2,2'-AZOBISISOBUTYRONITRILE

(CAS #78-67-1)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: August 18, 2021

Expiration Date: August 18, 2026

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GreenScreen® Executive Summary for 2,2'-Azobisisobutyronitrile (CAS #78-67-1)

2,2'-Azobisisobutyronitrile is an azo-containing organic compound that is used as a blowing agent for elastomers and plastics and as an initiator for free radical reactions. It is also a vinyl polymerization catalyst and a curing agent for unsaturated polyester resins. 2,2'-Azobisisobutyronitrile is an indirect food additive (21 CFR § 175.105, 21 CFR § 176.170, 21 CFR § 177.2420). It is readily ignited by sparks or flames.

2,2'-Azobisisobutyronitrile 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 2c
  - High Persistence-P + Moderate Group I Human Toxicity (carcinogenicity-C)
  - High P + Moderate Group II Human Toxicity (acute toxicity-AT and systemic toxicity-single dose-STs)
  - High P + High Ecotoxicity (acute aquatic-AA)
  - High P + Moderate Ecotoxicity (chronic aquatic-CA)
- Benchmark 2e
  - Moderate Group I Human Toxicity (C)
- Benchmark 2g
  - High Physical Hazards (reactivity-R)

A data gap (DG) exists for endocrine activity-E. As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), 2,2'-azobisisobutyronitrile meets requirements for a GreenScreen Benchmark™ Score of 2 despite the hazard data gap. In a worst-case scenario, if 2,2'-azobisisobutyronitrile 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 carcinogenicity, endocrine activity, respiratory sensitization, and bioaccumulation, and *in vitro* assays for endocrine activity and genotoxicity. 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 2,2'-azobisisobutyronitrile's NAMs dataset include no *in vivo* experimental or human data for carcinogenicity, endocrine activity, and respiratory sensitization, and lack of validated test methods for respiratory sensitization. 2,2'-Azobisisobutyronitrile's Type II (extrapolation output) uncertainties include uncertain *in vivo* relevance of *in silico* predictions and *in vitro* high throughput receptor binding assays of endocrine activity, the limitation of *in vitro* genotoxicity assays in mimicking metabolic systems, the lack of applicability domains for ToxCast models for endocrine activity, and the limitation of OECD Toolbox and Toxtree in identifying structural alerts without defining the applicability domain, and OECD Toolbox not accounting for non-immunologic mechanisms of respiratory sensitization. Some of 2,2'-azobisisobutyronitrile's type II uncertainties can be alleviated by the use of *in vitro* and/or in combination with *in vivo* data, and ECHA's decision framework to evaluate respiratory sensitization.

GreenScreen® Hazard Summary Table for 2,2'-Azobisisobutyronitrile

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	**L**	*L*	**L**	DG	**M**		*M*		*L*	**L**	*L*	**L**	**L**	**H**	*M*	*H*	**vL**	**H**	*L*

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 2,2'-Azobisisobutyronitrile (CAS #78-67-1)

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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Organization: ToxServices LLC
Date: August 13, 2021, November 15, 2021

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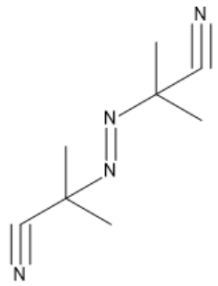
Organization: ToxServices LLC
Date: August 18, 2021, November 16, 2021

Expiration Date: November 16, 2026[2]

Chemical Name: 2,2'-Azobisisobutyronitrile

CAS Number: 78-67-1

Chemical Structure(s):



(PubChem 2021)

Also called:
2,2'-Azobis(2-methylpropionitrile); AIBN; Azobisisobutyronitrile; 2,2'-Azobisisobutyronitrile; Genitron; Aivn; Vazo; Azobisisobutylonitrile; Porofor N; 2,2'-AZOBIS(ISOBUTYRONITRILE);Azdh; Pianofor an; Porophor N; Aceto AZIB; Azobis(isobutyronitrile); Porofor 57; Chkhz 57; 2,2'-Azodiisobutyronitrile; VAZO 64; Poly-Zole AZDN; Propanenitrile, 2,2'-azobis[2-methyl-2,2'-Azobis(2-methylpropanenitrile); 2,2-Azodiisobutyronitrile; AZODIISOBUTYRONITRILE; 2,2'-Dimethyl-2,2'-azodipropiononitrile; Azodiisobutyrodinitrile; (E)-Azobis(isobutyronitrile); azo-bis-isobutyronitrile; alpha,alpha'-Azodiisobutyronitrile; UNII-FZ6PX8U5YB; 2,2'-Dicyano-2,2'-azopropane; NSC 1496; alpha,alpha-azoisobutyronitrile; 2,2'-Dimethyl-2,2'-azodipropionitrile; azo-bisisobutyronitrile; Azodi(isobutyronitrile); Propionitrile, 2,2'-azobis(2-methyl-; Propanenitrile, 2,2'-azobis(2-methyl-2-(2-cyanopropan-2-yldiazenyl)-2-methylpropanenitrile; Azobis(2-methylpropionitrile); 2,2'-Azodi(isobutyronitrile); 2,2'-Azobis[isobutyronitrile]; 34241-39-9; 2,2'-Dicyano-2,2'-azopropane; 2,2'-Dimethyl-2,2'-azodipropionitrile; alpha,alpha'-Azodiisobutyronitrile; Azodiisobutyrodinitrile; Azobisisobutyronitrile (PubChem 2021).

[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).

Suitable surrogates or moieties of chemicals used in this assessment (CAS #'s):
No surrogates were identified as sufficient data were available to assign a benchmark score for this chemical, and no surrogates with data were available to fill the data gap for endocrine activity.

Identify Applications/Functional Uses: (PubChem 2021)
1. Blowing agent for elastomers and plastics
2. Initiator for free radical reactions
3. Vinyl polymerization catalyst
4. Curing agent for unsaturated polyester resins.

Known Impurities[3]:
No information is available. The screen is performed on the theoretical pure substance.

GreenScreen® Summary Rating for 2,2'-Azobisisobutyronitrile[4,5 6,7]: 2,2'-Azobisisobutyronitrile 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 2c
  - High Persistence-P + Moderate Group I Human Toxicity (carcinogenicity-C)
  - High P + Moderate Group II Human Toxicity (acute toxicity-AT and systemic toxicity-single dose-STs)
  - High P + High Ecotoxicity (acute aquatic-AA)
  - High P + Moderate Ecotoxicity (chronic aquatic-CA)
- Benchmark 2e
  - Moderate Group I Human Toxicity (C)
- Benchmark 2g
  - High Physical Hazards (reactivity-R)

A data gap (DG) exists for endocrine activity-E. As outlined in GreenScreen® Guidance (CPA 2018b) Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), 2,2'-azobisisobutyronitrile meets requirements for a GreenScreen Benchmark™ Score of 2 despite the hazard data gap. In a worst-case scenario, if 2,2'-azobisisobutyronitrile 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 2,2'-Azobisisobutyronitrile

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	**L**	*L*	**L**	DG	**M**		*M*		*L*	**L**	*L*	**L**	**L**	**H**	*M*	*H*	**vL**	**H**	*L*

[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.

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.

Environmental Transformation Products

2,2'-Azobisisobutyronitrile does not readily undergo biodegradation, as shown by less than 10% degradation in a 28-day biodegradation test (OECD 301B) (Klimisch 2, reliable with restrictions) (ECHA 2021a). Additionally, in an OECD Guideline 111 test of hydrolysis properties as function of pH, 2,2'-azobisisobutyronitrile was considered to be stable and did not undergo hydrolysis (Klimisch score 2 - reliable with restrictions) (ECHA 2021a). Simulation using OECD Toolbox predicted the production of two autoxidation products: butanedinitrile, 2,2,3,3-tetramethyl- (CAS #3333-52-6) and nitrogen gas (CAS #7727-37-9) (OECD 2020a), as listed in Table 1, below. Nitrogen gas is naturally occurring in the air, and therefore not a relevant environmental transformation product. The other autoxidation product, butanedinitrile, 2,2,3,3-tetramethyl- is not an LT-1 chemical. Therefore, the Benchmark Score for 2,2'-azobisisobutyronitrile was not modified by transformation products.

Table 1: Environmental Transformation Product Summary

Life Cycle Stage	Transformation Pathway	Environmental Transformation Product	CAS #	Feasible (Yes or No)	Relevant (Yes or No)	GreenScreen® List Translator Score or GreenScreen® Benchmark™ Score[8,9]
Any	Autoxidation	Butanedinitrile, 2,2,3,3-tetramethyl-	3333-53-6	Yes	Yes	LT-P1
Any	Autoxidation	Nitrogen gas	7727-37-9	Yes	No	LT-U

Introduction

2,2'-Azobisisobutyronitrile is an azo-containing organic compound that is used as a blowing agent for elastomers and plastics and as an initiator for free radical reactions. It is also a vinyl polymerization catalyst and a curing agent for unsaturated polyester resins. 2,2'-Azobisisobutyronitrile is an indirect food additive (21 CFR § 175.105, 21 CFR § 176.170, 21 CFR § 177.2420) (PubChem 2021). ToxServices assessed 2,2'-azobisisobutyronitrile against GreenScreen® Version 1.4 (CPA 2018b) following procedures outlined in ToxServices' SOPs (GreenScreen® Hazard Assessment) (ToxServices 2020).

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 2021a). 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).

2,2'-Azobisisobutyronitrile is not listed on the SCIL.

[8] The GreenScreen® List Translator identifies specific authoritative or screening lists that should be searched to screen for GreenScreen Benchmark™ 1 chemicals (CPA 2018b). Pharos (Pharos 2021) is an online list-searching tool that is used to screen chemicals against the lists in the List Translator electronically.
[9] A GreenScreen® assessment of a transformation product depends on the Benchmark score of the parent chemical (see GreenScreen® Guidance).

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 2021) 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),[10] 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 2,2'-azobisisobutyronitrile can be found in Appendix C.

- 2,2'-Azobisisobutyronitrile is an LT-P1 chemical when screened using Pharos, and therefore a full GreenScreen® is required.
- 2,2'-Azobisisobutyronitrile is listed on the U.S. DOT list as a Hazard Class 4.1 chemical, Packing Group II.
- 2,2'-Azobisisobutyronitrile is on the following lists for multiple endpoints.
    - ChemSec – SIN List – Persistent, Mobile and Toxic
    - German FEA – Substances Hazardous to Waters – Class 2 – Hazard to Waters
- Specified lists for single endpoints are reported in individual hazard endpoints in the hazard assessment section below.

Hazard Statement and Occupational Control

European Union (EU) harmonized Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard statements were identified for 2,2'-azobisisobutyronitrile, as indicated in Table 2. General personal protective equipment (PPE) recommendations are presented in Table 3, below. No occupational exposure limits (OELs) were identified.

Table 2: GHS H Statements for 2,2'-Azobisisobutyronitrile (CAS #78-67-1) (ECHA 2021b)

H Statement	H Statement Details
H242	Self-react. C – Heating may cause fire
H302	Acute Tox. 4 – Harmful if swallowed
H332	Acute Tox. 4 – Harmful if inhaled
H412	Aquatic Chronic 3 – Harmful to aquatic life with long-lasting effects

Table 3: Occupational Exposure Limits and Recommended Personal Protective Equipment for 2,2'-Azobisisobutyronitrile (CAS #78-67-1)

Personal Protective Equipment (PPE)	Reference	Occupational Exposure Limits (OEL)	Reference
Respiratory protection – dust mask (Filter type P2), gloves (PVC; thickness 0.6 mm), safety glasses with side shields	ECHA 2021a	None identified	

Physicochemical Properties of 2,2'-Azobisisobutyronitrile

2,2'-Azobisisobutyronitrile is a white needle-like crystalline solid under standard temperature and pressure. It is not volatile, decomposes before boiling, and is moderately soluble in water (317.8 mg/L). 2,2'-Azobisisobutyronitrile will self-decompose or self-ignite when triggered by sparks or flames.

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

Table 4: Physical and Chemical Properties of 2,2'-Azobisisobutyronitrile (CAS #78-67-1)		
Property	**Value**	**Reference**
Molecular formula	C8H12N4	PubChem 2021
SMILES Notation	CC(C)(C#N)N=NC(C)(C)C#N	PubChem 2021
Molecular weight	164.21	PubChem 2021
Physical state	Solid	ECHA 2021a
Appearance	White needle-like crystals	ECHA 2021a
Melting point	103°C	ECHA 2021a
Boiling point	Decomposition occurs before boiling	ECHA 2021a
Vapor pressure	0.81 Pa at 25°C	ECHA 2021a
Water solubility	317.8 mg/L at 20°C (OECD Guideline 105)	ECHA 2021a
Dissociation constant	Non-ionizable functional groups	ECHA 2021a
Density/specific gravity	1.098 g/cm^3 at 20°C	ECHA 2021a
Partition coefficient	Log K_{ow} = 1.1 at 25°C (OECD Guideline 107)	ECHA 2021a

Toxicokinetics

In living organisms, 2,2'-azobisisobutyronitrile is metabolized to form hydrogen cyanide which is found in the blood, liver and brain (HSDB 2003). No *in vivo* toxicokinetic or metabolism studies were identified for 2,2'-azobisisobutyronitrile. In a GLP-compliant *in vitro* skin absorption study conducted according to OECD Guideline 428, viable human skin layers were exposed to 5 mg of 2,2'-azobisisobutyronitrile for 24 hours followed by rinsing each skin sample nine times with 1 mL extraction solution. Conductivity across the skin samples was measured before and after treatment to assess skin penetration potential. No changes in conductivity were observed after treatment with 2,2'-azobisisobutyronitrile, indicating that there was no loss of the barrier properties of the skin. Samples were analyzed by LC-MS/MS for the presence of 2,2'-azobisisobutyronitrile. Under the conditions of the study, 2,2'-azobisisobutyronitrile was shown to penetrate into the viable skin layers by its detection in the rinse samples (Klimisch score 1 - reliable without restriction) (ECHA 2021a).

Hazard Classification Summary

Group I Human Health Effects (Group I Human)

Carcinogenicity (C) Score (H, M, or L): *M*

2,2'-Azobisisobutyronitrile was assigned a score of Moderate for carcinogenicity based mixed predictions from Danish (Q)SAR and VEGA. GreenScreen® criteria classify chemicals as a Moderate hazard for carcinogenicity when there is marginal evidence for carcinogenicity (CPA 2018b). The confidence in the score is low as it is based on modeling and no experimental data were identified.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- DTU 2021 (Appendix D)
  - QSAR modeling with the Danish QSAR database resulted in the following predictions for 2,2'-azobisisobutyronitrile:
    - The Leadscope model predicted the compound to be negative for carcinogenicity with 4/7 FDA RCA Cancer models, positive with 2/7 models, and inconclusive with 1/7 models. The compound was within the applicability domain for 1 of the 4

negative models (female rat) and in the applicability domain for 2 of the 2 positive models (rat, rodent).
    - The E Ultra model predicted the compound to be inconclusive for carcinogenicity with all 7 FDA RCA Cancer models and the compound was outside the applicability domain for all 7.
    - For liver specific cancer in the rat or mouse, results were as follows:
        - Battery – inconclusive, and out of the applicability domain
        - CASE Ultra – inconclusive, and out of the applicability domain
        - Leadscope – negative, but out of the applicability domain
        - SciQSAR - inconclusive, and out of the applicability domain
- Toxtree 2018
    - Toxtree predicts 2,2'-azobisisobutyronitrile will not be a nongenotoxic carcinogen using the rulebase by ISS, but contains a structural alert for genotoxic carcinogenicity (aliphatic azo and azoxy) (Appendix E)
- VEGA 2021
    - 2,2'-Azobisisobutyronitrile was predicted to be a carcinogen by the Carcinogenicity model (CAESAR) 2.1.9 with moderate reliability (Global applicability domain (AD) Index = 0.735, similarity index = 0.747, accuracy index = 1, concordance index = 0.523) (Appendix F).
    - 2,2'-Azobisisobutyronitrile was predicted to be a carcinogen by the Carcinogenicity model (ISS) 1.0.2 with moderate reliability (Global AD Index = 0.863, similarity index = 0.744, accuracy index = 1, concordance index = 1) (Appendix F).
    - 2,2'-Azobisisobutyronitrile was predicted to be a non-carcinogen by the Carcinogenicity model (IRFMN/Antares) 1.0.0 with moderate reliability (Global AD Index = 0.77, similarity index = 0.738, accuracy index = 1, concordance index = 0.646) (Appendix F).
    - 2,2'-Azobisisobutyronitrile was predicted to be a non-carcinogen by the Carcinogenicity model (IRFMN/ISSCAN-CGX) 1.0.0 with low reliability (Global AD Index = 0, similarity index = 0.707, accuracy index = 1, concordance index = 0) (Appendix F).
    - 2,2'-Azobisisobutyronitrile was predicted to be a carcinogen by the oral Carcinogenicity model (IRFMN) 1.0.0 with moderate reliability (Global AD Index = 0.705, similarity index = 0.688, accuracy index = 1, concordance index = 1) (Appendix F).
    - 2,2'-Azobisisobutyronitrile was predicted to be a carcinogen by the inhalation Carcinogenicity model (IRFMN) 1.0.0 with low reliability (Global AD Index = 0.593, similarity index = 0.688, accuracy index = 1, concordance index = 0.499) (Appendix F).
- U.S. EPA 2021b
    - The carcinogenic potential of 2,2'-azobisisobutyronitrile was evaluated using OncoLogic™ (v9.0). 2,2'-Azobisisobutyronitrile was evaluated as an aliphatic azo and azoxy compound. Carcinogenic potential is dependent on metabolic activation of the azo containing compound. According to OncoLogic, the carcinogenic potential of aliphatic azo and azoxy compounds is reduced based on the presence of bulky substituents, highly hydrophilic substituents, and steric hindrance at the alpha-carbon. 2,2'-Azobisisobutyronitrile, an aliphatic azo compound with bulky cyano substituent groups which may block the metabolic activation pathway, has a level of concern of low (Appendix G).
- Based on a weight of evidence, a score of Moderate was assigned. Toxtree predicted that 2,2'-azobisisobutyronitrile will not be a nongenotoxic carcinogen but it contains a structural alert for genotoxic carcinogenicity – aliphatic azo and azoxy. However, OncoLogic evaluation of this alert indicates that the overall structure has a low cancer concern based on structural hindrance of the cyano group which may prevent metabolic activation of aliphatic azo compounds and carcinogenic activation. VEGA models produced mixed results. Two of the six models predicted 2,2'-

azobisisobutyronitrile to be a non-carcinogen with the global AD index >0.7 in one of the two models, indicating a reliable prediction. However, four of the six models predicted it to be a carcinogen, and the reliability was acceptable (global AD index >0.7) in 3 of the 4 models. Danish (Q)SAR Database models also produced mixed results. Of the in-domain predictions, it was predicted to be negative in one model and positive in two models. Based on the mixed predictions from VEGA and Danish (Q)SAR, ToxServices conservatively assigned a score of Moderate.

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

2,2'-Azobisisobutyronitrile was assigned a score of Low for mutagenicity/genotoxicity based on negative results in *in vitro* genotoxicity assays. GreenScreen® criteria classify chemicals as a Low hazard for mutagenicity/genotoxicity when negative data are available for both gene mutations and chromosome aberrations, 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 chemical.

- 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 2021a
  - *In vitro:* In a GLP-compliant bacterial reverse mutation assay, conducted in accordance with OCED Guideline 471, *Salmonella typhimurium* test strains TA98, TA100, TA1535, TA1537, and TA97 were tested at concentrations of 0, 313, 625, 1,250, 2,500, or 5,000 µg/plate 2,2'-azobisisobutyronitrile (99.9% purity) in DMSO, with and without metabolic activation. S9 metabolic activation mix was derived from rat liver induced with phenobarbital and 5,6-benzoflavone. The positive controls without metabolic activation were sodium azide (TA1535), 9-aminoacridine (TA1537 and TA97), and 2-aminoanthracene (TA1535, TA1537, TA98, and TA100) and with metabolic activation, the positive control was 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (TA98 and TA100). No mutagenic activity was observed under the conditions of this study (Klimisch score 1 – reliable without restriction).
  - *In vitro:* In a bacterial reverse mutation assay (GLP status not specified), conducted in a manner similar to OCED Guideline 471, *S. typhimurium* test strains TA98, TA100, TA1535, TA1537, and TA1538 were tested at concentrations of 0, 100, 200, 500, 1,000, 2,000, 5,000, and 10,000 µg/plate 2,2'-azobisisobutyronitrile (98% purity) in DMSO, with and without metabolic activation. S9 metabolic activation mix was derived from rat liver induced with sodium phenobarbital and 5,6-benzoflavone. The positive controls without metabolic activation were sodium azide (TA1535), 9-aminoacridine (TA1537), 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (TA98 and TA100), 2-nitrofluorene (TA1538) and 2-aminoanthracene (TA1535, TA1537, TA98, TA100, and TA1538) and with metabolic activation, the positive control was 4-nitroquinoline-N-oxide (TA1538). No mutagenic activity was observed under the conditions of this study (Klimisch score 2 – reliable with restriction).
  - *In vitro:* In a bacterial reverse mutation assay (GLP status not specified), conducted in a manner similar to OCED Guideline 471, *S. typhimurium* test strains TA98, TA100, TA1535, TA1537, and TA1538 were tested at concentrations of 0, 100, 333, 1,000, 3,333, and 10,000 µg/plate 2,2'-azobisisobutyronitrile (purity not specified) in DMSO, with and without metabolic activation. S9 metabolic activation mix was derived from rat liver induced with Aroclor 1254. The positive controls were not specified. No mutagenic activity was observed under the conditions of this study (Klimisch score 2 – reliable with restriction).
  - *In vitro:* In a bacterial reverse mutation assay (GLP status not specified), conducted in a manner similar to OCED Guideline 471, *S. typhimurium* test strains TA98, TA100, TA1535,

TA1537, and TA1538 were tested at concentrations of 0, 100, 333, 1,000, 3,333, and 10,000 µg/plate 2,2'-azobisisobutyronitrile (purity not specified) in DMSO, with and without metabolic activation. S9 metabolic activation mix was derived from rat and hamster liver induced with Aroclor 1254. The positive controls were not specified. No mutagenic activity was observed under the conditions of this study (Klimisch score 2 – reliable with restriction).

- *In vitro:* In a mammalian cell gene mutation assay, conducted in a manner similar to OECD Guideline 476, mouse lymphoma L5178Y cells were tested at concentrations of 600, 700, 800, 900, and 1000 µg/mL 2,2'-azobisisobutyronitrile (purity not reported) in DMSO, with and without metabolic activation. S9 metabolic activation mix was derived from rat liver induced with Aroclor 1254. Positive controls with and without metabolic activation were 3-methylcholantrene and ethylmethanesulphonate, respectively. No cytotoxicity was observed. 2,2'-Azobisisobutyronitrile did not induce any significant increase in the mutation frequency and no toxicity was observed (Klimisch score 2 – reliable with restriction).
- *In vitro:* In a GLP-compliant mammalian chromosome aberration test, conducted in accordance with OECD Guideline 473, Chinese hamster lung cells (CHL/IU) were tested at concentrations of 0, 0.40, 0.80, and 1.6 mg/L 2,2'-azobisisobutyronitrile (99.9% purity) in a 0.5% carboxymethylcellulose sodium solution, with and without metabolic activation. S9 metabolic activation mix was derived from rat liver induced with phenobarbital and 5,6-benzoflavone. Positive controls with and without metabolic activation were cyclophosphamide and mitomycin C, respectively. Under the conditions of the study, 2,2'-azobisisobutyronitrile did not induce any significant increase in the number of cells with chromosome aberrations, in the number of polyploid cells, nor in the number of cells with endoreduplicated chromosomes, and no toxicity was observed (Klimisch score 1 – reliable without restriction)**.**

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

2,2'-Azobisisobutyronitrile was assigned a score of Low for reproductive toxicity based on lack of specific effect on reproduction in an OECD 422 combined repeated dose toxicity and reproduction/developmental toxicity screening test in rats. Difficulty nursing offspring was observed at the highest dose of 50 mg/kg/day, but UNEP and U.S. EPA both attributed this effect to maternal toxicity rather than reproductive toxicity. GreenScreen® criteria classify chemicals as a Low hazard for reproductive toxicity when adequate data are available and they are not classified under GHS (CPA 2018b). The confidence in the score is low as it is based on a screening study with limited relevant endpoints examined.

- 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 2021a
  - In a GLP-compliant combined repeated dose toxicity and reproduction/developmental toxicity screening test, conducted in accordance with OECD Guideline 422, Sprague-Dawley rats (13/sex/dose) received doses of 2, 10, or 50 mg/kg/day 2,2'-azobisisobutyronitrile (99.9% purity) via gavage. Males were treated for 42 days and females were treated for 14 days prior to mating until day 3 of lactation. Reproductive indices, including copulation index, fertility index, gestation index, and duration of pregnancy, were evaluated. Effects of treatment on the estrus cycle and sperm measures and morphology were not examined, although testis and epididymis weights were recorded. One female in the 50 mg/kg/day group died on post-partum day 3. Decreased body weight and

food consumption was measured during early administrations in males at 50 mg/kg/day and in females at ≥ 10 mg/kg/day. No treatment-related effects on reproductive performance were observed, including copulation, fertility, duration of pregnancy, gestation index, and parturition. At 50 mg/kg/day, 3 of 12 dams had difficulty nursing and two of those animals let their offspring die within the first four days after birth. Live pup delivery index, overall delivery index, sex ratio, and body weight gain were comparable to controls. Viability of pups was not affected by treatment at doses less than 50 mg/kg/day. Study investigators identified a NOAEL of 10 mg/kg/day for reproductive and developmental toxicity in females and in pups (Klimisch score 2 – reliable with restriction). United States Environmental Protection Agency (U.S. EPA) assigned a NOAEL of 2 mg/kg/day and LOAEL of 10 mg/kg/day for maternal toxicity based on decreased body weight gain, and a NOAEL of 50 mg/kg/day for reproductive toxicity. U.S. EPA did not assign a developmental NOAEL/LOAEL due to insufficient information available to determine the significance of effects on viability of pups at birth and pup body weight on postnatal day (PND) 4 (U.S. EPA 2010). Similarly, United Nations Environment Programme (UNEP) considered difficulty taking care of the offspring to be due to maternal toxicity, and identified a NOAEL of 50 mg/kg/day for reproductive toxicity (OECD 1999).

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): L
2,2'-Azobisisobutyronitrile was assigned a score of Low for developmental toxicity based on lack of specific developmental toxicities observed in rats and rabbits in prenatal developmental toxicity studies. Decreased pup viability, post-implantation loss, and abortions were observed in developmental toxicity studies in rats and rabbits at maternally toxic doses, and study authors attributed these effects to maternal toxicity. GreenScreen® criteria classify chemicals as a Low hazard for developmental toxicity when adequate data are available and negative (CPA 2018b). The confidence in the score is high as it is based on reliable data on two animal species.

- 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 2021a
  - In a GLP-compliant prenatal developmental toxicity study, conducted in accordance with OECD Guideline 414, Crl:CD(SD) rats (22/group) were administered 0, 1, 5, or 20 mg/kg/day 2,2'-azobisisobutyronitrile (99% purity) via oral gavage. Females were treated on gestation days (GD) 6-19. One animal in low dose group died on GD7 and another in the mid dose group at GD 20 due to dosing error. Slight body weight loss was measured in all of the dose groups between GD 6 and GD 8 and a statistically significant reduced body weight gain was measured at GD 7 and GD 8 in the mid and high dose groups. A statistically significant reduction in food intake was observed in the mid and high dose groups between GD 6 and GD 8. The mean number of corpora lutea, mean incidence of pre- and post-implantation loss and mean litter size were not affected by treatment. Sex ratio, mean litter weight, mean placental weight and mean fetal weight were not affected by treatment, either. A NOAEL of 1 mg/kg/day was identified for maternal toxicity. Malformations were observed in 3 fetuses from 3 control litters (1 external/visceral, 2 skeletal), 4 fetuses from 3 low dose litters (skeletal), 5 fetuses from 2 mid dose litters (skeletal), and 9 fetuses from 8 high dose litters (3 external/visceral, 6 skeletal). The study authors conclude that the incidence and intergroup distribution of these fetal malformations are not indicative of an adverse treatment effect and when they reach statistical significance, these findings are not biologically significant because they are within the background range

or there is no clear treatment-related increase across treatment groups. A developmental toxicity NOAEL was estimated to be greater than 20 mg/kg/day (Klimisch score 1– reliable without restriction).

- In a GLP-compliant prenatal developmental toxicity study, conducted in accordance with OECD Guideline 414, New Zealand White rabbits (24/group) were administered 0, 3, 9, or 24 mg/kg/day 2,2'-azobisisobutyronitrile (99.5% purity) via gavage. Females were treated on GD 6-28. There were no treatment-related clinical findings in the low and mid dose groups. In the high dose group, females were emaciated, along with an absence of feces and urine, and blood in the bedding (correlated with low water consumption). Two females in the high dose group were euthanized following abortions on GD22 or GD23 and all other animals in the group were euthanized between GD17 and GD25 due to poor health condition. Abortions and deaths were considered by the study authors to be treatment related. There were treatment-related reductions in mean body weight, mean body weight change, and mean food consumption in the high dose group, resulting in premature euthanasia of the animals. A possible treatment related necropsy finding on the pyloric mucosa was observed in the mid dose group and necropsy findings in the stomach (thickened pyloric mucosa) and in the cecum were noted in the high dose group, contributing to poor condition and premature euthanasia. In the high dose group, 13/22 females had 90-100% post-implantation loss at premature euthanasia and there were 2 abortions. There were no treatment-related fetal external malformations or statistically significant treatment-related effect on internal malformations (comparable to historical control data). A NOAEL of 9 mg/kg/day for fetal effects (all females in the high dose group were sacrificed due to maternal toxicity) and a developmental NOAEL of 24 mg/kg/day (as effects were considered secondary to maternal toxicity) were identified (Klimisch score 1– reliable without restriction).
- In a GLP-compliant developmental toxicity study, which was conducted as a dose range finding study for the full study above, Crl:CD(SD) rats (7/group) were administered 0, 5, 25, or 75 mg/kg/day 2,2'-azobisisobutyronitrile (99% purity) via gavage. Females were treated on GD 6-19. Maternal toxicity was observed in the high dose group was observed as increased incidence of death, lack of appetite, and body weight loss. Embryo-fetal effects were also observed in the high dose group, including fetal loss, reduced fetal weight, and increased incidence of fetal variations. There were no significant treatment-related fetal defects, although there was an increase in the number and mean incidence of fetal variations (not specified) in the high dose group. An embryo-fetal NOEL of 25 mg/kg/day was identified (Klimisch score 1– reliable without restriction).
- In a non-GLP-compliant developmental toxicity study, which appears to be a dose range finding study for the full study above, New Zealand White rabbits (5/group) were administered 0, 7, 15, or 30 mg/kg/day 2,2'-azobisisobutyronitrile (99.5% purity) via gavage. Females were treated on GD 6-28. There were no treatment-related clinical findings in the low and mid dose groups. In the high dose group, females were emaciated, along with an absence of feces and urine, and blood in the bedding (correlated with low water consumption). All females in the high dose group were euthanized prematurely at GD13 due to excessive maternal toxicity, including body weight loss, and absence of food and water intake. In the high dose group, there were macroscopic findings in the stomach (dilatation with abnormal presence of dehydrated food and thickening of the pyloric mucosa). At the low and mid doses, there were no treatment-related effects at external examination of the fetuses. The high dose of 30 mg/kg/day was considered to exceed the Maximum Tolerated Dose (MTD) due to maternal toxicity (Klimisch score 1 – reliable without restriction).

  - In a GLP-compliant combined repeated dose toxicity and reproduction/developmental toxicity screening test, conducted in accordance with OECD Guideline 422, Sprague-Dawley rats(13/sex/dose) received doses of 2, 10, or 50 mg/kg/day 2,2'-azobisisobutyronitrile (99.9% purity) via gavage. Males were treated for 42 days and females were treated for 14 days prior to mating until day 3 of lactation. One female in the 50 mg/kg/day group died on post-partum day 3. Decreased body weight and food consumption was measured during early administrations in males at 50 mg/kg/day and in females at ≥ 10 mg/kg/day. Decreased body weight and food consumption was measured during pregnancy at 50 mg/kg/day. At 50 mg/kg/day, 3 of 12 dams had difficulty nursing and two of those animals let their offspring die within the first four days after birth. Live pup delivery index, overall delivery index, sex ratio, and body weight gain were comparable to controls. Viability of pups was not affected by treatment at doses less than 50 mg/kg/day. Study investigators identified a NOAEL of 10 mg/kg/day for reproductive and developmental toxicity in females and in pups (Klimisch score 2 – reliable with restriction).

- Based on the weight of evidence, a score of Low was assigned. For each of the full prenatal developmental toxicity study in rats and rabbits, range-finding studies were also described. ToxServices mainly relied on the full studies to score this endpoint as more animals and more appropriate doses were used. All developmental effects in these studies were attributed to maternal toxicity by the study authors. Therefore, GHS classification is not warranted.

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

2,2'-Azobisisobutyronitrile was assigned a score of DG for endocrine activity based on insufficient data. While the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) indicated that 2,2'-azobisisobutyronitrile was active in only 1/10 estrogen receptor assays, it was not active in the androgen receptor, steroidogenesis, and thyroid receptor assays. Danish QSAR predicted it to be negative for estrogen, androgen, and thyroid pathways, as well. No *in vivo* data were identified.

- 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 2021c
  - 2,2'-Azobisisobutyronitrile was active in 1/10 estrogen receptor (ER) assays, 0/9 androgen receptor (AR) assays, 0/2 steroidogenesis assays, and 0/9 thyroid receptor assays performed as part of the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) in the 21st Century.
  - 2,2'-Azobisisobutyronitrile was predicted to be inactive for androgen receptor agonism, antagonism, and binding using the COMPARA (consensus) model in ToxCast.
- DTU 2021
  - Modeling in the Danish QSAR database provides the following results that are within the applicability domains of the models (see Appendix H).
    - 2,2'-Azobisisobutyronitrile is predicted to be negative for estrogen receptor activation (CERAPP data *in vitro*) by the Leadscope model.
    - 2,2'-Azobisisobutyronitrile is predicted to be negative for androgen receptor binding (CoMPARA data *in vitro*), androgen receptor inhibition (CoMPARA data *in vitro*), and androgen receptor activation (CoMPARA data *in vitro*) by the Leadscope model.
    - 2,2'-Azobisisobutyronitrile is predicted to be negative for thyroperoxidase (TPO) inhibition (QSAR2 rat data *in vitro*) by the Leadscope model.

Group II and II* Human Health Effects (Group II and II* Human)

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): M

2,2'-Azobisisobutyronitrile was assigned a score of Moderate for acute toxicity based on GHS Harmonized EU classifications of Category 4 for oral and inhalation exposure routes and a reported oral LD_{50} value between 300 and 2,000 mg/kg, which corresponds to GHS Category 4 classification. GreenScreen® criteria classify chemicals as a Moderate hazard for acute toxicity when data for the most sensitive route of exposure meets the criteria for GHS Category 4 classification (CPA 2018b). The confidence in the score is high as it is based on Harmonized EU classifications of Category 4 for two routes of exposure and experimental data.

- Authoritative and Screening Lists
  - *Authoritative:*
    - EU – GHS – Acute Toxicity (oral) – Category 4 [H302]
    - EU – GHS – Acute Toxicity (inhalation) – Category 4 [H332]
  - *Screening:*
    - GHS – New Zealand – 6.1D (oral) – Acutely Toxic
    - GHS – New Zealand – 6.1D (inhalation) – Acutely Toxic
    - GHS – Japan – Acute Toxicity (oral) – Category 4 [H301]
    - GHS – Australia – Acute Toxicity (oral) – Category 4 [H302]
    - GHS – Australia – Acute Toxicity (inhalation) – Category 4 [H332]
- ChemIDplus 2021
  - *Oral*: LD_{50} (mouse) = 700 mg/kg
  - *Oral*: LD_{50} (rat) = 100 mg/kg (this study was given a Klimisch score of 4 (not assignable) in the REACH dossier)
- ECHA 2021a
  - *Oral:* 2,2'-Azobisisobutyronitrile (99.2% purity) was evaluated for acute oral toxicity according to OECD Guideline 423 (GLP compliant). Female Sprague-Dawley rats (3/low dose, 3/high dose) were orally exposed by gavage to the test substance at 300 and 2,000 mg/kg, followed by a 14-day observation period. No mortality occurred at the 300 mg/kg dose level. At 2,000 mg/kg, animals were observed to have signs of poor clinical condition, including hypoactivity, sedation, piloerection, tremors, dyspnea, hypersensitivity to touch and tonic clonic convulsions. The animals in the high dose group were euthanized on day 1 for ethical reasons. Hypoactivity, piloerection and rhinorrhea were observed in all of the low dose animals on days 1 and/or 2 and the rhinorrhea continued to be observed in 2/6 animals on day 3 and tremors and hypersensitivity to noises and being touched were observed in 3/6 animals on day 2. A lower body weight gain was measured between days 1 and 8 in 1/6 animals in the 300 mg/kg dose group, returning to normal after day 8. No abnormalities were observed in macroscopic examination of the main organs of treated animals. An LD_{50} between 300 and 2,000 mg/kg was estimated (Klimisch score 1 – reliable without restriction).
  - *Dermal:* 2,2'-Azobisisobutyronitrile (99.2% purity) was evaluated for acute dermal toxicity according to OECD Guideline 402 (GLP compliant). Male and female Sprague-Dawley rats (5/sex/group) were administered 2,000 mg/kg under semiocclusive conditions for 24 hours, followed by a 14-day observation period. No mortality, clinical signs, or cutaneous reactions, and no abnormalities in main organs during macroscopic examination were

observed in the study. An LD_{50} greater than 2,000 mg/kg was estimated for both males and females (Klimisch score 1 – reliable without restriction).

- OECD 1999
    - *Inhalation:* In an acute inhalation study, rats (strain and sex unspecified) were exposed to 2,2'-azobisisobutyronitrile for 4 hours at 12 g/m^3. Animals exhibited weight loss/decreased weight gain, exciting behavior, and conjunctival irritation. Study authors identified a 4-hour inhalation LC_{50} > 12 g/m^3, or 12 mg/L. No additional details were provided.

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

2,2'-Azobisisobutyronitrile was assigned a score of Data Gap for systemic toxicity (single dose) due to insufficient data identified. Japan classified it to GHS Category 1 based on lesions of the liver, lung, kidney, and stomach in rats after oral dosing and effects on thymus and kidney after an inhalation exposure to 12 mg/L. Limited details are available for these two studies, and ECHA dossier authors considered the oral study unreliable. Nevertheless, liver, lung, kidney and stomach effects in that study was reported at lethal doses. Therefore, they are not appropriate to serve as the basis for classification for this endpoint. Animals that survived in that study did not exhibit any lasting effects other than body weight loss, but the dose at which this effect was measured was not reported. In the inhalation study, the form of the test substance was not reported. Based on the low vapor pressure (i.e., 0.81 Pa, equivalent to 0.006 mmHg), it is likely to be an aerosol/dust exposure rather than vapor exposure. The exposure concentration of 12 mg/L is above the GHS dust cutoff of 5 mg/L for Category 2. Therefore, effects observed at this high concentration could not be used to classify it under GHS, either. Other studies available did not find significant systemic toxicities at relatively low doses, and it is unknown if at higher, but non-lethal doses, adverse systemic effect could occur.

- 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]
            - Category 1 classification based on lesions of the liver, lung, kidney, and stomach observed in rats administered at oral doses of 50 – 670 mg/kg 2,2'-azobisisobutyronitrile. In addition, aspiration of an inhalation dose (> 12 mg/L 2,2'-azobisisobutyronitrile resulted in slight atrophy of the thymus medulla and hyaline droplets in the renal tubule in rats (NITE 2015).
- ECHA 2021a.
    - *Oral:* 2,2'-Azobisisobutyronitrile (99.2% purity) was evaluated for acute oral toxicity according to OECD Guideline 423 (GLP compliant). Female Sprague-Dawley rats (3/low dose, 3/high dose) were orally exposed by gavage to the test substance at 300 and 2,000 mg/kg, followed by a 14-day observation period. No mortality occurred at the 300 mg/kg dose level. At 2,000 mg/kg, animals were observed to have signs of poor clinical condition, including hypoactivity, sedation, piloerection, tremors, dyspnea, hypersensitivity to touch and tonic clonic convulsions. The animals in the high dose group were euthanized on day 1 for ethical reasons. Hypoactivity, piloerection and rhinorrhea were observed in all of the low dose animals on days 1 and/or 2 and the rhinorrhea continued to be observed in 2/6 animals on day 3 and tremors and hypersensitivity to noises and being touched were observed in 3/6 animals on day 2. A lower body weight gain was measured between days 1 and 8 in 1/6 animals in the 300 mg/kg dose group, returning to normal after day 8. No

abnormalities were observed in macroscopic examination of the main organs of treated animals (Klimisch score 1 – reliable without restriction).

- *Oral:* In a non-GLP, non-guideline acute toxicity study, male ChR-CD rats (1/dose) received a single oral dose of 130, 200, 300, 450, 670, 1,000 1,500 or 2,250 mg/kg 2,2'-azobisisobutyronitrile and female ChR-CD rats (1/dose) received a single oral dose of 200, 300, 450 or 670 mg/kg 2,2'-azobisisobutyronitrile. All animals were observed until sacrifice, which was 11 days after dosing. Mortality occurred at 670 mg/kg and above in both sexes. Animals that survived to the scheduled sacrifice exhibited discomfort, irritability and weight loss (no additional details provided). Gross pathology findings were unremarkable in these animals. Animals that died during the study period exhibited discomfort, irritability, inactivity, convulsions, tremors, weight loss, polyurea, liver, lung, stomach and/or kidney damage, and brain congestion (Klimisch score 3 – not reliable).
  - *The REACH dossier authors assigned this study Klimisch score of 3 as it was conducted prior to GLP or establishment of guidelines, and were poorly reported. ToxServices considered this study weight of evidence as Japan used data from this study to classify 2,2'-azobisisobutyronitrile to GHS Cat. 1.*
- *Dermal:* 2,2'-Azobisisobutyronitrile (99.2% purity) was evaluated for acute dermal toxicity according to OECD Guideline 402 (GLP compliant). Male and female Sprague-Dawley rats (5/sex/group) were administered 2,000 mg/kg under semiocclusive conditions for 24 hours, followed by a 14-day observation period. No mortality, clinical signs, or cutaneous reactions, and no abnormalities in main organs during macroscopic examination were observed in the study (Klimisch score 1 – reliable without restriction).

- OECD 1999
  - *Inhalation:* In an acute inhalation study, rats (strain and sex unspecified) were exposed to 2,2'-azobisisobutyronitrile for 4 hours at 12 g/m^3. Animals exhibited weight loss/decreased weight gain, exciting behavior, and conjunctival irritation. Study authors identified a 4-hour inhalation LC_{50} > 12 g/m^3, or 12 mg/L. No additional details were provided.

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

2,2'-Azobisisobutyronitrile was assigned a score of Moderate for systemic toxicity (repeated dose) based on a NOAEL of 10 mg/kg/day in a 90-day oral toxicity study (which was the highest dose tested, and indicated that it was not classified to GHS Category 1) and a LOAEL of 50 mg/kg/day in an OECD 422 study (the NOAEL and LOAEL straddled the duration-adjusted GHS cutoff values, indicating the compound could be at least classified to GHS Category 2). Japan also classified it to GHS Category 2 based on a dog study that was considered to be not reliable by the REACH dossier authors due to poor reporting. GreenScreen® criteria classify chemicals as a Moderate hazard for systemic toxicity (repeated dose) when they are classified to GHS Category 2 (CPA 2018b). The confidence in the score is reduced due to uncertain significance (i.e., adaptive or adverse) of liver hypertrophy consistently observed in rat studies.

- 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]
      - In a 90-day feeding toxicity test with male and female dogs, animals were dosed at 300 ppm (male: 97.2 mg/kg/day, female: 94.2 mg/kg/day), an

increase of intracytoplasmic eosinophilic bodies in the liver cells, an increase in serum alkaline phosphatase activity, and an increase in relative liver weight were observed, which corresponds to a Category 2 classification (NITE 2015).

- ECHA 2021a
  - *Oral:* 2,2'-Azobisisobutyronitrile was evaluated in a repeated dose subchronic oral toxicity study according to OECD 408 (GLP-compliant). Male and female Crl:CD(SD) rats (10/sex/dose) were exposed to the test substance (99% purity) by gavage in corn oil, once daily for 92 days at 0, 0.5, 2, or 10 mg/kg/day. Animals were evaluated for cage side and clinical observations, body weight, food consumption, ophthalmoscopic examination, hematology, clinical chemistry, urinalysis, neurobehavioral examination, gross pathology, and histopathology. Additionally, locomotor activity was assessed during week 13 of the study and immunohistochemical staining for alpha-2-microglobulin was carried out to evaluate hyaline in the kidney. Six animals died during the study (3 control animals and 3 animals in the low dose group) due to dosing errors. Study authors considered the deaths to be unrelated to 2,2'-azobisisobutyronitrile treatment. No treatment-related clinical signs were observed and no toxicologically significant effects on ophthalmoscopy, functional observation battery, locomotor activity, body weights, food consumption, or hematology. A treatment-related increase in creatinine was measured in males and females had high levels of calcium and lower levels of chloride in all dose groups. Group mean adjusted liver weights were increased in all dose groups in males and in females in the mid and high dose groups. Equivalent liver to body weight ratios were also increased in all treated males and in females in the mid and high groups, as were liver to brain weight ratios when compared to controls. Increases in group mean adjusted kidney weights, equivalent kidney to body weight ratios, and kidney to brain weight ratios were observed in all treated male groups when compared to controls. There were no treatment related effects on kidney weights in treated females compared to controls. Hepatocyte hypertrophy, characterized by large clear vacuoles in hepatocytes primarily in the centrilobular region, was observed in all treated male groups and in females in the mid and high dose groups. Higher levels of hyaline droplets and tubular basophilia/focal nephropathy, along with increased immunohistochemical staining for alpha-2-microglobulin, were found in all treated male groups compared to control. Hyaline droplets were not observed in treated females. The liver hypertrophy was considered to be adaptive and the kidney changes, specific to male rats. Authors assigned the NOAEL at 10 mg/kg/day and considered 2,2'-azobisisobutyronitrile to be well-tolerated (Klimisch score 1 – reliable without restriction).
    - *While a LOAEL is not reported in this study for accurate GHS classification, the NOAEL of 10 mg/kg/day indicates that 2,2'-azobisisobutyronitrile is not classified to GHS Category 1.*
  - *Oral:* In a GLP-compliant combined repeated dose toxicity and reproduction/developmental toxicity screening test, conducted in accordance with OECD Guideline 422, Sprague-Dawley rats (13/sex/dose) received doses of 2, 10, or 50 mg/kg/day 2,2'-azobisisobutyronitrile (99.9% purity) via gavage. Males were treated for 42 days and females were treated for 14 days prior to mating until day 3 of lactation. Temporary salivation was observed after each administration in males at doses greater than 10 mg/kg/day. One female in the 50 mg/kg/day group died on post-partum day 3. Decreased body weight and food consumption was observed during early administrations in males at 50 mg/kg/day and in females at the mid and high doses. Decreased body weight and food consumption was measured during pregnancy at 50 mg/kg/day. Increased platelet and white

blood cell counts were measured in males at 50 mg/kg/day. Increased total protein and concentrations of albumin, total cholesterol, calcium, and inorganic phosphorus were measured at 50 mg/kg/day. Increased kidney weight was measured in all treated males and increased liver weight was measured in males at the mid and high doses. Liver and kidney weights were increased in females at 50 mg/kg/day. No abnormalities were observed during gross pathological examination. Increased eosinophilic bodies and basophilic changes of the renal tubular epithelial cells were observed in the kidneys of treated males. Granular casts were observed in lower nephrons as well. Centrilobular hypertrophy of hepatocytes was observed in males and females at the mid and high doses. Study investigators identified a NOAEL of 2 mg/kg/day for systemic toxicity based on pathological changes in the liver of both sexes (Klimisch score 2 – reliable with restriction).

- *ToxServices notes that liver hypertrophy was considered to be an adaptive, rather than adverse effect in the more comprehensive subchronic toxicity study described above. Kidney effects observed in males are also likely attributable to alpha-2μ-globulin and hence not relevant to humans. Therefore, ToxServices assigned a NOAEL of 10 mg/kg/day and LOAEL of 50 mg/kg/day based on decreased body weight and altered hematological and clinical chemistry parameters. As the study is approximately half the duration of a 90-day study, the NOAEL and LOAEL is compared to the doubled GHS cutoff values of 20 and 200 mg/kg/day.*

- *Oral:* In a short-term repeated dose (maximum tolerated dose) toxicity study (non-guideline, non-GLP), three female New Zealand White rabbits were administered doses of 10 and 30 mg/kg/day 2,2'-azobisisobutyronitrile (99.5% purity) in 1% methylcellulose via gavage for 7 days. The same animals were used for both doses. Clinical signs, body weight, food consumption, and gross pathology were evaluated. No concurrent controls were included in the study. None of the animal died during the study. No treatment-related effects were observed at 10 mg/kg/day. At 30 mg/kg/day, food consumption was reduced along with body weight and an absence of feces at the end of the study period. A dose of 30 mg/kg/day was considered to be close to the Maximum Tolerated Dose (MTD) (Klimisch score 1 – reliable without restriction).

Neurotoxicity (single dose, N-single) (Group II) Score (vH, H, M, or L): DG
2,2'-Azobisisobutyronitrile was assigned a score of Data Gap for neurotoxicity (single dose) based on insufficient data. Transient neurological signs such as hypoactivity, piloerection, tremors, hypersensitivity to touch in an acute oral study in rats at a non-lethal dose level of 300 mg/kg. Irritability was observed at unspecified non-lethal doses in another acute oral toxicity study. Exciting behavior (reversibility unspecified) was observed at 12 mg/L in an inhalation study in rats. These seem to suggest some neurological effects, but are not consistent with narcotic effects. It is unclear if these effects are specific to the test article or nonspecific effects reflecting the general discomfort of the animals after bolus dosing.

- 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 2021a
  - *Oral:* 2,2'-Azobisisobutyronitrile (99.2% purity) was evaluated for acute oral toxicity according to OECD Guideline 423 (GLP compliant). Female Sprague-Dawley rats (3/low dose, 3/high dose) were orally exposed by gavage to the test substance at 300 and 2,000 mg/kg, followed by a 14-day observation period. No mortality occurred at the 300 mg/kg dose level. At 2,000 mg/kg, animals were observed to have signs of poor clinical condition,

including hypoactivity, sedation, piloerection, tremors, dyspnea, hypersensitivity to touch and tonic clonic convulsions. The animals in the high dose group were euthanized on day 1 for ethical reasons. Hypoactivity, piloerection and rhinorrhea were observed in all of the low dose animals on days 1 and/or 2 and the rhinorrhea continued to be observed in 2/6 animals on day 3 and tremors and hypersensitivity to noises and being touched were observed in 3/6 animals on day 2. A lower body weight gain was measured between days 1 and 8 in 1/6 animals in the 300 mg/kg dose group, returning to normal after day 8. No abnormalities were observed in macroscopic examination of the main organs of treated animals (Klimisch score 1 – reliable without restriction).

- *Oral:* In a non-GLP, non-guideline acute toxicity study, male ChR-CD rats (1/dose) received a single oral dose of 130, 200, 300, 450, 670, 1,000 1,500 or 2,250 mg/kg 2,2'-azobisisobutyronitrile and female ChR-CD rats (1/dose) received a single oral dose of 200, 300, 450 or 670 mg/kg 2,2'-azobisisobutyronitrile. All animals were observed until sacrifice, which was 11 days after dosing. Mortality occurred at 670 mg/kg and above in both sexes. Animals that survived to the scheduled sacrifice exhibited discomfort, irritability and weight loss (no additional details provided). Gross pathology findings were unremarkable in these animals. Animals that died during the study period exhibited discomfort, irritability, inactivity, convulsions, tremors, weight loss, polyurea, liver, lung, stomach and/or kidney damage, and brain congestion (Klimisch score 3 – not reliable).
- *Dermal:* 2,2'-Azobisisobutyronitrile (99.2% purity) was evaluated for acute dermal toxicity according to OECD Guideline 402 (GLP compliant). Male and female Sprague-Dawley rats (5/sex/group) were administered 2,000 mg/kg under semiocclusive conditions for 24 hours, followed by a 14-day observation period. No mortality, clinical signs, or cutaneous reactions, and no abnormalities in main organs during macroscopic examination were observed in the study (Klimisch score 1 – reliable without restriction).

- OECD 1999
  - *Inhalation:* In an acute inhalation study, rats (strain and sex unspecified) were exposed to 2,2'-azobisisobutyronitrile for 4 hours at 12 g/m^3. Animals exhibited weight loss/decreased weight gain, exciting behavior, and conjunctival irritation. Study authors identified a 4-hour inhalation LC_{50} > 12 g/m^3, or 12 mg/L. No additional details were provided.

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

2,2'-Azobisisobutyronitrile was assigned a score of Low for neurotoxicity (repeated dose) based on ToxServices not classifying it as a repeated dose systemic toxicant due to neurotoxicity under GHS. GreenScreen® criteria classify chemicals as a Low hazard for neurotoxicity (repeated dose) when adequate data are available and they are not classified under GHS for systemic toxicity (repeated dose) due to neurotoxicity (CPA 2018b). The confidence in the score is low as the highest dose tested is below the GHS Category 2 cutoff of 100 mg/kg/day.

- 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 2021a
  - *Oral:* 2,2'-Azobisisobutyronitrile was evaluated in a repeated dose subchronic oral toxicity study according to OECD 408 (GLP-compliant). Male and female Crl:CD(SD) rats (10/sex/dose) were exposed to the test substance (99% purity) by gavage in corn oil, once daily for 92 days at 0, 0.5, 2, or 10 mg/kg/day. Animals were evaluated for cage side and clinical observations, body weight, food consumption, ophthalmoscopic examination, hematology, clinical chemistry, urinalysis, neurobehavioral examination, gross pathology,

and histopathology. Additionally, locomotor activity was assessed during week 13 of the study. Six animals died during the study (3 control animals and 3 animals in the low dose group) due to dosing errors. Study authors considered the deaths to be unrelated to 2,2'-azobisisobutyronitrile treatment. No treatment-related clinical signs were observed and no toxicologically significant effects on ophthalmoscopy, functional observation battery, locomotor activity, body weights, food consumption, or hematology. Authors assigned the NOAEL at 10 mg/kg/day and considered 2,2'-azobisisobutyronitrile to be well-tolerated with no indication of neurotoxicity (Klimisch score 1 – reliable without restriction).

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

2,2'-Azobisisobutyronitrile was assigned a score of Low for skin sensitization based on negative results in a guinea pig maximization test. GreenScreen® criteria classify chemicals as a Low hazard for skin sensitization 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 experimental data of good quality.

- 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 2021a
  - 2,2'-Azobisisobutyronitrile (99.2% purity) was not sensitizing in a GLP-compliant guinea pig maximization test conducted according to OECD Guideline 406. Male and female Dunkin-Hartley guinea pigs (5/sex in control, 10/sex treated group) were intradermally and subcutaneously induced with 0.1% and 100% w/w 2,2'-azobisisobutyronitrile, respectively, and intradermally and subcutaneously challenged with 0.1% and 100% w/w 2,2'-azobisisobutyronitrile, respectively. 2,4-Dinitrochlorobenzene was used as a positive control. No clinical signs and no treatment-related deaths were observed during the study. No positive reactions were observed. The authors concluded that 2,2'-azobisisobutyronitrile is not sensitizing under the conditions of the assay (Klimisch score 1 – reliable without restriction).
- OECD 1999
  - In an allergic patch test in humans (173 patients) with suspected occupational dermatoses, 1.0% 2,2'-azobisisobutyronitrile was applied to the skin for two days with occlusion and three readings were recorded. No allergic reactions were observed; therefore 2,2'-azobisisobutyronitrile was not considered to be sensitizing to the skin. No further study details were provided.

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

2,2'-Azobisisobutyronitrile was assigned a score of Low for respiratory sensitization based on absence of structural alerts and guidance from ECHA regarding assessment of respiratory sensitization potential. GreenScreen® criteria classify chemicals as a Low hazard for respiratory sensitization when they are not classifiable under GHS in the presence of adequate data (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 2020a
  - 2,2'-Azobisisobutyronitrile does not contain any structural alerts for respiratory sensitization (Appendix I)

- 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 2,2'-azobisisobutyronitrile 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 2,2'-azobisisobutyronitrile, and as 2,2'-azobisisobutyronitrile does not contain any structural alerts for respiratory sensitization (OECD 2020a), 2,2'-azobisisobutyronitrile is not expected to be a respiratory sensitizer.

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

2,2'-Azobisisobutyronitrile was assigned a score of Low for skin irritation/corrosivity based on the lack of dermal irritation observed in a rabbit study. GreenScreen® criteria classify chemicals as a Low hazard for skin irritation/corrosivity when they have negative data and are not GHS classified (CPA 2018b). The confidence in the score is high as it is based reliable experimental 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 2021a
  - 2,2'-Azobisisobutyronitrile (99.2% purity) was not irritating to the clipped skin of New Zealand White rabbits in a GLP-compliant study conducted in accordance with OECD Guideline 404. Three animals were administered on clipped skin a dressing containing 500 mg 2,2'-azobisisobutyronitrile, under semiocclusive conditions, for 4 hours. Treated skin was examined 1, 24, 48, and 72 hours after removal of test chemical for signs of erythema and edema. No positive reactions were observed at any of the time points (all erythema and edema time point scores were 0/4) (Klimisch score 1 – reliable without restriction).
- OECD 1999
  - In a skin irritation study in humans (173 patients) with suspected occupational dermatoses, 0.1% 2,2'-azobisisobutyronitrile was applied to the skin for two days with occlusion and three readings were recorded. A positive skin reaction was observed in one patient; therefore 2,2'-azobisisobutyronitrile was not considered to be irritating to the skin. No further study details were provided.

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

2,2'-Azobisisobutyronitrile was assigned a score of Low for eye irritation/corrosivity based on minimal ocular irritation effects that were insufficient for classification as a GHS eye irritant that were observed in rabbits. GreenScreen® criteria classify chemicals as a Low hazard for eye irritation/corrosivity when they have negative data and are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on reliable experimental 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 2021a
  - A GLP-compliant ocular irritation test conducted according to OECD 405 was performed with New Zealand rabbits (3 without rinsing; sex not specified) administered eye instillations of 100 mg 2,2'-azobisisobutyronitrile (99.2% purity) for approximately 1

second. The animals were observed for 21 days following installation without rinsing, with reactions scored at 1, 24, 48, and 72 hours. The mean chemosis score was 0.3/4 and the effects were fully reversible within 24 – 72 hours. The mean conjunctivae score was 0.6/3 and the effects were fully reversible within 48 – 72 hours. The mean iris score was 0.1/2 (1 animal) and the effects were fully reversible within 48 hours. The mean cornea opacity score (opacity and area) was 0.3/4 and the effects were fully reversible within 48 – 72 hours. The study authors concluded that 2,2'-azobisisobutyronitrile is not irritating to the eyes (Klimisch score 1 – reliable without restriction).

- *The effects observed in this study are not sufficient to classify 2,2'-azobisisobutyronitrile as a GHS Category 2 eye irritant. The criteria for a GHS Category 2 eye irritant are as follows: corneal opacity ≥ 1, and/or iritis ≥ 1, and/or conjunctival redness ≥ 2, and/or chemosis ≥ 2 (UN 2019).*

Ecotoxicity (Ecotox)

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

2,2'-Azobisisobutyronitrile was assigned a score of High for acute aquatic toxicity based on the most conservative EC_{50} of 4.5 mg/L in green algae for growth. GreenScreen® criteria classify chemicals as a High hazard for acute aquatic toxicity when acute aquatic toxicity values are >1 to 10 mg/L (CPA 2018b). The confidence in the score is high as it is based on reliable measured data for the target substance.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* New Zealand - GHS - 9.1B (fish) – Very ecotoxic in the aquatic environment (based on an estimated Log LC_{50} (mM/L) = 0.69 - 0.73 (log K_{ow} = 6.461 mg/L) in sheepshead minnow, NZ EPA 2021)
  - *Screening:* New Zealand - GHS - 9.1C (crustacean) - Harmful in the aquatic environment (based on an estimated Log LC_{50} (mM/L) = 1.72 - 0.91 (log K_{ow} = 21.072 mg/L) in daphnia, NZ EPA 2021).
  - *Screening:* New Zealand - GHS - 9.1C (algal) - Harmful in the aquatic environment (based on an estimated Log 96-h EC_{50} (mM/L) = 1.466 - 0.885 (log K_{ow} = 13.85 mg/L) in green algae, NZ EPA 2021).
- ECHA 2021a
  - 96-hour LC_{50} (*Danio rerio*, zebrafish) > 100 mg/L (GLP-compliant, OECD 203) (Klimisch score 1 – reliable without restriction)
  - 48-hour mobility EC_{50} (*Daphnia magna*) > 367 mg/L (GLP-compliant, OECD 202) (Klimisch score 2 – reliable with restriction)
  - 72-hour growth rate and biomass EC_{50} (*Desmodesmus subspicatus*, green algae) (GLP-compliant, OECD 201) (Klimisch score 1 – reliable without restriction):
    - 72-hour - EC_{50} - cell growth: 4.5 mg/L
    - 72-hour - EC_{50} - biomass: 3.9 mg/L
- OECD 1999
  - 96-hour LC_{50} (*Oryzias latipes*, Japanese rice fish) > 10 mg/L (GLP and guideline status not specified)
  - 72-hour biomass EC_{50} and NOEC (*Selenastrum capricornutum*, green algae) > 9.4 mg/L and 4.2, respectively (GLP and guideline status not specified)

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

2,2'-Azobisisobutyronitrile was assigned a score of Moderate for chronic aquatic toxicity based on a 21-day NOECr of 2.2 mg/L in daphnia and a 72-hour NOEC of 1.48 mg/L in algae. GreenScreen® criteria classify chemicals as a Moderate hazard for chronic aquatic toxicity when chronic aquatic toxicity values are >1.0 to 10 mg/L (CPA 2018b). The confidence in the score is low as it is based on experimental data from only two of 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.
  - *Other:*
    - EU – GHS (H-Statements) – H412 – Harmful to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) – Category 3)
    - GHS – Australia – H412 – Harmful to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) – Category 3)
- ECHA 2021a
  - 14-day LC_{50} (*O. latipes*, Japanese rice fish) > 10 mg/L (GLP-compliant, OECD 204) (Klimisch score 2 – reliable with restriction)
  - 21-day (rather than 14) acute immobilization test, followed by reproduction inhibition study (*D. magna*) (GLP-compliant, OECD 202) (Klimisch score 2 – reliable with restriction):
    - NOECr (maximum no-observed-effect concentration, reproduction) = 2.2 mg/L
    - LOECr (lowest-observed-effect concentration, reproduction) = 4.6 mg/L
  - 72-hour growth rate and biomass NOEC (*D. subspicatus*, green algae) (GLP-compliant, OECD 201) (Klimisch score 1 – reliable without restriction):
    - 72-hour NOEC - cell growth: 1.48 mg/L
    - 72-hour NOEC - biomass: 1.48 mg/L
- OECD 1999
  - 21-day reproduction test (*D. magna*) NOEC of 2.2 mg/L (GLP and guidance status not specified)

Environmental Fate (Fate)

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

2,2'-Azobisisobutyronitrile was assigned a score of High for persistence based on it degrading less than 10% in an OECD 301B test, and on being predicted to partition to soil with a half-life of 120 days. GreenScreen® criteria classify chemicals as a High hazard for persistence when the half-life in soil is > 60 to 180 days (CPA 2018b). The confidence in the score is low as it is based on modeled data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* EC – CEPA DSL – Persistent.
- ECHA 2021a
  - A GLP-compliant ready biodegradation test conducted according to OECD Guideline 301B (CO_2 Evolution Test) study was performed with domestic activated sludge exposed to 2,2'-azobisisobutyronitrile (99.2% purity) at 15 mg/L for 28 days. At the end of the exposure period, the level of degradation was less than 10%. 2,2'-Azobisisobutyronitrile was not readily biodegradable within the 28-day test period (Klimisch score 2 – reliable with restriction).
- OECD 1999

  - 2,2'-Azobisisobutyronitrile was not biodegradable in an OECD Guideline 301C study with 0% degradation after 28 days (no further study details provided).
- U.S. EPA 2017
  - The BIOWIN modeling Ready Biodegradable Predictor indicates that 2,2'-azobisisobutyronitrile is not expected to be readily biodegradable. Fugacity modeling (MCI method) predicts 61.6% will partition to soil with a half-life of 120 days, 35.2% will partition to water with a half-life of 60 days, 3.14% will partition to air with a half-life of 384 hours, and 0.111% will partition to the sediment with a half-life of 541.6 days (Appendix J).

Bioaccumulation (B) Score (vH, H, M, L, or vL): vL
2,2'-Azobisisobutyronitrile was assigned a score of Very Low for bioaccumulation based on a measured log K_{ow} 0f 1.1 and estimated BCF values ≤ 2.47. GreenScreen® criteria classify chemicals as a Very Low hazard for bioaccumulation when measured log K_{ow} values are ≤ 4 and the BCF is ≤100 (CPA 2018b). The confidence in the score is high as it is based on a measured value 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 2021a
  - 2,2'-Azobisisobutyronitrile has a log K_{ow} of 1.1.
- U.S. EPA 2017
  - BCFBAF predicts a BCF of 2.47 using the regression-based model based on a measured log K_{ow} of 1.1, and a BCF 1.98 using the Arnot-Gobas model for the upper trophic level, taking metabolism into consideration (Appendix J).

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M, or L): H
2,2'-Azobisisobutyronitrile was assigned a score of High for reactivity based on classification as a Type C self-reactive substance and Type 1.1, 1.2 or 1.3 explosive. GreenScreen® criteria classify chemicals as a High hazard for reactivity when they are classified as GHS Type C or D self-reactive substances or GHS Division 1.1, 1.2 or 1.3 explosives (CPA 2018b). The confidence in the score was high as it is based on reliable experimental data and an authoritative list.

- Authoritative and Screening Lists
  - *Authoritative:*
    - EU – GHS (H-Statements) – H242 – Heating may cause a fire [Self-reactive substances and mixtures; and Organic peroxides – Types C or D or E or F]
  - *Screening:*
    - GHS – Japan – H242 – Heating may cause a fire [Self-reactive substance and mixtures – Type C]
    - GHS – New Zealand – 4.1.2C – Self Reactive Substance: type C
    - GHS – Japan – H242 – Heating may cause a fire [Organic peroxides – Type C]
    - GHS – Australia – H242 – Heating may cause a fire [Self-reactive substances and mixtures; and Organic peroxides – Types C or D or E or F]
- Cameo Chemicals 2021

  - A chemical safety sheet for 2,2'-azobisisobutyronitrile reports an instability rating of 3 from NFPA ("Materials that are easily capable of explosive decomposition, but require an ignition source or will react explosively with water").
- ECHA 2021a
  - Explosiveness of 2,2'-azobisisobutyronitrile was determined using the EU Method A.14 on four batches of samples, as detailed below. The results of these test indicate that 2,2'-azobisisobutyronitrile is sensitive to heat and shock, and hence is classified as an R2 explosive substance under Directive 67/548/EEC (i.e., "risk of explosion by shock, friction, fire or other sources of ignition[11]", equivalent to GHS explosives Divisions 1.1, 1.2 or 1.3[12]) and self-reactive type C under EC Regulation 1272/2008.
    - The explosiveness of 2,2'-azobisisobutyronitrile (99.5% purity) was assessed in a GLP-compliant study according to guideline EU Method A.14. In a thermal sensitivity test (Koenen test), 2,2'-azobisisobutyronitrile was heated under defined confinement and one explosion occurred in 4 tests. In a mechanical sensitivity test (shock, BAM Falhammer), 2,2'-azobisisobutyronitrile was sensitive at 40 J (3 explosions in 3 tests) and at 7.5 J (3 explosions in 6 tests). In a mechanical sensitivity test (friction, BAM friction apparatus) loading 360N, 2,2'-azobisisobutyronitrile was not sensitive at a loading of 360 N (no explosions in 6 tests) (Klimisch score 1 – reliable without restriction).
    - The explosiveness of 2,2'-azobisisobutyronitrile (98.5% purity) was assessed in a GLP-compliant study according to guideline EU Method A.14. In a thermal sensitivity test (Koenen test), 2,2'-azobisisobutyronitrile was heated under defined confinement and two explosions occurred in one test. In a mechanical sensitivity test (shock, BAM Falhammer), 2,2'-azobisisobutyronitrile was sensitive at 40 J (1 explosion in 3 tests) and at 7.5 J (1 explosion in 6 tests). In a mechanical sensitivity test (friction, BAM friction apparatus) loading 360N, 2,2'-azobisisobutyronitrile was not sensitive at a loading of 360 N (Klimisch score 1 – reliable without restriction).
    - The explosiveness of 2,2'-azobisisobutyronitrile (85.4% purity) was assessed in a GLP-compliant study according to guideline EU Method A.14. In a thermal sensitivity test (Koenen test), 2,2'-azobisisobutyronitrile was heated under defined confinement and one explosion occurred in one test. In a mechanical sensitivity test (shock, BAM Falhammer), 2,2'-azobisisobutyronitrile was sensitive at 40 J (1 explosion was observed). In a mechanical sensitivity test (friction, BAM friction apparatus) loading 360N, 2,2'-azobisisobutyronitrile was not sensitive at a loading of 360 N (Klimisch score 1 – reliable without restriction).
    - The explosiveness of 2,2'-azobisisobutyronitrile (99% purity) was assessed in a GLP-compliant study according to guideline EU Method A.14. In a thermal sensitivity test (Koenen test), 2,2'-azobisisobutyronitrile was heated under defined confinement and one explosion occurred in four tests. In a mechanical sensitivity test (shock, BAM Falhammer), 2,2'-azobisisobutyronitrile was sensitive at 40 J (1 explosion in 2 tests) and at 7.5 J (1 explosion in 6 tests). In a mechanical sensitivity test (friction, BAM friction apparatus) loading 360N, 2,2'-azobisisobutyronitrile was not sensitive at a loading of 360 N (Klimisch score 1 – reliable without restriction).

[11] https://ec.europa.eu/environment/archives/dansub/pdfs/annex6_en.pdf
[12] https://osha.europa.eu/en/file/40573/

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

2,2'-Azobisisobutyronitrile was assigned a score of Low for flammability based on lack of additional flammability hazards, and its self-reactive and explosive properties that have already been captured in the reactivity endpoint above. GreenScreen® criteria classify chemicals as a Low hazard for flammability when available data indicate that the chemical does not warrant GHS classification as a flammable solid and the chemical is not present on authoritative or screening lists (CPA 2018b). The confidence in the score was low as it is not based on 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 2021b
  - According to its REACH dossier, the flammability study (required in Section 7.10) is not required because 2,2'-azobisisobutyronitrile is a solid which has explosive properties.

Use of New Approach Methodologies (NAMs)[13] in the Assessment, Including Uncertainty Analyses of Input and Output

New Approach Methodologies (NAMs) used in this GreenScreen® include *in silico* modeling for carcinogenicity, endocrine activity, respiratory sensitization, and bioaccumulation, and *in vitro* assays for endocrine activity and genotoxicity. NAMs are non-animal alternative 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 2020b). 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 2020b):

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

As shown in Table 5, Type I (input data) uncertainties in 2,2'-azobisisobutyronitrile's NAMs dataset include no *in vivo* experimental or human data for carcinogenicity, endocrine activity, and respiratory sensitization, and lack of validated test methods for respiratory sensitization. 2,2'-Azobisiso-butyronitrile's Type II (extrapolation output) uncertainties include uncertain *in vivo* relevance of *in silico* predictions and *in vitro* high throughput receptor binding assays of endocrine activity, the limitation of *in vitro* genotoxicity assays in mimicking metabolic systems, the lack of applicability domains for ToxCast models for endocrine activity, and the limitation of OECD Toolbox and Toxtree in identifying structural alerts without defining the applicability domain, and OECD Toolbox not accounting for non-immunologic mechanisms of respiratory sensitization. Some of 2,2'-azobisisobutyronitrile's type II uncertainties can be alleviated by the use of *in vitro* and/or in combination with *in vivo* data, and ECHA's decision framework to evaluate respiratory sensitization.

Table 5: Summary of NAMs Used in the GreenScreen® Assessment, Including Uncertainty Analyses	
Uncertainty Analyses (OECD 2020b)	
Type I Uncertainty: Data/Model Input	**Carcinogenicity**: No experimental data are available. **Endocrine activity**: No *in vivo* experimental data are available. **Respiratory sensitization**: No experimental data are available, and there are no validated test methods.
Type II Uncertainty: Extrapolation Output	**Carcinogenicity**: Toxtree only identifies structural alerts (SAs), and no applicability domain can be defined (Toxtree 2018). **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 system does not entirely mimic *in vivo* conditions[14].

[13] 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).

[14] https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1614097593&id=id&accname=guest&checksum=89925F80B9F4BD2FFC6E90F94A0EE427

	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).[15] The *in vitro* chromosome aberration assay (OECD 473) does not measure aneuploidy and it only measures structural chromosomal aberrations. The exogenous metabolic activation system does not entirely mirror *in vivo* metabolism[16]. **Endocrine activity:** The *in vivo* relevance of *in silico* receptor binding activity prediction and *in vitro* high throughput receptor binding assays is unclear due to lack of sufficient data on toxicokinetics. ToxCast models have no defined applicability domain/do not report reliability of the predictions. **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	Y	*In silico* modeling: VEGA/Toxtree/OncoLogic/ Danish QSAR
Mutagenicity	Y	*In vitro* data: Bacterial reverse mutation assay/*in vitro* gene mutation assay/*in vitro* chromosome aberration assay
Reproductive toxicity	N	
Developmental toxicity	N	
Endocrine activity	Y	*In vitro* high throughput data: EDSP Tox 21 screening assays *In silico* modeling: ToxCast models/ Danish QSAR
Acute mammalian toxicity	N	
Single exposure systemic toxicity	N	
Repeated exposure systemic toxicity	N	
Single exposure neurotoxicity	N	
Repeated exposure neurotoxicity	N	

[15] https://www.oecd-ilibrary.org/docserver/9789264264809-en.pdf?expires=1614097800&id=id&accname=guest&checksum=C0DE371FB9C5A878E66C9AB7F84E6BBE
[16] https://www.oecd-ilibrary.org/docserver/9789264264649-en.pdf?expires=1614098015&id=id&accname=guest&checksum=6A4F9CE52EA974F5A74793DD54D54352

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	N	
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 2,2'-Azobisisobutyronitrile (CAS #78-67-1)

TOXSERVICES
TOXICOLOGY RISK ASSESSMENT CONSULTING

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	2,2'-Azobisisobutyronitrile	78-67-1	*M*	L	*L*	L	DG	M		*M*		*L*	L	*L*	L	L	H	*M*	*H*	vL	H	*L*

Table 3: Hazard Summary Table

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

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
2,2'-Azobisisobutyronitrile	2

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

Table 6

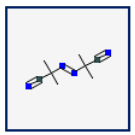
Chemical Name	Final GreenScreen® Benchmark Score
2,2'-Azobisisobutyronitrile	2

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 2,2'-Azobisisobutyronitrile (CAS #78-67-1)



78-67-1

2,2'-Azobisisobutyronitrile

ALSO CALLED .alpha.,.alpha.-Azobisisobutyronitrile, .alpha.,.alpha.'-Azobis(isobutylonitrile), .alpha.,.alpha.'-...

View all synonyms (154)

Share Profile

Hazards | Properties | Functional Uses | Process Chemistry | 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	-	-	-	-	-	M	-	-	-	-	-	-	-	-	-	-	-	vH-H	-	H	-	vH	-	-	-	R

ENDPOINT	HAZARD LEVEL	GS SCORE	LIST NAME	HAZARD DESCRIPTION	OTHER LISTS
Acute Mammalian Toxicity	M	LT-UNK	EU - GHS (H-Statements)	H302 - Harmful if swallowed [Acute toxicity (oral) - Category 4]	+9
	M	LT-UNK	EU - GHS (H-Statements)	H332 - Harmful if inhaled [Acute toxicity (inhalation) - Category 4]	
	H	LT-UNK	GHS - Japan	H301 - Toxic if swallowed [Acute Toxicity (oral) - Category 3]	
	H-M	LT-UNK	GHS - Australia	H302 - Harmful if swallowed [Acute toxicity (oral) - Category 4]	
	H-M	LT-UNK	GHS - Australia	H332 - Harmful if inhaled [Acute toxicity (inhalation) - Category 4]	
	M	LT-UNK	GHS - New Zealand	6.1D (inhalation) - Acutely toxic	
	M	LT-UNK	GHS - New Zealand	6.1D (oral) - Acutely toxic	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H301 - Toxic if swallowed (unverified) [Acute toxicity (oral) - Category 3]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H302 - Harmful if swallowed (unverified) [Acute toxicity (oral) - Category 4]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H332 - Harmful if inhaled (unverified) [Acute toxicity (inhalation) - Category 4]	

Persistence	vH-H	LT-UNK	EC - CEPA DSL	Persistent	+1
	pC	NoGS	ChemSec - SIN List	Persistent, Mobile and Toxic	
Reactivity	H	LT-UNK	GHS - Japan	H242 - Heating may cause a fire [Self-reactive substances and mixtures - Type C]	+5
	H	LT-UNK	GHS - New Zealand	4.1.2C - Self Reactive Substances: type C	
	H	LT-UNK	GHS - Japan	H242 - Heating may cause a fire [Organic peroxides - Type C]	
	vH-M	LT-UNK	EU - GHS (H-Statements)	H242 - Heating may cause a fire [Self-reactive substances and mixtures; and Organic peroxides - Types C or D or E or F]	
	vH-M	LT-UNK	GHS - Australia	H242 - Heating may cause a fire [Self-reactive substances and mixtures; and Organic peroxides - Types C or D or E or F]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H242 - Heating may cause a fire (unverified) [Self-reactive substances and mixtures; and Organic peroxides - Types C or D or E or F]	

T & P and/or B [(Chronic Aquatic Toxicity and Persistence) or (Acute Aquatic Toxicity and Persistence and/or Bioaccumulation)]	M	LT-UNK	GHS - Australia	H412 - Harmful to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) - Category 3]	+6
	U	LT-UNK	EU - GHS (H-Statements)	H412 - Harmful to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) - Category 3]	
	U	LT-P1	GHS - New Zealand	9.1B (fish) - Very ecotoxic in the aquatic environment	
	U	LT-UNK	GHS - New Zealand	9.1C (algal) - Harmful in the aquatic environment	
	U	LT-UNK	GHS - New Zealand	9.1C (crustacean) - Harmful in the aquatic environment	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H410 - Very toxic to aquatic life with long lasting effects (unverified) [Hazardous to the aquatic environment (chronic) - Category 1]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H412 - Harmful to aquatic life with long lasting effects (unverified) [Hazardous to the aquatic environment (chronic) - Category 3]	
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	

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]
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]

Restricted Substance Lists (2)

- CA SCP - Candidate Chemicals: Candidate Chemical List ✱
- EU - PACT-RMOA Substances: Substances selected for RMOA or hazard assessment

APPENDIX D: Danish QSAR Carcinogenicity Modeling Results for 2,2'-Azobisisobutyronitrile (CAS #78-67-1)

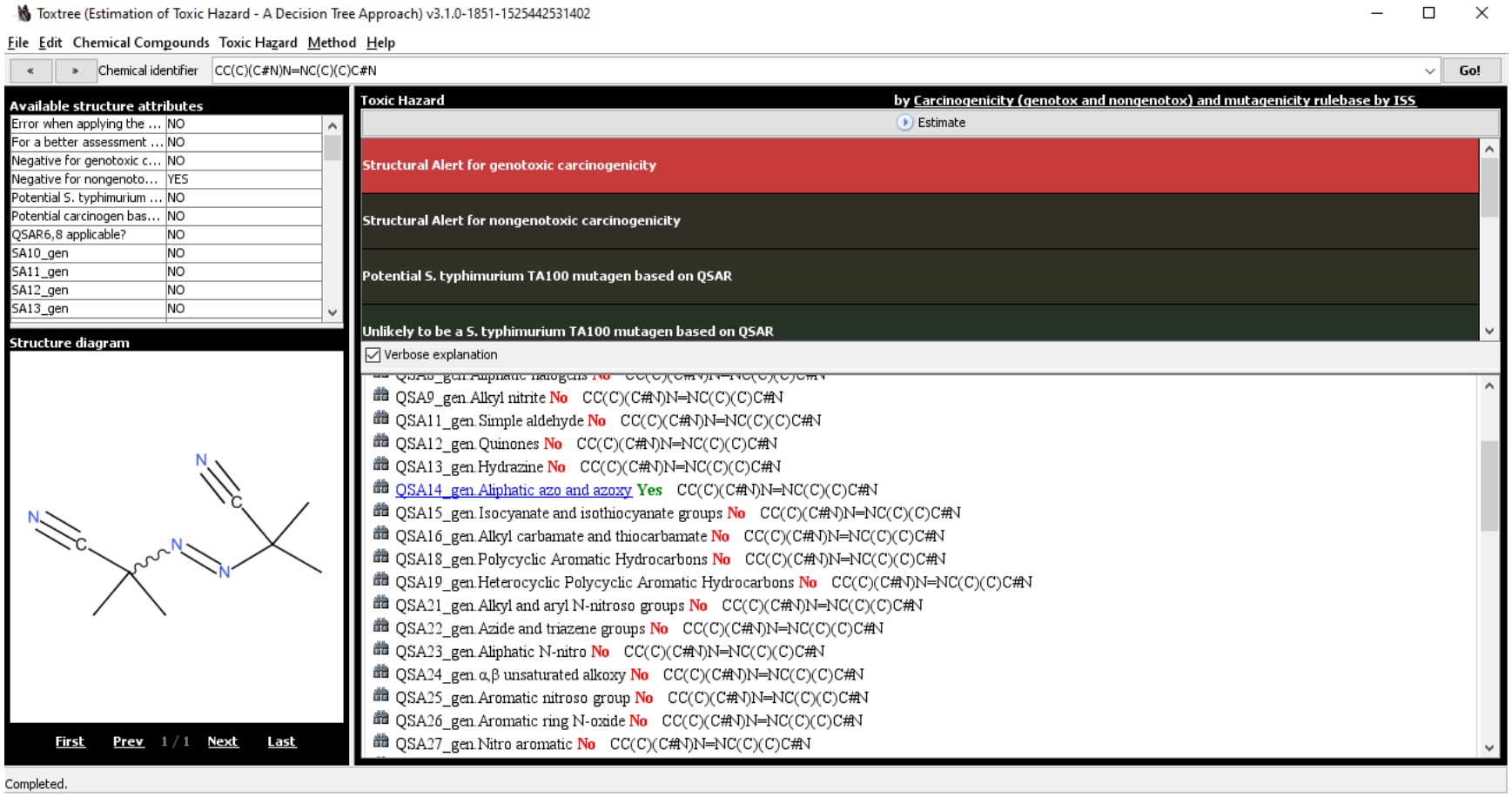
Carcinogenicity

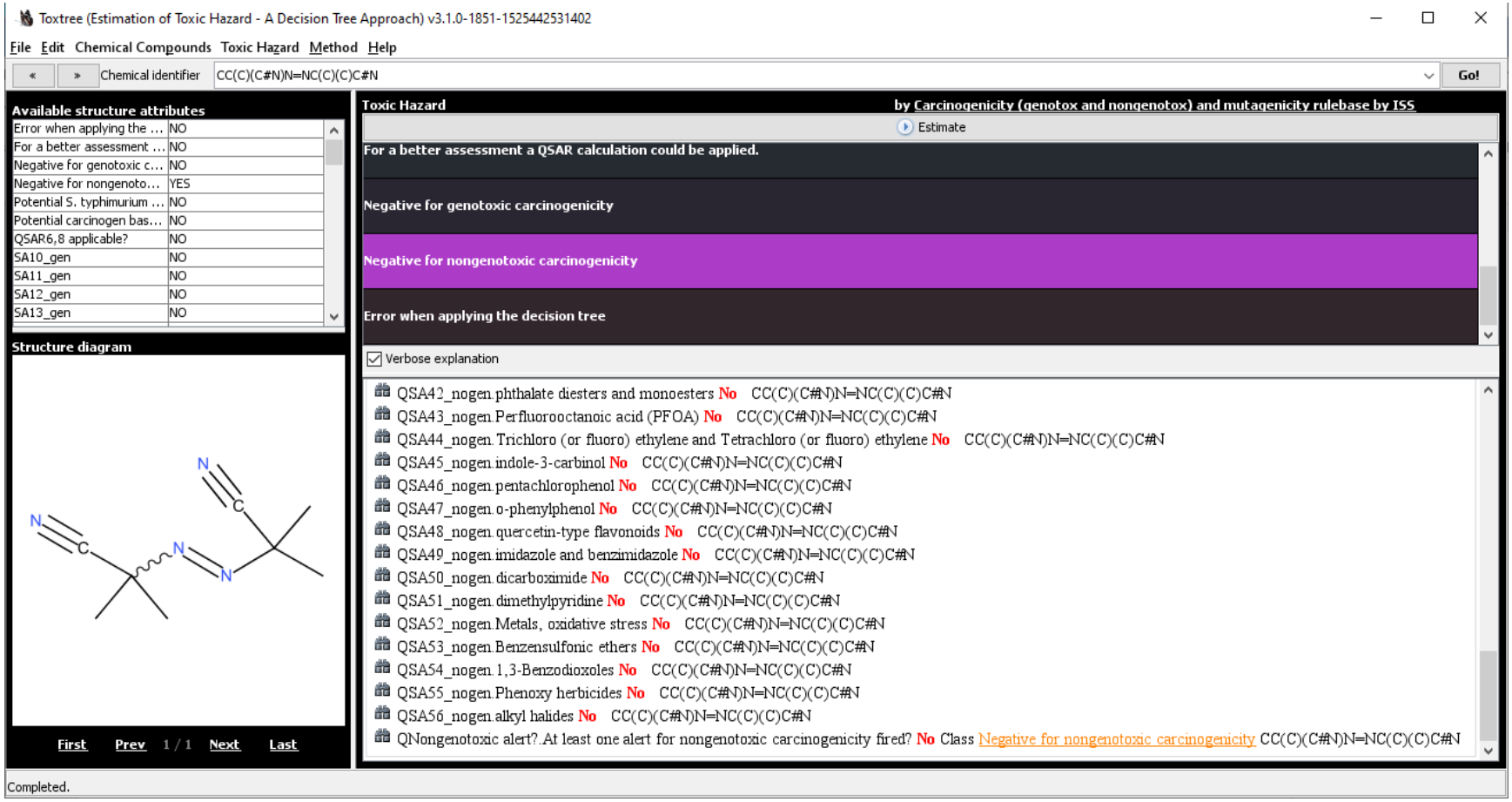
	E Ultra	Leadscope
FDA RCA Cancer Male Rat	INC_OUT	INC_OUT
FDA RCA Cancer Female Rat	INC_OUT	NEG_IN
FDA RCA Cancer Rat	INC_OUT	POS_IN
FDA RCA Cancer Male Mouse	INC_OUT	NEG_OUT
FDA RCA Cancer Female Mouse	INC_OUT	NEG_OUT
FDA RCA Cancer Mouse	INC_OUT	NEG_OUT
FDA RCA Cancer Rodent	INC_OUT	POS_IN
Commercial models from CASE Ultra and Leadscope		
FDA RCA: Data from US Food and Drug Administration as part of Research Cooperation Agreement		

Carcinogenicity (genotox and nongenotox) alerts by ISS, alerts in:	
- parent only	Aliphatic azo and azoxy (Genotox); Structural alert for genotoxic carcinogenicity
Oncologic Primary Classification, alerts in:	
- parent only	Aliphatic Azo and Azoxy Type Compounds
OECD QSAR Toolbox v.4.2 profilers	
Profiler predictions are supporting information to be used together with the relevant QSAR predictions	

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Liver Specific Cancer in Rat or Mouse		INC_OUT	INC_OUT	NEG_OUT	INC_OUT
DTU-developed models					

APPENDIX E: Toxtree Carcinogenicity Results for 2,2'-Azobisisobutyronitrile (CAS #78-67-1)

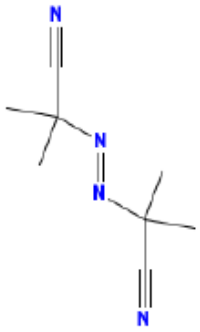




APPENDIX F: VEGA Carcinogenicity Results for 2,2'-Azobisisobutyronitrile (CAS #78-67-1)

1. Prediction Summary

Prediction for compound Molecule 0

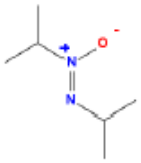


Prediction: Reliability:

Prediction is Carcinogen, but the result shows some critical aspects, which require to be checked:
- only moderately similar compounds with known experimental value in the training set have been found
- some similar molecules found in the training set have experimental values that disagree with the predicted value

Compound: Molecule 0
Compound SMILES: N#CC(N=NC(C#N)(C)C)(C)C
Experimental value: -
Predicted Carcinogen activity: Carcinogen
P(Carcinogen): 0.997
P(NON-Carcinogen): 0.003
Reliability: the predicted compound could be out of the Applicability Domain of the model
Remarks:
none

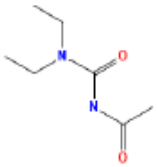
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 17967-53-9
Dataset id: 72 (Training set)
SMILES: [O-][N+](=NC(C)C)C(C)C
Similarity: 0.773

Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #2

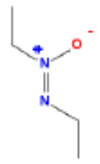
CAS: N.A.
Dataset id: 239 (Test set)
SMILES: O=C(NC(=O)C)N(CC)CC
Similarity: 0.725

Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen

Compound #3

CAS: 17697-55-1
Dataset id: 71 (Training set)
SMILES: [O-][N+](=NCCC)CCC
Similarity: 0.72

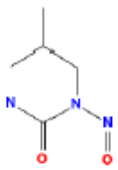
Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #4

CAS: 16301-26-1
Dataset id: 304 (Test set)
SMILES: [O-][N+](=NCC)CC
Similarity: 0.72

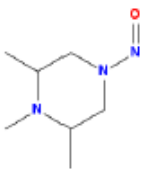
Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #5

CAS: 760-60-1
Dataset id: 553 (Test set)
SMILES: O=NN(C(=O)N)CC(C)C
Similarity: 0.702

Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #6

CAS: 75881-18-4
Dataset id: 566 (Training set)
SMILES: O=NN1CC(N(C)C(C)C1)C
Similarity: 0.697

Experimental value: Carcinogen
Predicted value: Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

Global AD Index
AD index = 0.735
Explanation: the predicted compound could be out of the Applicability Domain of the model.

Similar molecules with known experimental value
Similarity index = 0.747
Explanation: only moderately 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 = 0.523
Explanation: some similar molecules found in the training set have experimental values that disagree with the predicted value.

Model's descriptors range check
Descriptors range check = True
Explanation: descriptors for this compound have values inside 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.

Model class assignment reliability
Pos/Non-Pos difference = 0.994
Explanation: model class assignment is well defined.

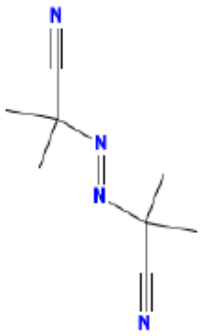
Neural map neurons concordance
Neurons concordance = 1
Explanation: predicted value agrees with experimental values of training set compounds laying in the same neuron.

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

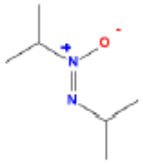
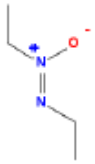
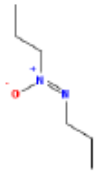
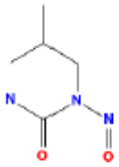
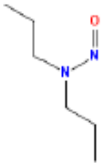


Prediction: Reliability:

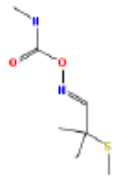
Prediction is Carcinogen, but the result shows some critical aspects, which require to be checked:
- only moderately similar compounds with known experimental value in the training set have been found
The following alerts have been found: SA14 Aliphatic azo and azoxy

Compound: Molecule 0
Compound SMILES: N#CC(N=NC(C#N)(C)C)(C)C
Experimental value: -
Predicted Carcinogen activity: Carcinogen
Structural alerts: SA14 Aliphatic azo and azoxy
Reliability: the predicted compound could be out of 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: 795 (Training set) SMILES: [O-][N+](=NC(C)C)C(C)C Similarity: 0.773 Experimental value: Carcinogen Predicted value: Carcinogen Alerts (found also in the target): SA14 Aliphatic azo and azoxy
	Compound #2 CAS: 16301-26-1 Dataset id: 431 (Training set) SMILES: [O-][N+](=NCC)CC Similarity: 0.72 Experimental value: Carcinogen Predicted value: Carcinogen Alerts (found also in the target): SA14 Aliphatic azo and azoxy
	Compound #3 CAS: 17697-55-1 Dataset id: 794 (Training set) SMILES: [O-][N+](=NCCC)CCC Similarity: 0.72 Experimental value: Carcinogen Predicted value: Carcinogen Alerts (found also in the target): SA14 Aliphatic azo and azoxy
	Compound #4 CAS: 760-60-1 Dataset id: 575 (Training set) SMILES: O=NN(C(=O)N)CC(C)C Similarity: 0.702 Experimental value: Carcinogen Predicted value: Carcinogen Alerts (not found in the target): SA21 Alkyl and aryl N-nitroso groups
	Compound #5 CAS: 621-64-7 Dataset id: 520 (Training set) SMILES: O=NN(CCC)CCC Similarity: 0.688 Experimental value: Carcinogen Predicted value: Carcinogen Alerts (not found in the target): SA21 Alkyl and aryl N-nitroso groups

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



Compound #6

CAS: 116-06-3
Dataset id: 93 (Training set)
SMILES: O=C(ON=CC(C)(C)SC)NC
Similarity: 0.685

Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

⚠	**Global AD Index** AD index = 0.863 Explanation: the predicted compound could be out of the Applicability Domain of the model.
⚠	**Similar molecules with known experimental value** Similarity index = 0.744 Explanation: only moderately 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.

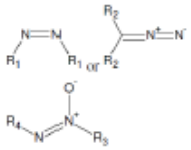
Symbols explanation:

✓ 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.

4.1 Reasoning:
Relevant Chemical Fragments and Moieties

(Molecule 0) Reasoning on fragments/structural alerts:

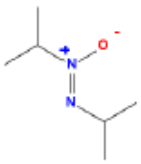
Fragment found: SA14 Aliphatic azo and azoxy



R1= Aliphatic carbon or hydrogen
R2, R3 = Any atom/group
R4 = Aliphatic carbon

Aliphatic azo and azoxy. Chemicals fired by alert SA22 should be excluded from this alert.

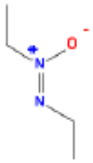
Following, the most similar compounds from the model's dataset having the same fragment.



CAS: N.A.
Dataset id: 795 (Training set)
SMILES: [O-][N+](=NC(C)C)C(C)C
Similarity: 0.773

Experimental value: Carcinogen
Predicted value: Carcinogen

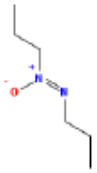
Alerts (found also in the target): SA14 Aliphatic azo and azoxy



CAS: 16301-26-1
Dataset id: 431 (Training set)
SMILES: [O-][N+](=NCC)CC
Similarity: 0.72

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): SA14 Aliphatic azo and azoxy



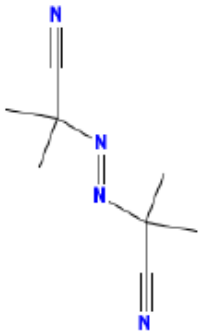
CAS: 17697-55-1
Dataset id: 794 (Training set)
SMILES: [O-][N+](=NCCC)CCC
Similarity: 0.72

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (found also in the target): SA14 Aliphatic azo and azoxy

1. Prediction Summary

Prediction for compound Molecule 0

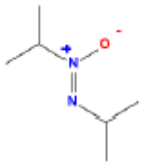
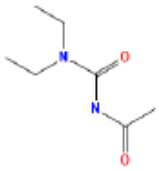
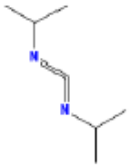
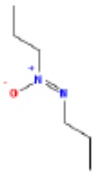
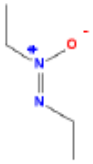


Prediction: Reliability:

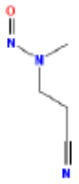
Prediction is Possible NON-Carcinogen, but the result shows some critical aspects, which require to be checked:
- only moderately similar compounds with known experimental value in the training set have been found
- some similar molecules found in the training set have experimental values that disagree with the predicted value

Compound: Molecule 0
Compound SMILES: N#CC(N=NC(C#N)(C)C)(C)C
Experimental value: -
Predicted Carcinogenic activity: Possible NON-Carcinogen
No. alerts for carcinogenicity: 0
Structural alerts: -
Reliability: the predicted compound could be out of 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: 72 (Training set) SMILES: [O-][N+](=NC(C)C)C(C)C Similarity: 0.773 Experimental value: Carcinogen Predicted value: Carcinogen Alerts (not found in the target): Carcinogenity alert no. 64; Carcinogenity alert no. 92; Carcinogenity alert no. 126
	Compound #2 CAS: N.A. Dataset id: 239 (Training set) SMILES: O=C(NC(=O)C)N(CC)CC Similarity: 0.725 Experimental value: NON-Carcinogen Predicted value: Possible NON-Carcinogen
	Compound #3 CAS: N.A. Dataset id: 960 (Training set) SMILES: C(=NC(C)C)=NC(C)C Similarity: 0.723 Experimental value: NON-Carcinogen Predicted value: Possible NON-Carcinogen
	Compound #4 CAS: N.A. Dataset id: 71 (Training set) SMILES: [O-][N+](=NCCC)CCC Similarity: 0.72 Experimental value: Carcinogen Predicted value: Carcinogen Alerts (not found in the target): Carcinogenity alert no. 64; Carcinogenity alert no. 92; Carcinogenity alert no. 126
	Compound #5 CAS: N.A. Dataset id: 304 (Training set) SMILES: [O-][N+](=NCC)CC Similarity: 0.72 Experimental value: Carcinogen Predicted value: Carcinogen Alerts (not found in the target): Carcinogenity alert no. 64; Carcinogenity alert no. 92; Carcinogenity alert no. 126

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



Compound #6

CAS: N.A.
Dataset id: 873 (Training set)
SMILES: N#CCCN(N=O)C
Similarity: 0.714

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): Carcinogenity alert no. 8; Carcinogenity alert no. 15; Carcinogenity alert no. 50; Carcinogenity alert no. 51; Carcinogenity alert no. 54; Carcinogenity alert no. 55; Carcinogenity alert no. 63

3.2 Applicability Domain:
Measured Applicability Domain Scores

⚠	**Global AD Index** AD index = 0.77 Explanation: the predicted compound could be out of the Applicability Domain of the model.
⚠	**Similar molecules with known experimental value** Similarity index = 0.738 Explanation: only moderately 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 = 0.646 Explanation: some 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:

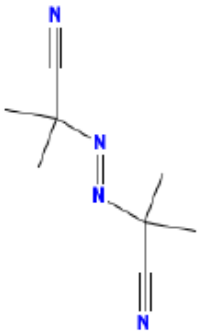
✓ The feature has a good assessment, model is reliable regarding this aspect.

⚠ 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.

1. Prediction Summary

Prediction for compound Molecule 0

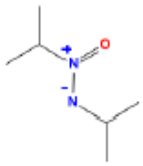


Prediction: Reliability:

Prediction is Possible NON-Carcinogen, 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:
- only moderately similar compounds with known experimental value in the training set have been found
- similar molecules found in the training set have experimental values that disagree with the predicted value

Compound: Molecule 0
Compound SMILES: N#CC(N=NC(C#N)(C)C)(C)C
Experimental value: -
Predicted Carcinogenic activity: Possible NON-Carcinogen
No. alerts for carcinogenicity: 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

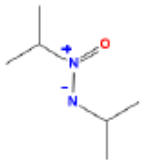


Compound #1

CAS: N.A.
Dataset id: 729 (Training set)
SMILES: O=[N+]([N-]C(C)C)C(C)C
Similarity: 0.709

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): Carcinogenity alert no. 1; Carcinogenity alert no. 14; Carcinogenity alert no. 27

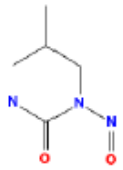


Compound #2

CAS: 17697-53-9
Dataset id: 797 (Training set)
SMILES: O=[N+]([N-]C(C)C)C(C)C
Similarity: 0.709

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): Carcinogenity alert no. 1; Carcinogenity alert no. 14; Carcinogenity alert no. 27

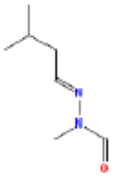


Compound #3

CAS: 760-60-1
Dataset id: 485 (Training set)
SMILES: O=NN(C(=O)N)CC(C)C
Similarity: 0.702

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): Carcinogenity alert no. 1; Carcinogenity alert no. 3; Carcinogenity alert no. 14; Carcinogenity alert no. 27; Carcinogenity alert no. 28

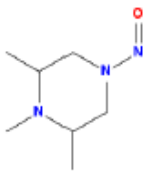


Compound #4

CAS: 57590-21-3
Dataset id: 862 (Training set)
SMILES: O=CN(N=CCC(C)C)C
Similarity: 0.697

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): Carcinogenity alert no. 3; Carcinogenity alert no. 28



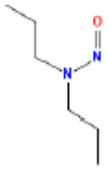
Compound #5

CAS: 75881-18-4
Dataset id: 892 (Training set)
SMILES: O=NN1CC(N(C)C(C)C1)C
Similarity: 0.697

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): Carcinogenity alert no. 1; Carcinogenity alert no. 14; Carcinogenity alert no. 27

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



Compound #6

CAS: 621-64-7
Dataset id: 432 (Training set)
SMILES: O=NN(CCC)CCC
Similarity: 0.688

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): Carcinogenity alert no. 1; Carcinogenity alert no. 14; Carcinogenity alert no. 27

3.2 Applicability Domain:
Measured Applicability Domain Scores

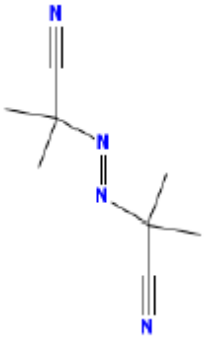
✖	**Global AD Index** AD index = 0 Explanation: the predicted compound is outside the Applicability Domain of the model.
⚠	**Similar molecules with known experimental value** Similarity index = 0.707 Explanation: only moderately similar compounds with known experimental value in the training set have 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 = 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.

1. Prediction Summary

Prediction for compound Molecule 0

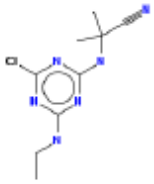
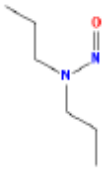
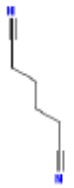
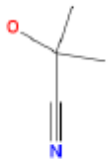
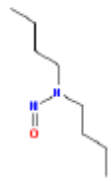


Prediction: Reliability:

Prediction is Carcinogen, but the result shows some critical aspects, which require to be checked:
- only moderately similar compounds with known experimental value in the training set have been found
- some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (1 infrequent fragments found)

Compound: Molecule 0
Compound SMILES: N#CC(N=NC(C#N)(C)C)(C)C
Experimental value: -
Predicted Oral Carcinogenic class: Carcinogen
Reliability: the predicted compound could be out of the Applicability Domain of the model
Remarks:
none

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

	Compound #1 CAS: 21725-46-2 Dataset id: 84 (Training set) SMILES: N#CC(Nc1nc(nc(n1)Cl)NCC)(C)C Similarity: 0.689 Experimental value: Carcinogen Predicted value: Carcinogen
	Compound #2 CAS: 621-64-7 Dataset id: 225 (Training set) SMILES: O=NN(CCC)CCC Similarity: 0.688 Experimental value: Carcinogen Predicted value: Carcinogen
	Compound #3 CAS: 116-06-3 Dataset id: 328 (Training set) SMILES: O=C(ON=CC(C)(C)SC)NC Similarity: 0.685 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen
	Compound #4 CAS: 111-69-3 Dataset id: 327 (Training set) SMILES: N#CCCCCC#N Similarity: 0.684 Experimental value: NON-Carcinogen Predicted value: Carcinogen
	Compound #5 CAS: 75-86-5 Dataset id: 322 (Training set) SMILES: N#CC(O)(C)C Similarity: 0.679 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen
	Compound #6 CAS: 924-16-3 Dataset id: 224 (Test set) SMILES: O=NN(CCCC)CCCC Similarity: 0.678 Experimental value: Carcinogen Predicted value: Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

⚠	**Global AD Index** AD index = 0.705 Explanation: the predicted compound could be out of the Applicability Domain of the model.
⚠	**Similar molecules with known experimental value** Similarity index = 0.688 Explanation: only moderately 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 = True Explanation: descriptors for this compound have values inside the descriptor range of the compounds of the training set.
⚠	**Atom Centered Fragments similarity check** ACF index = 0.85 Explanation: some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (1 infrequent fragments found).

Symbols explanation:

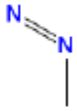
✓ The feature has a good assessment, model is reliable regarding this aspect.

⚠ 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.

4.1 Reasoning:
Relevant Chemical Fragments and Moieties

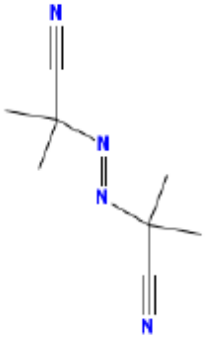
(Molecule 0) Reasoning on rare and missing Atom Centered Fragments.
The following Atom Centered Fragments have been found in the molecule, but they are not found or rarely found in the model's training set:



Fragment defined by the SMILES: N=NC
The fragment has less than 3 occurrences in the model's training set

1. Prediction Summary

Prediction for compound Molecule 0



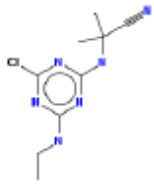
Prediction: Reliability:

Prediction is Carcinogen, 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:
- only moderately similar compounds with known experimental value in the training set have been found
- similar molecules found in the training set have experimental values that disagree with the predicted value
- some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (1 infrequent fragments found)

Compound: Molecule 0
Compound SMILES: N#CC(N=NC(C#N)(C)C)(C)C
Experimental value: -
Predicted Inhalation Carcinogenic class: Carcinogen
Reliability: the predicted compound is outside the Applicability Domain of the model
Remarks:
none

3.1 Applicability Domain:

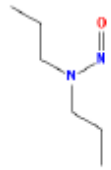
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 21725-46-2
Dataset id: 372 (Training set)
SMILES: N#CC(Nc1nc(nc(n1)Cl)NCC)(C)C
Similarity: 0.689

Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #2

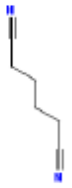
CAS: 621-64-7
Dataset id: 193 (Test set)
SMILES: O=NN(CCC)CCC
Similarity: 0.688

Experimental value: Carcinogen
Predicted value: Carcinogen

Compound #3

CAS: 116-06-3
Dataset id: 275 (Test set)
SMILES: O=C(ON=CC(C)(C)SC)NC
Similarity: 0.685

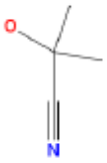
Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #4

CAS: 111-69-3
Dataset id: 273 (Training set)
SMILES: N#CCCCCC#N
Similarity: 0.684

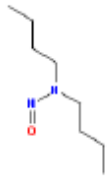
Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #5

CAS: 75-86-5
Dataset id: 268 (Training set)
SMILES: N#CC(O)(C)C
Similarity: 0.679

Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #6

CAS: 924-16-3
Dataset id: 192 (Training set)
SMILES: O=NN(CCCC)CCCC
Similarity: 0.678

Experimental value: Carcinogen
Predicted value: Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

✖	**Global AD Index** AD index = 0.593 Explanation: the predicted compound is outside the Applicability Domain of the model.
⚠	**Similar molecules with known experimental value** Similarity index = 0.688 Explanation: only moderately 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 = 0.499 Explanation: similar molecules found in the training set have experimental values that disagree with the predicted value.
✔	**Model's descriptors range check** Descriptors range check = True Explanation: descriptors for this compound have values inside the descriptor range of the compounds of the training set.
⚠	**Atom Centered Fragments similarity check** ACF index = 0.85 Explanation: some atom centered fragments of the compound have not been found in the compounds of the training set or are rare fragments (1 infrequent fragments found).

Symbols explanation:

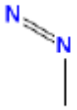
✔ The feature has a good assessment, model is reliable regarding this aspect.

⚠ 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.

4.1 Reasoning:
Relevant Chemical Fragments and Moieties

(Molecule 0) Reasoning on rare and missing Atom Centered Fragments.
The following Atom Centered Fragments have been found in the molecule, but they are not found or rarely found in the model's training set:



Fragment defined by the SMILES: N=NC
The fragment has less than 3 occurrences in the model's training set

APPENDIX G: Oncologic 9.0 Carcinogenicity Results for 2,2'-Azobisisobutyronitrile (CAS #78-67-1)

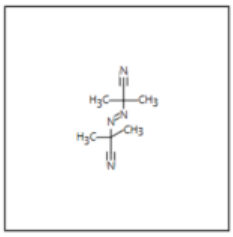
EPA OncoLogic 9.0

Target Report

Coded by OASIS Help

Chemical class	Level of concern
Diazene and Triazene Compounds	
Aliphatic Azo and Azoxy Compounds	Low

OncoLogic Justification Report



The level of concern for this compound, disregarding any highlighted substituents, is LOW.
The effect of any highlighted substituents is uncertain.

JUSTIFICATION

Aliphatic azo and azoxy compounds are, in many respects, quite similar to 1,2-dialkyl hydrazines in terms of carcinogenic activity and metabolic activation. Most of the aliphatic azo and azoxy compounds that have been tested are carcinogenic. Factors that are known to iminish or abolish the carcinogenic activity of aliphatic azo and azoxy compounds include:

(i) bulky substituents,
(ii) highly hydrophilic substituents, and
(iii) steric hindrance at the alpha-carbon.

Both the nature and the position of the substituent(s) are considered in the overall assessment of the carcinogenic potential of aliphatic azo and azoxy compounds.

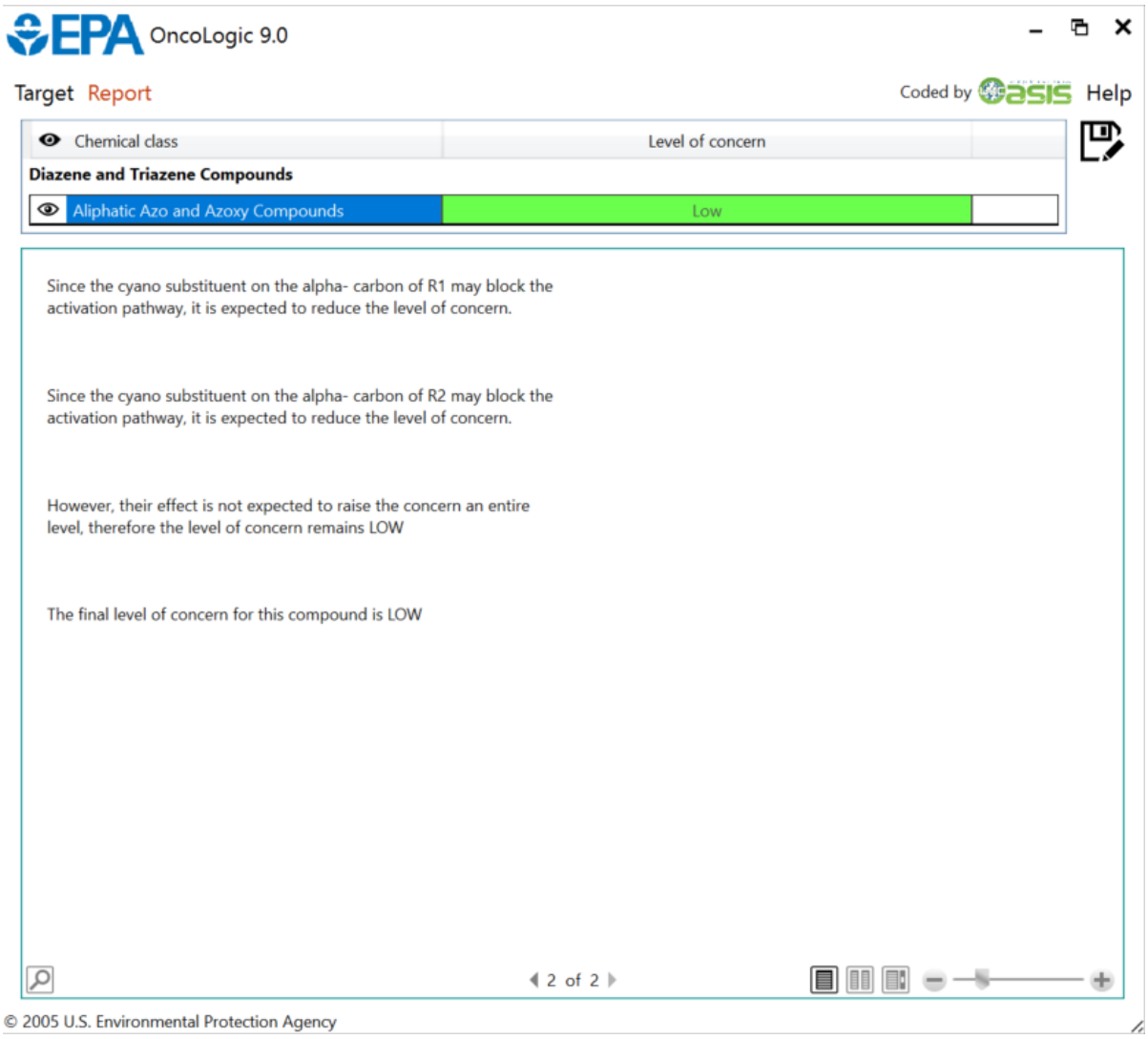
An aliphatic azo compound containing two R-groups, where R1 is s-propyl and R2 is s-propyl has a baseline level of concern of LOW-MODERATE

Since the cyano substituent on the alpha- carbon of R1 may block the activation pathway, it is expected to reduce the level of concern.

Since the cyano substituent on the alpha- carbon of R2 may block the activation pathway, it is expected to reduce the level of concern.

Therefore, the level of concern is reduced to LOW

1 of 2



APPENDIX H: Danish QSAR Endocrine Activity Modeling for 2,2'-Azobisisobutyronitrile (CAS #78-67-1)

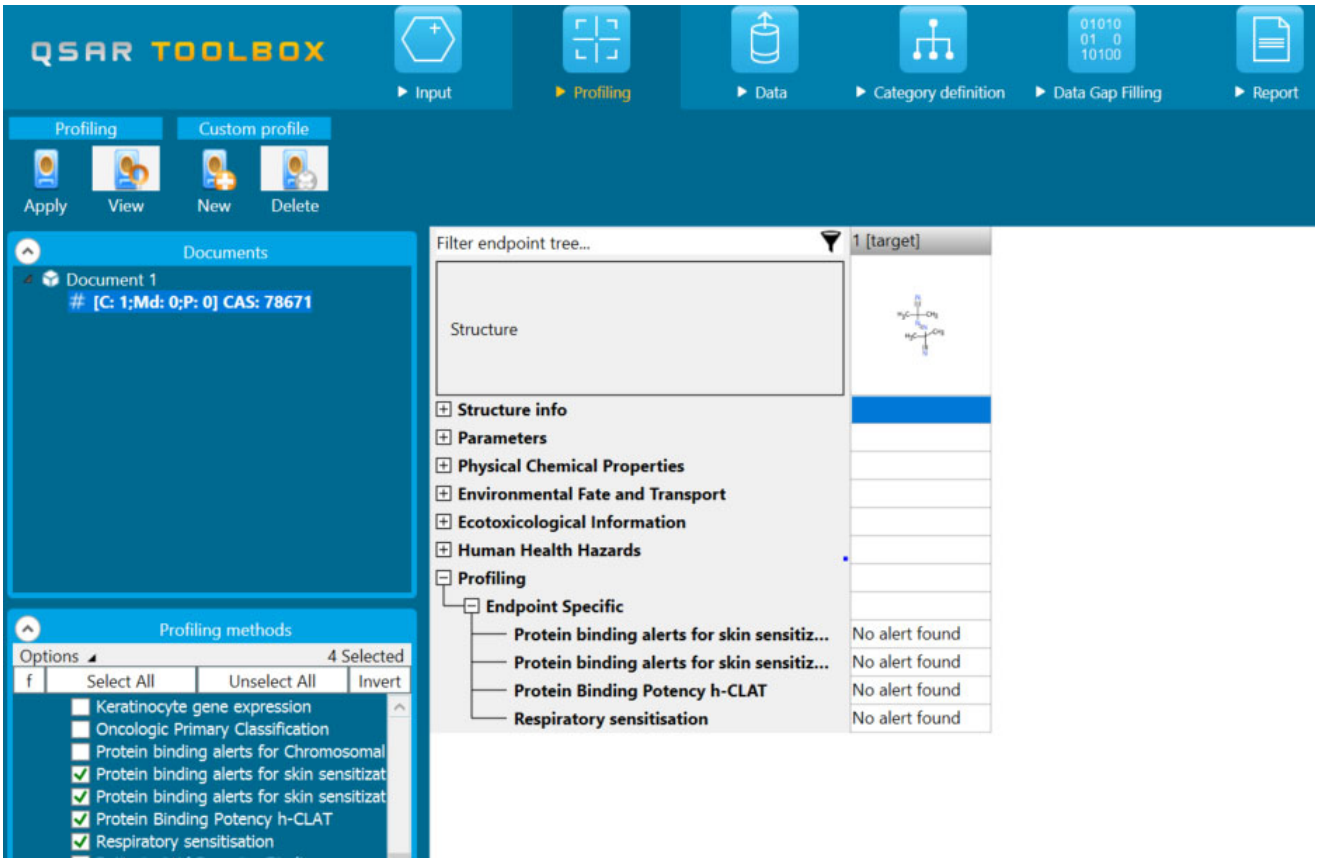
Endocrine and Molecular Endpoints

	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	NEG_IN	N/A
Androgen Receptor Inhibition, CoMPARA data (*in vitro*)		N/A	N/A	NEG_IN	N/A
Androgen Receptor Activation, CoMPARA data (*in vitro*)		N/A	N/A	NEG_IN	N/A
Thyroperoxidase (TPO) inhibition QSAR1 (Rat *in vitro*)		N/A	N/A	NEG_OUT	N/A
Thyroperoxidase (TPO) inhibition QSAR2 (Rat *in vitro*)		N/A	N/A	NEG_IN	N/A
Thyroid Receptor α Binding (Human *in vitro*)					
- mg/L			26266.34	2934.132	21.35009
- µM			159955.8	17868.17	130.017
- 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			5313.734	51.1069	82.81319
- µM			32359.38	311.2289	504.3127
- 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 –		N/A	N/A	NEG_IN	N/A

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Random final model (Human *in vitro*)					
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
Constitutive Androstane Receptor (CAR) Activation at max. 20 µM (*in vitro*)		N/A	N/A	NEG_IN	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	INC_OUT	N/A
Constitutive Androstane Receptor (CAR) Inhibition at max. 50 µM (*in vitro*)	NEG	N/A	N/A	NEG_IN	N/A
CYP3A4 Induction (Human *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	
- metabolites from Rat liver S9 metabolism simulator only	
rtER Expert System - USEPA, alerts in:	
- parent only	No alert found
- metabolites from *in vivo* Rat metabolism simulator only	
- metabolites from Rat liver S9 metabolism simulator only	
OECD QSAR Toolbox v.4.2 profilers	
Profiler predictions are supporting information to be used together with the relevant QSAR predictions	

APPENDIX I: OECD QSAR Respiratory Sensitization Modeling Results



APPENDIX J: EPI Suite™ Modeling Results for 2,2'-Azobisisobutyronitrile (CAS #78-67-1)

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

CAS Number: 78-67-1
SMILES : CC(C)(C(#N))N=NC(C)(C)C(#N)
CHEM : 2,2'-Azobisisobutyronitrile
MOL FOR: C8 H12 N4
MOL WT : 164.21
---------- EPI SUMMARY (v4.11) ----------
Physical Property Inputs:
Log Kow (octanol-water): 1.10
Boiling Point (deg C) : ------
Melting Point (deg C) : 103.00
Vapor Pressure (mm Hg) : 0.006
Water Solubility (mg/L): 317.8
Henry LC (atm-m3/mole) : ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = 2.87
Log Kow (Exper. database match) = 1.10
Exper. Ref: Revised OECD HPV Form 1 (1997)

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 273.64 (Adapted Stein & Brown method)
Melting Pt (deg C): 43.85 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 0.00135 (Modified Grain method)
VP (Pa, 25 deg C) : 0.181 (Modified Grain method)
MP (exp database): 101.5 deg C
VP (exp database): 6.70E-03 mm Hg (8.93E-001 Pa) at 25 deg C
Subcooled liquid VP: 0.0354 mm Hg (25 deg C, user-entered VP)
: 4.73 Pa (25 deg C, user-entered VP)

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 824.5
log Kow used: 1.10 (user entered)
melt pt used: 103.00 deg C
Water Sol (Exper. database match) = 350 mg/L (25 deg C)
Exper. Ref: Revised OECD HPV Form 1 (1997)

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 2653.7 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found:
Nitriles, Polyaliphatic

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 1.24E-010 atm-m3/mole (1.26E-005 Pa-m3/mole)

Group Method: Incomplete
Exper Database: 4.14E-06 atm-m3/mole (4.19E-001 Pa-m3/mole)
For Henry LC Comparison Purposes:
User-Entered Henry LC: not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 4.079E-006 atm-m3/mole (4.133E-001 Pa-m3/mole)
VP: 0.006 mm Hg (source: User-Entered)
WS: 318 mg/L (source: User-Entered)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 1.10 (user entered)
Log Kaw used: -3.771 (exp database)
Log Koa (KOAWIN v1.10 estimate): 4.871
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.6737
Biowin2 (Non-Linear Model) : 0.1543
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 1.9469 (months)
Biowin4 (Primary Survey Model) : 3.1207 (weeks)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.3881
Biowin6 (MITI Non-Linear Model): 0.0000
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): -0.1507
Ready Biodegradability Prediction: NO

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

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled): 4.72 Pa (0.0354 mm Hg)
Log Koa (Koawin est): 4.871
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 6.36E-007
Octanol/air (Koa) model: 1.82E-008
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 2.3E-005
Mackay model : 5.08E-005
Octanol/air (Koa) model: 1.46E-006

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 0.6691 E-12 cm3/molecule-sec
Half-Life = 15.985 Days (12-hr day; 1.5E6 OH/cm3)
Ozone Reaction:
No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi):

3.69E-005 (Junge-Pankow, Mackay avg)
1.46E-006 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 24.81 L/kg (MCI method)
Log Koc: 1.395 (MCI method)
Koc : 560.9 L/kg (Kow method)
Log Koc: 2.749 (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 = 0.393 (BCF = 2.47 L/kg wet-wt)
Log Biotransformation Half-life (HL) = -0.9281 days (HL = 0.118 days)
Log BCF Arnot-Gobas method (upper trophic) = 0.297 (BCF = 1.98)
Log BAF Arnot-Gobas method (upper trophic) = 0.297 (BAF = 1.98)
log Kow used: 1.10 (user entered)

Volatilization from Water:
Henry LC: 4.14E-006 atm-m3/mole (Henry experimental database)
Half-Life from Model River: 182.5 hours (7.605 days)
Half-Life from Model Lake : 2099 hours (87.45 days)

Removal In Wastewater Treatment:
Total removal: 2.13 percent
Total biodegradation: 0.09 percent
Total sludge adsorption: 1.80 percent
Total to Air: 0.23 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model: (MCI Method)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	**3.14**	**384**	**1000**
Water	**35.2**	**1.44e+003**	**1000**
Soil	**61.6**	**2.88e+003**	**1000**
Sediment	**0.111**	**1.3e+004**	**0**

Persistence Time: 962 hr

Level III Fugacity Model: (MCI Method with Water percents)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	3.14	384	1000
Water	35.2	1.44e+003	1000
water	(35.2)		
biota	(2.21e-005)		
suspended sediment	(0.00131)		

Soil 61.6 2.88e+003 1000
Sediment 0.111 1.3e+004 0
Persistence Time: 962 hr

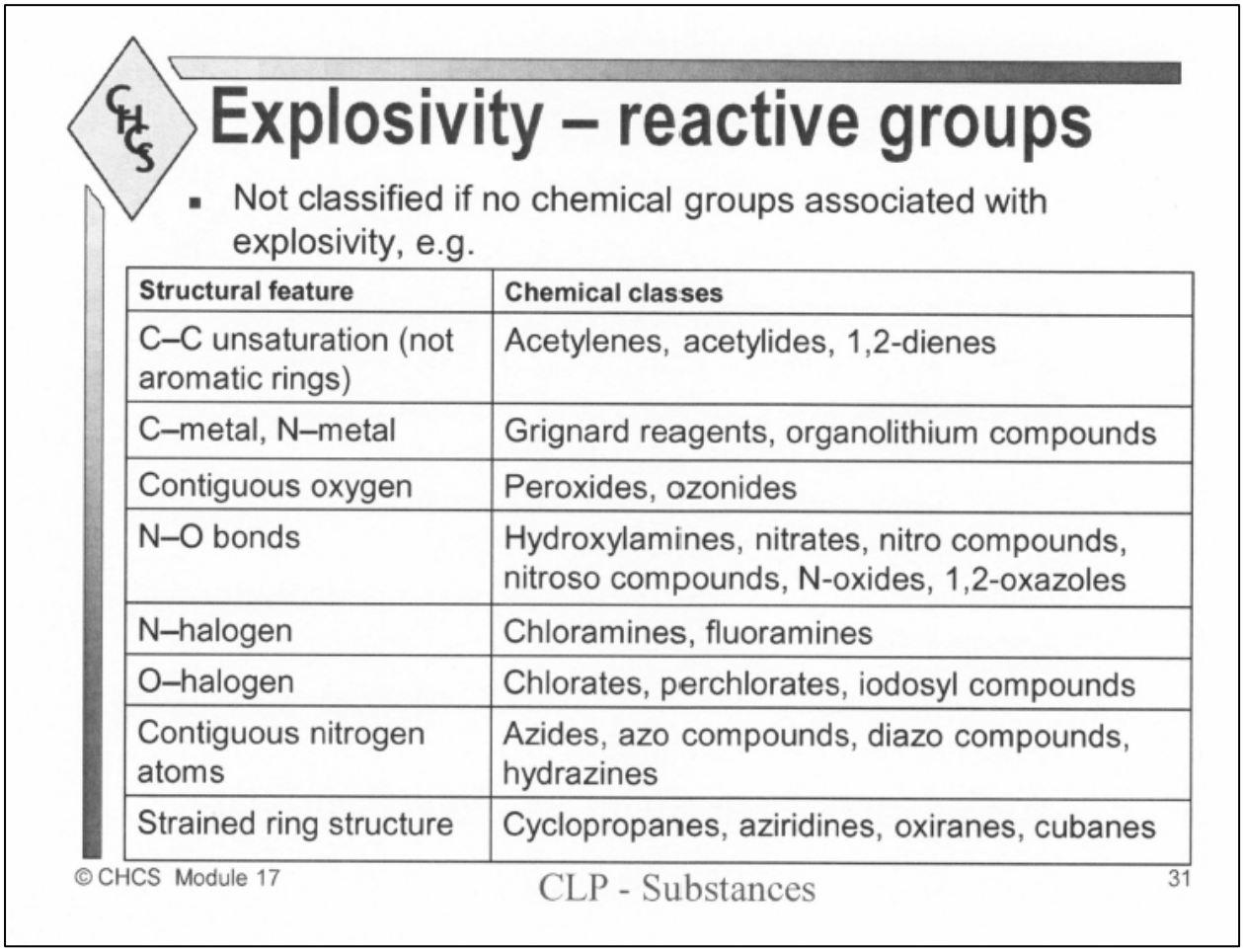
Level III Fugacity Model: (EQC Default)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	4.12	384	1000
Water	48.6	1.44e+003	1000
water	(48.6)		
biota	(3.06e-005)		
suspended sediment	(0.000377)		
Soil	47.1	2.88e+003	1000
Sediment	0.107	1.3e+004	0

Persistence Time: 758 hr

APPENDIX K: Known Structural Alerts for Reactivity

Explosivity – Abbreviated List



Explosivity – reactive groups

- Not classified if no chemical groups associated with explosivity, e.g.

Structural feature	Chemical classes
C–C unsaturation (not aromatic rings)	Acetylenes, acetylides, 1,2-dienes
C–metal, N–metal	Grignard reagents, organolithium compounds
Contiguous oxygen	Peroxides, ozonides
N–O bonds	Hydroxylamines, nitrates, nitro compounds, nitroso compounds, N-oxides, 1,2-oxazoles
N–halogen	Chloramines, fluoramines
O–halogen	Chlorates, perchlorates, iodosyl compounds
Contiguous nitrogen atoms	Azides, azo compounds, diazo compounds, hydrazines
Strained ring structure	Cyclopropanes, aziridines, oxiranes, cubanes

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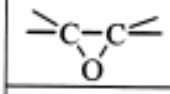
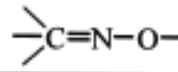
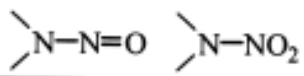
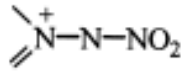
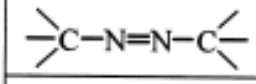
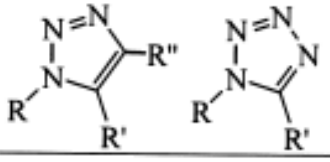
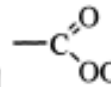
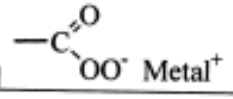
Explosivity – Full List

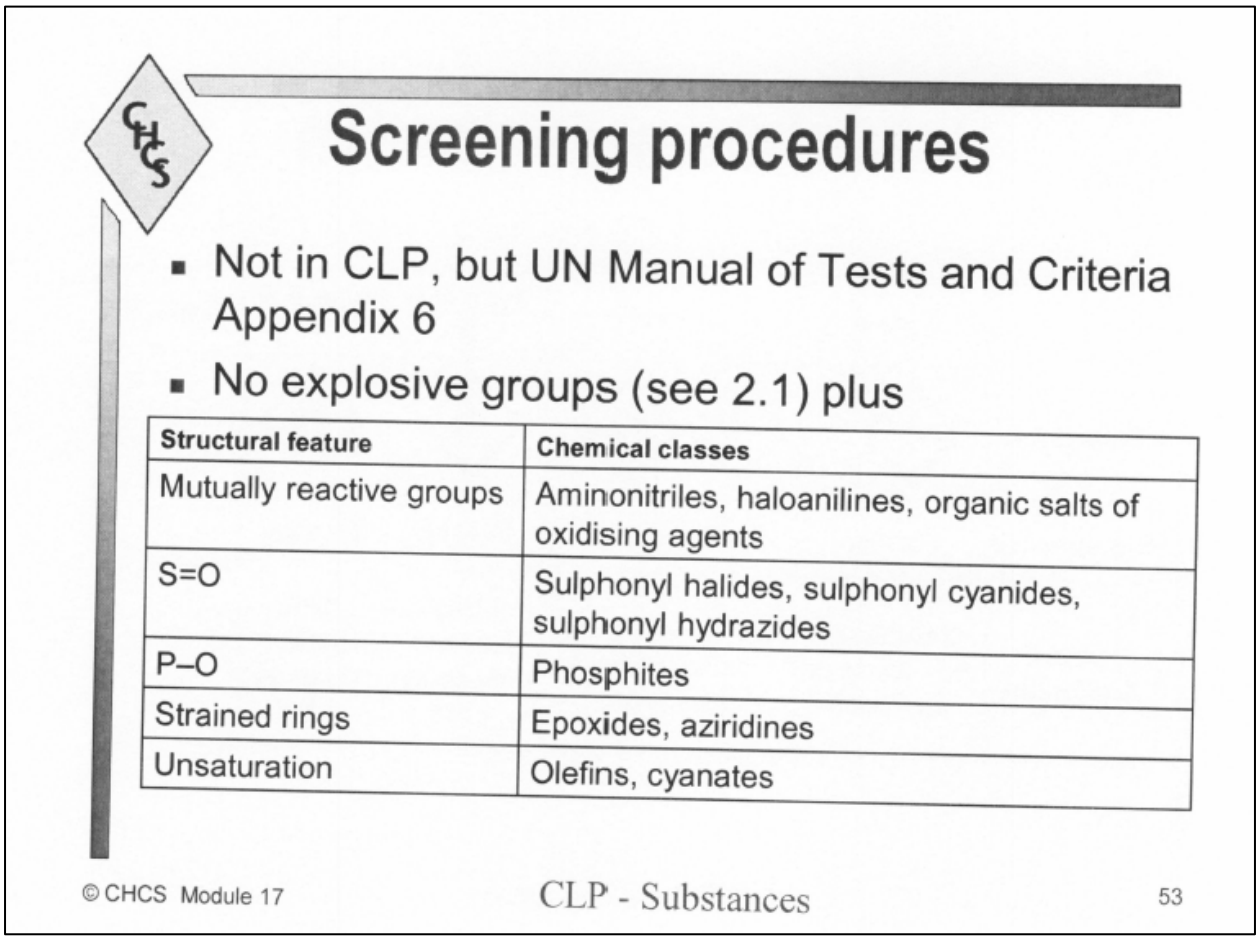
Table R.7.1-28 Chemical groups associated with explosive properties

Chemical group	Chemical Class
-C≡C- -C≡C-Metal -C≡C-Halogen	Acetylenic Compounds Metal Acetylides Haloacetylene Derivatives
	Diazo Compounds
-N=O -NO_2	Nitroso and Nitro Compounds,
R-O-N=O R-O-NO_2	Acyl or Alkyl Nitrites and Nitrates
	1,2-Epoxides
	Metal Fulminates or *aci*-Nitro Salts
	N-Metal Derivatives (especially heavy metals)
	N-Nitroso and N-Nitro Compounds
	N-Azolium Nitroimidates
	Azo Compounds
Ar-N=N-O-Ar	Arene Diazoates
$(ArN=N)_2O$, $(ArN=N)_2S$	Bis-Arenediazo Oxides and Sulfides
RN=N-NR'R''	Triazines
	High-nitrogen Compounds: e.g. Triazoles, Tetrazoles

Chemical group	Chemical Class
[1] ROOR', [2] 	Peroxy Compounds: [1] Alkyl hydroperoxides (R'=H), Peroxides (R'=organic); [2] Peroxo acids (R'=H), Peroxyesters (R'=organic)
[1] ROOMetal, [2] 	Metal peroxides, Peroxoacids salts
$-N_3$	Azides e.g. PbN_6, CH_3N_3
$^{-}O-C-N_2^{+}$	Arenediazonium oxides i.e. inner diazonium salts in which the counter ion is an oxide
Ar-N=N-S- Ar-N=N-S-Ar	Diazonium sulfides and derivatives, Arenediazo Aryl Sulfides
XO_n	Halogen Oxide: e.g. percholrates, bromates, etc
NX_3 e.g. $NC1_3$, $RNC1_2$	N-Halogen Compounds

Adapted from Bretherick (Bretherick's Handbook of Reactive Chemical Hazards 6th Ed., 1999, Butterworths, London)

Self-Reactive Substances



Screening procedures

- Not in CLP, but UN Manual of Tests and Criteria Appendix 6
- No explosive groups (see 2.1) plus

Structural feature	Chemical classes
Mutually reactive groups	Aminonitriles, haloanilines, organic salts of oxidising agents
S=O	Sulphonyl halides, sulphonyl cyanides, sulphonyl hydrazides
P–O	Phosphites
Strained rings	Epoxides, aziridines
Unsaturation	Olefins, cyanates

APPENDIX M: Change in Benchmark Score

Table 6 provides a summary of changes to the GreenScreen® Benchmark™ for 2,2'-azobisisobutyronitrile. This GreenScreen® assessment has undergone one round of updates and the benchmark score remains the same.

Table 6: Change in GreenScreen® Benchmark™ for 2,2'-Azobisisobutyronitrile

Date	GreenScreen® Benchmark™	GreenScreen® Version	Comment
August 18, 2021	BM-2	v. 1.4	New assessment
November 16, 2021	BM-2	v. 1.4	Minor updates without affecting hazard scores for any endpoint

Licensed GreenScreen® Profilers

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