them.
  - o The key difference between the target substance and the read-across analog is that the target has a 6-carbon alkene chain (hex-2-ene) alcohol portion of the ester while the read-across has a methyl group at the similar position. This structure difference between the target substance and the read-across analog do not raise additional structural alerts, so the structure differences are not relevant from a toxicoloigical endpoint perspective.
  - o The target substance and the read-across analog have a Tanimoto score as mentioned in the above table. The Tanimoto score is mainly driven by the methyl anthranilate fragment. The differences in the structure which are responsible for Tanimoto score < 1 are not relevant from a toxicoloigical endpoint perspective.
  - o The physical–chemical properties of the target substance and the read-across analog are sufficiently similar to enable comparison of their toxicological properties.
  - o The target substance and the read-across analog have ER binding alerts. ER Binding is a molecular initiating event. ER binding is not necessarily predictive of endocrine disruption given the complex pre- and post-receptor events that determine activity. The data described in the reproductive and developmental toxicity section show that the read-across analog has an adequate margin of exposure at the current level of use. Therefore, the alert will be superseded by the availability of data.
  - o The read-across analog is predicted to be a toxicant by the CAESAR model for developmental toxicity. The data described in the developmental toxicity section above show that the read-across analog has an adequate margin of exposure at the current level of use. Therefore, the alert will be superseded by the availability of the data.
  - o The target substance and the read-across analog are expected to be metabolized similarly as shown by metabolism simulator.
  - o The structural alerts for the reproductive and developmental toxicity and the repeated dose toxicity endpoints are consistent between the metabolites of the read-across analog and the target substance.
  - o The structural differences between the target substance and the read-across analog are deemed to be toxicologically insignificant.

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 and either (a) a single fused non-aromatic carbocyclic ring or (b) aliphatic substituent chains longer than 5 carbon atoms or (c) a polyoxyethylene [(-OCH2CH2-)x, with x = 4] chain either on the aromatic ring or on an aliphatic side chain? **No** Q22. Common component of food? **Yes**, Class Intermediate (Class II)

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