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Short review

RIFM fragrance ingredient safety assessment, p-methoxybenzonitrile, CAS Registry Number 874-90-8

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1. Identification

1. **Chemical Name:** p-Methoxybenzonitrile
2. CAS Registry Number: 874-90-8
3. **Synonyms:** Benzonitrile, 4-methoxy-; 4-Cyanoanisole; 1-Cyano-4-methoxybenzene; p-Methoxybenzonitrile; p-Methoxyphenyl cyanide; 4-Methoxybenzonitrile
4. Molecular Formula: C_8H_7NO
5. Molecular Weight: 133.5
6. RIFM Number: 5217

2. Physical data

1. **Boiling Point:** 230.92 °C [EPI Suite]
2. **Flash Point:** 135.00 °F. TCC (57.22 °C)*
3. **Log K_{OW}:** 1.62 [EPI Suite]
4. **Melting Point:** 27.05 °C [EPI Suite]
5. **Water Solubility:** 1715 mg/L [EPI Suite]
6. Specific Gravity: Not Available

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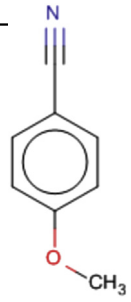
Version: 021717. This version replaces any previous versions.

Name: p-Methoxybenzonitrile
CAS Registry Number: 874-90-8



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) 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 Developmen
OECD TG- Organisation for Economic Co-operation and Development Testing Guidelines
OECD TG- Organisation for Economic Co-operation and Development Testing Guidelines
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
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.

(*continued*)

Summary: The use of this material under current conditions is supported by existing information. This material was evaluated for genotoxicity, repeated dose toxicity, developmental and reproductive toxicity, local respiratory toxicity, phototoxicity/photoallergenicity, skin sensitization, as well as environmental safety. Data from the suitable read across analog benzonitrile (CAS # 100-47-0) show that this material is not genotoxic, and provided a MOE > 100 for the repeated dose endpoint. The skin sensitization endpoint was completed by utilizing the non-reactive DST. 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 suitable UV spectra. The environmental endpoint was completed as described in the RIFM Framework.

Human Health Safety Assessment

Genotoxicity: Not genotoxic. (Zeiger et al., 1988; RIFM, 2007)
Repeated Dose Toxicity: NOAEL = 37.5 mg/kg/day. (ECHA, REACH Dossier on benzonitrile)
Developmental and Reproductive Toxicity: No data available. Exposure is below the TTC.
Skin Sensitization: Not a sensitization concern. Exposure is below 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: 2.76 (Biowin 3) (EpiSuite ver 4.1)
Bioaccumulation: Screening Level: 6.17 l/kg (EpiSuite ver 4.1)
Ecotoxicity: Screening Level: Fish LC50: 385.5 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)
Critical Ecotoxicity Endpoint: Fish LC50: 385.5 mg/l (RIFM Framework; Salvito et al., 2002)
RIFM PNEC is: 0.3855 μg/L
•**Revised PEC/PNECs (2011 IFRA VoU):** North America and Europe: Not Applicable; cleared at screening level

7. **Vapor Pressure:** 0.00488 mmHg @ 20 °C [EPI Suite 4.0], 0.0087 mm Hg @ 25 °C [EPI Suite]
8. **UV Spectra:** Minor absorbance between 290 and 700 nm; molar extinction coefficient is below the benchmark (1000 L mol^{-1} cm^{-1})
9. **Appearance/Organoleptic:** A white crystalline powder with a medium sweet, floral, hawthorn, hay, coumarin odor while at 10% or less in dipropylene glycol.*

*http://www.thegoodscentscompany.com/data/rw1005651.html#toorgano, retrieved 1/14/2016.

3. Exposure

1. **Volume of Use (worldwide band):** <0.1 metric tons per year (IFRA, 2011)
2. 95th Percentile Concentration in Shampoo (no reported use in hydroalcoholics): 0.0030% (RIFM, 2014b)
3. **Inhalation Exposure*:** 0.00023 mg/kg/day or 0.016 mg/day (RIFM, 2014b)
4. **Total Systemic Exposure**:** 0.00042 mg/kg/day (RIFM, 2014b)

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

**95th percentile calculated exposure; assumes 100% absorption

unless modified by dermal absorption data as reported in Section 4. 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).

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	Toxtree v 2.6	OECD QSAR Toolbox v 3.2
III	III	III

2. Analogues selected:
   a. **Genotoxicity:** Benzonitrile (CAS # 100-47-0)
   b. **Repeated Dose Toxicity:** Benzonitrile (CAS # 100-47-0)
   c. Developmental and Reproductive Toxicity: None
   d. **Skin Sensitization:** Benzonitrile (CAS# 100-47-0)
   e. Phototoxicity/Photoallergenicity: None
   f. Local Respiratory Toxicity: None
   g. Environmental Toxicity: None
3. Read-across Justification: 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)

p-Methoxybenzonitrile 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

None.

9. REACH dossier

Pre-registered for 2010, no dossier available as of 2/17/2017.

10. Summary

10.1. Human health endpoint summaries

10.1.1. Genotoxicity

Based on the current existing data and use levels, p-methoxybenzonitrile does not present a concern for genetic toxicity.

10.1.1.1. Risk assessment. p-Methoxybenzonitrile was assessed in the BlueScreen assay and found negative for genotoxicity, with and without metabolic activation, indicating a lack of concern regarding genotoxicity (RIFM, 2014a). There are no studies assessing the mutagenic/clastogenic activity of p-methoxybenzonitrile however, read across can be made to benzonitrile (CAS # 100-47-0; see Section 5). The mutagenic activity of benzonitrile (CAS # 100-47-0) has been evaluated in a bacterial reverse mutation assay using the preincubation method. *Salmonella typhimurium* strains TA1535, TA97, TA98 and TA100 were treated with benzonitrile in DMSO (dimethyl sulfoxide) at concentrations up to 3333 μg/plate. No increases in the mean number of revertant colonies were observed at any tested dose in the presence or absence of S9 (Zeiger et al., 1988). Under the conditions of the study, benzonitrile was not mutagenic in the Ames test.

The clastogenic activity of benzonitrile was evaluated in an in vivo micronucleus test conducted in compliance with GLP regulations and in accordance with OECD TG 474. The test material was administered in corn oil via oral route, to groups of male and female NMRI mice (5/sex/dose). Doses of 250, 500, or 1000 mg/kg were administered. Mice from each dose level were euthanized at 24 or 48 h post treatment period, the bone marrow was extracted and examined for polychromatic erythrocytes. The test material did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow (RIFM, 2007). Under the conditions of the study, benzonitrile was not considered to be clastogenic in the in vivo micronucleus test.

In an *in vitro* chromosomal aberration study (ECHA dossier: benzonitrile, accessed 06/27/2016), benzonitrile produced a weakly positive response (11% increase vs 3%increase in vehicle control) in presence of metabolic activation, but these increase was not dose dependent and thus not considered to be biologically relevant. Furthermore an in vivo micronucleus study showed negative results for clastogenicity both in presence and absence of metabolic activation. Thus benzonitrile is expected to be non-clastogenic.

Based on the data available, benzonitrile does not present a concern for genotoxic potential and this can be extended to p-methoxybenzonitrile.

Additional References: Osgood and Cyr, 1998; Bonacker et al., 2004; RIFM, 2009; Wu et al., 2009; RIFM, 2013.

Literature Search and Risk Assessment Completed on: 06/27/2016.

10.1.2. Repeated dose toxicity

The margin of exposure for p-methoxybenzonitrile 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 p-methoxybenzonitrile. Read across material, benzonitrile (CAS# 100-47-0; see section 5) has sufficient repeated dose toxicity data. A 13 week gavage study was conducted with test material, benzonitrile administered to a group of 10 Fischer 344 rats/sex/group at doses of 0 (corn oil), 19, 37.5, 75, 150 and 300 mg/kg/day. Hyperactivity and aggressiveness was reported among the 150 and 300 mg/kg/day animals. Hind leg strength

was also reduced along with delayed response to thermal stimuli among the high dose females. The kidney weight was increased in males of the 75 mg/kg/day and higher groups. The NOAEL was determined to be 37.5 and 75 mg/kg/day for females and males, respectively (ECHA, REACH Dossier on benzonitrile). In another study, a 13 week gavage toxicity study was conducted with test material, benzonitrile administered to a group of 10 B6C3F1/sex/group at doses of 0 (corn oil), 0, 37.5, 75, 150, 300, 600 mg/kg. Females of the high dose group showed startled responses to acoustic signals. A significant reduction in body weight gain was reported among the high dose animals. Liver weights among the animals treated with 75 mg/kg/day and higher were significantly higher than controls. Centrilobular hypertrophy of liver cells, increase of Kupffer cells, mineralization and necrosis was reported among the 300 and 600 mg/kg/day treated males and the 600 mg/kg/day treated females. Thus the NOAEL was determined to be 37.5 mg/kg/day both for males and females (ECHA, REACH Dossier on benzonitrile). The most conservative NOAEL of 37.5 mg/kg/day was selected for the repeated dose toxicity endpoint. **Therefore, the MOE for p-methoxybenzonitrile can be calculated by dividing the benzonitrile NOAEL by the total systemic exposure for p-methoxybenzonitrile, 37.5/0.00042 or 89286.**

In addition, the total systemic exposure for p-methoxybenzonitrile (0.42 μg/kg bw/day) is below the TTC (1.5 μg/kg bw/day) at the current level of use.

Additional References: ECHA, REACH Dossier on benzonitrile.

Literature Search and Risk Assessment Completed on: 7/6/2016.

10.1.3. Developmental and reproductive toxicity

There are insufficient developmental or reproductive toxicity data on p-methoxybenzonitrile or any read across materials. The exposure is below the Threshold of Toxicological Concern (TTC).

10.1.3.1. Risk assessment. There are no developmental or reproductive toxicity data on p-methoxybenzonitrile or any read across materials that can be used to support the developmental or reproductive toxicity endpoints. The total systemic exposure for p-methoxybenzonitrile (0.42 μg/kg/day) is below the TTC (1.5 μg/kg bw/day) at the current level of use.

Additional References: ECHA, REACH Dossier on benzonitrile.

Literature Search and Risk Assessment Completed on: 7/6/2016.

10.1.4. Skin sensitization

Based on the application of DST, p-methoxybenzonitrile does not present a concern for skin sensitization.

10.1.4.1. Risk assessment. No skin sensitization studies are available on p-methoxybenzonitrile. The chemical structure of this material indicates that it would not be expected to react with skin proteins directly (Toxtree 2.6.6; OECD toolbox v3.3). There exist no predictive test on this chemical or any of its possible read across materials. Similarly, no confirmatory human studies are available on this material. However, in confirmatory human maximization tests read across material 2% (1380 μg/cm^2) benzonitrile (CAS# 100-47-0; See Section 5) did not result in sensitization reactions (RIFM, 1977). Acting conservatively, due to limited data on read across, the reported exposure was benchmarked utilizing the non-reactive Dermal Sensitization Threshold (DST) of 900 μg/cm^2. Utilizing 900 μg/cm^2 for p-methoxybenzonitrile, the application of the Quantitative Risk Assessment (QRA) described by Api et al. (2008) results in the acceptable exposure limits summarized in Table 1. The current 95 t h percentile dermal exposure is below the DST for non-reactive materials when evaluated in all QRA categories. Based on application of DST, p-methoxybenzonitrile does not present a concern for skin sensitization.

Table 1
Acceptable exposure limits for p-methoxybenzonitrile based on DST non-reactive −.

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

[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: 10/11/16.

10.1.5. Phototoxicity/photoallergenicity. Based on available UV/Vis spectra, p-methoxybenzonitrile 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 p-methoxybenzonitrile 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^{-} (Henry et al., 2009). Based on lack of significant absorbance in the critical range, p-methoxybenzonitrile does not present a concern for phototoxicity or photoallergenicity.

Additional References: None.

Literature Search and Risk Assessment Completed on: 06/29/16.

10.1.6. Local respiratory toxicity. The margin of exposure could not be calculated due to lack of appropriate data. The material, p-methoxybenzonitrile, exposure level 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 p-methoxybenzonitrile. Based on the Creme RIFM model, the inhalation exposure is 0.016 mg/day. This exposure is 29.4 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: 07/08/2016.

10.2. Environmental endpoint summary

10.2.1. Screening-level assessment

A screening level risk assessment of p-methoxybenzonitrile 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, p-methoxybenzonitrile 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 did not identify p-methoxybenzonitrile 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 EPISUITE ver.4.1).

10.2.2. Risk assessment

Based on current Volume of Use (2011), p-methoxybenzonitrile does not present a risk to the aquatic compartment in the screening level assessment.

Biodegradation: No data available.

Ecotoxicity: No data available.

Other available data: p-Methoxybenzonitrile 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).

	LC50 (Fish)	EC50 (Daphnia)	EC50 (Algae)	AF	PNEC	Chemical Class
RIFM Framework Screening Level **(Tier 1)**	385.5 mg/L			1,000,000	0.3855μg/L	

Endpoints used to calculate PNEC are underlined.

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

Exposure	Europe (EU)	North America (NA)
Log K_{ow} used	1.62	1.62
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 0.3855 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: 6/20/2016.

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.2017.07.004.

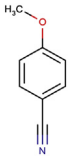
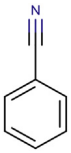
Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.fct.2017.07.004.

Appendix

Methods:

- The identified read-across analogs were confirmed by using expert judgment.
- Tanimoto structure similarity scores were calculated using ECFC 6 fingerprints (Rogers and Hahn, 2010).
- The physicochemical properties of target and analogs were calculated using EPI Suite™ v4.11 developed by US EPA (USEPA, 2012).
- 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 generated 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.2.1.7 and 2.1.6 respectively (Cassano et al., 2010).
- Protein binding was 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	p-Methoxybenzonitrile	Benzonitrile
CAS No.	874-90-8	100-47-0
Structure		
Similarity (Tanimoto score)		0.44578
Read across endpoint		• Genotoxicity, • Repeated dose, • Skin sensitization
Molecular Formula	C_8H_7NO	C_7H_5N
Molecular Weight	133.15	103.12
Melting Point (°C, EPISUITE)	27.05	−7.49
Boiling Point (°C, EPISUITE)	230.92	191.43
Vapor Pressure (Pa @ 25 °C, EPISUITE)	1.16	74.8
Log Kow (KOWWIN v1.68 in EPISUITE)	1.70	1.56
Water Solubility (mg/L, @ 25 °C, WSKOW v1.42 in EPISUITE)	1715	2893
J_{max} (mg/cm^2/h, SAM)	35.89652	101.9247
Henry's Law (Pa·m^3/mol, Bond Method, EPISUITE)	3.12E-001	5.27E+000
Genotoxicity		
	• No alert found	• No alert found

(*continued*)

	Target material	Read across material
DNA binding (OASIS v 1.1 QSAR Toolbox 3.4)		
DNA binding by OECD QSAR Toolbox (3.4)	• No alert found	• No alert found
Carcinogenicity (genotox and non-genotox) alerts (ISS)	• No alert found	• No alert found
DNA alerts for Ames, MN, CA by OASIS v 1.1	• No alert found	• No alert found
In-vitro Mutagenicity (Ames test) alerts by ISS	• No alert found	• No alert found
In-vivo mutagenicity (Micronucleus) alerts by ISS	• No alert found	• No alert found
Oncologic Classification	• Not classified	• Not classified
Repeated dose toxicity		
Repeated Dose (HESS)	• Not categorized	• Not categorized
Sensitization		
Protein binding by OASIS v1.1	• No alert found	• No alert found
Protein binding by OECD	• No alert found	• No alert found
Protein binding potency	• Not possible to classify according to these rules (GSH)	• Not possible to classify according to these rules (GSH)
Protein binding alerts for skin sensitization by OASIS v1.1	• No alert found	• No alert found
Skin Sensitization model (CAESAR) (version 2.1.6)	• Sensitizer (low reliability)	• Sensitizer (low reliability)
Metabolism		
OECD QSAR Toolbox (3.4)	See Supplemental Data 1	See Supplemental Data 2[1]
Rat liver S9 metabolism simulator		

1 Markus and Kwon, 2010

Summary

There are insufficient toxicity data on p-methoxybenzonitrile (CAS # 874-90-8). Hence *in-silico* evaluation was conducted by determining suitable read across analogues for this material. Based on structural similarity, reactivity, metabolism data, physicochemical properties and expert judgment, suitable analogue benzonitrile (CAS # 100-47-0) was identified as a proper read across material with data for its respective toxicity endpoints.

Conclusion/Rationale:

- Benzonitrile (CAS # 100-47-0) could be used as structurally similar read across analogue for the target material p-methoxybenzonitrile (CAS # 874-90-8) for the genotoxicity, repeated dose, and skin sensitization toxicological endpoints.
  - The target substance and the read across analogue are structurally similar and belong to the structural class of benzonitriles.
  - The key difference between the target substance and the read across analogue is that the target has a methoxy group on the para position and the read across does not have any substituent on its benzene ring. The differences in structure 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.
  - 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 benzonitrile fragment. The differences in the structure which are responsible for

Tanimoto score <1 are not relevent from a toxic endpoint perspective.

- The target substance and the read across analogue have similar physical chemical properties. Any differences in some of the physical chemical properties of the target substance and the read across analogue are estimated to be toxicologically insignificant for the genotoxicity, repeated dose, and skin sensitization endpoints.
- According to the QSAR OECD Toolbox (V3.4), structural alerts for the genotoxicity, repeated dose, and skin sensitization endpoints are consistent between the target substance and the read across analogue as seen in the table above.
- The target substance and the read across analogue are expected to be metabolized similarly as shown by the metabolism simulator. The target substance is predicted to metabolize to 4-cyanophenol through oxidative o-demethylation while the read across analog is predicted to metabolize to the same metabolite via aromatic C-hydroxylation with 0.9 intrinsic probability. Therefore the reactivity and toxicity of the target substance and the read across analog is expected to be comparable.
- The structural alerts for the genotoxicity, repeated dose, and skin sensitization endpoints are consistent between the metabolites of the read across analogue and the target substance.
- The structural differences between the target substance and the read across analogue are deemed to be toxicologically insignificant.

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