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Short review

RIFM fragrance ingredient safety assessment, isobutyl *N*-methylanthranilate, CAS Registry Number 65505-24-0

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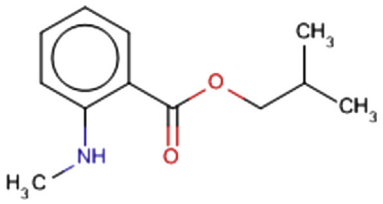
CAS Registry Number: 65505-24-0



Abbreviation 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; Safford et al., 2015; Safford et al., 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
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
Significant – Unless otherwise specified, statistically significant differences in reported results with a P-value of <0.05 using appropriate test for significance.
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 under the limits 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 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).
*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 guidance relevant to human health and environmental protection.

Summary: The use of this material under current conditions is supported by existing information.
This material was evaluated for genotoxicity, repeated dose toxicity, reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, as well as environmental safety. Data show that this material is not genotoxic. Data from the read across analogue methyl *N*-methylanthranilate (CAS # 85-91-6) show that this material does not have skin sensitization potential and provided a MOE > 100 for the repeated dose toxicity endpoint. The developmental and reproductive and local respiratory toxicity endpoints were completed using the TTC (Threshold of Toxicological Concern) for a Cramer Class III material (0.0015 mg/kg/day and 0.47 mg/day, respectively). The phototoxicity/photoallergenicity endpoint was completed based on UV spectra. The environmental endpoints were evaluated and the material was not found 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, 2015a; RIFM, 2016)
Repeated Dose Toxicity: NOAEL = 244 mg/kg/day (Gaunt et al., 1970)
Developmental and Reproductive Toxicity: No NOAEL available. The exposure is below the TTC.

(*continued*)

Skin Sensitization: Not sensitizing	(RIFM, 1981; Klecak et al., 1977; Klecak, 1985; RIFM, 1974)
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: 2.74 (Biowin 3)	(USEPA, 2012)
Bioaccumulation: Screening Level: 279 L/kg	(USEPA, 2012)
Ecotoxicity: Screening Level: Fish LC50: 3.34 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: Fish LC50: 3.34 mg/L	(Salvito et al., 2002)
RIFM PNEC is: 0.00334 μg/L	
• **Revised PEC/PNECs (2011 IFRA VoU):** North America and Europe: not applicable; cleared at screening level	

1. Identification

1 **Chemical Name:** Isobutyl *N*-methylanthranilate
2 **CAS Registry Number**: 65505-24-0
3 **Synonyms**: Benzoic acid, 2-(methylamino)-, 2-methylpropyl ester; Isobutyl *N*-methylanthranilate; Isobutyl 2-(methylamino)benzoate; Benzoic acid, 2-(methylamino)-, 2-methylpropyl ester; *N*-アルキル(C = 1 ~ 4)-*o*-アミノ安息香酸アルキル
4 **Molecular Formula**: $C_{12}H_{17}NO_2$
5 **Molecular Weight**: 207.27
6 **RIFM Number**: 1161

2. Physical data

1 **Boiling Point**: 341 °C [Private communication to FEMA], 289.22 °C [USEPA, 2012]
2 **Flash Point**: >200 °F; CC [FMA database]
3 **Log KOW**: 4.21 [USEPA, 2012]
4 **Melting Point**: 70 °C [Private communication to FEMA], 62.99 °C [USEPA, 2012]
5 **Water Solubility**: 10.15 mg/L [USEPA, 2012]
6 **Specific Gravity**: Not Available
7 **Vapor Pressure**: 0.000824 mmHg @ 20 °C [USEPA, 2012], 0.00153 mm Hg @ 25 °C [USEPA, 2012]
8 **UV Spectra:** Minor absorbance between 290 and 700 nm; molar absorption coefficient is below the benchmark of concern (1000 L mol^{-1} · cm^{-1})
9 **Appearance/Organoleptic:** A pale yellow solid with a medium fruity, grapefruit odor.*

* http://www.thegoodscentscompany.com/data/rw1045201.html#toorgano, retrieved 12/2/2015.

3. Exposure

1 Volume of Use (worldwide band): <0.1 metric tons per year (IFRA, 2011)
2 95th Percentile Concentration in Hydroalcoholics: 0.047% (RIFM, 2015b)
3 Inhalation Exposure*: 0.0000037 mg/kg/day or 0.00027 mg/day (RIFM, 2015b)
4 Total Systemic Exposure**: 0.00054 mg/kg/day (RIFM, 2015b)

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

Expert Judgment	Toxtree v 2.6	OECD QSAR Toolbox v 3.2
III*	III	II

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

2 **Analogues Selected:**
a **Genotoxicity:** None
b **Repeated Dose Toxicity:** Methyl *N*-methylanthranilate (CAS # 85-91-6)
c **Developmental and Reproductive Toxicity:** None
d **Skin Sensitization:** Methyl *N*-methylanthranilate (CAS # 85-91-6)
e **Phototoxicity/Photoallergenicity:** None
f **Local Respiratory Toxicity:** None
g **Environmental Toxicity:** None
3 **Read-across Justification:** See Appendix below

6. Metabolism

Metabolism of the target substance was not considered for this risk assessment.

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

Isobutyl *N*-methylanthranilate is not reported to occur in food 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, contains information on published volatile compounds which have been found in natural (processed) food products. Includes FEMA GRAS and EU-Flavis data.

8. IFRA standard

The material has been identified for having the potential of forming nitrosamines in nitrosating systems. Downstream users therefore have to be notified of the presence of the material and its potential to be able to consider adequate protective measures.

9. REACH Dossier

Pre-registered for 2010; no dossier available as of 056/01/2017.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data and use levels, isobutyl *N*-methylanthranilate does not present a concern for genetic toxicity.

10.1.1.1. Risk assessment. Isobutyl *N*-methylanthranilate was assessed in the BlueScreen assay and found negative for genotoxicity, with and without metabolic activation, indicating a lack of concern regarding genotoxicity (RIFM, 2013). The mutagenic activity of isobutyl *N*-methylanthranilate 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* strains WP2uvrA were treated with isobutyl *N*-methylanthranilate in DMSO (dimethyl sulfoxide) 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, 2015a). Under the conditions of the study, isobutyl *N*-methylanthranilate was not mutagenic in the Ames test.

The clastogenic activity of isobutyl *N*-methylanthranilate 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 isobutyl *N*-methylanthranilate in solvent DMSO (dimethyl sulfoxide) at concentrations up to 250 μg/mL in the presence and absence of metabolic activation (S9) at 3 h and 24-h time points. Isobutyl *N*-methylanthranilate did not induce binucleated cells with micronuclei when tested up to cytotoxic levels in either non-activated or S9-activated test systems at the 3-h time point. A statistically significant increase in binucleated cells with micronuclei was observed in the 24-h h non-activated test system at the highest dose treatment group however, the increase was well with in historical vehicle control range and was not considered to be biologically relevant (RIFM, 2016). Under the conditions of the study, isobutyl *N*-methylanthranilate was considered to be non-clastogenic in the *in vitro* micronucleus test.

Based on the data available, isobutyl *N*-methylanthranilate does not present a concern for genotoxic potential.

Additional References: None.

Literature Search and Risk Assessment Completed on: 08/17/2016.

10.1.2. Repeated dose toxicity

The margin of exposure for isobutyl *N*-methylanthranilate is adequate for the repeated dose toxicity endpoint at the current level of use.

10.1.3. Risk assessment

There are no repeated dose toxicity data on isobutyl *N*-methylanthranilate. There are sufficient repeated dose toxicity data on read across material methyl *N*-methylanthranilate (CAS # 85-91-6; see section V). A 13-week dietary study was conducted on a group of 15 CFE rats/sex/group administered test material methyl *N*-methylanthranilate at doses of 0, 300, 1200, or 3600 ppm (equivalent to 0, 21, 82 or 244 mg/kg/day in males and 0, 24, 95 or 280 mg/kg/day in females). There were no toxicologically relevant adverse effects reported among the animals up to the highest dose tested, thus the NOAEL was determined to be 3600 ppm or 244 mg/kg/day for males and 280 mg/kg/day for females (Gaunt et al., 1970). In another study, a group of 15 FDRL rats/sex/group were administered test material, methyl *N*-methylanthranilate for 90 days at doses of 19.9 and 22.2 mg/kg/day in males and females, respectively via diet. There were no reports on any adverse effects up to the highest dose tested (Oser et al., 1965, data also available in Bar and Griepentrog, 1967). Thus, the NOAEL for repeated dose toxicity endpoint was determined to be 244 mg/kg/day.

Therefore, the isobutyl *N*-methylanthranilate MOE can be calculated by dividing the methyl *N*-methylanthranilate NOAEL by the total systemic exposure to isobutyl *N*-methylanthranilate, 244/0.00054 or 451852.

In addition, the total systemic exposure to isobutyl *N*-methylanthranilate (0.54 μg/kg bw/day) is below the TTC (1.5 μg/kg bw/day) for the repeated dose toxicity endpoint of a Cramer Class III material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed on: 03/10/2016.

10.1.4. Developmental and reproductive toxicity

There are insufficient developmental and reproductive toxicity data on isobutyl *N*-methylanthranilate or any read across materials. The total systemic exposure to isobutyl *N*-methylanthranilate is below the TTC for the developmental and reproductive toxicity endpoints of a Cramer Class III material at the current level of use.

10.1.4.1. Risk assessment. There are no developmental or reproductive toxicity data on isobutyl *N*-methylanthranilate or any read across materials that can be used to support the developmental and reproductive toxicity endpoints. The total systemic exposure to isobutyl *N*-methylanthranilate (0.54 μg/kg/day) is below the TTC (1.5 μg/kg bw/day) for the developmental and reproductive toxicity endpoints of a Cramer Class III material at the current level of use.

Additional References: None.

Literature Search and Risk Assessment Completed on: 03/10/2016.

10.1.5. Skin sensitization

Based on the existing data and read across to methyl *N*-methylanthranilate (CAS # 85-91-6), isobutyl *N*-methylanthranilate does not present a concern for skin sensitization.

10.1.5.1. Risk assessment. Based on the available data and read across to methyl *N*-methylanthranilate (CAS # 85-91-6; see Section V), isobutyl *N*-methylanthranilate does not present a concern for

skin sensitization. The chemical structures of these materials indicate that they would not be expected to react with skin proteins (Roberts et al., 2007; Toxtree 2.5.0; OECD toolbox v3.1). No *in chemico*, *in vitro* or animal studies are available for isobutyl *N*-methylanthranilate. However, in guinea pig test methods, there were no reactions indicative of sensitization with read across analogue methyl *N*-methylanthranilate (Klecak et al., 1977, 1985). In a human maximization test, no reactions were observed with isobutyl *N*-methylanthranilate (RIFM, 1981). Similarly, in a human maximization test, two reactions were observed with 10% (6900 μg/cm^2) methyl *N*-methylanthranilate in petrolatum, on a panel of 25 subjects; however these were considered questionable due to the presence of concurrent test materials for which numerous strong reactions were observed (RIFM, 1974). The human maximization test was repeated with same concentration and no reactions (0/25) indicative of sensitization were observed (RIFM, 1974). Based on weight of evidence isobutyl *N*-methylanthranilate does not present a concern for skin sensitization.

Additional References: None.

Literature Search and Risk Assessment Completed on: 09/26/16.

10.1.6. Phototoxicity/photoallergenicity

Based on available UV/Vis spectra, isobutyl *N*-methylanthranilate would not be expected to present a concern for phototoxicity or photoallergenicity.

10.1.6.1. Risk assessment. There are no phototoxicity studies available for isobutyl *N*-methylanthranilate in experimental models. UV/Vis absorption spectra indicate minor absorbance between 290 and 700 nm. Corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity, 1000 L mol^{-1} · cm^{-1} (Henry et al., 2009). Based on lack of significant absorbance in the critical range, isobutyl *N*-methylanthranilate does not present a concern for phototoxicity or photoallergenicity.

Additional References: None.

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

10.1.7. Local Respiratory Toxicity

The margin of exposure could not be calculated due to lack of appropriate data. The material, isobutyl *N*-methylanthranilate, exposure level is below the Cramer Class III TTC value for inhalation exposure local effects.

10.1.7.1. Risk assessment. There are no inhalation data available on isobutyl *N*-methylanthranilate. Based on the Creme RIFM model, the inhalation exposure is 0.00027 mg/day. This exposure is 1741 times lower than the Cramer Class III TTC value of 0.47 mg/day (based on human lung weight of 650 g; Carthew et al., 2009); therefore, the exposure at the current level of use is deemed safe.

Additional References: None.

Literature Search and Risk Assessment Completed on: 9/2016.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening level risk assessment of isobutyl *N*-methylanthranilate was 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). Provided in the table below are the data necessary to calculate both the PEC and the PNEC determined within this Safety Assessment. For the PEC, while the actual regional tonnage is not provided, the range from the most recent IFRA Volume of Use Survey is reported. The PEC is calculated based on the actual tonnage and not the extremes noted for the range. Following the RIFM Environmental Framework, isobutyl *N*-methylanthranilate 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 EPI SUITE v4.1 did not identify isobutyl *N*-methylanthranilate as either being possibly persistent nor 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 EPI SUITE v4.1).

10.2.2. Risk assessment

Based on current Volume of Use (2011), isobutyl *N*-methylanthranilate does not presents a risk to the aquatic compartment in the screening level assessment.

Biodegradation: No data available.

Ecotoxicity: No data available.

Other available data:

Isobutyl *N*-methylanthranilate 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)	EC50 (Daphnia)	EC50 (Algae)	AF	PNEC	Chemical Class
RIFM Framework Screening Level **(Tier 1)**	<u>3.34 mg/L</u>			1,000,000	0.00334 μg/L	

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} used	4.21	4.21
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 0.00334 μg/L. The revised PEC/PNECs for EU and NA: not applicable; cleared at screening level and therefore, the material does not present a risk to the aquatic environment at the current reported volumes of use.

Literature Search and Risk Assessment Completed on: 1/25/16.

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/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.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.fct.2017.07.045.

Transparency document

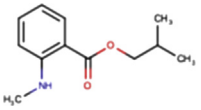
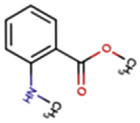
Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.fct.2017.07.045.

Appendix

Read across justification

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 Jmax 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 (v3.4) (OECD, 2012).
- ER binding and repeat dose categorization were estimated using OECD QSAR Toolbox (v3.4) (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 (v3.4) (OECD, 2012).
- The major metabolites for the target and read-across analogs were determined and evaluated using OECD QSAR Toolbox (v3.4) (OECD, 2012)

	Target material	Read across material
Principal Name	Isobutyl *N*-methylanthranilate	Methyl *N*-methylanthranilate
CAS No.	65505-24-0	85-91-6
Structure		
Similarity (Tanimoto score)[1]		0.803
Read across endpoint		• Repeate dose • Skin Sensitization
Molecular Formula	$C_{12}H_{17}NO_2$	$C_9H_{11}NO_2$
Molecular Weight	207.27	165.19
Melting Point (°C, EPI SUITE)	62.99	42.10
Boiling Point (°C, EPI SUITE)	289.22	249.86
Vapor Pressure (Pa @ 25 °C, EPI SUITE)	0.203	2.78
Log Kow (KOWWIN v1.68 in EPI SUITE)	4.21	2.81
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPI SUITE)	10.15	257
J_{max} (mg/cm^2/h, SAM)	26.911	74.151

(*continued*)

	Target material	Read across material
Henry's Law ($Pa \cdot m^3$/mol, Bond Method, EPI SUITE)	6.30E-008	2.69E-008
Repeated dose toxicity		
Repeated Dose (HESS)	• Not categorized	• Not categorized
Skin Sensitization		
Protein binding by OASIS v1.1	• Acylation • AN2 • Michael type addition	• Acylation • AN2 • Michael type addition
Protein binding by OECD	• No alert found	• No alert found
Protein binding potency	• Not possible to classify	• Not possible to classify
Protein binding alerts for skin sensitization by OASIS v1.1	• No alert found	• No alert found
Skin Sensitization model (CAESAR) (version 2.1.6)	• Sensitizer (low reliability)	• Non sensitizer (good reliability)
Metabolism		
OECD QSAR Toolbox (3.4)	See Supplemental Data 1	See Supplemental Data 2
Rat liver S9 metabolism simulator		

Summary

There are insufficient toxicity data on isobutyl *N*-methylanthranilate (CAS # 65505-24-0). Hence *in silico* evaluation was conducted by determining suitable read across analogs for this material. Based on structural similarity, reactivity, metabolism data, physicochemical properties and expert judgment, suitable analogue methyl *N*-methylanthranilate (CAS # 85-91-6) was identified as a proper read across material with data for its respective toxicity endpoints.

Conclusion/Rationale

- Methyl *N*-methylanthranilate (CAS # 85-91-6) could be used as structurally similar read across analogue for target material isobutyl *N*-methylanthranilate (CAS # 65505-24-0) for the skin sensitization and repeated dose toxicity endpoints.
  - o The target substance and the read across analogue are structurally similar and belong to the structural class of anthranilates.
  - o The target substance and the read across analogue have a methylanthranilate fragment common among them.
  - o The key difference between the target substance and the read across analogue is that the target has an isobutyl alcohol portion of the ester while the read across analogue has a methyl group at the similar position. This structural difference between the target substance and the read across analogue do not raise additional structural alerts so the structural differences are not relevant from a toxicological endpoint perspective.
  - o The target substance and the read across analogue have a Tanimoto score as mentioned in the above table. The Tanimoto score is mainly driven by the methylanthranilate fragment. The differences in the structure which are responsible for Tanimoto score <1 are not relevent from a toxic endpoint perspective.
  - o The physical chemical properties of the target substance and the read across analogue are sufficiently similar to enable comparison of their toxicological properties.
  - o According to the QSAR OECD Toolbox (V3.4), structural alerts for skin sensitization and repeated dose toxicity endpoints are consistent between the target substance and the read across analogue.
  - o The target substance and the read across analogue are expected to be metabolized similarly as shown by metabolism simulator. The target substance and the read across analogue, both are secondary amines, they are expected to form primary amines and formaldehyde. They will be substrate for MAO (monoamine oxidase).
  - o The structural alerts for skin sensitization and repeated dose toxicity endpoints are consistent between the metabolites of the read across analogue and the target substance.
  - o The structural differences between the target substance and the read across analogue are deemed to be toxicologically insignificant.

Explanation of Cramer Classification:

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 those listed below. **No**.
Q22 Common component of food **No** Class High (Class III)

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