ACETONITRILE

(CAS #75-05-8)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: April 17, 2023

Expiration Date: April 17, 2028

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GreenScreen® Executive Summary for Acetonitrile (CAS #75-05-8)

Acetonitrile is a highly polar nitrile compound that is a clear, colorless flammable liquid at standard temperature and pressure. It functions as a solvent, chemical intermediate, catalyst, starting material in organic synthesis, and stabilizer, and is used in pharmaceutical manufacturing and in fatty acid extraction/separation. Based on its vapor pressure of 73.99 mm Hg and its boiling point of 81.65℃ it is a volatile organic compound (VOC).

Acetonitrile was assigned a **GreenScreen Benchmark™ Score of 2** ("Use but Search for Safer Substitutes"). This score is based on the following hazard score combinations:

- Benchmark 2e
  - Moderate Group I Human Toxicity (carcinogenicity-C, mutagenicity-M, and developmental toxicity-D)
- Benchmark 2g
  - High Flammability-F

Data gaps (DG) exist for endocrine activity-E and neurotoxicity (repeated dose)-Nr*. As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), acetonitrile meets requirements for a GreenScreen Benchmark™ Score of 2 despite the hazard data gaps. In a worst-case scenario, if acetonitrile were assigned a High score for the data gap E, it would be categorized as a Benchmark 1 Chemical.

New Approach Methodologies (NAMs) used in this GreenScreen® include *in silico* modeling for endocrine activity, respiratory sensitization, persistence, and bioaccumulation, and *in vitro* testing for mutagenicity and endocrine activity. The quality, utility, and accuracy of NAM predictions are greatly influenced by two primary types of uncertainties:

- Type I: Uncertainties related to the input data used
- Type II: Uncertainties related to extrapolations made

Type I (input data) uncertainties in acetonitrile's NAMs dataset include lack of sufficient experimental data for endocrine activity, and respiratory sensitization, using a genotoxicity assay that has been deleted from the OECD test guidelines, and lack of validated test methods for respiratory sensitization. Acetonitrile's Type II (extrapolation output) uncertainties include the lack of defined applicability domains in some modeling programs, limitation of *in vitro* genotoxicity assays in mimicking *in vivo* metabolism, their focusing on one or only a few types of genotoxicity events, unclear *in vivo* relevance of *in vitro* testing and *in silico* prediction of receptor binding due to lack of toxicokinetic considerations, and the limitation of OECD Toolbox in identifying structural alerts for respiratory sensitization without accounting for non-immunologic mechanisms of respiratory sensitization. Some of acetonitrile's type II uncertainties were alleviated by the use of *in vitro* test batteries and/or in combination of *in vivo* data.

GreenScreen® Hazard Summary Table for Acetonitrile

Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	M	R	D	E	AT	ST		N		SnS	SnR	IrS	IrE	AA	CA	P	B	Rx	F
						s	r*	s	r*	*	*								
M	*M*	**L**	*M*	DG	**H**	*M*	**M**	*M*	DG	**L**	*L*	**L**	**H**	**L**	**L**	**vL**	**vL**	*L*	**H**

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect lower confidence in the hazard classification while hazard levels in **BOLD** font reflect higher confidence in the hazard classification. Group II Human Health endpoints differ from Group II* Human Health endpoints in that they have four hazard scores (i.e., vH, H, M, and L) instead of three (i.e., H, M, and L), and are based on single exposures instead of repeated exposures. Group II* Human Health endpoints are indicated by an * after the name of the hazard endpoint or after "repeat" for repeated exposure sub-endpoints. Please see Appendix A for a glossary of hazard acronyms.

GreenScreen® Chemical Assessment for Acetonitrile (CAS #75-05-8)

Method Version: GreenScreen® Version 1.4
Assessment Type[1]: Certified
Assessor Type: Licensed GreenScreen® Profiler

GreenScreen® Assessment (v.1.4) Prepared By:
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Date: March 28, 2023

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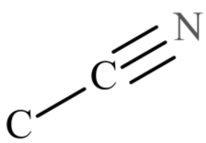
Organization: ToxServices LLC
Date: April 17, 2023

Expiration Date: April 17, 2028[2]

Chemical Name: Acetonitrile

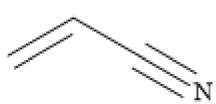
CAS Number: 75-05-8

Chemical Structure(s):



Also called: Cyanomethane; methyl cyanide; Ethanenitrile; Ethyl nitrile; Methanecarbonitrile; Methane, cyano-; Methylcyanide; Methylnitrile (PubChem 2023)

Suitable surrogates or moieties of chemicals used in this assessment (CAS #'s):
In order to fill the data gap for reproductive toxicity (oral route of exposure), data for acrylonitrile (CAS #107-13-1) were used. Structurally, acrylonitrile is a monounsaturated 3-carbon chain bonded to the nitrogen, whereas acetonitrile is a saturated two-carbon chain bonded to the nitrogen. The ECHA REACH dossier also used acrylonitrile as a surrogate for acetonitrile. The dossier states acetonitrile is a byproduct of the production of acrylonitrile. Additionally, acrylonitrile has a higher degree of systemic toxicity than acetonitrile, suggesting a lack of response in the two-generation study with acrylonitrile is indicative of low hazard for this endpoint for acetonitrile (ECHA 2023a). The maximum common substructure (MCS) Tanimoto coefficient for acetonitrile and acrylonitrile is 0.7500, indicating a high degree of structural similarity (ChemMine 2023).



Acrylonitrile (CAS #107-13-1)

[1] GreenScreen® reports are either "UNACCREDITED" (by unaccredited person), "AUTHORIZED" (by Authorized GreenScreen® Practitioner), or "CERTIFIED" (by Licensed GreenScreen® Profiler or equivalent).
[2] Assessments expire five years from the date of completion starting from January 1, 2019. An assessment expires three years from the date of completion if completed before January 1, 2019 (CPA 2018a).

Identify Applications/Functional Uses: (HSDB 2015)
1. Solvent
2. Chemical intermediate
3. Catalyst
4. Pharmaceutical manufacturing
5. Fatty acid extraction/separation
6. Starting material in organic synthesis
7. Stabilizer

Known Impurities[3]:
Common impurities include propionitrile and allyl alcohol (HSDB 2015). The screen is performed on the theoretical pure substance.

GreenScreen® Summary Rating for Acetonitrile[4,5 6,7]: Acetonitrile was assigned a **GreenScreen Benchmark™ Score of 2** ("Use but Search for Safer Substitutes") (CPA 2018b). This score is based on the following hazard score combinations:

- Benchmark 2e
    - Moderate Group I Human Toxicity (carcinogenicity-C, mutagenicity-M, and developmental toxicity-D)
- Benchmark 2g
    - High Flammability-F

Data gaps (DG) exist for endocrine activity-E and neurotoxicity (repeated dose)-Nr*. As outlined in GreenScreen® Guidance (CPA 2018b) Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), acetonitrile meets requirements for a GreenScreen Benchmark™ Score of 2 despite the hazard data gaps. In a worst-case scenario, if acetonitrile were assigned a High score for the data gap E, it would be categorized as a Benchmark 1 Chemical.

Figure 1: GreenScreen® Hazard Summary Table for Acetonitrile

Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	M	R	D	E	AT	ST		N		SnS	SnR	IrS	IrE	AA	CA	P	B	Rx	F
						s	r*	s	r*	*	*								
M	*M*	**L**	*M*	*DG*	**H**	*M*	**M**	*M*	*DG*	**L**	*L*	**L**	*H*	**L**	**L**	**vL**	**vL**	*L*	**H**

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect lower confidence in the hazard classification while hazard levels in **BOLD** font reflect higher confidence in the hazard classification. Group II Human Health endpoints differ from Group II* Human Health endpoints in that they have four hazard scores (i.e., vH, H, M, and L) instead of three (i.e., H, M, and L), and are based on single exposures instead of repeated exposures. Group II* Human Health endpoints are indicated by an * after the name of the hazard endpoint or after "repeat" for repeated exposure sub-endpoints. Please see Appendix A for a glossary of hazard acronyms.

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[3] Impurities of the chemical will be assessed at the product level instead of in this GreenScreen®.
[4] For inorganic chemicals with low human and ecotoxicity across all hazard endpoints and low bioaccumulation potential, persistence alone will not be deemed problematic. Inorganic chemicals that are only persistent will be evaluated under the criteria for Benchmark 4.
[5] See Appendix A for a glossary of hazard endpoint acronyms.
[6] For inorganic chemicals only, see GreenScreen® Guidance v1.4 Section 12 (Inorganic Chemical Assessment Procedure).
[7] For Systemic Toxicity and Neurotoxicity, repeated exposure data are preferred. Lack of single exposure data is not a Data Gap when repeated exposure data are available. In that case, lack of single exposure data may be represented as NA instead of DG. See GreenScreen® Guidance v1.4 Annex 2.

Environmental Transformation Products

Per GreenScreen® guidance (CPA 2018b), chemicals that degrade rapidly and completely (i.e., meet criteria for a Very Low for persistence) are not likely to form persistent biodegradation intermediates because the degradation intermediates will not persist long enough to be encountered after use or release of the parent chemical (i.e., relevant). As acetonitrile is readily biodegradable, it is not expected to have relevant transformation products.

Introduction

Acetonitrile is a highly polar nitrile compound that is a clear, colorless flammable liquid at standard temperature and pressure. It functions as a solvent, chemical intermediate, catalyst, starting material in organic synthesis, and stabilizer, and is used in pharmaceutical manufacturing and in fatty acid extraction/separation. It is prepared by the dehydration of acetamide with glacial acetic acid or by reacting acetic acid with ammonia in the presence of a dehydration catalyst (HSDB 2015).

ToxServices assessed acetonitrile against GreenScreen® Version 1.4 (CPA 2018b) following procedures outlined in ToxServices' SOPs (GreenScreen® Hazard Assessment) (ToxServices 2021).

U.S. EPA Safer Choice Program's Safer Chemical Ingredients List

The SCIL is a list of chemicals that meet the Safer Choice standard (U.S. EPA 2023a). It can be accessed at: http://www2.epa.gov/saferchoice/safer-ingredients. Chemicals on the SCIL have been assessed for compliance with the Safer Choice Standard and Criteria for Safer Chemical Ingredients (U.S. EPA 2015).

Acetonitrile is not currently present on the SCIL.

GreenScreen® List Translator Screening Results

The GreenScreen® List Translator identifies specific authoritative or screening lists that should be searched to identify GreenScreen Benchmark™ 1 chemicals (CPA 2018b). Pharos (Pharos 2023) is an online list-searching tool that is used to screen chemicals against all of the lists in the List Translator electronically. ToxServices also checks the U.S. Department of Transportation (U.S. DOT) lists (U.S. DOT 2008a,b),[8] which are not considered GreenScreen® Specified Lists but are additional information sources, in conjunction with the Pharos query. The output indicates benchmark or possible benchmark scores for each human health and environmental endpoint. The output for acetonitrile can be found in Appendix C.

- Acetonitrile is an LT-P1 chemical when screened using Pharos, and therefore a full GreenScreen® is required.
- Acetonitrile is listed on the U.S. DOT list as a Hazard Class 3 chemical, Packing Group II.
- Acetonitrile is on the following lists for multiple endpoints. Specified lists for single endpoints are reported in individual hazard endpoints in the hazard assessment section below.
    - Quebec CSST – WHMIS 1988 – Class D2B – Toxic material causing other toxic effects.
    - EC – CEPA DSL – Inherently Toxic to Humans (iTH).
    - German FEA – Substances Hazardous to Waters – Class 2 – Hazard to Waters.

[8] DOT lists are not required lists for GreenScreen® List Translator v1.4. They are reference lists only.

Hazard Statement and Occupational Control

Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard statements that are harmonized in the European Union (EU) as reported by ECHA for acetonitrile are reported in Table 1. General personal protective equipment (PPE) recommendations and occupational exposure limits (OELs) are presented in Table 2, below.

Table 1: GHS H Statements for Acetonitrile (CAS #75-05-8) (ECHA 2023b)

H Statement	H Statement Details
H225	Flammable liquid and vapor
H302*	Harmful if swallowed
H312*	Harmful in contact with skin
H319	Causes serious eye irritation
H332*	Harmful if inhaled

* Indicates that manufacturers or importers must apply at least this minimum classification, but must classify in a more severe hazard category in the even that further information is available which show that the hazard(s) meet the criteria for classification in the more severe category.

Table 2: Occupational Exposure Limits and Recommended Personal Protective Equipment for Acetonitrile (CAS #75-05-8)

Personal Protective Equipment (PPE)	Reference	Occupational Exposure Limits (OEL)	Reference
Half or full facepiece respirator, self-contained breathing apparatus, supplier air respirator; impervious gloves; safety goggles or face shield; impervious protective clothing; protective boots	TCI 2018	ACGIH TLV: 8h TWA: 20 ppm (skin)	HSDB 2015, NIOSH 2011, TCI 2018, OSHA 2023
		NIOSH REL: 10h TWA: 20 ppm (34 mg/m^3); IDLH: 500 ppm	HSDB 2015, OSHA 2023
		MAK: 10 ppm (17 mg/m^3)	NIOSH 2011
		EU-OEL: 40 ppm (70 mg/m^3) as TWA (skin)	NIOSH 2011
		OSHA PEL: 8h TWA: 40 ppm (70 mg/m^3)	TCI 2018
		Cal/OSHA PEL 8h TWA: 40 ppm (70 mg/m^3) STEL: 60 ppm (105 mg/m^3)	OSHA 2023

ACGIH: American Conference of Governmental Industrial Hygienists
IDLH: Immediately Dangerous to Life or Health
MAK: Maximum Workplace Concentration
NIOSH: National Institute for Occupational Safety and Health
OEL: Occupational Exposure Limit
OSHA: Occupational Safety and Health Administration
PEL: Permissible Exposure Limit
REL: Recommended Exposure Limits
STEL: Short-term Exposure Limit
TLV: Threshold Limit Value
TWA: Time Weighted Average

Physicochemical Properties of Acetonitrile

Acetonitrile is a clear colorless liquid at standard temperature and pressure. It is highly soluble in water, and based on its vapor pressure of 73.99 mm Hg at 20℃, it is volatile. Its log K_{ow} of -0.34 indicates it is unlikely to bioaccumulate.

Table 3: Physical and Chemical Properties of Acetonitrile (CAS #75-05-8)

Property	Value	Reference
Molecular formula	C_2H_3N	PubChem 2023
SMILES Notation	CC#N	PubChem 2023
Molecular weight	41.05 g/mol	PubChem 2023
Physical state	Liquid	ECHA 2023a
Appearance	Clear, colorless	ECHA 2023a
Melting point	-45.7ºC	ECHA 2023a
Boiling point	81.65ºC	ECHA 2023a
Vapor pressure	98.64 hPa (73.99 mmHg) at 20℃	ECHA 2023a
Water solubility	1,000,000 mg/L at 25ºC	ECHA 2023a
Dissociation constant	N/A	ECHA 2023a
Density/specific gravity	0.79 g/mL at 20℃	ECHA 2023a
Partition coefficient	Log K_{ow} = -0.34	ECHA 2023a

Toxicokinetics

Absorption

Acetonitrile is readily absorbed from the gastrointestinal tract and lungs. When male and female subjects (n=16) inhaled cigarette smoke and held it in the mouth for 2 seconds, 74% acetonitrile was absorbed when not inhaled while 91% acetonitrile was absorbed when the smoke was inhaled (U.S. EPA 1999). Beagle dogs exposed to 16,000 ppm acetonitrile vapor for 4 hours displayed rapid absorption with a steady state of blood cyanide concentration of 305-433 µg/100 mL reached at 3 hours into the 4 hour exposure period (U.S. EPA 1999, ECHA 2023a).

The dermal absorption of acetonitrile was measured in an *in vitro* assay similar to OECD Guideline 428 with human cadaver skin. For determination of a permeability coefficient, 1,200 µL/cm^2 [2-14C]acetonitrile was applied under occlusive conditions for approximately 8 hours. Only a small portion, approximately 0.24% had penetrated the skin and the permeability coefficient was calculated to be 1.82 x 10^{-4} cm/h. To determine the short-term absorption rate, 10 µL/cm^2 [2-14C]acetonitrile was applied to the cadaver skin for 10 and 60 minutes. Similarly, only a small portion, ≤0.74%, had penetrated the skin and the short-term penetration rates were calculated to be 375.6 and 66 µg equiv/cm^2/h for the 10 and 60 minute exposures, respectively (Klimisch 1 – Reliable without restriction) (Unnamed 2005 study report, ECHA 2023a).

Distribution

Whole body autoradiography of male mice injected intravenously with 684 µCi/kg [2-14C]acetonitrile indicated that radioactivity was widely distributed throughout the body with high levels in the liver, kidney, and gastrointestinal tissues (ECHA 2023a, U.S. EPA 1999). Hamsters exposed to a single dose of 100, 200, 300, and 400 mg/kg acetonitrile via gavage or intraperitoneal injection had measurable values of cyanide and thiocyanate in the blood, liver, brain and kidneys 2.5 hours after exposure, with levels of thiocyanate up to 10-fold higher in the blood, liver and kidneys compared to the brain (ECHA

2023a, U.S. EPA 1999). Following inhalation of acetonitrile vapor in rats, hydrocyanic acid was detected in the brain, heart, kidney, and spleen (U.S. EPA 1999).

An autopsy of a subject that died following inhalation of acetonitrile vapor indicated that it was distributed to the spleen lungs and kidneys as cyanide; however, cyanide was not detected in the liver (U.S. EPA 1999).

Metabolism

Acetonitrile is metabolized to inorganic cyanide via an oxygen- and NADPH-dependent conversion mediated by P450 isozyme. Studies in rats have shown the cytochrome-P450 reaction produces cyanohydrin which is then decomposed by a catalase enzyme to release cyanide (U.S. EPA 1999, ECHA 2023a). Cyanide is further oxidized to thiocyanate via a rhodanese, a type of sulfotransferase found in the liver and nasal respiratory mucosa (U.S. EPA 1999), with the nasal tissue being capable of metabolizing acetonitrile to cyanide at rates exceeding those of the liver (ECHA 2023a).

Formaldehyde and formic acid are also potential by-products of acetonitrile metabolism, as cyanide can be oxidized to the cyanate ion with further oxidation to formic acid (U.S. EPA 1999).

Studies performed in mouse hepatic microsomes incubated in contact with acetonitrile reported Km and Vmax values of 4.19 mM and 14.3 ng CN formed/15 min/mg, respectively, while rats Vmax and Km values with non-induced microsomes were 7.9 +/- 1.8 nmol cyanide/mg protein/20 min and 21.3 ± 4.2 mM, respectively (ECHA 2023a).

Excretion

Following ingestion, serum levels of acetonitrile were higher than serum levels of cyanide, with elimination half lives of 32 and 15 hours for acetonitrile and cyanide, respectively (U.S. EPA 1999). The major metabolite thiocyanate is excreted in the urine, with minor metabolites such as 2-aminothiolazine-4-carboxylic acid (U.S. EPA 1999).

Rats exposed to 166 or 300 ppm acetonitrile, 7 hours/day, 5 days/week, for 90 days had almost complete urinary excretion of thiocyanate after the 2.5-day rest period each week (U.S. EPA 1999). Monkeys given 0.1 mL/kg acetonitrile by a single intravenous injection excreted more than 12% of the dose as the urinary thiocyanate (ECHA 2023a).

When male and female Sprague-Dawley rats (3/sex) were administered 10 µCi radiolabeled acetonitrile in distilled water via gavage, radioactivity in expired air as carbon dioxide and volatiles was 24.1% and 47.8%, respectively, in males and 19.9% and 53.3%, respectively, in females. Radioactivity in the urine and feces was 6.25% and 0.59%, respectively, in males, and 7.21% and 0.41%, respectively, in females. The radioactivity found in tissues (liver, kidney, and composite fat) averaged 1.95% and 1.36% in males and females, respectively. Thus, recovery expressed as the total radioactivity eliminated, averaged 80.8% of dose for males and 82.3% of dose for females, and there were no apparent sex differences in elimination of 14C-acetonitrile by rats (ECHA 2023a).

Pharmacokinetics analysis of acetonitrile in tissues indicated that the distribution and excretion kinetics of the acetonitrile parent molecule in various tissues follows one compartment, first order kinetics. The half-life of elimination of [2-14C]acetonitrile from blood and most tissues ranged from 5.52 hours in the liver to 8.45 hours in the blood (ECHA 2023a).

Hazard Classification Summary

<u>Group I Human Health Effects (Group I Human)</u>

Carcinogenicity (C) Score (H, M, or L): *M*
Acetonitrile was assigned a score of Moderate for carcinogenicity based on equivocal evidence of carcinogenicity in male rats in a 2-year inhalation carcinogenicity study conducted by National Toxicology Program (NTP). GreenScreen® criteria classify chemicals as a Moderate hazard for carcinogenicity when there is limited or marginal evidence of carcinogenicity in animals (CPA 2018b). The confidence in the score is low as the inhalation studies produced equivocal results and there were no oral or dermal experimental data identified.

- Authoritative and Screening Lists
    - *Authoritative:* US EPA – IRIS Carcinogens – (1986) Group D – Not classifiable as to human carcinogenicity.
    - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a, NTP 1996
    - *Inhalation:* In an inhalation chronic toxicity/carcinogenicity study conducted by the NTP, male and female B6C3F1 mice (60/sex/concentration) were exposed to acetonitrile (>99% purity) via whole body inhalation at 0, 50, 100, and 200 ppm 6 hours/day, 5 days/week for 103 weeks. Ten animals/sex were evaluated at 15 months. Survival rate of treated animals was similar to controls and there were no adverse effects on body weights or organ weights. The incidence of squamous hyperplasia of the epithelium of the forestomach was significantly increased at 15 months in 200 ppm females. At 2 years, the increased incidence of this lesion was dose related in the 100 and 200 ppm male groups and in all exposed female groups. There were no increases in the incidences of neoplasms. NTP concluded there was no evidence of carcinogenic activity in male and female mice (Klimisch 1, Reliable without restriction) (Unnamed 1996 study, 001 Weight of evidence).
    - *Inhalation:* In a second inhalation chronic toxicity/carcinogenicity study conducted by the NTP, male and female Fisher 344 rats (56/sex/concentration) were exposed to acetonitrile (>99% purity) via whole body inhalation at 0, 100, 200, and 400 ppm 6 hours/day, 5 days/week for 103 weeks. Eight animals/sex were evaluated at 15 months. Survival rate of treated animals was similar to controls and there were no adverse effects on body weights or organ weights. There was a slight increase in the incidence of basophilic, eosinophilic, and mixed cell hepatic foci in 400 ppm males, and there was a statistically significant increase in basophilic foci in the 200 and 400 ppm groups, but the foci were not atypical in appearance. Thus, authors stated it is uncertain if these lesions were preneoplastic. The incidences of hepatocellular adenoma (3/48), hepatocellular carcinoma (3/48), and hepatocellular adenoma and carcinoma combined (5/48) were greater in male rats exposed to 400 ppm; however, the incidences of hepatocellular adenoma and hepatocellular carcinoma were within the range of historical controls. The incidence of hepatocellular adenoma or carcinoma combined slightly exceeded the range of historical controls (2-8%). There were no exposure-related liver lesions in treated female rats. No significant dose-related trend was present after incidences were adjusted for survival using the life table test. NTP concluded there was equivocal evidence of carcinogenic activity in male rats based on the marginally increased incidences of hepatocellular adenoma and carcinoma, and there was no evidence of carcinogenic activity in female rats (Klimisch 1, Reliable without restriction) (Unnamed 1996 study, 002 Weight of evidence).

Mutagenicity/Genotoxicity (M) Score (H, M, or L): *M*

Acetonitrile was assigned a score of Moderate for mutagenicity/genotoxicity based on ToxServices classifying acetonitrile as a GHS Category 2 mutagen due to positive results in clastogenicity assays and other genotoxicity assays. GreenScreen® criteria classify chemicals as a Moderate hazard for mutagenicity/genotoxicity when a GHS Category 2 classification for mutagenicity is warranted (CPA 2018b). The confidence in the score is low as *in vivo* results were mixed and positive effects were generally marginal.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a, NTP 1996
    - *In vitro:* Acetonitrile was negative for mutagenicity in a bacterial reverse mutation assay reported by the NTP. *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 were exposed to acetonitrile (>99% purity, vehicle not specified) at 0, 100, 333, 1,000, 3,333, and 10,000 µg/plate with and without metabolic activation (Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver). Positive controls were sodium azide, 9-aminoacridine, 4-nitro-o-henylenedimaine, and 2-aminoanthracene. There were no increases in the frequency of revertants in any strain at any dose with or without metabolic activation. Positive controls were valid (Klimisch 1, Reliable without restriction) (Mortelmans et al 1986, Unnamed 1996 study, 004 Key).
    - *In vitro:* Acetonitrile was equivocal for clastogenicity in a mammalian cell chromosome aberration assay reported by NTP. Chinese hamster ovary (CHO) cells were exposed to acetonitrile (purity and vehicle not reported) at concentrations of 500, 1,600, and 5,000 µg/mL with and without metabolic activation (Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix). Positive controls were triethylenemelamine and cyclophosphamide. In the presence of metabolic activation, there was a slight, non-significant, increase in aberrations at the highest dose; there were no increases in chromosome aberrations in the absence of metabolic activation. The authors concluded acetonitrile to be equivocal under the conditions of this assay. Positive and vehicle controls were valid (Klimisch 1, Reliable without restriction) (Galloway et al. 1985/1987, Unnamed 1996 study, 002 Key).
    - *In vitro:* Acetonitrile was weakly positive in a sister chromatid exchange (SCE) assay conducted according to EPA OPPTS 870.5900 (GLP not specified). CHO cells were exposed to acetonitrile (purity and vehicle not reported) at concentrations of 160, 500, 1,600, and 5,000 µg/mL with and without metabolic activation (Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix). Positive controls were triethylenemelamine and cyclophosphamide. A weak positive response (20% increase in SCE) occurred at 5,000 µg/mL in the absence of metabolic activation; there was no dose-response and no increase in SCE in the presence of metabolic activation. Positive and untreated negative controls were valid (Klimisch 1, Reliable without restriction) (Galloway et al. 1985/1987, Unnamed 1996 study, 003 Key)
    - *In vivo:* Acetonitrile produced ambiguous results for clastogenicity in a micronucleus assay conducted by the NTP. Male and female B6C3F1 mice (10/sex/dose, except 9 females at 400 ppm and 6 females at 800 ppm) were exposed to acetonitrile vapor (99% purity) at 0, 100, 200, 400, and 800 ppm 6 hours/day 5 days/week for 13 weeks. Peripheral blood samples were taken at the end of the exposure period. A positive control was not specified. There was a small statistically significant increase in micronucleated normochromatic erythrocytes in males exposed to 400 ppm; the trend-test was positive but there was no clear

dose-response. There were no increases in micronucleated normochromatic erythrocytes in females (Klimisch 1, Reliable without restriction) (Unnamed 1996 study, 002 Key).

- ECHA 2023a (note: only studies reported with a Klimisch 1 or 2 were included below)
    - *In vitro:* Acetonitrile was negative for mutagenicity in a bacterial reverse mutation assay conducted according to Japan Ministry of Labor guidelines (GLP not specified). *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 and *Escherichia coli* WP_2 *uvr*A and WP_2 *uvr*A/pKM101 were exposed to acetonitrile (purity not reported) in water at 0, 100, 250, 500, 1,000, 2,500, and 5,000 µg/plate with and without metabolic activation (PCB (KC-500)-induced male Sprague-Dawley rat liver). Positive controls were 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, N-ethyl-N-nitro-N-nitrosoguanidine, 9-aminoacridine, 2-nitrofluorene, 2-aminoanthracene, and benzo(a)pyrene. There were no increases in the frequency of revertants in any strain at any dose with or without metabolic activation. Validity of controls was not specified (Klimisch 1, Reliable without restriction) (Unnamed 1980 study, 007 Supporting).
    - *In vitro:* Acetonitrile was negative for mutagenicity in a bacterial reverse mutation assay (GLP and guideline not specified). *S. typhimurium* strains TA98 and TA100 were exposed to acetonitrile (purity and vehicle not reported) at 0.27, 1.35, 2.71, 13.6, 27.1, 136, 271, 1,350, and 2,710 mM with and without metabolic activation (phenobarbitone or autoinduced rat S9). Positive controls were daunomycin and NaN_3. There were no increases in the frequency of revertants in either strain at any dose with or without metabolic activation. Validity of controls was not specified (Klimisch 2, Reliable with restrictions) (Schlegelmilch et al. 1988, 008 Supporting).
    - *In vitro:* Acetonitrile was negative for mutagenicity in a GLP-compliant mammalian cell gene mutation (HGPRT locus) assay conducted according to US EPA TSCA 48 FR 50942 (similar to EPA OTS 798.5300). CHO cells were exposed to acetonitrile (99% purity, vehicle not specified) at 0.1-30 mg/mL without metabolic activation and 4-16 mg/mL with metabolic activation (Aroclor 1254-inducced male rat liver S9) in experiment 1 and 1-25 mg/mL without metabolic activation and 8-20 mg/mL with metabolic activation in experiment 2. Positive controls were ethylmethanesulphonate and benzo(a)pyrene. There were no toxicologically significant increases in the mutation frequency in either experiment with or without metabolic activation. Positive and vehicle controls were valid (Klimisch 1, Reliable without restriction) (Unnamed 1984 study, 001 Key).
    - *In vitro:* Acetonitrile was negative for mutagenicity in a GLP-compliant mammalian cell gene mutation ($TK^{+/-}$ locus) assay conducted similar to OECD Guideline 476. Mouse lymphoma L5178Y cells were exposed to acetonitrile (purity not reported) in water at 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, and 50 µL/mL with and without metabolic activation (S9 from rats) in the initial toxicity test and 5, 12, 18, and 24 µg/mL with and without metabolic activation in the definitive assay (24 µL/mL tested without metabolic activation only). Positive controls were 7,12-dimethylbenz(a)anthracene and ethylmethanesulphonate. There were no toxicologically significant increases in the mutation frequency with or without metabolic activation. Positive and vehicle controls were valid (Klimisch 1, Reliable without restriction) (Unnamed 1983/1984 study, 006 Key).
    - *In vitro:* Acetonitrile was positive for clastogenicity in a yeast cytogenic assay conducted according to Parry and Zemmermann 1976 (GLP not specified). *Saccharomyces cerevisiae* strain D61.M cells were exposed to acetonitrile (>99% purity, vehicle not reported) at concentrations of 0.05-4.76% (≤ 37.4 mg/mL) without metabolic activation. The use of positive controls was not stated. Acetonitrile induced chromosome loss at relatively high concentrations. Authors stated it is possible that microtubules formed during growth are

somehow weakened and function poorly during mitosis resulting in the observed chromosomal malsegregation (Klimisch 2, Reliable with restrictions) (Whittaker et al. 1989, Zimmermann et al 1985, 005 Key).

- *In vitro:* Acetonitrile produced ambiguous results in a gene mutation assay in fungi (GLP and guideline not specified). *S. cerevisiae* strain D7 cells were exposed to acetonitrile (purity and vehicle not reported) at concentrations of 360, 405, 450, 495, 540, 585, 630, 675, 720, and 768 mM with and without metabolic activation (phenobarbitone or autoinduced rat S9). Positive controls were not specified. There was no effect on mutation rate. A weak recombinogenic effect occurred at very high concentrations in the presence of metabolic activation. Authors stated this response is an indication of repair of DNA damage rather than persistent DNA damage (Klimisch 2, Reliable with restrictions) (Shlegelmilch et al 1988, 012 Supporting).
- *In vitro:* Acetonitrile was positive in an *in vitro* comet assay (GLP and guideline not specified). Peripheral human blood lymphocytes and HepG2 cells were exposed to acetonitrile (purity not reported) in 0.5% DMSO at concentrations of 50, 100, 250, and 500 µM without metabolic activation. The positive controls was hydrogen peroxide. There was an increase in comet tail intensity indicative of DNA damage in blood lymphocytes at all concentrations and in HepG2 cells at ≥ 100 mM. Positive and vehicle controls were valid (Klimisch 2, Reliable with restrictions) (Wu et al. 2009, 016 Weight of evidence)
- *In vitro:* Acetonitrile caused near complete inhibition of microtubule assembly in an *in vitro* mouse brain and drosophila assay (GLP and guideline not specified). Mouse brain and drosophila microtubules were exposed to acetonitrile (purity and vehicle not reported) at concentrations of 5 and 50 mM without metabolic activation. The use of positive controls was not stated. Acetonitrile caused near complete inhibition of microtubule assembly at both concentrations (Klimisch 2, Reliable with restrictions) (Sehgal et al. 1990, 013 Supporting).
- *In vitro:* Acetonitrile completely inhibited microtubule assembly in an *in vitro* porcine brain assay (GLP and guideline not specified) in which porcine brain microtubules were exposed to acetonitrile (purity and vehicle not reported) at 0.5% v/v. No further details were provided (Klimisch 2, Reliable with restrictions) (Groschel-Stewart et al. 1985, 014 Supporting).
- *In vivo:* Acetonitrile was negative for clastogenicity in a GLP-compliant micronucleus assay conducted according to OECD Guideline 474 and EU Method B.12. Male and female NMRI mice (5/sex/sampling interval) were administered a single intraperitoneal dose of acetonitrile (99.93% purity) in corn oil at 100 (males) or 125 (female) mg/kg/day. Blood marrow was sampled at 18, 24, and 36 hours and peripheral blood was sampled at 24, 48, 72, and 96 hours. The positive control was cyclophosphamide. There were no statistically or biologically significant increases in the incidence of micronucleated polychromatic erythrocytes in the bone marrow or peripheral blood cells at any of the sampling times. Positive and vehicle controls were valid (Klimisch 1, Reliable without restriction) (Jones 2001, unnamed 1998 study, 001 Key).
- *In vivo:* Acetonitrile was positive in an *in vivo* germ line aneuploidy test conducted according to Zimmering et al. 1990 (GLP not specified). Female *Drosophila melanogaster* were administered acetonitrile (purity not reported) in the feed at 2,000, 5,000, 20,000 or 50,000 ppm as early third instar larvae (LF3), late third instar (LF4), or young adults (AF3). Two genetic test systems (FIX and ZESTE) were used in this assay. Acetonitrile induced both chromosomal loss and gain in both assays. With FIX, acetonitrile was strongly positive only after feeding to third instar larvae (LF3); with ZESTE, the chemical induced

aneuploidy after adult feeding (AF) with no discernable effect after LF3 (Klimisch 2, Reliable with restrictions) (Osgood et al. 1991, Zimmering et al. 1986, 003 Supporting).
    - *In vivo:* Acetonitrile was positive in an *in vivo* germ line aneuploidy test conducted according to Osgood et al. 1990 (GLP not specified). Female *D. melanogaster* were administered acetonitrile (purity not reported) via inhalation at 131 ppm for 0 – 70 minutes. One genetic test system (ZESTE) was used in this assay. Acetonitrile induced both chromosomal loss and gain following brief exposure (Klimisch 2, Reliable with restrictions) (Osgood et al. 1991, 004 Supporting).
    - *In vivo:* Acetonitrile did not inhibit mitochondrial respiration in an *in vivo* germ line aneuploidy test conducted according to Osgood et al. 1991 (GLP not specified). Male and female *D. melanogaster* were administered acetonitrile (purity not reported) via inhalation at 131 ppm for 30 minutes for 2 days. Based on the results of this assay, authors suggested the induction of aneuploidy by acetonitrile may not be attributable solely to cyanide release (Klimisch 2, Reliable with restrictions) (Osgood et al. 1998, 005 Supporting).
    - *In vivo:* Acetonitrile produced ambiguous results for clastogenicity in a micronucleus assay conducted by the NTP. Male and female NMRI mice (4/sex/dose) were administered a single intraperitoneal injection of acetonitrile (purity not reported) at 40% and 60% of the LD_{50}, and sacrificed at 24, 48 and 22 hours after injection. A positive control was not specified. A weakly positive response was detected 24 hours after injection of 60% of the LD_{50} (Klimisch 2, Reliable with restrictions) (Schlegelmilch et al 1988, 006 Supporting).
- Based on the weight of evidence, a score of Moderate was assigned. Multiple genotoxicity assays were identified for acetonitrile with mixed results. Acetonitrile does not induce gene mutation based on negative results in three bacterial reverse mutation assays and two mammalian cell gene mutation assays. Although ambiguous results were noted in a gene mutation assay in fungi, authors stated the weak recombinogenic effect at high concentrations was likely an indication of repair of DNA damage rather than persistent DNA damage. Acetonitrile produced equivocal results in an *in vitro* chromosome aberration assay and positive results in yeast cytogenic assay. Positive results were also reported in an *in vitro* SCE assay and *in vitro* comet assay, and acetonitrile inhibited microtubule assembly in *in vitro* assays with mouse brain, drosophila, and porcine brain. Acetonitrile was negative in one *in vivo* micronucleus assay but produced ambiguous results in two other *in vivo* micronucleus assays. Finally, acetonitrile was positive in two *in vivo* germ line aneuploidy tests in *Drosophila melanogaster* flies, but did not inhibit mitochondrial respiration in another. Based on the positive results in clastogenicity assays and other genotoxicity assays, ToxServices classified acetonitrile as a GHS Category 2 mutagen.

Reproductive Toxicity (R) Score (H, M, or L): L

Acetonitrile was assigned a score of Low for reproductive toxicity based on ToxServices not classifying it as a reproductive toxicant under GHS based on a lack of significant reproductive effects in an OECD Guideline 422 screening study in rats exposed at up to 1,200 ppm acetonitrile, and in an OECD Guideline 416 two-generation reproductive toxicity with the surrogate acrylonitrile in rats exposed to concentrations up to 90 ppm. GreenScreen® criteria classify chemicals as a Low hazard for reproductive toxicity when adequate negative data are available and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data for the target substance and a conservative surrogate.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.

- ECHA 2023a
    - *Inhalation:* In a GLP-compliant OECD Guideline 422 combined repeated dose toxicity study with the reproduction/developmental toxicity screening test, male and female Sprague-Dawley rats (10/sex/dose) were exposed to acetonitrile vapor (100% purity) via whole body exposure at concentrations of 0, 150, 300, 600, and 1,200 ppm 6 hours/day. Males were exposed for 42 days (from 2 weeks prior to mating until 2 weeks after the end of the mating period) and females were exposed for 35-41 days (from two weeks prior to mating until day 19 of pregnancy). Three high concentration females had prolonged estrous stages. Fertility was slightly but non-significantly reduced at the high concentration. The authors identified a NOEC of 600 ppm for this study (Klimisch 1, Reliable without restriction) (Unnamed 2000 study, 001 Key).
        - Based on the molecular weight of 41.05 g/mol, the NOEC of 600 ppm is equivalent to 600 ppm * 41.05 / 24,450 = 1.0 mg/L.
    - *Inhalation:* In an inhalation study conducted by the NTP, male and female B6C3F1 mice (10/sex/dose) were exposed to acetonitrile vapor (>99.6% purity) via whole body inhalation at concentrations of 0, 100, 200, and 400 ppm 6 hours/day, 5 days/week for 13 weeks. Males were examined for sperm motility, sperm drive range, sperm count, and sperm morphology. The right caudal epididymis and testis were weighed. There were no effects on any of the parameters evaluated at any of the concentrations tested (Klimisch 2, Reliable with restrictions) (Morrisey et al. 1988, Unnamed 1983 study, 004 & 005 Supporting).
        - Based on the molecular weight of 41.05 g/mol, the high dose of 400 ppm is equivalent to 400 ppm * 41.05 / 24,450 = 0.67 mg/L; adjusting for less than daily exposure 0.67 mg/L * 5 days / 7 days = 0.48 mg/L.
    - *Inhalation: <u>Surrogate: Acrylonitrile (CAS #107-13-1)</u>*: In a GLP-compliant OECD Guideline 416 two-generation reproduction toxicity study, male and female Sprague-Dawley rats (25/sex/dose) were exposed to acrylonitrile (99.94-99.98% purity) via whole body inhalation at concentrations of 0, 5, 15, 45, and 90 ppm 6 hours/day, 7 days/week for 10 weeks through F2 weanlings in postnatal day 28. Exposure to F1 animals at 90 ppm was terminated due to excessive systemic toxicity in males. In the F0 generation, body weight and food consumption was reduced at ≥45 ppm, and relative liver weight was increased without gross pathological changes at the high dose. There was a slight reduction in sperm motility in the F0 males at 90 ppm; however, there was no corresponding impact on spermatogenesis or other fertility parameters. There were no effects on reproductive parameters in F1 animals. The authors identified a reproductive NOAEC of 90 ppm based on a lack of adverse reproductive effects (Klimisch 1, Reliable without restriction) (Unnamed 2008 publication, 002 Key & 003 Key).
        - Based on the molecular weight of 53.05 g/mol, the NOAEC of 90 ppm is equivalent to 90 ppm * 53.06 / 24,450 = 0.19 mg/L.
- Based on the weight of evidence, a score of Low was assigned. ToxServices did not classify acetonitrile as a reproductive toxicant under GHS. Although an OECD Guideline 422 screening study in rats with acetonitrile reported prolonged estrous cycle and slight decrease in fertility at the high concentration of 1,200 ppm, these effects were non-significant. An OECD Guideline 416 two-generation reproductive toxicity with the surrogate acrylonitrile reported no adverse effects on reproduction in rats exposed to concentrations up to 90 ppm. Therefore, ToxServices did not classify acetonitrile as a reproductive toxicant.

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): *M*

Acetonitrile was assigned a score of Moderate for developmental toxicity based on equivocal evidence of adverse effects to fetal development in the presence of maternal toxicity in oral and inhalation studies and its listing as an MAK Pregnancy Risk Group C. GreenScreen® criteria classify chemicals as a Moderate hazard for developmental toxicity when there is limited or marginal evidence of developmental toxicity in animal studied (CPA 2018b). The confidence in the score is low as adverse developmental effects occurred in the presence of maternal toxicity and it is not all apparent that these effects are secondary to maternal toxicity.

- Authoritative and Screening Lists
  - *Authoritative:* MAK – Pregnancy Risk Group C.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a
  - *Oral:* In a GLP-compliant prenatal developmental toxicity study according to US EPA TSCA 48 FR 50942 (similar to EPA OPPTS 870.3700) pregnant female New Zealand white rabbits (25/dose) were administered acetonitrile (99% purity) in deionized water at 0, 2, 15, and 30 mg/kg/day via gavage on gestation days (GD) 6-18. At the high dose, there were 5 deaths and 2 abortions, and weight gain was reduced during treatment. There was a decrease in average number of live fetuses per litter and a slight (non-significant) increase in resorptions at the high dose. There were no other adverse effects on developmental parameters. Authors identified a maternal toxicity and developmental toxicity NOAEL of 15 mg/kg/day and concluded developmental effects are likely secondary to maternal toxicity (Klimisch 1, Reliable without restriction) (Unnamed 1984 study, Unnamed 2002 company data, 001 Key).
  - *Oral:* In a GLP-compliant prenatal developmental toxicity study comparable to OECD Guideline 414, pregnant female Sprague-Dawley rats (25/dose) were administered acetonitrile (>94% purity) in water at 0, 125, 190, and 275 mg/kg/day via gavage on GD 6-19. At the high dose, four animals appeared emaciated and two others died and animals had reduced weight gain. Also at the high dose, there was an increase in post-implantation loss due to early resorptions that corresponded to a decrease in the mean number of fetuses. There was also a slightly increased incidence of unossified sternebrae. There were no other adverse effects on developmental parameters. Authors identified a maternal toxicity and developmental toxicity NOAEL of 190 mg/kg/day and concluded developmental effects are likely secondary to maternal toxicity (Klimisch 2, Reliable with restrictions) (Johannsen et al. 1986, Unnamed 1981 study, 003 Key).
  - *Oral:* In a prenatal developmental toxicity study according to a modified Chernoff and Kavlock design (1982) (GLP status not specified), pregnant female Long-Evans rats (20-22/dose) were administered acetonitrile (>99% purity) in tricaprylin oil at 0, 50, 150, 300, and 500 mg/kg/day via gavage on GD 7-21. Offspring were observed through day 41-42 postpartum. Maternal deaths occurred at the top two dose levels but there were no adverse developmental effects. The authors identified a maternal toxicity NOAEL of 150 mg/kg/day and a developmental toxicity NOAEL of 500 mg/kg/day for this study (Klimisch 2, Reliable with restrictions) (Smith 1987, 005 Supporting).
  - *Oral:* In a prenatal developmental toxicity study (GLP not specified, no guideline followed), pregnant Syrian hamsters (6/dose except for the high dose which had 12 animals) were administered a single dose of acetonitrile (purity not reported) in water at 0, 100, 200, 300, and 400 mg/kg via gavage on GD 8. An additional 12 animals were administered 400 mg/kg acetonitrile plus an intraperitoneal injection of 300 mg/kg sodium thiosulfate prior to exposure and at 2 hour intervals for 10 hours. Animals were sacrificed on GD 15. At 300

and 400 mg/kg 1/6 and 4/12 maternal animals died, and high dose dams displayed overt signs of toxicity. Weight gain was reduced in the 100-300 mg/kg groups but not at 400 mg/kg. Resorptions were significantly increased in the 200 and 400 mg/kg groups. The incidence of malformations was significantly increased at ≥300 mg/kg and grossly malformed fetuses were recovered from dams in these dose groups that did not show overt signs of toxicity. Skeletal malformations included rib fusions and severe axial skeleton dysrapic disorders. Mean fetal body weight was significantly decreased in all treatment groups. Administration of sodium thiosulfate completely blocked acetonitrile poisoning and mortality, and reduced the number of malformed offspring and resorption sites. The REACH dossier stated NOAELs cannot be clearly established based on the findings of this study (Klimisch 2, Reliable with restrictions) (Willhite 1983, 007 Supporting).

- *Inhalation:* In the previously described GLP-compliant OECD Guideline 422 combined repeated dose toxicity study with the reproduction/developmental toxicity screening test, male and female Sprague-Dawley rats (10/sex/dose) were exposed to acetonitrile vapor (100% purity) via whole body exposure at concentrations of 0, 150, 300, 600, and 1,200 ppm 6 hours/day. Males were exposed for 42 days (from 2 weeks prior to mating until 2 weeks after the end of the mating period) and females were exposed for 35-41 days (from two weeks prior to mating until day 19 of pregnancy). There were no treatment-related effects on clinical signs, mortality, body weight and weight gain, or gross pathology of the offspring. The authors identified a NOAEC of 1,200 ppm, for this study based on a lack of developmental effects at the highest concentration tested (Klimisch 1, Reliable without restriction) (Unnamed 2000 study, 001 Key).
  - Based on the molecular weight of 41.05 g/mol, the NOEC of 1,200 ppm is equivalent to 1,200 ppm * 41.05 / 24,450 = 2.0 mg/L.
- *Inhalation:* In a prenatal developmental toxicity study conducted by the NTP, female Sprague-Dawley rats (10 pregnant females, 10 non-pregnant females, and 33 positively mated females per concentration) were exposed to acetonitrile vapor (purity not reported) via whole body inhalation at 0, 100, 400, and 1,200 ppm 6 hours/day on GD 6-19. Mortality occurred in the mid and high concentration groups (3 and 1 animals total, respectively), and high concentration dams displayed hypoactivity, emaciation, dyspnea, abnormal posture, and bloody vaginal discharge. There were no treatment related effects on body weight or reproduction indices. Treatment had no effects on number of implants, litter size, resorptions, and fetal weight or sex ratio, and there were no increases in fetal malformations or variations. The authors identified a maternal toxicity NOAEC of 100 ppm (reported to be 160 mg/m^3) based on mortality, and a developmental toxicity NOAEC of 1,200 ppm (reported to be 2,015 mg/m^3), the highest concentration studied, due to the lack of adverse effects (Klimisch 1, Reliable without restriction) (Unnamed 1994 NTP abstract, 002 Key).
- *Inhalation:* In a prenatal developmental toxicity study comparable to OECD Guideline 414 (GLP status not specified) female Sprague-Dawley rats (20/group) were exposed to acetonitrile (>99% purity) via whole body inhalation at concentrations of 0, 900, 1,200, 1,500 and 1,800 ppm 6 hours/day on GD 6-20. Maternal mortality occurred at the high concentration (“a number” of deaths occurred; specific value not reported) and body weight gain was reduced at the top two concentrations. At the high concentration, mean resorptions were increased and there was an increase in the mean percentage of non-surviving implants and early embryonic resorptions associated with a non-significant decrease in the mean number of live fetuses per letter. One litter from the high concentration group was completely resorbed. There were no other adverse developmental effects. The authors identified a maternal toxicity NOAEC of 1,200 ppm (reported to be 2,015 mg/m^3), based on mortality and reduced maternal body weight, and a developmental toxicity NOAEC of 1,500

ppm (reported to be 2,518 mg/m^3), based on increased resorptions, non-surviving implants, and early embryonic resorptions associated with a decrease in live fetuses per litter (Klimisch 2, Reliable with restrictions) (Sailenfait et al. 1993, 004 Supporting).

- *Inhalation:* In a prenatal developmental toxicity study (GLP not specified, no guideline followed), pregnant Syrian hamsters (6/group except for the high concentration which had 12 animals) were exposed to acetonitrile (>99% purity) via whole body inhalation at 0, 1,800, 3,800, 5,000 and 8,000 ppm for 60 minutes on GD 8. An additional 9 animals were exposed to 8,000 ppm acetonitrile plus an intraperitoneal injection of 300 mg/kg sodium thiosulfate prior to exposure and at 2 hour intervals for 10 hours. Animals were sacrificed on GD 14. At 3,800, 5,000 and 8,000 ppm 1/6, 1/6, and 3/12 maternal animals died, respectively, displayed clinical signs of dyspnea, tremors, hypersalivation, ataxia, and hypothermia. Resorptions were significantly increased at 5,000 ppm. Fetal abnormalities, including exencephaly, encephalocele, and rib fusions, occurred at ≥5,000 ppm. Severe axial skeleton dysrapic disorders and significantly reduced fetal body wights occurred at 8,000 ppm. Administration of sodium thiosulfate completely blocked acetonitrile poisoning and mortality, and reduced the number of malformed offspring. The authors established a maternal toxicity NOAEC of 1,800 ppm (reported to be 3,022 mg/m^3), based on maternal mortality and clinical signs of toxicity, and a developmental toxicity NOAEC of 3,800 ppm (reported to be 6,380 mg/m^3), based on increased resorptions, fetal abnormalities, skeletal disorders, and reduced fetal body weight, for this study (Klimisch 2, Reliable with restrictions) (Willhite 1983, 006 Supporting).
- *Inhalation: Surrogate: Acrylonitrile (CAS #107-13-1)*: In the previously described GLP-compliant OECD Guideline 416 two-generation reproduction toxicity study, male and female Sprague-Dawley rats (25/sex/dose) were exposed to acrylonitrile (99.94-99.98% purity) via whole body inhalation at concentrations of 0, 5, 15, 45, and 90 ppm 6 hours/day, 7 days/week for 10 weeks through F2 weanlings in postnatal day 28. Exposure to F1 animals at 90 ppm was terminated due to excessive systemic toxicity in males. In the F1 generation, there were no treatment-related effects on the number of pups, live litter sizes, sex ratios, postnatal survival, clinical signs (other than irritation), or gross pathology. There were “weight decrements” in the first 3-4 weeks of direct exposure after weaning at 90 ppm, but the authors did not attribute this effect to treatment (details not provided). There were also “slight” delays in balanopreputial separation and vaginal patency and reduced body weights on the day of acquisition in males at 45 ppm and 90 ppm and females at 90 ppm, which the authors also stated was unrelated to exposure because the effects were not seen in the F2 offspring. There were no developmental effects noted in the F2 generation. The authors identified a NOAEC of 90 ppm for the F1 generation and 45 ppm for the F2 generation based on a lack of adverse effects at the highest parental doses tested (Klimisch 1, Reliable without restriction) (Unnamed 2008 publication, 002 Key & 003 Key).
  - Based on the molecular weight of 53.05 g/mol, the NOAEC of 90 ppm is equivalent to 90 ppm * 53.06 / 24,450 = 0.19 mg/L.

- Based on the weight of evidence, a score of Moderate was assigned. One oral study and one inhalation study, both in rats, reported no adverse developmental effects at oral doses up to 500 mg/kg/day and inhalation concentrations up to 1,200 ppm (2,015 mg/m^3 or 2.015 mg/L). However, slight increases in resorptions and decreased numbers of live fetuses per litter were reported in the presence of maternal toxicity in oral studies in rabbits, rats, and hamsters. Rats and hamsters also had slight increases in variations and malformations. Authors attributed the developmental effects in rabbits and rats to maternal toxicity, and maternal toxicity and developmental toxicity oral NOAELs of 15 mg/kg/day and 190 mg/kg/day were reported in rabbits and rats, respectively. An inhalation study in rats reported an increase in the number of resorptions and mean percentage of non-surviving implants associated with a decrease in mean number of live fetuses per litter in the presence of maternal toxicity. The maternal toxicity inhalation NOAEC was 1,200 ppm (2,015 mg/m^3 or 2.015 mg/L) and the developmental toxicity NOAEC was 1,500 ppm (2,518 mg/m^3 or 2.518 mg/L). A study in hamsters also identified increased resorption and fetal abnormalities as well as reduced fetal body weights in the presence of maternal toxicity. A maternal toxicity inhalation NOAEC of 1,800 ppm (3,022 mg/m^3 or 3.022 mg/L) and a developmental toxicity NOAEC of 3,800 ppm (6,380 mg/m^3 or 6.380 mg/L) was reported for this study. Effects secondary to maternal toxicity are not classified under GHS. However, it is not entirely clear if all the observed developmental effects in all species are secondary to maternal toxicity.

Endocrine Activity (E) Score (H, M, or L): DG

Acetonitrile was assigned a score of Data Gap for endocrine activity based on insufficient data identified for this endpoint.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- U.S. EPA 2023b
  - Acetonitrile was inactive in 12/12 estrogen receptor (ER) assays (gene symbols: ESR1 – estrogen receptor α; ESR2 – estrogen receptor β and ESRRA – estrogen related receptor α), 7/7 androgen receptor (AR) assays (gene symbol: AR – androgen receptor), 2/2 steroidogenesis assays (i.e. assay names contain “aromatase”), and 7/7 thyroid receptor assays (gene symbols: TRHR – thyrotropin releasing hormone receptor; THRA – thyroid hormone receptor α; THRB – thyroid hormone receptor β; TSHR – thyroid stimulating hormone receptor) performed as part of the U.S. EPA’s Endocrine Disruptor Screening Program (EDSP) in the 21st Century (i.e., Tox21).
  - Acetonitrile was predicted to be inactive for estrogen receptor agonism, antagonism and binding in the CERAPP Potency Level (From literature) model in ToxCast.
- ECHA 2023a
  - *Inhalation: <u>Surrogate: Acrylonitrile (CAS #107-13-1)</u>*: In a GLP-compliant OECD Guideline 416 two-generation reproduction toxicity study, male and female Sprague-Dawley rats (25/sex/dose) were exposed to acrylonitrile (99.94-99.98% purity) via whole body inhalation at concentrations of 0, 5, 15, 45, and 90 ppm 6 hours/day, 7 days/week for 10 weeks through F2 weanlings in postnatal day 28. Exposure to F1 animals at 90 ppm was terminated due to excessive systemic toxicity in males. There were no effects on estrous cycle or sperm measures in parental (F1) animals. There were “slight” delays in balanopreputial separation and vaginal patency and reduced body weights on the day of acquisition in males at 45 ppm and 90 ppm and females at 90 ppm, and a slight increase in anogenital distance (AGD) in males at 45 ppm and 90 ppm which the authors also stated was unrelated to exposure because the effects were not seen in the F2 offspring. There were no effects on sexual maturation in this generation. There were no effects on sexual maturation, AGD, or nipple retention in male pups noted in the F2 generation (Klimisch 1, Reliable without restriction) (Unnamed 2008 publication, 002 Key & 003 Key).
- VEGA 2021
  - ToxServices predicted the endocrine potential of acetonitrile using the following three VEGA v1.1.5 models: Estrogen Receptor Relative Binding Affinity Model (IRFMN) v1.0.1, Estrogen Receptor-mediated effect (IRFMN/CERAPP) v1.0.0, and Androgen Receptor-mediated effect (IRFMN/COMPARA) v1.0.0.

  - Acetonitrile is predicted to be inactive for estrogen receptor relative binding in the IRFMN affinity model with low reliability (Global AD Index = 0, similarity index = 0.628, accuracy index = 1, concordance index = 1); therefore, the prediction is not reliable (Appendix D).
  - Acetonitrile was not predicted for estrogen receptor-mediated effects in the IRFMN/CERAPP model (Appendix D).
  - Acetonitrile is predicted to be non-active for androgen receptor-mediated effect in the IRFMN/COMPARA model with high reliability (Global AD Index = 0.925, similarity index = 0.856, accuracy index = 1, concordance index = 1); therefore, the prediction is reliable (Appendix D).
- DTU 2023
  - Danish (Q)SAR Database reports acetonitrile is predicted to be negative for estrogen receptor activation, CERAPP data (*in vitro*) in one model (Leadscope). The remaining predictions are outside their applicability domains, considered unreliable, and not included in the weight of evidence (Appendix E).
- Based on the weight of evidence, a Data Gap was assigned. The weight of evidence from modeling on estrogen and androgen receptors binding suggests acetonitrile is not likely to be endocrine active. However, these results are insufficient to assign a Low score.

<u>**Group II and II* Human Health Effects (Group II and II* Human)**</u>
Note: Group II and Group II* endpoints are distinguished in the v 1.4 Benchmark system (the asterisk indicates repeated exposure). For Systemic Toxicity and Neurotoxicity, Group II and II* are considered sub-endpoints. See GreenScreen® Guidance v1.4, Annex 2 for more details.

Acute Mammalian Toxicity (AT) (Group II) Score (vH, H, M, or L): H
Acetonitrile was assigned a score of High for acute toxicity based on ToxServices classifying it as a GHS Category 3 acute oral, dermal, and inhalation toxicant based on an oral LD_{50} of 139 mg/kg in male rats, a dermal LD_{50} value of 980 mg/kg in rabbits, and a 4-hour vapor LC_{50} of 6.022 mg/L in mice. GreenScreen® criteria classify chemicals as a High hazard for acute toxicity when the oral LD_{50} is >50-300 mg/kg, the dermal LD_{50} is >200-1,000 mg/kg and/or the inhalation LC_{50} is >2-10 mg/L, and a GHS Category 3 classification is warranted (CPA 2018b). The confidence in the score is high as it is based on measured values for the target substance.

- Authoritative and Screening Lists
  - *Authoritative:* EU – GHS (H-Statements) Annex 6 Table 3-1 – H302 – Harmful if swallowed [Acute toxicity (oral) – Category 4].
  - *Authoritative:* EU – GHS (H-Statements) Annex 6 Table 3-1 – H312 – Harmful in contact with skin [Acute toxicity (dermal) – Category 4].
  - *Authoritative:* EU – GHS (H-Statements) Annex 6 Table 3-1 – H332 – Harmful if inhaled [Acute toxicity (inhalation) – Category 4].
  - *Screening:* GHS – New Zealand – Acute oral toxicity category 2.
  - *Screening:* GHS – Australia – H302 – harmful if swallowed [Acute toxicity (oral) – Category 4].
  - *Screening:* GHS – Malaysia – H302 – harmful if swallowed [Acute toxicity (oral) – Category 4].
  - *Screening:* GHS – New Zealand – Acute dermal toxicity category 3.
  - *Screening:* GHS – Japan – H311 – Toxic in contact with skin [Acute toxicity (dermal) – Category 3].

    - *Screening:* GHS – Australia – H312 – harmful in contact with skin [Acute toxicity (dermal) – Category 4].
    - *Screening:* GHS – Malaysia – H312 – harmful in contact with skin [Acute toxicity (dermal) – Category 4].
    - *Screening:* GHS – New Zealand – Acute inhalation toxicity category 3.
    - *Screening:* GHS – Australia – H332 – harmful if inhaled [Acute toxicity (inhalation) – Category 4].
    - *Screening:* GHS – Malaysia – H332 – harmful if inhaled [Acute toxicity (inhalation) – Category 4].
    - *Screening:* GHS – Japan – H332 – harmful if inhaled [Acute toxicity (inhalation) – Category 4].
    - *Screening:* Quebec CSST – WHMIS 1988 – Class D1A – Very toxic material causing immediate and serious toxic effects.
- ECHA 2023a
    - *Oral:* LD_{50} (male and female Crl:CD-1 (ICR) BR mice) = 617 mg/kg (469 mg/kg in males and 765 mg/kg in females) (GLP, similar to OECD Guideline 401) (Klimisch 1 – Reliable without restriction) (Unnamed 1998 study, 001 Key).
    - *Oral:* LD_{50} (male and female Wistar or Nelson rats) >1.68 - <4.49 mL/kg (males, reported to be >124 - <821 mg/kg) and >4.49-<8.53 mL/kg (females, reported to be >246-<1,190 mg/kg) (non-GLP, non-guideline) (Klimisch 2 – Reliable with restrictions) (Unnamed 1959 publication, 003 Supporting).
    - *Oral:* LD_{50} (male Sprague-Dawley rats) = 3,081 mg/kg (non-GLP, non-guideline) (Klimisch 2 – Reliable with restrictions) (Kimura et al. 1971, 004 Supporting).
    - *Oral:* LD_{50} (male guinea pigs) = 0.177 mL/kg (reported to be 139 mg/kg) (non-GLP, non-guideline) (Klimisch 2 – Reliable with restrictions) (Unnamed 1959 publication, 005 Supporting).
    - *Oral:* LD_{50} (Sherman rats, sex not specified) = 3,800 mg/kg (non-GLP, non-guideline) (Klimisch 2 – Reliable with restrictions) (Smyth and Carpenter 1948, 006 Supporting).
    - *Oral:* LD_{50} (male ddY mice) = 6.55 mmol/kg (reported to be 269 mg/kg) (non-GLP, non-guideline) (Klimisch 2 – Reliable with restrictions) (Tanii and Hashimoto 1984, 008 Supporting).
    - *Dermal:* LD_{50} (male and female New Zealand white rabbits) >2,000 mg/kg (GLP, similar to OECD Guideline 402) (Klimisch 1 – Reliable without restriction) (Unnamed 1997 study, 001 Key).
    - *Inhalation:* 4-hour vapor LC_{50} (male and female Crl: CD-1® (ICR) BR mice) = 3,587 ppm (reported to be 6,022 mg/m^3 or 6.022 mg/L) (GLP, similar to OECD Guideline 403) (Klimisch 1 – Reliable without restriction) (Unnamed 1998 study, 001 Key).
        - *Due to the large availability of acute inhalation toxicity studies in the ECHA REACH dossier for acetonitrile and the harmonized classification in the EU, only the Key study for the inhalation route of exposure was included.*
- ECHA 2023a, PubChem 2023
    - *Dermal:* LD_{50} (male rabbits, strain not specified) = 1.25 mL/kg (reported to be 980 mg/kg) for undiluted acetonitrile and 0.5 mL/kg (reported to be 392 mg/kg) for a 75% solution of acetonitrile in water (non-GLP, non-guideline) (Klimisch 2 – Reliable with restrictions) (Unnamed 1959 publication, 002 Supporting).
- Based on the weight of evidence, a score of High was assigned. Although acetonitrile has a harmonized classification to GHS Category 4 for the oral, dermal, and inhalation routes of exposure with the Hazard Statements H302 (harmful if swallowed), H312 (harmful in contact with skin), and

H332 (harmful if inhaled) in the EU, which corresponds to a score of Moderate, these classifications contain the caveat that these are minimum classifications, and acetonitrile can be classified in a more severe hazard category based on available toxicity information. Therefore, ToxServices classified acetonitrile as a GHS Category 3 acute oral toxicant based on the lowest identified LD_{50} of 139 mg/kg in male rats, which is within the oral guidance values of >50 to 300 mg/kg for acute oral toxicity Category 3 classification; a GHS Category 3 acute dermal toxicant due to the reported dermal LD_{50} value of 980 mg/kg in rabbits for undiluted acetonitrile, which is within the GHS Category 3 guidance values of >200 to ≤1,000 mg/kg for dermal acute toxicity; and a GHS Category 3 acute inhalation toxicant due to the 4-hour vapor LC_{50} of 3,587 ppm (reported to be 6,022 mg/m^3 or 6.022 mg/L) in mice, which falls within the GHS Category 3 guidance values of 2.0-10.0 mg/L (UN 2021).

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-single) (Group II) Score (vH, H, M, or L): *M*

Acetonitrile was assigned a score of Moderate for systemic toxicity (single dose) based on reports of it being irritating to mucus membranes in humans and labored breathing and respiration changes observed in an acute inhalation study in mice. GreenScreen® criteria classify chemicals as a Moderate hazard for systemic toxicity (single dose) when a GHS Category 3 classification for respiratory irritation is warranted (CPA 2018b). The confidence in the score is low due to limited reporting details for the irritative effects.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* GHS – Japan – H370 – Causes damage to organs [Specific target organs/systemic toxicity following single exposure – Category 1].
- ECHA 2023a
  - *Oral:* In a GLP-compliant acute oral toxicity study similar to OECD Guideline 401, male and female Crl:CD-1 (ICR) BR mice (5/sex/dose) were administered acetonitrile (99.9% purity) in distilled water at 300, 500, 650, 900, 1,200, and 2,000 mg/kg via gavage and were observed for 14 days. At 300, 500, 650, 900, 1,200, and 2,000 mg/kg mortality was 10, 30, 60, 80, 90, and 90%, respectively. Clinical signs included death, tremors, prostration, decreased activity, impaired righting reflex, labored breathing, convulsions, gasping, and increased salivation; surviving animals were judged to be normal by day 4. There were no treatment-related findings at necropsy. The LD_{50} was 617 mg/kg (469 mg/kg in males and 765 mg/kg in females) (Klimisch 1 – Reliable without restriction) (Unnamed 1998 study, 001 Key).
  - *Dermal:* In a GLP-compliant acute dermal toxicity study similar to OECD Guideline 402, male and female New Zealand white rabbits (5/sex/dose) were administered undiluted acetonitrile (99.9% purity) at 2,000 mg/kg under occlusive conditions for 24 hours and were observed for 14 days. There were no mortalities and no clinical signs of toxicity with the exception of decreased defecation in 3 females. There were no treatment-related findings at necropsy (Klimisch 1 – Reliable without restriction) (Unnamed 1997 study, 001 Key).
  - *Inhalation:* In a GLP-compliant acute inhalation toxicity study similar to OECD Guideline 403, male and female Crl: CD-1® (ICR) BR mice (5/sex/concentration) were exposed to acetonitrile vapor (99.9% purity) at 3,202, 3,747, 4,653 and 5,499 ppm via whole body inhalation for 4 hours and were observed for 14 days. At 3,202, 3,747, 4,653 and 5,499 ppm mortality was 20, 50, 90, and 80%, respectively. Clinical signs of toxicity included death, decreased activity, abnormal gait, loss of righting reflex, slow respiration, labored breathing, rapid respiration, gasping, cold to touch, limbs splayed, leaning to the right, and yellow body

surface staining. There were no treatment-related findings at necropsy (Klimisch 1 – Reliable without restriction) (Unnamed 1998 study, 001 Key).

- HSDB 2015
  - Acetonitrile can cause irritation of mucus membranes at concentrations up to 500 ppm.
- NITE 2017
  - Japan classified acetonitrile to GHS Category 1 for this endpoint based on effects on neurological system (discussed in single exposure neurotoxicity section below) and the respiratory organs in humans and animals. In humans case reports, there was irritation of the nose and throat. In animal experiments, respiratory effects included labored breathing, rapid respiration, gasping, dyspnea, and/or pulmonary hemorrhage and congestion in surviving and dead animals. However, all effects in animals occurred at doses within the range of Category 2, and it is not clear why Japan determined Category 1 classification.
- Based on the weight of evidence, a score of Moderate was assigned. Although there was a lack of indications of systemic toxicity at sublethal doses in acute oral, dermal, and inhalation toxicity studies with the target substance; ToxServices classified acetonitrile as GHS Category 3 for respiratory irritation based it being reported to be irritating to mucus membranes in humans and labored breathing and respiration changes observed in an acute inhalation study in mice. ToxServices discounted Japan's Category 1 classification as the effects observed may have been observed at lethal doses, and the LOAEL/LOAEC values reported did not support Category 1 classification.

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-repeat) (Group II*) Score (H, M, or L): M

Acetonitrile was assigned a score of Moderate for systemic toxicity (repeated dose) based on several inhalation NOAEC and LOAEC values within the guidance values of 0.2-1 mg/L (vapor) for Category 2 classification. GreenScreen® criteria classify chemicals as a Moderate hazard for systemic toxicity (repeated dose) when a GHS Category 2 classification is warranted for repeated dose systemic toxicity (CPA 2018b). The confidence in the score is high as it is based on multiple reliable experimental studies in multiple species for the target substance.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* GHS – Japan – H373 – May cause damage to organs through prolonged or repeated exposure [Specific target organs/systemic toxicity following repeated exposure – Category 2].
- ECHA 2023a, NTP 1996
  - *Inhalation:* In the inhalation chronic toxicity/carcinogenicity study conducted by the NTP described under carcinogenicity, male and female B6C3F1 mice (60/sex/concentration) were exposed to acetonitrile (>99% purity) via whole body inhalation at 0, 50, 100, and 200 ppm 6 hours/day, 5 days/week for 103 weeks (reported by NTP to be equivalent to 0, 84, 168, and 335 mg/m^3, respectively). Ten animals/sex were evaluated at 15 months. Survival rate of treated animals was similar to controls and there were no adverse effects on body weights or organ weights. The incidence of squamous hyperplasia of the epithelium of the forestomach was significantly increased at 15 months in 200 ppm females. At 2 years, the increased incidence of this lesion was dose related in the 100 and 200 ppm male groups and in all exposed female groups. The REACH dossier stated although the forestomach lesion was adverse, it was likely a result of grooming of contaminated fur or mucociliary clearance followed by ingestion rather than a result of inhalation. Therefore, a systemic toxicity NOAEC of 200 ppm was established for this study, equivalent to 0.24 mg/L when adjusted

for less than daily exposure (i.e., (200 ppm * 41.0527)/24,450 * (5 days/7 days) or 335 mg/m^3 * (5 days/7 days)) (Klimisch 1, Reliable without restriction) (Unnamed 1996 study, 001 Key).

  - *The NOAEC of 0.24 mg/L is above the duration-adjusted GHS Guidance value of 0.13 mg/L (1 mg/L x 13 weeks/103 weeks = 0.13 mg/L) for Category 2 classification for a 103-week study. Therefore, ToxServices did not classify acetonitrile as an inhalation repeated dose toxicant.*

- *Inhalation:* In the second inhalation chronic toxicity/carcinogenicity study conducted by the NTP described previously, male and female Fisher 344 rats (56/sex/concentration) were exposed to acetonitrile (>99% purity) via whole body inhalation at 0, 100, 200, and 400 ppm 6 hours/day, 5 days/week for 103 weeks (reported by NTP to be equivalent to 0, 168, 335 and 670 mg/m^3, respectively). Eight animals/sex were evaluated at 15 months. Survival rate of treated animals was similar to controls and there were no adverse effects on body weights or organ weights. Minor, non-significant anemia was reported in high concentration females at 15 months. There was a slight increase in the incidence of basophilic, eosinophilic, and mixed cell hepatic foci in 400 ppm males, and there was a statistically significant increase in basophilic foci in the 200 and 400 ppm groups, but the foci were not atypical in appearance and thus not considered a hepatotoxic effect. Therefore, a systemic toxicity NOAEC of 400 ppm was established for this study, equivalent to 0.48 mg/L when adjusted for less than daily exposure (i.e., (400 ppm * 41.0527)/24,450 * (5 days/7 days) or 670 mg/m^3 * (5 days/7 days)), (Klimisch 1, Reliable without restriction) (Unnamed 1996 study, 004 Key).
  - *The NOAEC of 0.48 mg/L is above the duration-adjusted GHS Guidance value of 0.13 mg/L (1 mg/L x 13 weeks/103 weeks = 0.13 mg/L) for Category 2 classification for a 103-week study. Therefore, ToxServices did not classify acetonitrile as an inhalation repeated dose toxicant.*
- *Inhalation:* In an inhalation subchronic toxicity study conducted by the NTP, male and female B6CF1 mice (10/sex/concentration) were exposed to acetonitrile (>99% purity) via whole body inhalation at 0, 100, 200, 400, 800, and 1,600 ppm 6 hours/day 5 days/week for 13 weeks. At 400, 800 and 1,600 ppm, 1, 5, and 20 animals died, respectively. The mean body weights of males in the 800 ppm group were slightly decreased. Liver weights were increased in all male exposure groups and in females exposed to ≥400 ppm. Necropsy revealed red or mottled lungs and pulmonary congestion in animals that died, ulcers in high concentration animals, forestomach lesions, including epithelial hyperplasia, in males (≥400 ppm) and females (≥200 ppm), vacuolization of the hepatocellular cytoplasm in surviving animas at 400 and 800 ppm. Liver vacuolization was reported to represent increased glycogen storage, and was not considered an adverse finding and the forestomach lesion was likely a result of grooming of contaminated fur or mucociliary clearance followed by ingestion rather than a result of inhalation. A NOAEC of 200 ppm, equivalent to 0.24 mg/L when adjusted for less than daily exposure (i.e., (200 ppm * 41.0527)/24,450 * (5 days/7 days)), was established for this study based on mortality; the LOAEC is 400 ppm (0.48 mg/L) (Klimisch 1, Reliable without restriction) (Unnamed 1996 study, 002 Key).
  - *The NOAEC and LOAEC of 0.24 and 0.48 mg/L, respectively, are both within the GHS Guidance values of 0.2-1 mg/L for Category 2 classification for a 13-week study. Therefore, ToxServices classified acetonitrile as a GHS Category 2 inhalation repeated dose toxicant.*
- *Inhalation:* In an inhalation subchronic toxicity study conducted by the NTP, male and female Fischer 344 rats (10/sex/concentration) were exposed to acetonitrile (>99% purity) via whole body inhalation at 0, 100, 200, 400, 800, and 1,600 ppm 6 hours/day 5 days/week for 13 weeks. At 800 and 1,600 ppm, 1 and 9 animals died, respectively. There were no

adverse changes to body weight. Females from the 800 ppm group and males from the 1,600 ppm group displayed anemia in the form of decreased erythrocyte counts, hemoglobin concentrations, and hematocrit; decreased triiodothyronine (T3) was also reported in 1,600 ppm females. Thymus weights were decreased at ≥800 ppm, and high concentration females had increased heart, kidney, and liver weights. Necropsy revealed red or mottled lungs and red foci in the brain in animals that died. There were no toxicologically significant adverse histopathological effects. A NOAEC of 400 ppm, equivalent to 0.48 mg/L when adjusted for less than daily exposure (i.e., (400 ppm * 41.0527)/24,450 * (5 days/7 days)), was established for this study based on mortality, anemia, and changes to organ weights; the LOAEC is 800 ppm (0.96 mg/L) (Klimisch 1, Reliable without restriction) (Unnamed 1996 study, 003 Key).

        - *The NOAEC and LOAEC of 0.48 and 0.96 mg/L, respectively, are both within the GHS Guidance values of 0.2-1 mg/L for Category 2 classification for a 13-week study. Therefore, ToxServices classified acetonitrile as a GHS Category 2 inhalation repeated dose toxicant.*

- ECHA 2023a
    - *Inhalation:* In GLP-compliant OECD Guideline 422 combined repeated dose toxicity study with the reproduction/developmental toxicity screening test described under reproductive and developmental toxicity, male and female Sprague-Dawley rats (10/sex/dose) were exposed to acetonitrile vapor (100% purity) via whole body exposure at concentrations of 0, 150, 300, 600, and 1,200 ppm 6 hours/day. Males were exposed for 42 days (from 2 weeks prior to mating until 2 weeks after the end of the mating period) and females were exposed for 35-41 days (from two weeks prior to mating until day 19 of pregnancy). At the high concentration, clinical signs included piloerection and soiled progenitalia and there were 7 mortalities (5 males and 2 females). Spleen weights and heart weights were increased in high concentration males. Red lungs with congestion, edema, and thrombus, atrophic and karyorrhexis thymus and enlargement, pale and white zones of the heart, congestion of bone marrow, and atrophy and deposit of hemosiderin of the spleen were reported in animals that died; there were no histopathological changes in surviving animals. The authors identified a systemic NOEC of 600 ppm equivalent to 1.01 mg/L (i.e., (600 ppm * 41.0527)/24,450) for this study based on mortality; the LOAEC is 1,200 ppm (2.01 mg/L) (Klimisch 1, Reliable without restriction) (Unnamed 2000 study, 005 Supporting).
        - *The NOAEC and LOAEC of 1.01 and 2.01 mg/L, respectively, are both within the duration-adjusted GHS Guidance values of 0.43-2.17 mg/L (0.2-1 mg/L x 13 weeks/6 weeks = 0.43-2.17 mg/L) for Category 2 classification for a 13-week study. Therefore, ToxServices classified acetonitrile as a GHS Category 2 inhalation repeated dose toxicant.*
    - *Inhalation:* In a non-GLP-compliant inhalation subchronic toxicity study (guideline not specified), male and female Wistar rats (15/sex/concentration) were exposed to acetonitrile (purity not reported) at 0, 166, 330, and 655 ppm 7 hours/day 5 days/week for 90 days. There were no treatment-related mortalities and adverse effects on body weight, hematology parameters, or organ weights. Significant histopathological effects (not specified) were reported in the lungs, kidneys, and liver of rats in the high concentration group. A NOAEC of 330 ppm, equivalent to 0.40 mg/L when adjusted for less than daily exposure (i.e., (330 ppm * 41.0527)/24,450 * (5 days/7 days)), can be established for this study; the LOAEC is 655 ppm (0.78 mg/L) (Klimisch 2, Reliable with restrictions) (Unnamed 1959 study, 006 Supporting).

        - *The NOAEC and LOAEC of 0.4 and 0.78 mg/L, respectively, are both within the GHS Guidance values of 0.2-1 mg/L for Category 2 classification for a 13-week study. Therefore, ToxServices classified acetonitrile as a GHS Category 2 inhalation repeated dose toxicant.*
    - *Inhalation:* In a non-GLP-compliant inhalation subchronic toxicity study (guideline not specified), male mixed breed dogs (3 exposed, 2 control) were exposed to acetonitrile (purity not reported) at 0 and 350 ppm 7 hours/day 5 days/week for 91 days. Mean body weight was reduced on individual days during the exposure period. Hematocrit and hemoglobin values were decreased in the fifth week but values returned to normal by the end of the exposure. Histopathology showed some focal emphysema and proliferation of alveolar septa. A NOAEC was not established for this study due to the lack of details and endpoints examined (Klimisch 2, Reliable with restrictions) (Unnamed 1959 study, 009 Supporting).
    - *Inhalation:* In a non-GLP-compliant inhalation subchronic toxicity study (guideline not specified), male Rhesus monkey (n=3) were exposed to acetonitrile (purity not reported) at 350 ppm 7 hours/day 5 days/week for 91 days. Mean body weight was reduced and erythrocyte counts were increased in one animal. Slight to moderate hemorrhage of the superior and inferior sagittal sinuses was reported in the brain of all three monkeys. Two monkeys had small discrete nodules in the lungs and a pale liver. Focal emphysema and proliferation of alveolar septa was reported at histopathological evaluation. A LOAEC of 350 ppm equivalent to 0.42 mg/L when adjusted for less than daily exposure (i.e., (350 ppm * 41.0527)/24,450 * (5 days/7 days)), was reported for this study (Klimisch 2, Reliable with restrictions) (Unnamed 1959 study, 010 Supporting).
        - *The LOAEC of 0.42 mg/L is within the GHS Guidance values of 0.2-1 mg/L for Category 2 classification for a 13-week study. Therefore, ToxServices classified acetonitrile as a GHS Category 2 inhalation repeated dose toxicant.*
- Based on the weight of evidence, a score of Moderate was assigned. Acetonitrile can be classified as a GHS Category 2 specific target organ toxicant following repeated inhalation exposures based on several NOAEC and LOAEC values within guidance values of 0.2-1 mg/L (vapor) for Category 2 classification (UN 2021). Adverse effects following inhalation exposure included increased mortality in mice exposed to ≥400 ppm (0.48 mg/L/6h/day) in a 13-week study, increased mortality, anemia, and changes to organ weights (decreased thymus weight and increased heart, kidney and liver weights) in rats exposed to ≥800 ppm (0.96 mg/L/6h/day) in a 13-week study, increased mortality and clinical signs and changes to organ weights (increased spleen and heart weights) in rats exposed to 1,200 ppm (2.01 mg/L/6h/day) in a 6-week exposure study, histopathological lesions in the lungs, kidneys, and liver in rats exposed to 655 ppm (0.78 mg/L/6h/day) in a 90-day study, and reduced body weight, increased erythrocyte count, brain hemorrhage, and focal emphysema in monkeys exposed to 350 ppm (0.42 mg/L/7h/day) in a 91-day study.

Neurotoxicity (single dose, N-single) (Group II) Score (vH, H, M, or L): *M*

Acetonitrile was assigned a score of Moderate for neurotoxicity (single dose) based on transient narcotic effects following acute oral and inhalation toxicity studies in mice. GreenScreen® criteria classify chemicals as a Moderate hazard for neurotoxicity (single dose) when a GHS Category 3 classification for transient narcotic effects is warranted (CPA 2018b). The confidence in the score is low as the clinical signs may also reflect general toxicity rather than specific neurotoxicity and because the inhalation study summary did not specifically report on reversibility.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* GHS – Japan – H370 – Causes damage to organs [Specific target organs/systemic

toxicity following single exposure – Category 1].

- ECHA 2023a
    - *Oral:* In a GLP-compliant acute oral toxicity study similar to OECD Guideline 401, male and female Crl:CD-1 (ICR) BR mice (5/sex/dose) were administered acetonitrile (99.9% purity) in distilled water at 300, 500, 650, 900, 1,200, and 2,000 mg/kg via gavage and were observed for 14 days. Clinical signs included death, tremors, prostration, decreased activity, impaired righting reflex, labored breathing, convulsions, gasping, and increased salivation; surviving animals were judged to be normal by day 4. There were no treatment-related findings at necropsy (Klimisch 1 – Reliable without restriction) (Unnamed 1998 study, 001 Key).
    - *Dermal:* In a GLP-compliant acute dermal toxicity study similar to OECD Guideline 402, male and female New Zealand white rabbits (5/sex/dose) were administered undiluted acetonitrile (99.9% purity) at 2,000 mg/kg under occlusive conditions for 24 hours and were observed for 14 days. There were no mortalities and no clinical signs of toxicity with the exception of decreased defecation in 3 females. There were no treatment-related findings at necropsy (Klimisch 1 – Reliable without restriction) (Unnamed 1997 study, 001 Key).
    - *Inhalation:* In a GLP-compliant acute inhalation toxicity study similar to OECD Guideline 403, male and female Crl: CD-1® (ICR) BR mice (5/sex/concentration) were exposed to acetonitrile vapor (99.9% purity) at 3,202, 3,747, 4,653 and 5,499 ppm via whole body inhalation for 4 hours and were observed for 14 days. Clinical signs of toxicity included death, decreased activity, abnormal gait, loss of righting reflex, slow respiration, labored breathing, rapid respiration, gasping, coldness to touch, splayed limbs, leaning to the right, and yellow body surface staining. There were no treatment-related findings at necropsy (Klimisch 1 – Reliable without restriction) (Unnamed 1998 study, 001 Key).
- NITE 2017
    - Japan classified acetonitrile to GHS Category 1 for this endpoint based on effects on neurological system and the respiratory organs (discussed above) in humans and animals. Neurological effects reported in humans included fatigue, nausea, vomiting, confusion, convulsions, and coma, leading to death in severe cases. Neurological effects reported in animals included hypoactivity, tremors, weakness, reduced righting reflex, convulsions, and salivation at oral doses of 300 – 2,000 mg/kg, hypoactivity, abnormal gait, loss of righting reflex, hypothermia, hindlimb extension, lateral position, tremors, and convulsions at inhalation concentrations of 250-5,000 ppm after 1-4 hours of exposure. However, all effects in animals occurred at doses within the range of Category 2, and it is not clear why Japan determined Category 1 classification.
- Based on the weight of evidence, a score of Moderate was assigned. Clinical signs of tremors, prostration, decreased activity, impaired righting reflex, and convulsions were observed in an acute oral toxicity study in mice, either leading to death at higher doses, or completely reversible at lower doses. Clinical signs of decreased activity, abnormal gait, loss of righting reflex, slow respiration, labored breathing, rapid respiration, gasping, coldness to touch, splayed limbs, and leaning to the right were observed in an acute inhalation toxicity study in mice. Therefore, ToxServices classified acetonitrile to GHS Category 3 (transient narcotic effects). ToxServices discounted Japan's Category 1 classification as the effects observed may have been observed at lethal doses, and the LOAEL/LOAEC values reported did not support Category 1 classification.

Neurotoxicity (repeated dose, N-repeated) (Group II*) Score (H, M, or L): DG
Acetonitrile was assigned a score of Data Gap for neurotoxicity (repeated dose) based on insufficient data identified for this endpoint.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a
  - *Inhalation:* In the inhalation subchronic toxicity study conducted by the NTP that is described under systemic toxicity, male and female Fischer 344 rats (10/sex/concentration) were exposed to acetonitrile (>99% purity) via whole body inhalation at 0, 100, 200, 400, 800, and 1,600 ppm 6 hours/day 5 days/week for 13 weeks. In the inhalation chronic toxicity/carcinogenicity study conducted by the NTP described under carcinogenicity and systemic toxicity, male and female Fisher 344 rats (56/sex/concentration) were exposed to acetonitrile (>99% purity) via whole body inhalation at 0, 100, 200, and 400 ppm 6 hours/day, 5 days/week for 103 weeks. In the subchronic study, 1 and 9 animals died at 800 and 1,600 ppm, respectively. Necropsy revealed red foci in the brain in animals that died. An independent pathology review of the brain sections from animals exposed to 800 and 1,600 ppm in the subchronic study and from the animals exposed to 400 ppm in the chronic study the failed to reveal any treatment-related neuropathologic alterations (Klimisch 2, Reliable with restrictions) (Unnamed 1999 pathology review, 001 Key).
  - *Inhalation:* In the non-GLP-compliant inhalation subchronic toxicity study (guideline not specified) that is described under systemic toxicity, male Rhesus monkey (n=3) were exposed to acetonitrile (purity not reported) at 350 ppm 7 hours/day 5 days/week for 91 days. Slight to moderate hemorrhage of the superior and inferior sagittal sinuses was reported in the brain of all three monkeys. An independent pathology review of the brain sections was performed. There was no evidence of any treatment-related neuropathologic alterations within any of the brain sections from the monkeys (Klimisch 2, Reliable with restrictions) (Unnamed 1999 study, 002 Key).
- Based on the weight of evidence, a Data Gap was assigned. Although histopathological evaluation of brain tissue from subchronic and chronic studies in rats and a subchronic study in monkeys revealed no treatment-related neuropathologic alterations, no repeated dose toxicity studies were available that specifically examined neurotoxicity in the form of a functional observation battery. Therefore, there are insufficient data to classify this endpoint.

Skin Sensitization (SnS) (Group II*) Score (H, M, or L): L

Acetonitrile was assigned a score of Low for skin sensitization based on negative results in an OECD Guideline 406 Buehler test in guinea pigs. GreenScreen® criteria classify chemicals as a Low hazard for skin sensitization when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on a reliable animal study for the target substance.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a
  - Acetonitrile was not a dermal sensitizer in a GLP-compliant Buehler test according to OECD Guideline 406. Male and female Hartley guinea pigs (n=20) were epicutaneously induced and challenged with undiluted acetonitrile (99.9% purity) under occlusive conditions. Distilled water control animals (n=10) were epicutaneously induced with distilled water and challenged with two patches, one containing undiluted test substance and one patch containing distilled water. Positive control animals (n=10) were epicutaneously induced with 2.5% hexyl cinnamic aldehyde in 95% ethanol and challenged with 1%, 2.5%,

and 5% hexyl cinnamic aldehyde formulated in w/v acetone. At 24 and 48 hours after challenge, 3/20 (15%) and 1/20 (5%) animals, respectively, had positive reactions to the test chemical, 2/10 (20%) and 2/10 (20%) control animals, respectively, displayed positive reactions, 1/10 (10%) and 2/10 (20%) distilled water control animals, respectively, displayed positive reactions, and 10/10 (100%) and 10/10 (100%) positive control animals challenged with 1%, respectively, displayed positive reactions. The authors concluded acetonitrile was not a dermal sensitizer under the conditions of this assay (Klimisch 1 – Reliable without restriction) (Unnamed 1997 study, 001 Key).

- *Based on GHS guidance (UN 2021) a GHS Category 1B classification is warranted when ≥15% respond at a >20% topical induction dose. Although 15% treated animals responded at 24 hours after challenge, there were also positive reactions reported in negative control animals. As negative control animals do not receive the test substance during induction but receive the challenge exposure, positive reactions in these animals generally demonstrate irritative properties of the test substance or vehicle. Therefore, it is appropriate to subtract the negative control response from the test substance response. At 24 hours: 15% response (3/20 positive test animals) – 20% response (2/10 positive control animals) = 0% response attributed to test substance, and at 48 hours: 5% response (1/20 positive test animals) – 20% response (2/10 positive control animals) = 0% response attributed to test substance. Therefore, a GHS classification for skin sensitization is not warranted.*

Respiratory Sensitization (SnR) (Group II*) Score (H, M, or L): *L*

Acetonitrile was assigned a score of Low for respiratory sensitization based on a lack of structural alerts for respiratory sensitization and extrapolation from negative results for skin sensitization in accordance with ECHA guidance on the evaluation of respiratory sensitization (ECHA 2017). GreenScreen® criteria classify chemicals as a Low hazard for respiratory sensitization when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is low as this evaluation does not include non-immunologic mechanisms of respiratory sensitization, and no specific data are available for respiratory sensitization.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- OECD 2022
  - Acetonitrile does not contain any structural alerts for respiratory sensitization (Appendix F)
- Based on the weight of evidence and guidance from ECHA regarding assessment of respiratory sensitization potential, a score of Low was assigned. The guidance from ECHA states that the mechanisms leading to respiratory sensitization are essentially similar to those leading to skin sensitization (ECHA 2017). ECHA recommended that if a chemical is not a dermal sensitizer based on high quality data, it is unlikely to be a respiratory sensitizer. ECHA also noted that this rationale does not cover respiratory hypersensitivity caused by non-immunological mechanisms, for which human experience is the main evidence of activity (ECHA 2017). As acetonitrile was not sensitizing to the skin (see skin sensitization section above), and a literature search did not find any human evidence of respiratory sensitization by acetonitrile, and as acetonitrile does not contain any structural alerts for respiratory sensitization (OECD 2022), acetonitrile is not expected to be a respiratory sensitizer.

Skin Irritation/Corrosivity (IrS) (Group II) Score (vH, H, M, or L): L

Acetonitrile was assigned a score of Low for skin irritation/corrosivity based on negative results in a dermal irritation study in rabbits. GreenScreen® criteria classify chemicals as a Low hazard for skin irritation/corrosivity when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data for the target substance.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a
    - Acetonitrile was not irritating to the skin in a GLP-compliant acute dermal irritation assay similar to OECD Guideline 404. New Zealand white rabbits (n=6) were administered 0.5 mL unchanged (no vehicle) acetonitrile (99.9% purity) to shaved skin under semi-occlusive conditions for 4 hours. The mean 24/48/72 hour erythema and edema scores were both 0/4. Acetonitrile was not a dermal irritant under the conditions of this assay (Klimisch 1 – Reliable without restriction) (Unnamed 1997 study, 001 Key).

Eye Irritation/Corrosivity (IrE) (Group II) Score (vH, H, M, or L): H

Acetonitrile was assigned a score of High for eye irritation/corrosivity based on its harmonized GHS Category 2A classification in the EU, with the associated H Statement of H319. GreenScreen® criteria classify chemicals as a High hazard for eye irritation/corrosivity when they are classified to GHS Category 2A for eye irritation (CPA 2018b). The confidence in the score is high as it is based on a harmonized classification.

- Authoritative and Screening Lists
    - *Authoritative:* EU – GHS (H-Statements) Annex 6 Table 3-1 – H319 – Causes serious eye irritation [Serious eye damage/eye irritation – Category 2A].
    - *Screening:* GHS – Australia – H319 – Causes serious eye irritation [Serious eye damage/eye irritation – Category 2A].
    - *Screening:* GHS – Malaysia – H319 – Causes serious eye irritation [Serious eye damage/eye irritation – Category 2A].
    - *Screening:* GHS – New Zealand – Eye irritation category 2.
    - *Screening:* GHS – Japan – H319 – Causes serious eye irritation [Serious eye damage/eye irritation – Category 2].
- ECHA 2023a
    - Acetonitrile was irritating to the eyes in a GLP-compliant acute ocular irritation assay similar to OECD Guideline 405. One eye of 6 New Zealand white rabbits was instilled with 0.1 mL unchanged (no vehicle) acetonitrile (99.9% purity) and remained unwashed. The individual mean 24, 48, 72 hour chemosis scores were 3, 3, 3, 2-3, 3, and 3; the individual mean 24, 48, 72 hour conjunctivae scores were 3 in all 6 animals; the individual mean 24, 48, 72 hour iris scores were 5, 5, 5, 0-5, 0-5, and 5; the individual mean 24, 48, 72 hour cornea opacity scores were 20, 15-20, 20-30, 15-30, 20, and 10-40 (the scoring scale for effects on the cornea was not described). The overall irritation score was >36.7-≤46 and some effects were not fully reversible within 21 days ("some" ocular irritation remained in 4/6 animals). The authors classified acetonitrile as a GHS Category 2 ocular irritant based on the results of this study (Klimisch 1, Reliable without restriction) (Unnamed 1997 study, 001 Key).
        - *ToxServices notes that the study authors stated that acetonitrile has a harmonized Category 2 classification and concluded that no change is proposed based on the*

available data. It is unclear based on the summary in the REACH dossier if the effects seen after 21 days support Category 1 classification for irreversible effects.

- Based on the weight of evidence, a score of High was assigned. The only reliable ocular irritation study in rabbits reported "some" ocular irritation present at day 21, which may correspond to Category 1 classification for irreversible effects. However, details were not provided regarding the severity and nature of the effects remaining at the end of the observation period. In addition, there are no details available regarding the full dataset considered by ECHA in determining the harmonized classification. Therefore, ToxServices relied mainly on the harmonized classification for this endpoint and classified acetonitrile as a GHS Category 2A ocular irritant based on its harmonized GHS Category 2A classification in the EU, with the associated H Statement of H319.

<u>**Ecotoxicity (Ecotox)**</u>

Acute Aquatic Toxicity (AA) Score (vH, H, M, or L): L

Acetonitrile was assigned a score of Low for acute aquatic toxicity based on L/EC_{50} values > 100 mg/L in fish, daphnia, and algae. GreenScreen® criteria classify chemicals as a Low hazard for acute aquatic toxicity when acute aquatic toxicity values are > 100 mg/L for all three trophic levels (CPA 2018b). The confidence in the score is high as it is based on measured data for the target chemical for all three trophic levels.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a
  - A 96-hour LC_{50} of 1,640 mg/L is reported in *Pimephales promelas* (fathead minnow) (GLP and guideline not specified) (Klimisch 2, Reliable with restrictions) (Unnamed 1984 study, 001 Key).
  - A 96-hour LC_{50} of >100 mg/L is reported in *P. promelas* (fathead minnow) (GLP and guideline not specified) (Klimisch 2, Reliable with restrictions) (Ewell et al. 1986, 005 Supporting).
  - A 96-hour LC_{50} of >100 mg/L is reported in *Oryzias latipes* (Japanese rice fish) (GLP, OECD Guideline 203) (Klimisch 1, Reliable without restriction) (Unnamed 199 study, 007 Supporting).
  - A 48-hour TL_m (medial tolerance limit) of ≥1,000 mg/L is reported in *O. latipes* (Japanese rice fish) (GLP not specified, Japanese Industrial Standards) (Klimisch 2, Reliable with restrictions) (Tonogai et al. 1982, 008 Supporting).
  - 24-hour LC_{50} values of 641 mg/L (24-hour larvae), 521 mg/L (48-hour larvae), and 400 mg/L (72-hour larvae) are reported in *Artemia salina* (brine shrimp) (GLP and guideline not specified) (Klimisch 2, Reliable with restrictions) (Barahona-Gomariz et al. 1994, 001 Key).
  - A 24-hour EC_{50} of >10,000 mg/L is reported in *Daphnia magna* (daphnia) (GLP not specified, DIN 38412 Part II) (Klimisch 2, Reliable with restrictions) (Bringmann and Kuhn 1982, 005 Supporting).
  - A 96-hour EC_{50} of >100 mg/L is reported in *D. magna* (daphnia) (GLP and guideline not specified) (Klimisch 2, Reliable with restrictions) (Ewell et al. 1986, 011 Supporting).
  - A 24- and 48-hour EC_{50} of >1,000 mg/L is reported in *D. magna* (daphnia) (GLP, OECD Guideline 202) (Klimisch 1, Reliable without restriction) (Unnamed 1996 study, 012 Supporting).
  - A 48-hour LC_{50} of 3,600 mg/L is reported in *D. magna* (daphnia) (GLP not specified, OECD Guidelines) (Klimisch 2, Reliable with restrictions) (Tong et al. 1996, 013 Supporting).

- A 96-hour EC_{50} of >100 mg/L is reported in *Dugesua tigrine* (flatworm) (GLP and guideline not specified) (Klimisch 2, Reliable with restrictions) (Ewell et al. 1986, 006 Supporting).
- A 96-hour EC_{50} of >100 mg/L is reported in *Asellus intermediums* (pillbug) (GLP and guideline not specified) (Klimisch 2, Reliable with restrictions) (Ewell et al. 1986, 007 Supporting).
- A 96-hour EC_{50} of >100 mg/L is reported in *Helisoma trivolvis* (ramshorn snail) (GLP and guideline not specified) (Klimisch 2, Reliable with restrictions) (Ewell et al. 1986, 008 Supporting).
- A 96-hour EC_{50} of >100 mg/L is reported in *Lumbriculus variegatus* (segmented worm) (GLP and guideline not specified) (Klimisch 2, Reliable with restrictions) (Ewell et al. 1986, 009 Supporting).
- A 96-hour EC_{50} of >100 mg/L is reported in *Gammarus fasciatus* (sideswimmer) (GLP and guideline not specified) (Klimisch 2, Reliable with restrictions) (Ewell et al. 1986, 010 Supporting).
- 72-hour EC_{50} values of 3,560 mg/L (yield) and 9,686 mg/L (growth rate) are reported in *Phaeodactylum tricornutum* (diatom) (GLP, ISO 10253) (Klimisch 1, Reliable without restriction) (Unnamed 2010 study, 001 Key).
- 48-hour EC_{50} values of 7,943 mg/L (growth rate) and 5,926 mg/L (based on dissolved oxygen production) are reported in *Raphidocelis subcapitata* (green algae) (GLP and guideline not specified) (Klimisch 2, Reliable with restrictions) (Chen et al. 2005, 005 Supporting).
- 72-hour EC_{50} values of 1,000 mg/L (growth rate and biomass) are reported in *R. subcapitata* (green algae) (GLP, OECD Guideline 201) (Klimisch 1, Reliable without restriction) (Unnamed 1996 study, 006 Supporting).

Chronic Aquatic Toxicity (CA) Score (vH, H, M, or L): L

Acetonitrile was assigned a score of Low for chronic aquatic toxicity based on NOEC values > 10 mg/L in fish, daphnia, and algae. GreenScreen® criteria classify chemicals as a Low hazard for chronic aquatic toxicity when chronic aquatic toxicity values are > 10 mg/L for all three trophic levels (CPA 2018b). The confidence in the score is high as it is based on experimental data for the target chemical for all three trophic levels.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a
  - A 21-day NOEC of 102 mg/L is reported in *O. latipes* (Japanese rice fish) (GLP, OECD Guideline 204) (Klimisch 1, Reliable without restriction) (Unnamed 1996 study, 001 Key).
  - A 21-day reproduction NOEC of 960 mg/L is reported in *D. magna* (daphnia) (GLP, OECD Guideline 202) (Klimisch 1, Reliable without restriction) (Unnamed 1996 study, 001 Weight of evidence).
  - A 21-day reproduction NOEC of 160 mg/L and ChV of 230 mg/L and a 21-day mortality NOEC of 640 mg/L and ChV of >900 mg/L are reported in *D. magna* (daphnia) (GLP, OECD Guideline 202) (Klimisch 2, Reliable with restrictions) (Tong et al. 1996, 002 Weight of evidence).
  - 72-hour NOEC values of 400 mg/L (yield and growth rate) are reported in *P. tricornutum* (diatom) (GLP, ISO 10253) (Klimisch 1, Reliable without restriction) (Unnamed 2010 study, 001 Key).

- 72-hour NOEC values of 1,000 mg/L (growth rate and biomass) are reported in *R. subcapitata* (green algae) (GLP, OECD Guideline 201) (Klimisch 1, Reliable without restriction) (Unnamed 1996 study, 006 Supporting).

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): vL

Acetonitrile was assigned a score of Very Low for persistence based on it being readily biodegradable and meeting the 10-day window in OECD Guideline 310 and 301C ready biodegradability studies. GreenScreen® criteria classify chemicals as a Very Low hazard for persistence when they are readily biodegradable and meet the 10-day window in ready biodegradation tests (CPA 2018b). The confidence in the score is high as it is based on high quality experimental data for the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* EC – CEPA DSL – Persistent.
- ECHA 2023a
  - Acetonitrile was readily biodegradable and met the 10-day window in a GLP-compliant OECD Guideline 310 ready biodegradability assay (CO_2 in sealed vessels (headspace test)). In this assay, 684 mg/L acetonitrile (100% purity) was exposed to non-adapted, activated sludge in sealed vessels for 21 days. The test substance degraded 11% in 4 days, 52% in 7 days, 62% in 14 days, and 70% in 21 days (Klimisch 1, Reliable without restriction) (Unnamed 2010 study, 001 Key).
  - Acetonitrile was readily biodegradable in a GLP-compliant OECD Guideline 301C ready biodegradability assay (modified MITI test). In this assay, 100 mg/L acetonitrile (100% purity) was exposed to aerobic non-adapted, activated sludge for 28 days. The test substance degraded 65% (by BOD), 84% (by TOC removal), and 88% (by GC analysis) in 28 days. An additional test with a lower concentration of test substance (30 mg/L) measured 100% biodegradation (by TOC and GC) after 14 days. Based on these results, the authors concluded acetonitrile to be readily biodegradable (Klimisch 1, Reliable without restriction) (Unnamed 1998 study, 002 Key).
    - *The OECD 301 Guideline (OECD 1992) states "specific chemical analysis can also be used to assess primary degradation of the test substance and to determine the concentration of any intermediate substances formed. It is obligatory in the MITI method (301 C)". Therefore, ToxServices used the percent degradation by GC analysis to conclude the test substance was readily biodegradable.*
- CCR 2023
  - Acetonitrile is listed on the Environment Canada (EC) Canadian Environmental Protection Act (CEPA) Domestic Substances List (DSL) as persistent based on an ozone reaction half-life of 76.38 days and an atmospheric oxidation half-life of 406.63 days.
- U.S. EPA 2017
  - The BIOWIN modeling Ready Biodegradable Predictor indicates that acetonitrile is expected to be readily biodegradable. Fugacity modeling (MCI method) predicts 47.8% will partition to soil with a half-life of 30 days, 40.3% will partition to water with a half-life of 15 days, 11.9% will partition to air with a half-life of 64.58 days, and 0.0816% will partition to sediment with a half-life of 135 days (Appendix G).
- Based on the weight of evidence, a score of Very Low was assigned. Acetonitrile is readily biodegradable based on 70% degradation in 21 days in an OECD Guideline 310 ready biodegradability test in which it met the 10-day window, and 88% and 100% biodegradation (based

on GC analysis) in 14 and 28 days, respectively, in an OECD Guideline 301C test, in which it also met the 10-day window. No data were found regarding environmental partitioning. Although acetonitrile is listed on the EC CEPA DSL as persistent, this is based on ozone reaction and atmospheric oxidation half-lives, and it is ToxServices' internal policy to assign the hazard score for persistence based on the dominant environmental compartment(s) (ToxServices 2021). Modeling using the U.S. EPA's EPI Suite™ v 4.11 program (U.S. EPA 2017) found that acetonitrile will mainly partition to soil at 50.8% with a half-life of 30 days. Based on ready degradability in an OECD 310 and 301C tests, the compound is likely to be readily biodegradable in soil/sediment as well.

Bioaccumulation (B) Score (vH, H, M, L, or vL): vL

Acetonitrile was assigned a score of Very Low for bioaccumulation based on a measured log K_{ow} of -0.34 and a predicted BCF of less than 100. GreenScreen® criteria classify chemicals as a Very Low hazard for bioaccumulation when the log K_{ow} is < 4 and the BCF of less than 100 (CPA 2018b). The confidence in the score is high as it is based on a measured log K_{ow} with support from modeling.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a
  - Acetonitrile has a measured log K_{ow} of -0.34 (GLP and guideline not specified) (Klimisch 2, Reliable with restrictions) (Lide 2005, 001 Key).
- U.S. EPA 2017
  - BCFBAF predicts a BCF of 3.162 L/kg wet-wt using the regression-based method, based on a log K_{ow} of -0.34. Taking metabolism into consideration, the Arnot-Goas model predicts a BCF of 0.9244 for the upper trophic level (Appendix G).

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M, or L): *L*

Acetonitrile was assigned a score of Low for reactivity based on its instability/physical hazard rating of 0 from NFPA and HMIS. GreenScreen® criteria classify chemicals as a Low hazard for reactivity when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is low due to the lack of measured data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a
  - Acetonitrile does not contain atoms in the structure capable of being reduced (and therefore imparting oxidizing properties to the chemical structure).
  - Acetonitrile does not contain chemical groups associated with explosive properties.
- TCI 2018
  - An SDS for acetonitrile (>99.5% purity) has an instability rating of 0 from the NFPA ("Normally stable, even under fire exposure conditions, and is not reactive with water") and physical hazard rating of 0 from HMIS ("Materials that are normally stable, even under fire conditions, and will not react with water, polymerize, decompose, condense, or self-react. Non-explosives").

Flammability (F) Score (vH, H, M, or L): H

Acetonitrile was assigned a score of High for flammability based on ToxServices classifying it as a GHS Category 2 flammable liquid based on a flash point of 6-12.8℃ and a boiling point of 81.65℃, and as the EU has classified acetonitrile as a GHS Category 2 flammable liquid (H225). In addition, acetonitrile is DOT Class 3 Group 2. GreenScreen® criteria classify chemicals as a High hazard for flammability when they are classified as a GHS Category 2 flammable liquid and when they are DOT Class 3 Group 2 (CPA 2018b). The confidence in the score is high as it is based on measured data and authoritative A listings.

- Authoritative and Screening Lists
    - *Authoritative:* EU – GHS (H-Statements) Annex 6 Table 3-1 – H225 – Highly flammable liquid and vapor [Flammable liquids – Category 2].
    - *Authoritative:* Quebec CSST – WHMIS 1988 – Class B2 – Flammable liquids
    - *Authoritative:* U.S. DOT Class 3 Group 2.
    - *Screening* GHS – Australia – H225 – Highly flammable liquid and vapor [Flammable liquids – Category 2].
    - *Screening* GHS – Japan – H225 – Highly flammable liquid and vapor [Flammable liquids – Category 2].
    - *Screening* GHS – Malaysia – H225 – Highly flammable liquid and vapor [Flammable liquids – Category 2].
    - *Screening* GHS – New Zealand – Flammable liquids Category 2.
- ECHA 2023a
    - Acetonitrile has a flash point of 12.8℃ as identified in a closed cup test (GLP and guideline not specified) (Klimisch 2, Reliable with restrictions) (Merck 1989, 001 Key).
    - Acetonitrile has a flash point of 6℃ as identified in an open cup test (GLP and guideline not specified) (Klimisch 2, Reliable with restrictions) (Quincy 2002, Lide 2005, 002 Supporting).
    - Acetonitrile has an auto-ignition temperature of 524℃ (GLP and guideline not specified) (Klimisch 2, Reliable with restrictions) (Lide 2005, 001 Key).
    - Acetonitrile has a lower explosion limit of 3% vol and an upper explosion limit of 16% vol (GLP and guideline not specified) (Klimisch 2, Reliable with restrictions) (Quincy 2002, 001 Key).
        - *Although GHS defines a Category 1A flammable gas as one that is ignitable when a mixture of 13% or less at 20℃ and standard pressure of 101.3 kPa (UN 2021), acetonitrile is a liquid at 20℃; therefore, it is not classified as a flammable gas.*
- Based on the weight of evidence, a score of High was assigned. Acetonitrile has a flash point of 6-12.8℃ and a boiling point of 81.65℃. GHS criteria define Category 2 flammable liquids as chemicals with flash points < 23℃ and an initial boiling point >35℃ (UN 2021).

Use of New Approach Methodologies (NAMs)[9] in the Assessment, Including Uncertainty Analyses of Input and Output

New Approach Methodologies (NAMs) used in this GreenScreen® include *in silico* modeling for endocrine activity, respiratory sensitization, persistence, and bioaccumulation, and *in vitro* testing for mutagenicity and endocrine activity. NAMs are non-animal alternatives that can be used alone or in combination to provide information for safety assessment (Madden et al. 2020). At present, there is not a uniformly accepted framework on how to report and apply individual NAMs (U.S. EPA 2020, OECD 2020). The expanded application of NAMs greatly amplifies the need to communicate uncertainties associated with their use. As defined by EFSA (2018), uncertainty is "a general term referring to all types of limitations in available knowledge that affect the range and probability of possible answers to an assessment question." The quality, utility, and accuracy of NAM predictions are greatly influenced by two primary types of uncertainties (OECD 2020):

- Type I: Uncertainties related to the input data used
- Type II: Uncertainties related to extrapolations made

As shown in Table 4, Type I (input data) uncertainties in acetonitrile's NAMs dataset include lack of sufficient experimental data for endocrine activity, and respiratory sensitization, using a genotoxicity assay that has been deleted from the OECD test guidelines, and lack of validated test methods for respiratory sensitization. Acetonitrile's Type II (extrapolation output) uncertainties include the lack of defined applicability domains in some modeling programs, limitation of *in vitro* genotoxicity assays in mimicking *in vivo* metabolism, their focusing on one or only a few types of genotoxicity events, unclear *in vivo* relevance of *in vitro* testing and *in silico* prediction of receptor binding due to lack of toxicokinetic considerations, and the limitation of OECD Toolbox in identifying structural alerts for respiratory sensitization without accounting for non-immunologic mechanisms of respiratory sensitization. Some of acetonitrile's type II uncertainties were alleviated by the use of *in vitro* test batteries and/or in combination of *in vivo* data.

Table 4: Summary of NAMs Used in the GreenScreen® Assessment, Including Uncertainty Analyses	
	Uncertainty Analyses (OECD 2020)
Type I Uncertainty: Data/Model Input	**Genotoxicity:** The yeast mitotic recombination assay test guideline (OECD Guideline 481) has been deleted from the recommended OECD test guidelines due to lack of use under regulatory settings, and inferior performance compared to other tests.[10] There are no validated *in vitro* comet assay protocols among OECD guidelines. **Endocrine Activity**: Only limited *in vivo* data are available. **Respiratory sensitization**: No experimental data are available and there are no validated test methods.
Type II Uncertainty: Extrapolation Output	**Genotoxicity:** The bacterial reverse mutation assay (as defined in OECD Guideline 471) only tests point-mutation inducing activity in non-mammalian cells, and the exogenous metabolic activation

[9] NAMs refers to any non-animal technology, methodology, approach, or combination thereof that inform chemical hazard and risk assessments. NAMs include *in silico*/computational tools, *in vitro* biological profiling (e.g., cell cultures, 2,3-D organotypic culture systems, genomics/transcriptomics, organs on a chip), and frameworks (i.e., adverse outcome pathways (AOPs), defined approaches (DA), integrated approaches to testing and assessment (IATA).

[10] https://www.oecd.org/env/ehs/testing/Draft_Intro_Genotoxicity%20TGs%20September%202014.pdf

	system does not entirely mimic *in vivo* conditions[11]. The mammalian cell gene mutation assay (as defined in OECD Guideline 476) only detects gene mutations, and the exogenous metabolic activation system does not entirely mirror *in vivo* metabolism (i.e., the liver S9 mix contains enzymes present in the endoplasmic reticulum but not the cytosol of liver cells).[12] The *in vitro* chromosome aberration assay (OECD Guideline 473) does not measure aneuploidy and it only measures structural chromosomal aberrations. The exogenous metabolic activation system does not entirely mirror *in vivo* metabolism[13]. The *in vitro* SCE assay (as defined in OECD Guideline 479, a guideline deleted in 2014) detects reciprocal exchange of DNA without providing the underlying mechanism of action[14]. Assays conducted in mammalian cells are preferred to those in yeasts, fungi or insects. The *in vitro* comet assay simply represents the ratio of the fragmented DNA in cells. Additional supportive information is required to thoroughly depict the cellular changes besides DNA damage[15]. **Endocrine activity:** ToxCast models don't define applicability domain; the *in vivo* relevance of EDSP Tox 21 screening assays and *in silico* modeling of receptor binding activities is unknown due to lack of consideration of metabolism and other toxicokinetic factors. EDSP Tox 21 assays do not cover all critical endocrine pathways. **Respiratory sensitization**: The OECD Toolbox only identifies structural alerts and does not define applicability domains. Additionally, the ECHA guidance (2017), on which the use of OECD Toolbox structural alerts is based, does not evaluate non-immunologic mechanisms for respiratory sensitization.	
Endpoint	**NAMs Data Available and Evaluated? (Y/N)**	**Types of NAMs Data (*in silico* modeling/*in vitro* biological profiling/frameworks)**
Carcinogenicity	N	
Mutagenicity	Y	*In vitro* data: Bacterial reverse mutation assay/*in vitro* gene mutation assay/*in vitro* chromosome aberration assay/*in vitro* yeast cytogenic assay/*in vitro* fungi gene mutation assay/*in vitro* sister chromatid exchange assay/*in vitro* comet assay/*in vitro* microtubule assembly assay

[11] https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1614097593&id=id&accname=guest&checksum=89925F80B9F4BD2FFC6E90F94A0EE427
[12] https://www.oecd-ilibrary.org/docserver/9789264264809-en.pdf?expires=1614097800&id=id&accname=guest&checksum=C0DE371FB9C5A878E66C9AB7F84E6BBE
[13] https://www.oecd-ilibrary.org/docserver/9789264264649-en.pdf?expires=1614098015&id=id&accname=guest&checksum=6A4F9CE52EA974F5A74793DD54D54352
[14] https://www.oecd.org/env/ehs/testing/Draft_Intro_Genotoxicity%20TGs%20September%202014.pdf
[15] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5752397/

Reproductive toxicity	N	
Developmental toxicity	N	
Endocrine activity	Y	*In vitro* high throughput data: EDSP Tox 21 screening assays *In silico* modeling: VEGA/Danish QSAR/ ToxCast models
Acute mammalian toxicity	N	
Single exposure systemic toxicity	N	
Repeated exposure systemic toxicity	N	
Single exposure neurotoxicity	N	
Repeated exposure neurotoxicity	N	
Skin sensitization	N	
Respiratory sensitization	Y	*In silico* modeling: OECD Toolbox structural alerts
Skin irritation	N	
Eye irritation	N	
Acute aquatic toxicity	N	
Chronic aquatic toxicity	N	
Persistence	Y	*In silico* modeling: EPI Suite™ Non-animal testing: OECD 310 and 301C Biodegradation tests
Bioaccumulation	Y	*In silico* modeling: EPI Suite™

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APPENDIX A: Hazard Classification Acronyms
(in alphabetical order)

(AA) Acute Aquatic Toxicity

(AT) Acute Mammalian Toxicity

(B) Bioaccumulation

(C) Carcinogenicity

(CA) Chronic Aquatic Toxicity

(D) Developmental Toxicity

(E) Endocrine Activity

(F) Flammability

(IrE) Eye Irritation/Corrosivity

(IrS) Skin Irritation/Corrosivity

(M) Mutagenicity and Genotoxicity

(N) Neurotoxicity

(P) Persistence

(R) Reproductive Toxicity

(Rx) Reactivity

(SnS) Sensitization- Skin

(SnR) Sensitization- Respiratory

(ST) Systemic/Organ Toxicity

APPENDIX B: Results of Automated GreenScreen® Score Calculation for Acetonitrile (CAS #75-05-8)

TOXSERVICES TOXICOLOGY RISK ASSESSMENT CONSULTING

GREEN SCREEN FOR SAFER CHEMICALS

GreenScreen® Score Inspector

Table 1: Hazard Table

			Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
			Carcinogenicity	Mutagenicity/Genotoxicity	Reproductive Toxicity	Developmental Toxicity	Endocrine Activity	Acute Toxicity	Systemic Toxicity		Neurotoxicity		Skin Sensitization*	Respiratory Sensitization*	Skin Irritation	Eye Irritation	Acute Aquatic Toxicity	Chronic Aquatic Toxicity	Persistence	Bioaccumulation	Reactivity	Flammability
Table 2: Chemical Details									S	R *	S	R *	*	*								
Inorganic Chemical?	**Chemical Name**	**CAS#**	**C**	**M**	**R**	**D**	**E**	**AT**	**STs**	**STr**	**Ns**	**Nr**	**SNS***	**SNR***	**IrS**	**IrE**	**AA**	**CA**	**P**	**B**	**Rx**	**F**
No	**Acetonitrile**	**75-05-8**	*M*	*M*	**L**	*M*	DG	**H**	*M*	**M**	*M*	DG	**L**	*L*	**L**	**H**	**L**	**L**	**vL**	**vL**	*L*	**H**

Table 3: Hazard Summary Table

Benchmark	a	b	c	d	e	f	g
1	No	No	No	No	No		
2	No	No	No	No	Yes	No	Yes
3	STOP						
4	STOP						

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
Acetonitrile	2

Note: Chemical has not undergone a data gap assessment. Not a Final GreenScreen™ Score

Table 6

Chemical Name	Final GreenScreen® Benchmark Score
Acetonitrile	2

After Data gap Assessment
Note: No Data gap Assessment Done if Preliminary GS Benchmark Score is 1.

Table 5: Data Gap Assessment Table

Datagap Criteria	a	b	c	d	e	f	g	h	i	j	bm4	End Result
1												
2	Yes	Yes	Yes	Yes	Yes							2
3												
4												

APPENDIX C: Pharos Output for Acetonitrile (CAS #75-05-8)

75-05-8
Acetonitrile
ALSO CALLED 200-835-2, 54841-72-4, Acetonitril, Acetonitril [German, Dutch], Cyanomethane, Cyanure de methyl, Cy...
View all synonyms (14)

Share Profile

Hazards | Properties | Functional Uses | Resources

All Hazards View

Show PubMed Results | Request Assessment | Add to Comparison

		Group I Human					Group II and II* Human									Ecotox			Fate		Physical		Mult	Non-GSLT			
	GS Score	C	M	R	D	E	AT	ST	ST	N	N	SnS	SnR	IrS	IrE	AA	CA	ATB	P	B	Rx	F	Mult	PBT	GW	O	Other
All Hazards	LT-P1	H-L	-	-	M-L	-	M	pC	-	pC	-	-	-	-	H	-	-	-	vH-H	-	-	H	vH	-	-	-	R

Hazard Lists

Download Lists

ENDPOINT	HAZARD LEVEL	GS SCORE	LIST NAME	HAZARD DESCRIPTION	OTHER LISTS
Carcinogenicity	H-L	LT-UNK	US EPA - IRIS Carcinogens	(1986) Group D - Not classifiable as to human carcinogenicity	+1
	H-L	NoGS	US EPA - IRIS Carcinogens	(1996) Carcinogenic potential cannot be determined	
Developmental Toxicity incl. developmental neurotoxicity	M-L	LT-UNK	MAK	Pregnancy Risk Group C	
Acute Mammalian Toxicity	M	LT-UNK	EU - GHS (H-Statements) Annex 6 Table 3-1	H302 - Harmful if swallowed [Acute toxicity (oral) - Category 4]	+19
	M	LT-UNK	EU - GHS (H-Statements) Annex 6 Table 3-1	H312 - Harmful in contact with skin [Acute toxicity (dermal) - Category 4]	
	M	LT-UNK	EU - GHS (H-Statements) Annex 6 Table 3-1	H332 - Harmful if inhaled [Acute toxicity (inhalation) - Category 4]	
	vH	LT-UNK	GHS - New Zealand	Acute oral toxicity category 2	
	vH-H	LT-UNK	Québec CSST - WHMIS 1988	Class D1A - Very toxic material causing immediate and serious toxic effects	
	H	LT-UNK	GHS - New Zealand	Acute dermal toxicity category 3	
	H	LT-UNK	GHS - New Zealand	Acute inhalation toxicity category 3	
	H	LT-UNK	GHS - Japan	H311 - Toxic in contact with skin [Acute Toxicity (dermal) - Category 3]	
	H-M	LT-UNK	GHS - Australia	H332 - Harmful if inhaled [Acute toxicity (inhalation) - Category 4]	
	H-M	LT-UNK	GHS - Malaysia	H332 - Harmful if inhaled [Acute toxicity (inhalation) - Category 4]	
	M	LT-UNK	GHS - Australia	H302 - Harmful if swallowed [Acute toxicity (oral) - Category 4]	
	M	LT-UNK	GHS - Australia	H312 - Harmful in contact with skin [Acute toxicity (dermal) - Category 4]	
	M	LT-UNK	GHS - Japan	H332 - Harmful if inhaled [Acute toxicity (inhalation: vapor) - Category 4]	
	M	LT-UNK	GHS - Malaysia	H302 - Harmful if swallowed [Acute toxicity (oral) - Category 4]	

	M	LT-UNK	GHS - Malaysia	H312 - Harmful in contact with skin [Acute toxicity (dermal) - Category 4]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H302 - Harmful if swallowed (unverified) [Acute toxicity (oral) - Category 4]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H311 - Toxic in contact with skin (unverified) [Acute toxicity (dermal) - Category 3]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H312 - Harmful in contact with skin (unverified) [Acute toxicity (dermal) - Category 4]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H331 - Toxic if inhaled (unverified) [Acute toxicity (inhalation) - Category 3]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H332 - Harmful if inhaled (unverified) [Acute toxicity (inhalation) - Category 4]	
Systemic Toxicity/Organ Effects-Single Exposure	pC	NoGS	EU - Manufacturer REACH hazard submissions	H335 - May cause respiratory irritation (unverified) [Specific target organ toxicity - single exposure; Respiratory tract irritation - Category 3]	
Neurotoxicity-Single Exposure	pC	NoGS	EU - Manufacturer REACH hazard submissions	H336 - May cause drowsiness or dizziness (unverified) [Specific target organ toxicity - single exposure; Narcotic effects - Category 3]	
Eye Irritation/Corrosivity	H	LT-UNK	EU - GHS (H-Statements) Annex 6 Table 3-1	H319 - Causes serious eye irritation [Serious eye damage/eye irritation - Category 2A]	+5
	vH	LT-UNK	GHS - Australia	H318 - Causes serious eye damage [Serious eye damage/eye irritation - Category 1]	
	H	LT-UNK	GHS - Malaysia	H319 - Causes serious eye irritation [Serious eye damage/eye irritation - Category 2A]	
	H	LT-UNK	GHS - New Zealand	Eye irritation category 2	
	H-M	LT-UNK	GHS - Japan	H319 - Causes serious eye irritation [Serious eye damage / eye irritation - Category 2]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H319 - Causes serious eye irritation (unverified) [Serious eye damage/eye irritation - Category 2A]	
Persistence	vH-H	LT-UNK	EC - CEPA DSL	Persistent	
Flammability	H	LT-UNK	EU - GHS (H-Statements) Annex 6 Table 3-1	H225 - Highly flammable liquid and vapour [Flammable liquids - Category 2]	+6
	H	LT-UNK	GHS - Australia	H225 - Highly flammable liquid and vapour [Flammable liquids - Category 2]	
	H	LT-UNK	GHS - Japan	H225 - Highly flammable liquid and vapour [Flammable liquids - Category 2]	
	H	LT-UNK	GHS - Malaysia	H225 - Highly flammable liquid and vapour [Flammable liquids - Category 2]	
	H	LT-UNK	GHS - New Zealand	Flammable liquids category 2	
	vH-M	LT-UNK	Québec CSST - WHMIS 1988	Class B2 - Flammable liquids	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H225 - Highly flammable liquid and vapour (unverified) [Flammable liquids - Category 2]	
Carcinogenicity,Mutagenicity/Genotoxicity Reproductive Toxicity, Developmental Toxicity, Acute Mammalian Toxicity, or System Toxicity/Organ Effects.	U	LT-UNK	Québec CSST - WHMIS 1988	Class D2B - Toxic material causing other toxic effects	+1
	U	LT-UNK	EC - CEPA DSL	Inherently Toxic to Humans (iTH)	
Systemic Toxicity/Organ Effects [Single Exposure] and/or Neurotoxicity [Single Exposure]	vH	LT-UNK	GHS - Japan	H370 - Causes damage to organs [Specific target organs/systemic toxicity following single exposure - Category 1]	
Systemic Toxicity/Organ Effects [Repeated Exposure] and/or Neurotoxicity [Repeated Exposure]	M	LT-UNK	GHS - Japan	H373 - May cause damage to organs through prolonged or repeated exposure [Specific target organs/systemic toxicity following repeated exposure - Category 2]	
Human and/or Aquatic toxicity and/or Persistence and/or Bioaccumulation	U	LT-P1	German FEA - Substances Hazardous to Waters	Class 2 - Hazard to Waters	

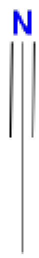
Restricted Substance Lists (10)

- C2C Certified v4 Product Standard Restricted Substances List (RSL) - Effective July 1, 2022: Cosmetics & Personal Care Products
- CA SCP - Candidate Chemicals: Candidate Chemical List
- EU - Cosmetics Regulation: Annex II - Prohibited Substances
- EU - PACT-RMOA Substances: Substances selected for RMOA or hazard assessment
- GSPI - Six Classes of Problematic Chemicals: Some Solvents
- Health Canada - Cosmetic Ingredient Hotlist: Ingredients that are Prohibited for Use in Cosmetic Products
- MA Toxics Use Reduction Act (TURA) listed substances: Reportable Chemicals
- MDH - Chemicals of High Concern and Priority Chemicals: Chemicals of High Concern
- TSCA Chemical Substance Inventory (Active-Inactive): TSCA Chemical Substance Inventory - Active
- US EPA - Hazardous Air Pollutants: HAPs subject to the Clean Air Act

APPENDIX D: VEGA Endocrine Results for Acetonitrile (CAS #75-05-8)

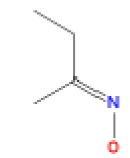
1. Prediction Summary

Prediction for compound Molecule 0

	Prediction: Reliability: **Prediction is Inactive, but the result may be not reliable. A check of the information given in the following section should be done, paying particular attention to the following issues:** **- no similar compounds with known experimental value in the training set have been found** **- 2 descriptor(s) for this compound have values outside the descriptor range of the compounds of the training set.**

Compound: Molecule 0
Compound SMILES: N#CC
Experimental value: -
Predicted activity: Inactive
Classification tree final node: 4
Reliability: the predicted compound is outside the Applicability Domain of the model
Remarks:
none

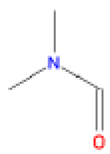
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 96-29-7
Dataset id: 7 (Training set)
SMILES: ON=C(C)CC
Similarity: 0.629

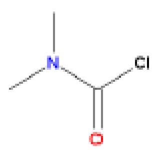
Experimental value: Inactive
Predicted value: Inactive



Compound #2

CAS: 68-12-2
Dataset id: 23 (Test set)
SMILES: O=CN(C)C
Similarity: 0.628

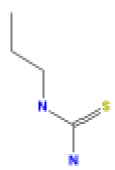
Experimental value: Inactive
Predicted value: Inactive



Compound #3

CAS: 79-44-7
Dataset id: 25 (Training set)
SMILES: O=C(N(C)C)Cl
Similarity: 0.597

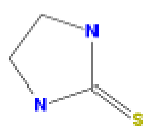
Experimental value: Inactive
Predicted value: Inactive



Compound #4

CAS: 927-67-3
Dataset id: 6 (Training set)
SMILES: NC(NCCC)=S
Similarity: 0.59

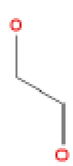
Experimental value: Inactive
Predicted value: Inactive



Compound #5

CAS: 96-45-7
Dataset id: 839 (Training set)
SMILES: N1C(NCC1)=S
Similarity: 0.581

Experimental value: Inactive
Predicted value: Inactive



Compound #6

CAS: 107-21-1
Dataset id: 22 (Training set)
SMILES: OCCO
Similarity: 0.573

Experimental value: Inactive
Predicted value: Inactive

3.2 Applicability Domain:

Measured Applicability Domain Scores

Global AD Index
AD index = 0
Explanation: the predicted compound is outside the Applicability Domain of the model.

Similar molecules with known experimental value
Similarity index = 0.628
Explanation: no similar compounds with known experimental value in the training set have been found.

Accuracy of prediction for similar molecules
Accuracy index = 1
Explanation: accuracy of prediction for similar molecules found in the training set is good.

Concordance for similar molecules
Concordance index = 1
Explanation: similar molecules found in the training set have experimental values that agree with the predicted value.

Model's descriptors range check
Descriptors range check = False
Explanation: 2 descriptor(s) for this compound have values outside the descriptor range of the compounds of the training set..

Atom Centered Fragments similarity check
ACF index = 1
Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

Symbols explanation:

The feature has a good assessment, model is reliable regarding this aspect.

The feature has a non optimal assessment, this aspect should be reviewed by an expert.

The feature has a bad assessment, model is not reliable regarding this aspect.

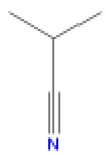
1. Prediction Summary

Prediction for compound Molecule 0

	Prediction: Reliability: **Prediction is Not predicted, but the result may be not reliable. A check of the information given in the following section should be done, paying particular attention to the following issues:** **- accuracy of prediction for similar molecules found in the training set is not optimal** **- similar molecules found in the training set have experimental values that disagree with the predicted value**

Compound: Molecule 0
Compound SMILES: N#CC
Experimental value: -
Predicted ER-mediated effect: Not predicted
No. alerts for activity: 0
No. alerts for possible activity: 0
No. alerts for non-activity: 0
No. alerts for possible non-activity: 0
Structural alerts: -
Reliability: the predicted compound is outside the Applicability Domain of the model
Remarks:
none

3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values

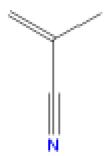


Compound #1

CAS: N.A.
Dataset id: 149 (Training set)
SMILES: N#CC(C)C
Similarity: 0.868

Experimental value: NON-active
Predicted value: NON-active

Alerts (not found in the target): ER non-activity alert no. 22

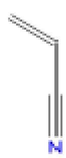


Compound #2

CAS: N.A.
Dataset id: 617 (Training set)
SMILES: N#CC(=C)C
Similarity: 0.844

Experimental value: NON-active
Predicted value: NON-active

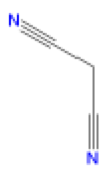
Alerts (not found in the target): ER non-activity alert no. 22



Compound #3

CAS: N.A.
Dataset id: 418 (Training set)
SMILES: N#CC=C
Similarity: 0.842

Experimental value: NON-active
Predicted value: Not predicted

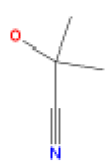


Compound #4

CAS: N.A.
Dataset id: 452 (Training set)
SMILES: N#CCC#N
Similarity: 0.76

Experimental value: NON-active
Predicted value: NON-active

Alerts (not found in the target): ER non-activity alert no. 22

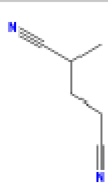


Compound #5

CAS: N.A.
Dataset id: 119 (Training set)
SMILES: N#CC(O)(C)C
Similarity: 0.733

Experimental value: NON-active
Predicted value: NON-active

Alerts (not found in the target): ER non-activity alert no. 22



Compound #6

CAS: N.A.
Dataset id: 1052 (Training set)
SMILES: N#CCCC(C#N)C
Similarity: 0.729

Experimental value: NON-active
Predicted value: NON-active

Alerts (not found in the target): ER non-activity alert no. 22

3.2 Applicability Domain:
Measured Applicability Domain Scores

✗	**Global AD Index** AD index = 0 Explanation: the predicted compound is outside the Applicability Domain of the model.
✓	**Similar molecules with known experimental value** Similarity index = 0.851 Explanation: strongly similar compounds with known experimental value in the training set have been found.
⚠	**Accuracy of prediction for similar molecules** Accuracy index = 0.671 Explanation: accuracy of prediction for similar molecules found in the training set is not optimal.
✗	**Concordance for similar molecules** Concordance index = 0 Explanation: similar molecules found in the training set have experimental values that disagree with the predicted value.
✓	**Atom Centered Fragments similarity check** ACF index = 1 Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

Symbols explanation:

✓ The feature has a good assessment, model is reliable regarding this aspect.
⚠ The feature has a non optimal assessment, this aspect should be reviewed by an expert.
✗ The feature has a bad assessment, model is not reliable regarding this aspect.

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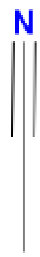
1. Prediction Summary

Prediction for compound Molecule 0

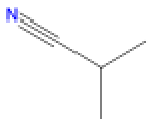


Prediction: Reliability:

Prediction is NON-active, the result appears reliable. Anyhow, you should check it through the evaluation of the information given in the following sections.

Compound: Molecule 0
Compound SMILES: N#CC
Experimental value: -
Predicted AR binding activity: NON-active
No. alerts for binding activity: 0
No. alerts for non-binding activity: 0
Structural alerts: -
Reliability: the predicted compound is into the Applicability Domain of the model
Remarks:
none

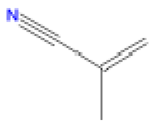
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 78-82-0
Dataset id: 874 (Training set)
SMILES: CC(C)C#N
Similarity: 0.868

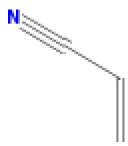
Experimental value: NON-active
Predicted value: NON-active



Compound #2

CAS: 126-98-7
Dataset id: 715 (Training set)
SMILES: CC(=C)C#N
Similarity: 0.844

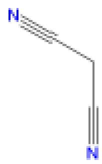
Experimental value: NON-active
Predicted value: NON-active



Compound #3

CAS: 107-13-1
Dataset id: 256 (Training set)
SMILES: C=CC#N
Similarity: 0.842

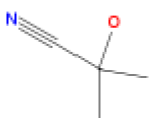
Experimental value: NON-active
Predicted value: NON-active



Compound #4

CAS: 109-77-3
Dataset id: 548 (Training set)
SMILES: N#CCC#N
Similarity: 0.76

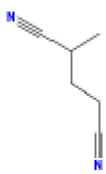
Experimental value: NON-active
Predicted value: NON-active



Compound #5

CAS: 75-86-5
Dataset id: 822 (Training set)
SMILES: CC(C)(O)C#N
Similarity: 0.733

Experimental value: NON-active
Predicted value: NON-active



Compound #6

CAS: 4553-62-2
Dataset id: 833 (Training set)
SMILES: CC(CCC#N)C#N
Similarity: 0.729

Experimental value: NON-active
Predicted value: NON-active

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3.2 Applicability Domain:

Measured Applicability Domain Scores

Global AD Index
AD index = 0.925
Explanation: the predicted compound is into the Applicability Domain of the model.

Similar molecules with known experimental value
Similarity index = 0.856
Explanation: strongly similar compounds with known experimental value in the training set have been found.

Accuracy of prediction for similar molecules
Accuracy index = 1
Explanation: accuracy of prediction for similar molecules found in the training set is good.

Concordance for similar molecules
Concordance index = 1
Explanation: similar molecules found in the training set have experimental values that agree with the predicted value.

Atom Centered Fragments similarity check
ACF index = 1
Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

Symbols explanation:

The feature has a good assessment, model is reliable regarding this aspect.

The feature has a non optimal assessment, this aspect should be reviewed by an expert.

The feature has a bad assessment, model is not reliable regarding this aspect.

APPENDIX E: Danish QSAR Endocrine Results for Acetonitrile (CAS #75-05-8)

Endocrine and Molecular Endpoints

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Estrogen Receptor α Binding, Full training set (Human *in vitro*)		INC_OUT	INC_OUT	NEG_OUT	NEG_OUT
Estrogen Receptor α Binding, Balanced Training Set (Human *in vitro*)		INC_OUT	INC_OUT	NEG_OUT	NEG_OUT
Estrogen Receptor α Activation (Human *in vitro*)		INC_OUT	INC_OUT	NEG_OUT	NEG_OUT
Estrogen Receptor Activation, CERAPP data (*in vitro*)		N/A	N/A	NEG_IN	N/A
Androgen Receptor Inhibition (Human *in vitro*)		INC_OUT	INC_OUT	NEG_OUT	NEG_OUT
Androgen Receptor Binding, CoMPARA data (*in vitro*)		N/A	N/A	INC_OUT	N/A
Androgen Receptor Inhibition, CoMPARA data (*in vitro*)		N/A	N/A	INC_OUT	N/A
Androgen Receptor Activation, CoMPARA data (*in vitro*)		N/A	N/A	INC_OUT	N/A
Thyroperoxidase (TPO) inhibition QSAR1 (Rat *in vitro*)		N/A	N/A	INC_OUT	N/A
Thyroperoxidase (TPO) inhibition QSAR2 (Rat *in vitro*)		N/A	N/A	INC_OUT	N/A
Sodium/iodide symporter (NIS), higher sensitivity		N/A	N/A	INC_OUT	N/A
Sodium/iodide symporter (NIS), higher specificity		N/A	N/A	INC_OUT	N/A
Thyroid Receptor α Binding (Human *in vitro*)					
- mg/L			6566.186	655.048	51.98921
- μM			159955.8	15957.32	1266.485
- Positive for $IC_{50} \leq 10$ μM					
- Positive for $IC_{50} \leq 100$ μM					
- Domain		OUT	OUT	OUT	OUT
Thyroid Receptor β Binding (Human *in vitro*)					
- mg/L			1328.353	11.46999	531.757
- μM			32359.38	279.4152	12953.89
- Positive for $IC_{50} \leq 10$ μM					
- Positive for $IC_{50} \leq 100$ μM					
- Domain		OUT	OUT	OUT	OUT
Arylhydrocarbon (AhR) Activation – Rational final model (Human *in vitro*)		N/A	N/A	INC_OUT	N/A
Arylhydrocarbon (AhR) Activation – Random final model (Human *in vitro*)		N/A	N/A	INC_OUT	N/A

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Pregnane X Receptor (PXR) Binding (Human *in vitro*)	N/A	INC_OUT	INC_OUT	NEG_OUT	NEG_OUT
Pregnane X Receptor (PXR) Binding (Human *in vitro*) NEW		N/A	N/A	INC_OUT	N/A
Pregnane X Receptor (PXR) Activation (Human *in vitro*)		N/A	N/A	INC_OUT	N/A
Pregnane X Receptor (PXR) Activation (Rat *in vitro*)		N/A	N/A	INC_OUT	N/A
CYP3A4 Induction (Human *in vitro*)		N/A	N/A	INC_OUT	N/A
Constitutive Androstane Receptor (CAR) Activation at max. 20 µM (*in vitro*)		N/A	N/A	INC_OUT	N/A
Constitutive Androstane Receptor (CAR) Activation at max. 50 µM (*in vitro*)		N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Inhibition at max. 20 µM (*in vitro*)		N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Inhibition at max. 50 µM (*in vitro*)		N/A	N/A	INC_OUT	N/A
DTU-developed models					

Estrogen Receptor Binding, alerts in:	
- parent only	Non binder, non cyclic structure
- metabolites from *in vivo* Rat metabolism simulator only	Non binder, non cyclic structure
- metabolites from Rat liver S9 metabolism simulator only	Non binder, non cyclic structure
rtER Expert System - USEPA, alerts in:	
- parent only	No alert found
- metabolites from *in vivo* Rat metabolism simulator only	No alert found
- metabolites from Rat liver S9 metabolism simulator only	No alert found
OECD QSAR Toolbox v.4.2 profilers	
Profiler predictions are supporting information to be used together with the relevant QSAR predictions	

APPENDIX F: OECD Toolbox Respiratory Sensitization Results for Acetonitrile (CAS #75-05-8)

Filter endpoint tree...	1 [target]
Structure	
Structure info	
Additional Ids	EC Number:2008352
CAS Number	75-05-8
CAS-SMILES relation	High
Chemical name(s)	Acetonitrile
Identity	Sources:33
Molecular formula	C2H3N
Predefined substance type	Mono constituent
SMILES	CC#N
Parameters	
Physical Chemical Properties	
Environmental Fate and Transport	
Ecotoxicological Information	
Human Health Hazards	
Profiling	
Endpoint Specific	
Respiratory sensitisation	No alert found

APPENDIX G: EPI Suite™ Modeling Results for Acetonitrile (CAS #75-05-8)

(Estimated values included in the GreenScreen® are highlighted and bolded)

CAS Number: 000075-05-8
SMILES : C(#N)C
CHEM : ACETONITRILE
MOL FOR: C2 H3 N1
MOL WT : 41.05
------------------------------ EPI SUMMARY (v4.11) --------------------------
Physical Property Inputs:
Log Kow (octanol-water): -0.34
Boiling Point (deg C) : 81.60
Melting Point (deg C) : -43.80
Vapor Pressure (mm Hg) : 88.8
Water Solubility (mg/L): 1E+006
Henry LC (atm-m3/mole) : 3.45E-005

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = -0.15
Log Kow (Exper. database match) = -0.34
Exper. Ref: HANSCH,C ET AL. (1995)

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 71.84 (Adapted Stein & Brown method)
Melting Pt (deg C): -83.79 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 81.6 (Mean VP of Antoine & Grain methods)
VP (Pa, 25 deg C) : 1.09E+004 (Mean VP of Antoine & Grain methods)
MP (exp database): -45 deg C
BP (exp database): 59.6 deg C
VP (exp database): 8.88E+01 mm Hg (1.18E+004 Pa) at 25 deg C

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 1.386e+005
log Kow used: -0.34 (user entered)
melt pt used: -43.80 deg C
Water Sol (Exper. database match) = 1e+006 mg/L (25 deg C)
Exper. Ref: RIDDICK,JA ET AL. (1986)

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 2.2266e+005 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found:
Neutral Organics

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 3.06E-005 atm-m3/mole (3.10E+000 Pa-m3/mole)
Group Method: 3.46E-005 atm-m3/mole (3.50E+000 Pa-m3/mole)

Exper Database: 3.45E-05 atm-m3/mole (3.50E+000 Pa-m3/mole)
For Henry LC Comparison Purposes:
User-Entered Henry LC: 3.450E-005 atm-m3/mole (3.496E+000 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 4.796E-006 atm-m3/mole (4.860E-001 Pa-m3/mole)
VP: 88.8 mm Hg (source: User-Entered)
WS: 1E+006 mg/L (source: User-Entered)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: -0.34 (user entered)
Log Kaw used: -2.851 (user entered)
Log Koa (KOAWIN v1.10 estimate): 2.511
Log Koa (experimental database): 2.310

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 1.0350
Biowin2 (Non-Linear Model) : 0.9992
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 3.0261 (weeks)
Biowin4 (Primary Survey Model) : 3.7233 (days-weeks)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.5579
Biowin6 (MITI Non-Linear Model): 0.7881
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): 0.7565
Ready Biodegradability Prediction: YES

Hydrocarbon Biodegradation (BioHCwin v1.01):
Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled): 1.18E+004 Pa (88.8 mm Hg)
Log Koa (Exp database): 2.310
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 2.53E-010
Octanol/air (Koa) model: 5.01E-011
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 9.15E-009
Mackay model : 2.03E-008
Octanol/air (Koa) model: 4.01E-009

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 0.0258 E-12 cm3/molecule-sec
Half-Life = 413.931 Days (12-hr day; 1.5E6 OH/cm3)
Ozone Reaction:
No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi):
1.47E-008 (Junge-Pankow, Mackay avg)

```
    4.01E-009 (Koa method)
  Note: the sorbed fraction may be resistant to atmospheric oxidation

 Soil Adsorption Coefficient (KOCWIN v2.00):
    Koc    :  4.67  L/kg (MCI method)
    Log Koc:  0.669       (MCI method)
    Koc    :  13.46  L/kg (Kow method)
    Log Koc:  1.129       (Kow method)

 Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
   Rate constants can NOT be estimated for this structure!

 Bioaccumulation Estimates (BCFBAF v3.01):
   Log BCF from regression-based method = 0.500 (BCF = 3.162 L/kg wet-wt)
   Log Biotransformation Half-life (HL) = -1.3475 days (HL = 0.04493 days)
   Log BCF Arnot-Gobas method (upper trophic) = -0.034 (BCF = 0.9244)
   Log BAF Arnot-Gobas method (upper trophic) = -0.034 (BAF = 0.9244)
       log Kow used: -0.34 (user entered)

 Volatilization from Water:
   Henry LC:  3.45E-005 atm-m3/mole  (entered by user)
   Half-Life from Model River:      11.53  hours
   Half-Life from Model Lake :      179.5  hours   (7.478 days)

 Removal In Wastewater Treatment:
   Total removal:               3.67  percent
   Total biodegradation:        0.09  percent
   Total sludge adsorption:     1.73  percent
   Total to Air:                1.85  percent
     (using 10000 hr Bio P,A,S)

 Level III Fugacity Model: (MCI Method)
           Mass Amount    Half-Life    Emissions
            (percent)        (hr)       (kg/hr)
   Air       11.9           1.55e+003    1000
   Water     40.3             360          1000
   Soil      47.8           720          1000
   Sediment  0.0816          3.24e+003    0
     Persistence Time: 347 hr

 Level III Fugacity Model: (MCI Method with Water percents)
           Mass Amount    Half-Life    Emissions
            (percent)        (hr)       (kg/hr)
   Air       11.9           1.55e+003    1000
   Water     40.3            360          1000
     water     (40.3)
     biota     (9.2e-007)
     suspended sediment (0.000282)
   Soil      47.8           720          1000
```

Sediment 0.0816 3.24e+003 0
Persistence Time: 347 hr

Level III Fugacity Model: (EQC Default)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	13	1.55e+003	1000
Water	44.1	360	1000
water	(44.1)		
biota	(1.01e-006)		
suspended sediment	(1.24e-005)		
Soil	42.8	720	1000
Sediment	0.0811	3.24e+003	0

Persistence Time: 327 hr

APPENDIX H: Change in Benchmark Score

Table 5 provides a summary of changes to the GreenScreen® Benchmark™ for acetonitrile. The original GreenScreen® assessment was performed in 2023 under version 1.4 criteria and ToxServices assigned a Benchmark 2 (BM-2) score.

Table 5: Change in GreenScreen® Benchmark™ for Acetonitrile			
Date	**GreenScreen® Benchmark™**	**GreenScreen® Version**	**Comment**
March 31, 2023	BM-2	v. 1.4	Original GreenScreen® assessment.

Licensed GreenScreen® Profilers

Acetonitrile GreenScreen® Evaluation Prepared by:

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Acetonitrile GreenScreen® Evaluation QC'd by:

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