CYCLOHEXANE

(CAS #110-82-7)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: September 6, 2019[a]

Expiration Date: September 6, 2024

TOXSERVICES
TOXICOLOGY RISK ASSESSMENT CONSULTING
1367 Connecticut Ave., N.W., Suite 300
Washington, D.C. 20036

[a] ToxServices incorporated January 2020 comments submitted by the Washington State Department of Ecology into this document.

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GreenScreen® Executive Summary for Cyclohexane (CAS #110-82-7)

Cyclohexane is a colorless liquid under standard temperature and pressure. It's highly volatile and flammable. Cyclohexane is produced via recovery from hydrocarbon streams or hydrogenation of benzene. The majority of cyclohexane produced (96%) is used as an intermediate to produce adipic acid and caprolactam, which are raw materials in nylon manufacturing. Two percent of cyclohexane produced is used as a solvent in chemical production processes, functioning as a precipitating and extraction agent and a reaction enhancer. The remaining 2% of cyclohexane produced is used as a solvent in adhesives and coatings. For this use, it is mainly found in neoprene-based adhesives in shoe and floor coatings manufacture and the automobile equipment industry. Smaller quantities are used as solvent in styrene-butadiene-styrene, styrene-isoprene-styrene and natural rubber-based adhesives in shoe and bedding equipment manufacturing and in adhesives used by craftsmen (shoe repairers, carpet layers, decorators). The usage level as a solvent in adhesives is 10 – 30%.

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

- Benchmark 2e
  - Moderate Group I Human Health Hazard (developmental toxicity-D).
- Benchmark 2f
  - Very High Ecotoxicity (acute aquatic toxicity-AA and chronic aquatic toxicity-CA).
- Benchmark 2g
  - High Flammability-F.

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), cyclohexane meets requirements for a GreenScreen Benchmark™ Score of 2 despite the hazard data gap. In a worst-case scenario, if cyclohexane were assigned a High score for the data gap E, it would be categorized as a Benchmark 1 Chemical.

GreenScreen® Hazard Summary Table for Cyclohexane

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**
						single	repeated*	single	repeated*											
L	**L**	**L**	*M*	DG	*M*	**H**	**L**	**M**	**L**	**L**	*L*	**H**	**L**	**vH**	*vH*	**vL**	*L*	*L*	**H**

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

GreenScreen® Chemical Assessment for Cyclohexane (CAS #110-82-7)

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

GreenScreen® Assessment Prepared By:
Name: Sara M. Ciotti, Ph.D.
Title: Toxicologist
Organization: ToxServices LLC
Date: July 31, 2014

Quality Control Performed By:
Name: Bingxuan Wang, Ph.D.
Title: Toxicologist
Organization: ToxServices LLC
Date: August 5, 2014

GreenScreen® Assessment Updated By:
Name: Zach Guerrette, Ph.D., D.A.B.T.
Title: Toxicologist
Organization: ToxServices LLC
Date: April 3, 2017

Update Quality Control Performed By:
Name: Bingxuan Wang, Ph.D., D.A.B.T.
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: April 6, 2017

GreenScreen® Assessment Prepared By:
Name: Bingxuan Wang, Ph.D., D.A.B.T.
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: August 22, 2019

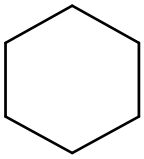
Quality Control Performed By:
Name: Jennifer Rutkiewicz
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: September 6, 2019

Expiration Date: September 6, 2024[2]

Chemical Name: Cyclohexane

CAS Number: 110-82-7

Chemical Structure(s):



Also called: Benzene, hexahydro-; Benzenehexahydride; Hexahydrobenzene; Hexamethylene; Hexanaphthene; Benzene, hexahydro- (ChemIDplus 2019).

[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 analogs or moieties of chemicals used in this assessment (CAS #'s):
A sufficiently complete database was identified for cyclohexane in order to assign a Benchmark Score. No appropriate surrogates with relevant data were identified to fill the only data gap (endocrine activity) for cyclohexane. Therefore, no surrogates were used.

Identify Applications/Functional Uses: (ECB 2004)
1. Chemical intermediate to make adipic acid for use in nylon manufacture (accounts for 96% of production volume)
2. Solvent in chemical production process (precipitating and extraction agent, reaction enhancer, accounts for 2% of production volume)
3. Solvent in adhesives and coatings at 10 – 30% (accounts for 2% of production volume)
4. Additive in printer inks (minor use)
5. Separation/dilution agent in analytical chemistry (minor use)
6. Azeotropic agent for alcohol dehydration (minor use)

Known Impurities[3]:
Linear and branched aliphatic hydrocarbons (<0.1%) (alkanes, in particular n-hexane ≤0.02%), alicyclic hydrocarbons (<0.12%) (cyclopentane and alkylcycloalkanes, in particular methylcyclohexane: 0.005 – 0.06%), benzene (0.002 – 0.012%), toluene (<0.001%) (ECB 2004).

GreenScreen® Summary Rating for Cyclohexane[4,5 6,7]: Cyclohexane was assigned a **GreenScreen Benchmark™ Score of 2** ("Use but Search for Safer Substitutes")(CPA 2018b). This score is based on the following hazard score combinations:
- Benchmark 2e
  - Moderate Group I Human Health Hazard (developmental toxicity-D).
- Benchmark 2f
  - Very High Ecotoxicity (acute aquatic toxicity-AA and chronic aquatic toxicity-CA).
- Benchmark 2g
  - High Flammability-F.

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), cyclohexane meets requirements for a GreenScreen Benchmark™ Score of 2 despite the hazard data gap. In a worst-case scenario, if cyclohexane were assigned a High score for the data gap E, it would be categorized as a Benchmark 1 Chemical.

[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.

Figure 1: GreenScreen® Hazard Summary Table for Cyclohexane

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
						single	repeated*	single	repeated*										
L	**L**	**L**	*M*	DG	*M*	**H**	**L**	**M**	**L**	**L**	*L*	**H**	**L**	**vH**	*vH*	**vL**	*L*	*L*	**H**

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

Environmental Transformation Products

Based on the structure, hydrolysis is not expected to occur (ECB 2004). Combustion of cyclohexane is expected to generate carbon monoxide and carbon dioxide, which are naturally occurring in the environment and not relevant to this assessment. Per GreenScreen® guidance (CPA 2018b), chemicals that degrade rapidly and completely (i.e. meet criteria for a Very Low for persistence) are not likely to form persistent biodegradation intermediates and are therefore not considered to be relevant because the transformation products will not persist long enough to be encountered after use or release of the parent chemical. As cyclohexane is readily biodegradable (see persistence section below), it is not expected to have relevant transformation products.

Introduction

Cyclohexane is produced via recovery from hydrocarbon streams or hydrogenation of benzene (ECB 2004). The majority of cyclohexane produced (96%) is used as an intermediate to produce adipic acid and caprolactam, which are used in nylon manufacturing. Two percent of cyclohexane produced is used as a solvent in chemical production processes, functioning as a precipitating and extraction agent and a reaction enhancer. The remaining 2% of cyclohexane produced is used as a solvent in adhesives and coatings. It is mainly used as a solvent in neoprene-based adhesives[8] in shoe and floor coatings manufacture and the automobile equipment industry. Smaller quantities are used as solvent in styrene-butadiene-styrene, styrene-isoprene-styrene and natural rubber-based adhesives in shoe and bedding equipment manufacturing and in adhesives used by craftsmen (shoe repairers, carpet layers, decorators) (ECB 2004). The usage level as a solvent in adhesives is 10 – 30% (ECB 2004).

ToxServices assessed cyclohexane against GreenScreen® Version 1.4 (CPA 2018b) following procedures outlined in ToxServices' SOPs (GreenScreen® Hazard Assessment) (ToxServices 2016).

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 2018). 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).

- Cyclohexane is not listed on the SCIL.

[8] It should be noted that cyclohexane is restricted in neoprene-based contact adhesives to < 0.1% by weight in package sizes greater than 350 g in the EU under Annex XVII (https://echa.europa.eu/documents/10162/ca2ec609-0177-402a-911f-6ac524275d6e).

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 2019) 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),[9] 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 cyclohexane can be found in Appendix C.

- Cyclohexane is an LT-P1 chemical when screened using Pharos, and therefore a full GreenScreen® is required.
- Cyclohexane is on the following lists for multiple endpoints.
  - Quebec CSST – WHMIS 1988 – Class D2B – Toxic material causing other toxic effects.
  - EC – CEPA DSL – Inherently toxic in the environment (iTE).
  - German FEA – Substances hazardous to waters – Class 2 – Hazard to Waters.
  - EU - GHS (H-Statements) - H410 - Very toxic to aquatic life with long lasting effects.
  - New Zealand - GHS - 9.1B (fish, crustacean) - Very ecotoxic in the aquatic environment
    - Based on a 96-hour LC_{50} of 8.3 mg/L in striped bass and a 48-hour mobility EC_{50} of 3.78 mg/L in daphnias (CCID 2019).
  - Japan - GHS - Hazardous to the aquatic environment (chronic) - Category 3
    - Based on H412 (NITE 2013).
  - Australia - GHS - H410 - Very toxic to aquatic life with long lasting effects.
- Specified lists for single endpoints are reported in individual hazard endpoints in the hazard assessment section below.

Hazard Statement and Occupational Control

An EU harmonized classification is available for cyclohexane (ECHA 2019a). The GHS hazard statements assigned to cyclohexane are listed in Table 1 below. Available occupational exposure limits and recommended personal protective equipment are presented in Table 2 below.

Table 1: H Statements for Cyclohexane (CAS #110-82-7) (ECHA 2019a)

H Statement	H Statement Details
H225	Highly flammable liquid and vapor
H315	Causes skin irritation
H304	May be fatal if swallowed and enters airways
H336	May cause drowsiness or dizziness
H400	Very toxic to aquatic life
H410	Very toxic to aquatic life with long lasting effects

Table 2: Occupational Exposure Limits and Recommended Personal Protective Equipment for Cyclohexane (CAS #110-82-7)

Personal Protective Equipment (PPE)	Reference	Occupational Exposure Limits (OEL)	Reference
Gloves, protective clothing, eye goggles or glasses, respirator	HSDB 2015	ACGIH TLV = 100 ppm (8-hour TWA)	HSDB 2015

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

Table 2: Occupational Exposure Limits and Recommended Personal Protective Equipment for Cyclohexane (CAS #110-82-7)			
Personal Protective Equipment (PPE)	**Reference**	**Occupational Exposure Limits (OEL)**	**Reference**
	Sigma-Aldrich 2017	NIOSH REL = 300 ppm (~1,050 mg/m^3) (10-hour TWA) NIOSH IDLH = 1,300 ppm OSHA OEL = 300 ppm (~1,050 mg/m^3) (8-hour TWA) California PEL = 300 ppm (~1,050 mg/m^3)	Sigma-Aldrich 2017

ACGIH: American Conference of Governmental Industrial Hygienists
IDLH: Immediately Dangerous to Life or Health
NIOSH: National Institute for Occupational Safety and Health value
OEL: Occupational Exposure Limit
OSHA: Occupational Safety and Health Administration
PEL: Permissible Exposure Limit
REL: Recommended Exposure Limit
TLV: Threshold Limit Value
TWA: Time Weighted Average

Physicochemical Properties of Cyclohexane

Cyclohexane is a colorless liquid under standard temperature and pressure. It has a high vapor pressure, indicating that it is volatile and exists as a mixture of liquid and vapor. It is slightly soluble in water (55 mg/L) and is more soluble in octanol than in water (log K_{ow} = 3.44). Its low log K_{ow} value indicates that it is unlikely to bioaccumulate in aquatic biota.

Table 3: Physical and Chemical Properties of Cyclohexane (CAS #110-82-7)		
Property	**Value**	**Reference**
Molecular formula	C6-H12	ChemIDplus 2019
SMILES Notation	C1CCCCC1	ChemIDplus 2019
Molecular weight	84.1608	ChemIDplus 2019
Physical state	Liquid	ECHA 2019b
Appearance	Colorless	HSDB 2015
Melting point	6.6ºC	ChemIDplus 2019
Boiling point	80.7°C	ECGA 2019b
Vapor pressure	96.9 mm Hg at 25ºC	ChemIDplus 2019
Water solubility	55 mg/L at 25ºC 52 mg/L at 23.5°C	ChemIDplus 2019 ECHA 2019b
Dissociation constant	N/A	
Density/specific gravity	0.7781 at 20ºC	HSDB 2015
Partition coefficient	Log K_{ow} = 3.44	ChemIDplus 2019

Toxicokinetics

- ECHA 2019b
  - Adult male Fischer 344 rats (3 total) were administered intravenous injections of 10 mg/kg ^{14}C-radiolabeled cyclohexane (5.3 mCi/mmole; Fischer reagent grade) in serum from male Fischer 344 rats. In the first hour after dosing, 54% of the dose was excreted in the exhaled breath. Subsequently, 80% and 83% of the dose was excreted in exhaled breath within 24

and 72 hours of dosing. Fourteen percent of the dose was excreted in the urine. No significant excretion of the dose was detected in the feces. Cyclohexane accounted for 93-99% of the dose in exhaled air, while 0.04-0.4% of the dose was exhaled as cyclohexanone and 0.09-0.6% was exhaled as cyclohexanol. Cyclohexane, cyclohexanone, and cyclohexanol each accounted for less than 0.1% of the radioactivity excreted in the urine. The specific chemical species representing the majority of radioactivity excreted in the urine were not identified, but eluted from the HPLC column in the region typical of metabolic conjugates. After 72 hours, the concentration of radioactivity in the adipose tissue was approximately 16 times that measured in the blood, and cyclohexane accounted for 79-84% of the radioactivity measured in the adipose tissue, but only 2-18% of the radioactivity measured in the liver, muscle, and skin. Low amounts of cyclohexanone and cyclohexanol were measured in all tissues. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study due to its non-GLP status and minor deficiencies in design and/or reporting.

- Adult male Fischer 344 rats (2-3/dose group) were administered oral doses of ^{14}C-radiolabeled cyclohexane (5.3 mCi/mmole; Fischer reagent grade) in sesame oil at 100, 200, 1,000, or 2,000 mg/kg via gavage. Over 72 hours, 63, 62, 76, and 78% of the dose was excreted in exhaled air in the 100, 200, 1,000, and 2,000 mg/kg dose groups, respectively. A maximum rate of excretion was observed at 2-8 hours after dosing. Urinary excretion of the dose was 29%, 29%, 15%, and 12%, respectively. No significant excretion of the dose was detected in the feces. Cyclohexane accounted for 93-99% of the dose in exhaled air, while 0.04-0.4% of the dose was exhaled as cyclohexanone and 0.09-0.6% was exhaled as cyclohexanol. Cyclohexane, cyclohexanone, and cyclohexanol each accounted for less than 0.1% of the radioactivity excreted in the urine. The specific chemical species representing the majority of radioactivity excreted in the urine were not identified, but eluted from the HPLC column in the region typical of metabolic conjugates. In the animals dosed with 200 mg/kg, the radioactivity present in the plasma was fairly constant from 2-12 hours after dosing but decreased to approximately 20% of these values by 24 hours after dosing. Cyclohexane was only a minor constituent of the radioactivity present in the plasma. Cyclohexanone represented approximately 10% of the radioactivity in total plasma 2-4 hours after dosing and 1-3% of the radioactivity in total plasma 6-24 hours after dosing. Cyclohexanol concentrations in plasma were generally 2-3 times greater than the cyclohexanone concentrations with the exception of 6 hours after dosing when the levels of cyclohexanol were eight times that of cyclohexanone. At least 5 other metabolites were detected in the plasma but they were not identified in this study. At 6, 24, and 72 hours after dosing with 200 mg/kg cyclohexane, the amount of radioactivity in the adipose tissue was approximately 16 times that measured in the blood. For animals dosed with 1,000 or 2,000 mg/kg, the levels in the adipose tissue were approximately three times greater relative to that observed in the animals dosed with 200 mg/kg. After 72 hours, the concentration of radioactivity in the adipose tissue was approximately 16 times that measured in the blood, and cyclohexane accounted for 79-84% of the radioactivity measured in the adipose tissue, but only 2-18% of the radioactivity measured in the liver, muscle, and skin. Low amounts of cyclohexanone and cyclohexanol were measured in all tissues. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study due to its non-GLP status and minor deficiencies in design and/or reporting.
- Female Chinchilla rabbits (4/dose group) were administered oral doses of ^{14}C-radiolabeled cyclohexane (98.5% purity) in water at 0.3 or 350-400 mg/kg (equivalent to 14 to 185 µCi/animal) via gavage. Within 2 days of dosing, 40% of the radioactivity was measured in expired air and 50% was detected in the urine for animals dosed with 350-400 mg/kg. In the

expired air, cyclohexane and carbon dioxide composed 30% and 10% of the radioactivity, respectively. Of the urinary metabolites detected for the high dose animals, cyclohexyl glucuronide (cyclohexanol glucuronide conjugate) represented 35-50% of the dose while trans-cyclohexane-1:2-diol glucuronide represented 3-8% of the dose. In animals dosed with 0.3 mg/kg, 90% of the radioactivity was measured in the urine and 5% was measured in expired air. Only carbon dioxide was detected in the respired air for the low dose animals. Of the urinary metabolites detected for the low dose animals, cyclohexyl glucuronide represented 61% of the dose while trans-cyclohexane-1:2-diol glucuronide represented 16.7% of the dose. The ratio of cyclohexanol/cyclohexane-diol in the urine was approximately the same in the two dose groups. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study as it was not conducted under GLP.

- A non-GLP, non-guideline study was performed with male Wistar rats (3/dose) receiving cyclohexane in drinking water at 0 or 2.5% for 5 consecutive days. At the end of the study, livers were excised to determine cytochrome P450 enzyme induction by immunoblot analyses. Additional groups of animals received classical inducers of CYP1A (3-methylcholanthrene) or CYP2B (phenobarbital) and served as positive controls. The results indicated that CYP2E1 and CYP2B1/2 were induced by cyclohexane, but CYP1A1/2 were not. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study as it was a non-GLP and non-guideline study.
- A GLP-compliant dermal absorption study was performed with Fischer 344 rats administered doses of cyclohexane (99.5% chemical purity, at least 98% radiochemical purity). The rats were separated into two groups: one group of rats (5/sex/dose group) was administered intravenous injections of ^{14}C radiolabeled cyclohexane at 10 mg/kg; the second group of rats (6/sex/dose group) was administered topical applications of undiluted ^{14}C radiolabeled cyclohexane at 1 or 100 mg/cm^2 (equivalent to approximately 6 and 600 mg/rat) to clipped skin for 6 hours under occlusive dressing. The results of the two exposures were evaluated to determine dermal absorption. Cyclohexane was rapidly excreted with expired air as the primary route of excretion (70%) followed by urinary elimination (29%). Essentially no radioactivity was measured in the feces. Male and female rats absorbed approximately 39% and 60% of the 1 mg/cm^2 dose, whereas only 4% of the 100 mg/cm^2 dose was absorbed in male and female rats. The average absorption rates at the low dose were 0.06 and 0.1 mg/cm^2 of exposed skin/hour for males and females, respectively. In contrast, increasing the dose to 100 mg/cm^2 only increased the dermal absorption to 0.6 and 0.7 mg/cm^2 of exposed skin/hour for males and females, respectively. The areas under the concentration of total radiolabel in blood vs. time curves AUC (0 - ∞) were similar between males and females administered the intravenous injections, but the value was 3 times greater for females than males in the low dermal dose group and 2 times greater in females for the high dermal dose group, indicating a sex difference in dermal absorption of cyclohexane. Less than 0.4% and 0.1% of the administered dose remained in the carcasses 72 hours after dermal exposures at the low and high doses, respectively. The identity of the metabolites was not determined in this study. Authors of the REACH dossier assigned a Klimisch score of 1 (reliable without restrictions) for this study.

- ECB 2004 (Klimisch scores not reported)
  - In a study on 5 workers and 3 volunteers, approximately 23% of inhaled cyclohexane was absorbed by the lung. At relatively high exposure levels (not specified), 40% the absorbed dose was excreted unchanged in the air, and 10% as carbon dioxide in the air. At lower

concentrations (unspecified), 10% of the absorbed dose was exhaled unchanged and 5% as carbon dioxide. Only 1% of the absorbed dose was excreted as metabolite in the urine (mainly as cyclohexanol). No additional information was provided.

- In an occupational study, the environmental air concentrations of cyclohexane in a shoe factory ranged from 17-2,484 mg/m^3. Alveolar concentrations of cyclohexane measured in 59 samples from 22 workers ranged from 16-1,929 mg/m^3. The mean alveolar concentration corresponded to 78% of the air concentrations of cyclohexane. Blood cyclohexane levels, determined 4 hours after exposure, ranged from 29-367 µg/L, which correlates to 53-78% of the alveolar concentrations. Urinary cyclohexanol corresponded to 0.1-0.2% of the cyclohexane dose absorbed. Overall, the excretion of cyclohexane metabolites was correlated with the blood levels of cyclohexane.
- In a survey of women (n=33) who either applied glue with cyclohexane being used almost exclusively as the solvent or worked in the vicinity of glue application, the geometric mean and maximum cyclohexane air concentrations were 93 and 943 mg/m^3. Quantitative estimates of the absorbed dose performed at the end of the work shift indicated that less than 1% of the cyclohexane was excreted in the urine as cyclohexanol, which was excreted almost exclusively as a glucuronide conjugate.
- In a closed exposure chamber study, volunteers (4/sex, aged 33-55 years) were exposed to cyclohexane and cyclohexanol at 1,010 and 236 mg/m^3, respectively, for 8 hours. The minute respiratory volume and mean retention time of the chemicals in the respiratory tract were calculated. The majority of the cyclohexane was metabolized as 1,2-cyclohexanediol and 1,4-cyclohexanediol, with only 1% of the dose being excreted as cyclohexanol. The peak level of cyclohexanol excretion was reached just following exposure and the elimination half-life was calculated as 1.5 hours. Maximum excretion of cyclohexanediols was reached a few hours following exposure and the elimination half-lives were calculated as 17 and 16.1 hours for 1,2- and 1,4-cyclohexanediol, respectively. The excretion curves were similar between cyclohexane and cyclohexanol and there were no differences between men and women in terms of metabolic yields or elimination half-lives.
- Only negligible binding of the cyclohexanediols to blood proteins was observed following incubation of human plasma in a dialysis casing.
- Volunteers were administered oral doses of cyclohexanediols pooled and dissolved in water at 2 mmol each (1,2- and 1,4-cyclohexanediol) (equivalent to 232.2 mg). Urine was collected for 72 hours and analyzed by gas chromatography to determine cyclohexanol and cyclohexanediol concentrations. The excretion peak was obtained within 4 hours of dosing, and 57% and 76% of the 1,2-diol and 1,4-diol, respectively, were excreted over a period of 72 hours. The 1,4-diol was excreted unconjugated while the 1,2-diol was excreted as a glucuronide conjugate.
- The cyclohexane exposures of 156 workers in shoe and leather factories were evaluated via urine samples collected on different days of the work week and air sampling performed on the same days. The individual exposures ranged from 7-617 mg/m^3, and the mean was 60 mg/m^3. A close correlation was observed between the air concentrations and urinary levels of the urinary diols measured in the post shift urinary samples collected on Monday. Data collected on Thursday or Friday show poor correlations. The authors suggest that these observations reflect accumulation of the diols during the work week as the urinary elimination half-lives for these metabolites are 16-18 hours.

- In summary, cyclohexane is rapidly absorbed and metabolized via the oral (100%), dermal (50% for vapor and 5% for liquid), and inhalation (100%) routes of exposure. Sex differences were measured in the dermal absorption rates when male and female rats were compared. Based on its low

molecular weight and structure features, cyclohexane is expected to cross the blood brain barrier and the placental barrier. Once in the circulation, cyclohexane distributes throughout the body but may accumulate in adipose tissue as demonstrated by the concentration of cyclohexane in the fat relative to blood levels following oral or intravenous dosing in rats. Cyclohexane is metabolized to cyclohexanone and cyclohexanol in the liver, and these metabolites may be excreted as glucuronide conjugates, or metabolized further to 1,2-cyclohexanediol and 1,4-cyclohexanediol and subsequently excreted in the urine as glucuronide conjugates. In animal studies, the majority of the dose was excreted via expired air as un-metabolized cyclohexane with the metabolites composing only minor amounts of the expired dose (ECB 2004).

Hazard Classification Summary

Group I Human Health Effects (Group I Human)

Carcinogenicity (C) Score (H, M, or L): *L*
Cyclohexane was assigned a score of Low for carcinogenicity based on negative predictions by OncoLogic, by VEGA models with high confidence, and the lack of structural alerts. GreenScreen® criteria classify chemicals as a Low hazard for carcinogenicity when adequate data are available and they are not classified under GHS (CPA 2018b). The confidence in the score is low as it is mostly based on modeling.

- Authoritative and Screening Lists
  - *Authoritative:* US EPA - IRIS Carcinogens - (1999) Data are inadequate for an assessment of human carcinogenic potential.
  - *Screening:* Not present on any screening lists.
- ECB 2004 (Klimisch score not assigned)
  - The tumorigenic potential of cyclohexane was tested in a multi-stage initiation-promotion test on mouse skin. Mice were topically initiated with 0.2 µmol of DMBA in acetone. After initiation, mice were separated into seven treatment groups. One week after initiation, mice in group 1 were promoted three times a week with 5 µg TPA in acetone for 2 weeks followed by application of 100 µL of cyclohexane three times per week; mice in group 2 were promoted with cyclohexane three times a week alone; groups 3 - 7 served as controls. However, there was not a control group treated with cyclohexane alone. The authors concluded that cyclohexane was a weak tumor promoter. However, the significance of the study was questioned due to uncertainties pertaining to the results and testing method.
- ECHA 2019b
  - The authors of the REACH dossier for cyclohexane determined that the carcinogenic concern is low, based on the lack of genotoxicity structural alerts on itself and its known metabolites, the negative genotoxicity data *in vitro* and *in vivo*, the lack of bioaccumulation in animals, and the lack of systemic toxicity that suggests a carcinogenic concern in subchronic toxicity studies.
- U.S. EPA 2003a
  - No animal or human carcinogenicity studies were identified for cyclohexane. The U.S. Environmental Protection Agency (U.S. EPA) noted that genotoxicity studies are generally negative. The U.S. EPA concluded there are inadequate data available to assess cyclohexane's human carcinogenic potential.
- U.S. EPA 2013a
  - Cyclohexane was evaluated as a saturated alkane in OncoLogic and was predicted to have low cancer concern (Appendix D).

- Toxtree 2018
    - Cyclohexane does not have structural alerts for genotoxic carcinogenicity or non-genotoxic carcinogenicity. See Appendix E for justification.
- VEGA 2019
    - The CAESAR model predicted cyclohexane to be a carcinogen with low confidence. See Appendix F for justification.
    - The ISS model predicted cyclohexane to be a non-carcinogen with low confidence. See Appendix F for justification.
    - The IRFMN/Antares model predicted cyclohexane to be a possible non-carcinogen with moderate confidence. See Appendix F for justification.
    - The IRFMN/ISSCAN-CGX model predicted cyclohexane to be a possible non-carcinogen with high confidence. See Appendix F for justification.
    - The IRFMN oral carcinogenicity classification model predicted cyclohexane to be a non-carcinogen with good reliability, based on experimental data (unspecified). See Appendix F for justification.
    - The IRFMN inhalation carcinogenicity classification model predicted cyclohexane to be a non-carcinogen with good reliability, based on experimental data (unspecified). See Appendix F for justification.
- Based on the weight of evidence, a score of Low was assigned. No chronic carcinogenicity studies were identified for cyclohexane. One tumor-promotion study reported that cyclohexane was a weak tumor-promoter. However, the significance of the study was questioned due to uncertainties about the results and testing methodology. The U.S. EPA concluded that there are insufficient data available to assess the carcinogenic potential of cyclohexane. The U.S. EPA also noted that cyclohexane is generally negative in genotoxicity tests. OncoLogic predicted it to be of low carcinogenic concern as a saturated alkane, although it is not clear if this category (saturated hydrocarbons) is applicable to cyclic hydrocarbons. Toxtree did not identify any structural alerts for genotoxic carcinogenicity or non-genotoxic carcinogenicity. Five of the six models in VEGA predicted cyclohexane to be a non-carcinogen, with three of the five models producing high confidence predictions. Based on negative predictions by OncoLogic, by VEGA models with high confidence, and the lack of structural alerts, a score of Low is appropriate.

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

Cyclohexane was assigned a score of Low for mutagenicity/genotoxicity based on negative mutagenicity studies *in vitro* and clastogenicity studies *in vitro* and *in vivo*. 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.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists.
    - *Screening:* Not present on any screening lists.
- ECB 2004, ECHA 2019b
    - *In vitro*: Cyclohexane (purity unspecified) was negative for mutagenicity in a non-GLP Ames assay in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, with and without metabolic activation at concentrations up to 10,000 µg/plate. Cytotoxicity was reported at 3,333 µg/plate in TA98 and TA1537 and at 10,000 µg/plate in TA100 and TA1535. Vehicle, negative and positive controls were valid. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study (justification not provided).

- *In vitro*: Cyclohexane (99 Mol% pure) was negative for mutagenicity in a mouse lymphoma assay, with and without metabolic activation at concentrations up to 7,800 µg/mL conducted under GLP in L5178Y cells. Cytotoxicity was reported at 7,800 µg/mL with and without metabolic activation. Vehicle, negative and positive controls were valid. Authors of the REACH dossier assigned a Klimisch score of 1 (reliable without restriction) for this study.
- *In vitro*: Cyclohexane (assumed 100% pure) was negative for mutagenicity in a second mouse lymphoma assay (GLP status unknown), with and without metabolic activation at doses up to 100 µg/mL. No cytotoxicity was observed. Vehicle, negative and positive controls were valid. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study as the GLP status is unknown and it has minor deficiencies in design and/or reporting.
- *In vitro*: Cyclohexane was negative for genotoxicity in a sister chromatic exchange test in Chinese hamster ovary (CHO) cells, with and without metabolic activation at concentrations up to 25 µg/mL. Complete growth inhibition was observed at the highest concentration tested. No additional details were provided. Authors of the REACH dossier did not assign a Klimisch score for this study.
- *In vitro*: Ambiguous results were reported in an unscheduled DNA synthesis test. Human lymphocytes were treated for 4 hours with cyclohexane at 0.1 – 10 mM in the presence or absence of metabolic activation. Treatment caused decreased [^{3}H]TdR uptake in the absence of metabolic activation. However, this effect was not dose related and was within the control ranges. No effects were reported in the presence of metabolic activation. Authors of the REACH dossier did not assign a Klimisch score for this study. The European Commission (EC) noted that the definition of positivity or negativity was not provided in this study and there was significant variability in solvent and negative controls. Therefore, EC could not draw any conclusion from this study.
- *In vivo*: Cyclohexane was negative for clastogenicity in a GLP-compliant bone marrow cytogenetic assay conducted in a manner similar to OECD Guideline 475. Male and female Sprague-Dawley rats (10/sex/group) were exposed to cyclohexane (purity not specified) vapor at up to 3,650 mg/m^3 (equivalent to 3.650 mg/L[10]) for 6 hours per day for 5 days. A significant increase in numerical aberrations was reported in low and medium dose females, and pooled data at the low dose of both sexes. The authors indicated that there was no dose-response and concluded that the increases were of no biological importance. Authors of the REACH dossier assigned a Klimisch score of 1 (reliable without restriction) for this study.

- ECB 2004 (no Klimisch scores assigned)
  - *In vitro*: Cyclohexane was negative for genotoxicity in a DNA cell binding assay at doses up to 100 µM when tested alone, with liver extract, and with lysozyme and liver extract. Cyclohexane tested positive (1.6%) with lysozyme at 100 µM. However, the result is questionable due to the slight extent of binding and the negative findings in other groups.
  - *In vivo*: Cyclohexane was negative for genotoxicity in a Drosophila sex linked recessive lethal assay. No additional details were provided.

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

Cyclohexane was assigned a score of Low for reproductive toxicity based on lack of adverse effects on fertility in a two-generation reproduction study. GreenScreen® criteria classify chemicals as a Low hazard for reproductive toxicity when adequate negative data are available and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on a reliable study.

- Authoritative and Screening Lists

[10] 3,650 mg/m^3 / 1,000 = 3.650 mg/L.

  - *Authoritative:* Not present on any authoritative lists.
  - *Screening:* Not present on any screening lists.
- ECB 2004, ECHA 2019b
  - In a GLP-compliant two-generation reproduction study conducted in a manner similar to OECD Guideline 416, Crl:CD BR rats (30/sex/concentration) were exposed by whole body inhalation to cyclohexane (greater than 99.9% purity) vapor at 0, 500, 2,000, or 7,000 ppm (equivalent to 0, 1,721, 6,886, 24,101 mg/m^3 and 0, 1.721, 6.886, 24.101 mg/L[11]) for 6 hours/day and 5 days/week, excluding holidays. The equivalent concentrations for a 7-day/week exposure frequency were 0, 1.23, 4.92, and 17.22 mg/L, respectively. After 10 weeks of exposure, the animals were bred within their respective treatment groups and allowed to deliver and rear their offspring until weaning. With the exception of gestation day (GD) 21 until day 4 of lactation, when they were not exposed, females were exposed daily after breeding throughout pregnancy and lactation. Neonate rats were not directly exposed to cyclohexane. At weaning, F1 rats were randomly selected to produce the next generation and were treated to the same exposure schedule as the P1 generation. At least 11 weeks after weaning, the F1 rats were bred to produce the F2 litters. Treatment with 6.886 mg/L caused a significant decrease in response to a sound stimulus in parental females, which was characterized as a transient sedation effect. Treatment with 24.101 mg/L caused significant decreases in body weight gain of F1 male rats, decreased body weights of P1 and F1 females during pre-mating, decreased mean food efficiency of P1 and F1 females during lactation, decreased food consumption of P1 females during lactation, and decreased mean body weight of F1 females during lactation. There were no significant treatment related effects on mating, fertility, gestation indices, implantation efficiency, or gestation length in P1 and F1 generations. In addition, there were no dose-related effects on number of implantation sites, number of pups/litter, sex ratio, percent born alive, 0-4-day viability, lactation index, and litter survival in F1 and F2 litters. The authors identified a reproductive NOAEC of 6.880 mg/L and a LOAEC of 24.101 mg/L, equivalent to 4.92 and 17.22 mg/L for a 7-day/week exposure frequency, respectively, based on decreased pup weight (attributed to exposure via lactation). Authors of the REACH dossier assigned a Klimisch score of 1 (reliable without restriction) for this study.
- Based on the weight of evidence, a score of Low was assigned. No effects on fertility were found in the GLP-compliant two-generation reproductive toxicity study. Decreased mean pup weight was found in this study and was attributed to pup exposure through lactation. Effects on/via lactation are classified as a special category rather than Category 1 or 2 under GHS, and are considered under developmental toxicity according to the GreenScreen® criteria.

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

Cyclohexane was assigned a score of Moderate for developmental toxicity based on decreased offspring body weight during lactation through transfer of the compound via milk. GreenScreen® criteria classify chemicals as a Moderate hazard for developmental toxicity when there is limited or marginal evidence of developmental toxicity (CPA 2018b). The confidence in the score is reduced as the low severity of the effects may not warrant GHS classification.

- Authoritative and Screening Lists
  - *Authoritative:* MAK - Pregnancy Risk Group D ("Either there are no data for an assessment of damage to the embryo or fetus or the currently available data are not sufficient for classification in one of the groups A–C").
  - *Screening:* Not present on any screening lists.

[11] 1,721 mg/m^3 / 1,000 L/m^3 = 1.721 mg/L.

- ECB 2004, ECHA 2019b
    - In the previously described GLP-compliant two-generation reproduction study conducted in a manner similar to OECD Guideline 416, rats (30/sex/concentration) were exposed by whole body inhalation to cyclohexane (greater than 99.9% purity) vapor at 0, 500, 2,000, or 7,000 ppm (equivalent to 0, 1,721, 6,886, 24,101 mg/m^3 and 0, 1.721, 6.886, 24.101 mg/L[12]) for 6 hours/day and 5 days/week, excluding holidays. The equivalent concentrations for a 7-day/week exposure frequency were 0, 1.23, 4.92, and 17.22 mg/L, respectively. After 10 weeks of exposure, the animals were bred within their respective treatment groups and allowed to deliver and rear their offspring until weaning. With the exception of GD 21 until day 4 of lactation, females were exposed daily after breeding throughout pregnancy and lactation. Neonate rats were not directly exposed to cyclohexane. At weaning, F1 rats were randomly selected to produce the next generation and were treated to the same exposure schedule as the P1 generation. At least 11 weeks after weaning, the F1 rats were bred to produce the F2 litters. Treatment with 6.886 mg/L caused a significant decrease in response to a sound stimulus in parental females, which was characterized as a transient sedation effect. Treatment with 24.101 mg/L caused significant decreases in body weight gain of F1 male rats, decreased body weights of P1 and F1 females during pre-mating, decreased mean food efficiency of P1 and F1 females during lactation, decreased food consumption of P1 females during lactation, and decreased mean body weight of F1 females during lactation. Authors of the REACH dossier assigned a Klimisch score of 1 (reliable without restriction) for this study. ToxServices identified a developmental NOAEC of 6.880 mg/L and a LOAEC of 24.101 mg/L, equivalent to 4.92 and 17.22 mg/L for a 7-day/week exposure frequency, respectively, based on decreased pup weight (attributed to exposure via lactation).
    - In a GLP-compliant prenatal developmental study conducted according to OECD Guideline 414/EPA OTS 798/4350, pregnant female Crl:CD BR rats (25/group) were administered whole body exposures to cyclohexane (greater than 99.9% purity) vapor at 0, 1,720, 6,880, or 24,080 mg/m^3 (equivalent to 1.720, 6.880, and 24.080 mg/L[13]) on GD 7-16. Treatment caused significant reductions in bodyweight gain, decreased food consumption, and a diminished response to a sound stimulus in dams. No adverse effects were reported in the pups. The authors identified a developmental NOAEC of 24.080 mg/L (highest concentration tested). Authors of the REACH dossier assigned a Klimisch score of 1 (reliable without restriction) for this study.
    - In a GLP-compliant prenatal developmental toxicity study conducted according to OECD Guideline 414/EPA OTS 798.4350, pregnant female New Zealand White rabbits (20/group) were administered whole body inhalation exposures to cyclohexane (greater than 99.9% purity) vapor at 0, 1,720, 6,880, or 24,080 mg/m^3 (equivalent to 1.720, 6.880, and 24.080 mg/L[14]) on GD 6-18. Treatment had no adverse effects on development. The authors identified a developmental NOAEC of 24.080 mg/L (highest concentration tested). Authors of the REACH dossier assigned a Klimisch score of 1 (reliable without restriction) for this study.
- ECB 2004
    - In a developmental study, pregnant female Crl:CD BR rats (8/group) were exposed to 0, 10,320, 20,640, or 30,960 mg/m^3 (equivalent to 10.320, 20.640, and 30.960 mg/L[15]) via

[12] 1,721 mg/m^3 / 1,000 L/m^3 = 1.721 mg/L.
[13] 1,720 mg/m^3 / 1,000 L/m^3 = 1.720 mg/L.
[14] 1,720 mg/m^3 / 1,000 L/m^3 = 1.720 mg/L.
[15] 10,320 mg/m^3 / 1,000 L/m^3 = 10.320 mg/L.

whole body inhalation on GD 7-16. Dams had decreased bodyweight gain, decreased food consumption, and a diminished response to a sound stimulus during exposure to 20.640 mg/L or greater. No adverse effects were reported in the pups. The authors identified a developmental NOAEC of 30.960 mg/L (highest concentration tested).
    - It was concluded that cyclohexane is not a developmental toxicant for rats and rabbits.
- Based on the evidence, a score of Moderate was assigned. The two-generation study reported decreased pup weights in animals exposed to cyclohexane via maternal milk. Lack of effects on offspring in prenatal developmental toxicity studies in rats and rabbits in which exposure only occurred during organogenesis periods of gestation on maternal animals suggests that cyclohexane only exerted adverse effects during post-natal development through transfer via maternal milk. Effects on/via lactation are classified as a special category rather than Category 1 or 2 under GHS and are only included to support a High score for developmental toxicity under GreenScreen® criteria. The severity of the effects seen with cyclohexane via lactational exposure does not warrant a GHS classification. ToxServices conservatively considered it limited/marginal evidence of developmental toxicity and assigned a score of Moderate for this endpoint.

Endocrine Activity (E) Score (H, M, or L): DG
Cyclohexane was assigned a score of Data Gap for endocrine activity based on the lack of data identified.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists.
    - *Screening:* Not present on any screening lists.
- No data were identified.

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

Acute Mammalian Toxicity (AT) Score (vH, H, M, or L): *M*
Cyclohexane was assigned a score of Moderate for acute toxicity based on an oral LD_{50} of 813 mg/kg in mice and a vapor LC_{50} of 13.9 mg/L in rats. GreenScreen® criteria classify chemicals as a Moderate hazard for acute toxicity when the vapor inhalation LC_{50} is between 10 and 20 mg/L or the oral LD_{50} is between 300 and 2,000 mg/kg (CPA 2018b). The confidence in the score is reduced as the reliability of the critical studies could not be determined, and other studies identified do not support classification.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists.
    - *Screening:* New Zealand - GHS - 6.1D (oral, inhalation) - Acutely toxic
        - Based on an oral LD_{50} of 813 mg/kg in mice, and a vapor LC_{50} of 13.9 mg/L in rats (CCID 2019).
- ECB 2004, ECHA 2019b
    - *Oral*: LD_{50} (rat) > 5,000 mg/kg (similar to OECD Guideline 401, Klimisch score = 2 (reliable with restrictions) due to unknown GLP status and deficiencies in design and/or reporting).
    - *Dermal*: LD_{50} (rabbit) > 2,000 mg/kg (similar to OECD Guideline 402, Klimisch score = 2 (reliable with restrictions) due to unknown GLP status and deficiencies in design and/or reporting).

  - *Inhalation*: 4-hour whole body vapor LC_{50} (rat) > 32.88 mg/L (non-GLP-compliant, similar to OECD Guideline 403, Klimisch score = 2 (reliable with restrictions) due to non-GLP status and deficiencies in design and/or reporting).
- ECHA 2019b
  - *Inhalation*: 4-hour whole body vapor LC_{50} (Sprague-Dawley rat) > 19,070 mg/m^3 (equivalent to 19.07 mg/L[16]) (GLP-compliant, similar to OECD Guideline 403, Klimisch score = 2 (reliable with restrictions) due to minor deficiencies in design and/or reporting).
- ECB 2000
  - *Inhalation*: 4-hour vapor LC_{50} (rat) = 13.9 mg/L (Klimisch score unassigned).
- RTECS 2018 (No Klimisch scores)
  - *Oral*: LD_{50} (mouse) = 813 mg/kg.
  - *Oral*: LD_{50} (rabbit) = 5.5 mg/kg (ToxServices noted that there may have been a typo in the unit of this dose, which should be g/kg instead of mg/kg).
  - *Oral*: LD_{50} (rat) = 6,240 mg/kg.
- U.S. EPA 2012 (No Klimisch scores)
  - *Oral:* LD_{50} (rat) = 29,800 mg/kg.
  - *Oral:* LD_{50} (young adult rat) = 30,420 mg/kg.
  - *Oral:* LD_{50} (adult rat) = 12,870 mg/kg.
- Based on the weight of evidence, a score of Moderate was assigned. GHS-New Zealand classified cyclohexane as acutely toxic via inhalation and oral exposure based on an LC_{50} of 13.9 mg/L and an LD_{50} of 813 mg/kg, respectively. A score of Moderate was assigned based on these values.

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-single) Score (vH, H, M, or L): H
Cyclohexane was assigned a score of High for systemic toxicity (single dose) based on association with the authoritative H304 for aspiration hazards. GreenScreen® criteria classify chemicals as a High hazard for systemic toxicity (single dose) when they are associated with H304 (CPA 2018b). The confidence in the score is high as it is based an authoritative list.

- Authoritative and Screening Lists
  - *Authoritative:* EU - GHS (H-Statements) - H304 - May be fatal if swallowed and enters airways.
  - *Screening:* Japan - GHS - Specific target organs/systemic toxicity following single exposure - Category 2 (cardiovascular system)
    - Based on vascular injury observed in rabbits following oral dosing (NITE 2006, 2013).
  - *Screening:* Japan - GHS - Specific target organs/systemic toxicity following single exposure - Category 3 (respiratory tract irritation)
    - Based on respiratory irritation observed in humans (NITE 2006, 2013).
  - *Screening:* Australia - GHS - H304 - May be fatal if swallowed and enters airways.
- ECHA 2019b
  - *Oral*: In the acute oral toxicity test that identified an oral LD_{50} greater than 5,000 mg/kg in rats, clinical signs of toxicity observed included salivation and soft feces 1 hour after dosing until day 1. The animals appeared normal by day 2 of the observation period. The animals gained weight during the observation period and no gross pathological abnormalities were observed at necropsy. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study due to unknown GLP status and deficiencies in design and/or reporting.

[16] 19,070 mg/m^3 / 1,000 L/m^3 = 19.070 mg/L.

- *Dermal*: In the acute dermal toxicity test that identified a dermal LD_{50} greater than 2,000 mg/kg in rabbits, clinical signs of toxicity included vocalizations by all rabbits as the cyclohexane was applied. All animals gained weight during the observation period and no gross pathological abnormalities were observed at necropsy. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study due to unknown GLP status and deficiencies in design and/or reporting.
- *Inhalation*: In the acute inhalation toxicity test that identified a 4-hour whole body vapor LC_{50} greater than 32.88 mg/L in rats, clinical signs of toxicity observed during the observation period were limited to localized alopecia up to day 10 and sores around the left eye on days 8-10 observed in one male. No treatment-related effects were reported on body weight or gross pathological findings. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study due to the non-GLP status and deficiencies in design and/or reporting.
- *Inhalation*: In the acute inhalation toxicity test that identified a 4-hour whole body vapor LC_{50} greater than 19.070 mg/L, no non-behavioral clinical signs of toxicity were observed during the exposure and observation periods. No treatment related effects were reported on body weight or gross pathological findings. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study due to minor deficiencies in design and/or reporting.

- ECB 2004 (No Klimisch scores assigned)
  - *Inhalation*: Human volunteers were exposed to cyclohexane in the air at 25 or 250 ppm (equivalent to 87.5 and 875 mg/m^3, respectively). The exposure duration was not specified. Volunteers exposed to the high concentration complained of throat irritation more frequently than those exposed to the low concentration.

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

Cyclohexane was assigned a score of Low for systemic toxicity (repeated dose) based on measured data not warranting GHS classification for effects other than neurotoxicity. GreenScreen® criteria classify chemicals as a Low hazard for systemic toxicity (repeated dose) when adequate data are available and negative at vapor inhalation concentrations of 1 mg/L/6-hours/day and above for 90-day studies, and the chemical is not GHS classified (CPA 2018b). The confidence in the score is high as it is based on reliable measured data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists.
  - *Screening:* Not present on any screening lists.
- ECB 2004, ECHA 2019b
  - *Inhalation*: In a GLP-compliant subchronic exposure study conducted according to EPA OPPTS 870.3465, CD BR rats (20/sex/group for controls and high concentration, 10/sex/group for intermediary concentration groups) were administered whole body inhalation exposures of 0, 500, 2,000, or 7,000 ppm (equivalent to 0, 1,720, 7,000, or 24,500 mg/m^3 and 0, 1.72, 6.88, and 24.5 mg/L[17]) cyclohexane (greater than 99.9% purity) for 6 hours per day, 5 days per week for 90 days. The equivalent concentrations for a 7-day/week exposure frequency were 0, 1.23, 4.91, and 17.5 mg/L, respectively. Ten rats per sex were allowed a one-month recovery in the control and the high-dose groups. Treatment did not alter body weight, body weight gain, food consumption, urine analysis, or clinical examinations. Treatment with 24.5 mg/L caused a significant increase in relative liver

[17] 1,750 mg/m^3 / 1,000 L/m^3 = 1.75 mg/L.

weight accompanied with hepatic hypertrophy in males, and there was also an increased incidence of centrilobular hypertrophy in both sexes. This was partially reversible in the one-month recovery period. Neurological examinations were performed, and the results are discussed in the repeated dose neurotoxicity section below. EC identified a systemic toxicity NOAEC and LOAEC of 6.88 (equivalent to 4.91 mg/L for a 7-day/week exposure frequency) and 24.5 mg/L (equivalent to 17.50 mg/L for a 7-day/week exposure frequency), respectively, based on changes of the liver. The authors of REACH dossier considered the liver changes to be adaptive rather than adverse and assigned a systemic toxicity NOAEC of 24.5 mg/L (17.50 mg/L/day) based on the lack of adverse systemic toxicity observed. The authors of the REACH dossier assigned a Klimisch score of 1 (reliable without restriction) for this study.

- *Inhalation*: In a GLP-compliant subchronic exposure study conducted according to EPA OPPTS 870.3465, Crl:CD-1 BR mice were administered whole body inhalation exposures of 0, 500, 2,000 or 7,000 ppm (equivalent to 0, 1,720, 7,000, or 24,500 mg/m^3, or 0, 1.72, 6.88, and 24.5 mg/L[18]) cyclohexane (greater than 99.9% purity) vapor 6 hours per day, 5 days per week for 90 days. The equivalent concentrations for a 7-day/week exposure frequency were 1.25, 4.91, and 17.50 mg/L, respectively. Treatment with 24.5 mg/L caused increased absolute and relative liver weight in males; this was not accompanied with histopathological findings. Females treated with 24.5 mg/L had increased relative liver weight. Neurological effects were observed and were discussed in repeated dose neurotoxicity section below. The EC authors identified a systemic NOAEC and LOAEC of 6.88 mg/L (equivalent to 4.91 mg/L for a 7-day/week exposure frequency) and 24.5 mg/L (equivalent to 17.5 mg/L for a 7-day/week exposure frequency), respectively, based on changes to the liver. The authors of REACH dossier considered the liver changes to be adaptive rather than adverse, but maintained the NOAEC assigned by EC. The authors of the REACH dossier assigned a Klimisch score of 1 (reliable without restriction) for this study.
- *Inhalation:* In a study that predates GLP and guidelines, rabbits were exposed to cyclohexane vapor (purity not specified) at 0, 434, 786, or 3,330 ppm (equivalent to 0, 1.47, 2.66, and 11.27 mg/L, according to the EC record) for 6 hours per day, 5 days per week for 10 weeks, at 435 ppm (1.47 mg/L) for 8 hours per day, 5 days per week for 26 weeks, or at 7,444 – 18,565 ppm (25 – 63 mg/L) for 6 hours per day, 5 days per week for 2 – 5 weeks. Animals exposed to 25 – 63 mg/L for 2 – 5 weeks exhibited dose-dependent increases in the severity of nose rubbing, conjunctival injection, weight loss, salivation, diarrhea, rapid labored respiration, and cyanosis. No clinical biochemistry examination was performed. There were no effects upon weekly hematological examinations. Barely discernable microscopic changes in the liver and kidneys were observed at 786 ppm for 10 weeks. Animals at higher concentrations exhibited generalized vascular endothelium injury, and widespread tissue inflammation and degenerative changes. EC authors assigned a NOAEC of 435 ppm (equivalent to 1.5 mg/L, or 1.4 mg/L/6h/day[19]) for 8 hours/day treatment for 26 weeks, and a LOAEC of 786 ppm (2.7 mg/L, or 1.9 mg/L/6h/day[20]) for 6 hours/day treatment for 10 weeks. Neither EC nor authors of the REACH dossier assigned a Klimisch score for this study.
- *Inhalation:* The same authors that conducted the rabbit study above exposed one monkey to 1,243 ppm (4.19 mg/L according to EC) cyclohexane vapor for 6 hours per day, 5 days per week for 10 weeks. The animal had decreased body weight, but there were no clinical signs

[18] 1,750 mg/m^3 / 1,000 L/m^3 = 1.75 mg/L.
[19] 1.5 mg/L * 8 hours/6 hours * 5 days/7 days = 1.4 mg/L.
[20] 2.7 mg/L * 5 days/7 days = 1.9 mg/L.

or histological findings. The information is too limited to derive a NOAEC/LOAEC for this study. Neither EC nor authors of the REACH dossier assigned a Klimisch score for this study.

- ECB 2004
    - Three 2-week inhalation studies in rats and/or mice and one 2-week dermal study in rabbits were described. However, due to the availability of longer-term studies by inhalation in rats and mice as described above, and due to limitations in the rabbit dermal study, these studies are not described in this assessment.
- In summary, the subchronic studies in rats and mice identified NOAEC and LOAEC values of 4.91 and 17.5 mg/L after adjustment to a 7-day/week exposure frequency. The 10 - 26-week rat study identified a NOAEC of 1.4 mg/L and a LOAEC of 1.9 mg/L. As these values are greater than the GHS guidance value of 1.0 mg/L/6-hours/day for vapors for a 90-day study, and the duration adjusted value of 1.3 mg/L for a 10-week study[21] (UN 2017), ToxServices did not classify cyclohexane as a systemic toxicant following repeated exposure under GHS criteria.

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

Cyclohexane was assigned a score of Moderate for neurotoxicity (single dose) based on data demonstrating transient narcotic effects and association with the authoritative H336. GreenScreen® criteria classify chemicals as a Moderate hazard for neurotoxicity (single dose) when classified as GHS Category 3 for transient narcotic effects (CPA 2018b). The confidence in the score is high as it is based on authoritative lists.

- Authoritative and Screening Lists
    - *Authoritative:* EU - GHS (H-Statements) - H336 - May cause drowsiness or dizziness.
    - *Screening:* Australia - GHS - H336 - May cause drowsiness or dizziness.
    - *Screening:* G&L Neurotoxic chemicals – Neurotoxic.
    - *Screening:* Japan - GHS - Specific target organs/systemic toxicity following single exposure - Category 3 (narcotic effects)
        - Based on central nerve inhibition (nausea, unconsciousness, loss of reflexes) and anesthetic actions in animal studies (NITE 2006, 2013).
- ECB 2004 (No Klimisch scores assigned)
    - *Inhalation*: The neurotoxicity of cyclohexane was studied with a schedule controlled operant behavior (SCOB) test. Male rats (10/group) were trained 5 times a week for 5 to 6 weeks prior to cyclohexane treatment. Mice were exposed to 0, 1,720, 6,880, or 24,080 mg/m^3 (equivalent to 1.720, 6.880, and 24.080 mg/L) cyclohexane via inhalation for 6 hours. Thirty minutes after exposure the SCOB test took place and measured the fixed ratio response rate, fixed ratio pause duration, fixed interval response rate, and fixed interval index of curvature. High dose animals had a slight decrease in the fixed ratio rate. The authors identified a NOAEC of 6.880 mg/L and a LOAEC of 24.08 mg/L.
    - *Inhalation*: The behavioral effects of cyclohexane in rats were evaluated in a series of experiments that evaluated motor activity, psychomotor effects, and brain and blood concentrations of cyclohexane. Male Crl BR rats were exposed to 1,400, 8,000, or 28,000 mg/m^3 (equivalent to 1.4, 8.0, and 28 mg/L[22]) via inhalation for one or three 8-hour exposures. A single 8-hour exposure to 28 mg/L caused slight ataxia-like movements in 1 of 8 animals, a slight tremor in 1 of 8 animals, and a significant decrease in body temperature. Animals treated with 8 mg/L had a significant treatment-by-time interaction for the number of repetitive errors. Treatment also caused a significant decrease in the mean number of

[21] 1 mg/L * 13 weeks/10 weeks = 1.3 mg/L.
[22] 1,400 mg/m^3 / 1,000 L/m^3 = 1.4 mg/L.

short S+ response latencies in animals treated with ≥ 8.0 mg/L. A significant increase in long S+ response latencies were reported in animals treated with ≥ 8.0 mg/L. High cyclohexane concentrations were measured in the lipophilic brain compartment. Concentrations were approximately 10 times the blood concentration. There was no accumulation of cyclohexane in the brain with fast and almost complete elimination between each exposure. The authors identified a NOAEC 1.4 mg/L and a LOAEC of 8 mg/L based on reported increases in the response latencies observed in the discrete-trial two-choice discrimination task.

- *Inhalation*: Twelve male volunteers (ages 20 – 39) were exposed to 860 mg/m^3 (equivalent to 0.860 mg/L[23]) cyclohexane or placebo (86 mg/m^3) for 4 hours. The experiment used a double-blind, two-way cross-over design. Neurobehavioral tests were conducted before and after treatment. Volunteers exposed to cyclohexane reported headaches, and irritation of the eyes and throat.

- ECHA 2019b
  - *Oral*: In the acute oral toxicity test that identified an oral LD_{50} greater than 5,000 mg/kg in rats, clinical signs of toxicity observed included slight depression or depression 1 hour after dosing until day 1. The animals appeared normal by day 2 of the observation period. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study due to unknown GLP status and deficiencies in design and/or reporting.
  - *Inhalation*: In the acute inhalation toxicity test that identified a 4-hour whole body vapor LC_{50} greater than 32.88 mg/L in rats, clinical signs of toxicity observed during the exposure period included tremors, hyperactivity, lying prostrate, and rapid respiration. After the exposure period, all animals exhibited normal behavior. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study due to the non-GLP status and deficiencies in design and/or reporting.
  - *Inhalation*: In the acute inhalation toxicity test that identified a 4-hour whole body vapor LC_{50} greater than 19.070 mg/L, clinical signs of toxicity included slightly depressed activity during exposure. The animals appeared normal during the observation period. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study due to minor deficiencies in design and/or reporting.
- Based on the weight of evidence, a score of Moderate was assigned. Acute inhalation exposure to cyclohexane caused reversible nervous system depression classifying it to GHS Category 3. While a LOAEC of 8 mg/L was identified in rats, which is below the cutoff of 10 mg/L for GHS Category 1 classification, the reversibility and the severity of the effects indicate that a GHS Category 3 classification is more appropriate. Cyclohexane has also been identified as a neurotoxicant by Grandjean and Landrigan (2006).

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

Cyclohexane was assigned a score of Low for neurotoxicity (repeated dose) based on measured data not warranting GHS classification. GreenScreen® criteria classify chemicals as a Low hazard for neurotoxicity (repeated dose) when 90-day effect concentrations for neurotoxicity are greater than 1 mg/L/6h/day for vapors (CPA 2018b). The confidence in the score is high as it is based on data from high quality studies.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists.
  - *Screening:* Not present on any screening lists.
- Not classified as a developmental neurotoxicant (Grandjean and Landrigan 2006, 2014).

[23] 860 mg/m^3 / 1,000 L/m^3 = 0.860 mg/L.

- U.S. EPA 2003b (No Klimisch score assigned)
    - *Inhalation*: In a previously described GLP-compliant, similar to OECD Guideline 416 two-generation reproduction inhalation toxicity study, male and female Crl:CD BR rats (Sprague-Dawley strain; 30/sex/concentration) were exposed by whole body inhalation to cyclohexane vapor at 0, 500, 2,000, or 7,000 ppm (0, 1,721, 6,886, or 24,101 mg/m^3). After 10 weeks of exposure (generally 6 hours/day and 5 days/week, excluding holidays), the animals were bred within their respective treatment groups and allowed to deliver and rear their offspring until weaning. With the exception of gestation day 21 until day 4 of lactation, females were exposed daily after breeding throughout pregnancy and lactation. Neonate rats were not directly exposed to cyclohexane. At weaning, F1 rats were randomly selected to produce the next generation and were treated to the same exposure schedule as the P1 generation. At least 11 weeks after weaning, the F1 rats were bred to produce the F2 litters. Clinical observations during exposure showed a diminished or absent response to a sound stimulus beginning at exposure 15 in animals exposed to 6,886 or 24,101 mg/m^3. Rats were evaluated for their response to an auditory altering stimulus prior to cyclohexane exposure, during cyclohexane exposure, and during the time required to clear the exposure chamber. Groups, rather than individual animals, were observed for normal, diminished, or hyper responsive behavior in response to the auditory stimulus. The sedation was transient and was no longer apparent shortly after the rats were removed from the chamber. The animals in these two groups also showed salivation, stained perioral area, and wet chin. These clinical signs may have been related to the sedation. At the 6,886 or 24,101 mg/m^3 level, diminished response to a sound stimulus or absent sound stimulus was observed during exposure. The principal study authors noted that the most suggestive evidence of maternal toxicity was the altered response to an altering sound stimulus. The authors indicate that the effects appeared to be transient and compound-related. They also noted that the effects would be expected and were consistent with overexposure to cyclohexane.
- ECB 2004 (No Klimisch score assigned)
    - *Inhalation*: Rats were exposed to 5,250 or 8,750 mg/m^3 (equivalent to 5.25 and 8.75 mg/L[24]) cyclohexane via inhalation for 9-10 hours per day, 5-6 days per week for up to 30 weeks. Treatment caused no outward manifestations of neuropathy, no changes in body weight, and no histopathological changes in nervous tissue. EC identified a NOAEC of 8.75 mg/L based on the lack of effects observed at up to the highest concentration tested.
- ECB 2004, ECHA 2019b
    - *Inhalation*: In a previously described GLP-compliant U.S. EPA Guideline study, CD BR rats (20/sex/group for controls and high concentration, 10/sex/group for intermediary concentration groups) were exposed to 0, 1,720, 7,000, or 24,500 mg/m^3 (equivalent to 1.72, 6.88, and 24.5 mg/L[25]) cyclohexane via inhalation for 6 hours per day, 5 days per week for 90 days. The equivalent concentrations for a 7-day/week exposure frequency were 1.23, 4.91, and 17.50 mg/L, respectively. Treatment with ≥ 1.72 mg/L caused a decreased or absent response to auditory stimulation. This response was dose-dependent. Sedation was reported in animals treated with ≥ 6.88 mg/L. The authors identified a NOAEC of 1.72 mg/L, equivalent to 1.23 mg/L for a 7-day/week exposure period, for neurological effects based on sedation and decreased response to auditory stimulation. These effects were transient and reversible. The authors of the REACH dossier assigned a Klimisch score of 1 (reliable without restriction) for this study.
    - *Inhalation*: In a previously described GLP-compliant U.S. EPA Guideline study, Crl:CD-1

[24] 5,250 mg/m^3 / 1,000 L/m^3 = 5.250 mg/L.
[25] 1,750 mg/m^3 / 1,000 L/m^3 = 1.75 mg/L.

BR mice were exposed to 1,720, 7,000, or 24,500 mg/m^3 (equivalent to 1.72, 6.88, and 24.5 mg/L[26]) cyclohexane via inhalation for 6 hours per day, 5 days per week for 90 days. The equivalent concentrations for a 7-day/week exposure frequency were 1.25, 4.91, and 17.50 mg/L, respectively. Mice treated with 6.88 mg/L had a decreased or absent response to auditory stimulation and a hyperactive state. The authors also reported treatment caused abnormal gait, excessive grooming, hyperactivity, hyper reactivity, spasms, aggressivity, hypo activity, and ruffled fur. The authors identified a NOAEC of 1.72 mg/L, equivalent to 1.23 mg/L for a 7-day/week exposure period, based on sedation in animals. The authors of the REACH dossier assigned a Klimisch score of 1 (reliable without restriction) for this study.

- Based on the weight of the evidence, a score of Low was assigned. Cyclohexane is classified as a neurotoxicant by Grandjean and Landrigan (2006), which is a screening B list that warrants a Low to Very High hazard score for either single or repeated exposure neurotoxicity. The data indicate that repeated exposure to cyclohexane causes effects to the central nervous system, but the NOAEC levels are greater than 1 mg/L/6h/day (90-day duration) for GHS Category 2 classification and the neurological signs are consistent with the reversible narcotic effects observed in acute toxicity studies (discussed in the single dose neurotoxicity section above) rather than repeated dose neurotoxicity. Therefore, no additional GHS classification is warranted for repeated dose neurotoxicity.

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

Cyclohexane was assigned a score of Low for skin sensitization based on the lack of dermal sensitization reactions observed in a Buehler test. GreenScreen® criteria classify chemicals as a Low hazard for skin sensitization when adequate data are available and negative, there are no structural alerts, and the chemical is not GHS classified (CPA 2018b). The confidence in the score is high as it is based on data from a high quality study.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists.
  - *Screening:* Not present on any screening lists.
- ECB 2004, ECHA 2019b
  - A GLP-compliant Buehler test conducted according to EU Method B.6 was performed with Hartley guinea pigs (20 in the test group, 9 males and 11 females, and 10 controls, 5/sex) administered dermal doses of cyclohexane (purity not specified). The induction doses were applied as a topical application of 10% cyclohexane in ethanol under occlusive dressing for 6 hours once per week for 3 weeks. The challenge dose was applied 15 days after the last induction dose as a topical application of 10% cyclohexane in acetone for 6 hours. The dermal reactions were scored at 24 and 48 hours. The tested concentration (10%) was determined from a range finding study as the minimally irritating concentration. No positive skin reactions were observed in the treatment group following the challenge dose. The positive and negative controls performed as expected. The study authors concluded that cyclohexane was not sensitizing to the skin in this study. Authors of the REACH dossier assigned a Klimisch score of 1 (reliable without restriction) to this study.
- ECB 2004
  - Cyclohexane is a widely-used, high tonnage chemical and no reports of skin sensitization reaction in humans have been reported.

[26] 1,750 mg/m^3 / 1,000 L/m^3 = 1.75 mg/L.

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

Cyclohexane was assigned a score of Low for respiratory sensitization based on the lack of dermal sensitization potential according to the ECHA guidance (2017). GreenScreen® criteria classify chemicals as a Low hazard for respiratory sensitization when they are not GHS classified (CPA 2018b). 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.
  - *Screening:* Not present on any screening lists.
- ECB 2004
  - Cyclohexane is a widely-used, high tonnage chemical and no reports of allergic effects on the airways in humans have been reported.
- OECD 2019
  - Cyclohexane contains no structural alerts for respiratory sensitization (Appendix G).
- 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 cyclohexane 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 cyclohexane, and as it does not contain any structural alerts for respiratory sensitization (OECD 2019), cyclohexane is not expected to be a respiratory sensitizer.

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

Cyclohexane was assigned a score of High for skin irritation/corrosivity based on association with the authoritative H315. GreenScreen® criteria classify chemicals as a High hazard for skin irritation/corrosivity when the chemical is classified to GHS Category 2 with H315 (CPA 2018b). Confidence in the score is high as it is based on an authoritative A list.

- Authoritative and Screening Lists
  - *Authoritative:* GHS (H-Statements) - H315 - Causes skin irritation.
  - *Screening:* Japan - GHS - Skin corrosion / irritation - Category 2
    - Based on dermal irritation observed in rabbits and humans (NITE 2006, 2013).
  - *Screening:* New Zealand - GHS - 6.3B - Mildly irritating to the skin
    - Based on R38 (CCID 2019).
  - *Screening:* Australia - GHS - H315 - Causes skin irritation.
- ECB 2004
  - Application of undiluted cyclohexane to the skin of human volunteers for 1 hour resulted in erythema and weal formation. No further details were provided.

- ECHA 2019b
    - A skin irritation test conducted in a manner similar to OECD Guideline 404 (abraded and non-abraded sites) was performed with rabbits (3/sex for abraded and 3/sex for non-abraded skin, strain not specified) administered topical applications of cyclohexane (purity not specified) to abraded or non-abraded skin. ECB (2003) specifies that semi-occlusive dressing was used in this study. The duration of treatment and vehicle used (if any) were not specified. An observation period of 72 hours followed the exposure period. At 24 and 72 hours, the primary dermal irritation index (PDII) was 0. No erythema, edema, or other dermal effects were observed following topical application of cyclohexane in this study. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study due to unknown GLP status and restrictions in design/reporting.
    - A skin irritation test conducted in a manner similar to EU Method B.4 was performed with New Zealand White rabbits (5-6, sex not specified) administered topical applications of 0.5 mL undiluted cyclohexane (purity not specified) to shaved skin under occlusive dressing for 4 hours. An observation period of 72 hours followed the exposure period. At 24, 48, and 72 hours, the mean erythema score was 1.93. A maximum erythema score of 2.56 was achieved at 119 hours. No signs of edema were observed with treatment. Study authors concluded that cyclohexane was not irritating to the skin. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study due to unclear GLP status.
        - Based on the results of the above test, ToxServices classified cyclohexane as a Category 3 skin irritant under GHS criteria (UN 2015). GHS criteria define Category 3 skin irritants as chemicals that produce mean erythema and/or edema scores of ≥ 1.5 and < 2.3 from gradings in at least 2 of 3 animals from grades at 24, 48, and 72 hours. Chemicals classified as GHS Category 2 skin irritants are associated with H315. It should be noted that erythema appeared to have worsened after exposure ceased, with maximum score achieved at 119 hours. Inflammation that persists to 14 days in at least 2 animals may be classified to GHS Category 2. However, limited information was provided in this study for accurate classification.
    - In the previously described acute dermal toxicity test that identified a dermal LD_{50} greater than 2,000 mg/kg in rabbits (3/sex/dose group), erythema was observed and ranged from very slight in 2 males to well-defined in 1 female. On day 3 of the observation period, very slight erythema was observed in 2 males and 2 females. All signs of erythema cleared by day 7. Edema was observed on day 1 and ranged from very slight in 1 male and 3 females to slight in 1 male. All signs of edema cleared by day 3. One female exhibited scaling of the skin on day 10. This study was a limit test and the only dose tested was 2,000 mg/kg. No information on the coverage used (if any) was provided. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study due to unknown GLP status and deficiencies in design and/or reporting.

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

Cyclohexane was assigned a score of Low for eye irritation/corrosivity based on studies reporting only slight eye irritation in rabbits that does not warrant classification under GHS. GreenScreen® criteria classify chemicals as a Low hazard for eye irritation/corrosivity when adequate data are available and negative, and they are not classifiable under GHS (CPA 2018b). The confidence in the score is high as it is based on reliable measured data.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists.

    - *Screening:* Japan - GHS - Serious eye damage / eye irritation - Category 2 (H319)
        - Based on reversible corneal clouding, iritis, conjunctival hyperemias, and chemosis observed in rabbits and cases of ocular irritation in humans (NITE 2006, 2013)
- ECB 2004 (No Klimisch scores assigned)
    - Human volunteers were exposed to cyclohexane vapor at 5 ppm (equivalent to 17.5 mg/m^3) for 90 seconds. Most subjects reported no ocular irritation, but some individuals reported very slight eye irritation. Details for this study were poorly reported.
    - Human volunteers were exposed to cyclohexane in the air at 25 or 250 ppm (equivalent to 87.5 and 875 mg/m^3, respectively). The exposure duration was not specified. Volunteers exposed to the high concentration complained of eye irritation more frequently than those exposed to the low concentration.
- ECHA 2019b
    - An ocular irritation test conducted in a manner similar to OECD Guideline 405 was performed with rabbits (6 total, sex and strain not specified) administered ocular instillations of cyclohexane (purity not specified). The volume instilled, duration of treatment, and use of a vehicle (if any) were not specified. An observation period of 7 days followed the instillation. At 1 hour, the mean corneal score was 2, the mean iris score was 0.8, the mean conjunctival score was 2, and the total irritation score (cornea, iris, conjunctiva) was 3.7. The ocular irritation effects were fully reversible within 24 hours. The study authors concluded that cyclohexane was slightly irritating to the eyes in this study. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study due to unknown GLP status and restrictions in design and/or reporting.
    - An ocular irritation test conducted in a manner similar to OECD Guideline 405 was performed in rabbits (6 total, sex and strain not specified) administered ocular instillations of cyclohexane (purity not specified). The eyes were washed following instillation (no further details provided). The volume instilled, duration of treatment, and use of a vehicle (if any) were not specified. An observation period of 7 days followed the instillation. At 1 hour, the mean corneal and iris scores were 0 and the conjunctival score was 1.3. The conjunctival effects were fully reversible within 24 hours. No signs of chemosis or discharge were observed following instillation. The study authors concluded that cyclohexane was slightly irritating to the eyes in this study. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study due to unknown GLP status and restrictions in design and/or reporting.
- Based on the weight of evidence, a score of Low was assigned. Instillation of cyclohexane to the eyes of rabbits caused slight eye irritation that was fully reversible within 24 hours. These observations are insufficient for a GHS category 2B classification (at least 2 of the 3 animals have the mean 24, 48 and 72 h scores of ≥ 1 for corneal opacity, ≥ 1 for iritis, ≥ 2 for conjunctival redness and/or ≥ 2 for chemosis, and the effects are reversible within 7 days) (UN 2017). The GHS-Japan classification was based on the statements "in rabbits corneal clouding, iritis, conjunctival hyperemias, and chemosis each are seen reversible" and "in animals and in humans irritation is in the eye". These statements are consistent with the studies described above, which do not warrant a GHS classification. Therefore, a score of Low was assigned.

Ecotoxicity (Ecotox)

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

Cyclohexane was assigned a score of Very High for acute aquatic toxicity based on an authoritative list and a 48h EC_{50} of 0.9 mg/L in daphnias. GreenScreen® criteria classify chemicals as a Very High

hazard for acute aquatic toxicity when they are associated with H400 or acute L/EC_{50} values are less than 1 mg/L (CPA 2018b). The confidence in the score is high as it is based on an authoritative list and reliable experimental data.

- Authoritative and Screening Lists
  - *Authoritative:* EU - GHS (H-Statements) - H400 - Very toxic to aquatic life.
  - *Screening:* Japan - GHS - Hazardous to the aquatic environment (acute) - Category 1
    - Based on a 48-hour EC_{50} of 0.9 mg/L in daphnia (NITE 2006, 2013).
- ECB 2004, ECHA 2019b
  - 96-hour LC_{50} (*Pimephales promelas*, fathead minnow) = 4.53 mg/L (measured) (similar to OECD Guideline 203, Klimisch score = 2 (reliable with restrictions) due to non-GLP and non-guideline status and lack of description of behavioral data).
  - Eleven additional acute fish studies were available from ECHA but were all discounted by REACH dossier authors due to methodological deficiencies and insufficient documentation. REACH dossier authors indicated that the key study described above was the only study that used a flow-through system with analytical monitoring, and reported the most conservative LC_{50}. Therefore, these additional studies were not included in this assessment.
  - 48-hour mobility EC_{50} (*Daphnia magna*) = 0.9 mg/L (measured), 2.4 mg/L (nominal) (non-GLP-compliant, similar to OECD Guideline 202, Klimisch score = 2 (reliable with restrictions) due to non-GLP status and notable limitations in design and reporting).
  - 48-hour mortality EC_{50} (*D. magna*) = 3.78 mg/L (estimated based on saturated solutions) (similar to OECD Guideline 202, Klimisch score = 2 (reliable with restrictions) due to non-GLP and non-guideline status).
  - Five additional acute aquatic invertebrate studies were recorded in ECHA, but were discounted by REACH dossier authors due to major methodological deficiencies. REACH dossier authors stated that the two studies described above in daphnias were the only studies that included analytical monitoring and reported the most conservative acute toxicity value. Therefore, these additional studies were not included in this assessment.
  - 72-hour biomass EC_{50} (*Pseudokirchneriella subcapitata*, algae) = 3.4 mg/L (measured), growth rate EC_{50} = 9.317 mg/L (measured) (GLP-compliant, similar to OECD Guideline 201) (Klimisch score = 2 (reliable with restrictions) due to deviations in study guideline and limitations in study design).
  - Five additional algae studies were available from ECHA but were all discounted by REACH dossier authors due to methodological deficiencies and/or insufficient documentation. Therefore, these additional studies were not included in this assessment.
- Based on acute aquatic toxicity values as low as 0.9 mg/L, ToxServices classified cyclohexane as a Category 1 acute aquatic toxicant under GHS criteria (UN 2017). GHS criteria define Category 1 acute aquatic toxicants as chemicals that have acute aquatic toxicity values no greater than 1 mg/L. Chemicals classified as GHS Category 1 acute aquatic toxicants are associated with H400, in agreement with the EU's classification for cyclohexane.

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

Cyclohexane was assigned a score of Very High for chronic aquatic toxicity based on an estimated chronic aquatic toxicity values of 0.09 mg/L for daphnias. GreenScreen® criteria classify chemicals as a Very High hazard for chronic aquatic toxicity when chronic aquatic toxicity values are 0.1 mg/L or below (CPA 2018b). The confidence in the score is low as no experimental data were identified for the fish or aquatic invertebrate trophic levels, and modeling and estimation were performed to predict toxicity in these two trophic levels.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists.[27]
  - *Screening:* Not present on any screening lists.[28]
- ECB 2004, ECHA 2019b
  - 72-hour biomass NOEC (*P. subcapitata*, algae) = 0.9 mg/L (measured), 72-hour growth rate NOEC = 0.94 mg/L (measured) (GLP-compliant, similar to OECD Guideline 201) (Klimisch score = 2 (reliable with restrictions) due to deviations in study guideline and limitations in study design)
    - According to GHS criteria (UN 2017), values based on growth rate are preferred over values based on biomass for classification purposes.
  - One additional algae study reported a 72h NOEC. However, authors of the REACH dossier assigned a Klimisch score of 3 (not reliable) for this study due to 99 - 100% of the test material being lost during the study. Therefore, this study was not included in this assessment.
- U.S. EPA 2017a
  - Cyclohexane belongs to the Neutral Organics ECOSAR chemical class. The predicted chronic values (ChV) are 0.675 mg/L in fish, 0.508 mg/L in daphnias, and 1.5 mg/L in algae (Appendix H).
- U.S. EPA 2013b
  - Applying neutral organics acute to chronic ratios (ACR) of 10, 10, and 4 to the lowest acute toxicity values of 4.53, 0.9, and 9.317 mg/L for fish, daphnias, and algae, respectively predicts ChVs of 0.453, 0.09 and 2.33 mg/L, respectively.
- Based on the weight of evidence, a score of Very High was assigned. Data were available only for algae, and the NOEC of 0.9 mg/L corresponds to a High. However acute aquatic toxicity data indicate that daphnias may be more sensitive than algae. Although the modeled ChV of 0.51 mg/L corresponds to a High, the modeled EC_{50} of 3.85 is higher than the experimental EC_{50} of 0.9 mg/L in daphnia. Therefore, ToxServices applied the ACR of 10 to the experimental EC_{50} to estimate a ChV of 0.09 mg/L which corresponds to a classification of Very High.

Environmental Fate (Fate)

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

Cyclohexane was assigned a score of Very Low for persistence based on it meeting the 10-day window in a ready biodegradability test and being predicted to have water as its dominant environmental compartment. GreenScreen® criteria classify chemicals as a Very Low hazard for persistence when the 10-day window is met (CPA 2018b). The confidence in the score is high as it is based on data from a high-quality study.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists.
  - *Screening:* Not present on any screening lists.
- ECB 2004, ECHA 2019b
  - A GLP-compliant ready biodegradability test conducted according to OECD Guideline 301 F (Manometric Respirometry Test) was performed with non-adapted, activated domestic sludge exposed to cyclohexane (99% purity) at 34 mg/L for 28 days. The level of

[27] Cyclohexane is present on multiple authoritative lists for chronic aquatic toxicity. However, as these lists cover multiple endpoints, they are listed under GreenScreen® List Translator Screening section above.

[28] Cyclohexane is present on multiple authoritative lists for chronic aquatic toxicity. However, as these lists cover multiple endpoints, they are listed under GreenScreen® List Translator Screening section above.

degradation was 10% on day 13, 68% on day 23, and 77% on day 28. The study authors concluded that cyclohexane met the 10-day window and was readily biodegradable in this study. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) to this study for unspecified reasons.

- ECB 2004 (No Klimisch scores assigned)
  - In a ready biodegradability test (OECD Guideline 301 F), cyclohexane achieved 6% degradation after 28 days. No further details were provided.
  - In a ready biodegradability test (OECD Guideline 301 C), cyclohexane achieved 0.6% degradation after 14 days. No further details were provided.
    - This test was not considered to be conclusive by the ECB (2004) authors due to the duration of only 14 days, especially given the long lag-phase observed in the GLP-compliant, OECD Guideline 301 F test.
- ECHA 2019b
  - A biodegradability test conducted according to ISO 14593 (Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium – CO_2 headspace test) performed with non-adapted, activated municipal sludge exposed to cyclohexane (greater than 99.5% purity) at 23 mg/L for 49 days. The level of degradation was less than 10% after 28 days and greater than 60% to less than 70% after 49 days. The study authors concluded that cyclohexane was biodegradable but not readily biodegradable under the conditions of this study. Authors of the REACH dossier assigned a Klimisch score of 2 (reliable with restrictions) for this study due to unknown GLP status and some limitations in design and/or reporting.
  - Five additional records were included in the REACH dossier for this endpoint. However, the dossier authors disregarded these studies due to major methodological deficiencies or insufficient documentation. Therefore, these studies are not included in this assessment.
- ECB 2004
  - Using the Level I Fugacity Model (Mackay), the European Chemicals Bureau (ECB) predicted that cyclohexane is mainly partitioning to the air (99.98%), and only minor fractions partition to water (0.01%) and soil (0.002%).
- U.S. EPA 2017b
  - The BIOWIN modeling Ready Biodegradable Predictor indicates that cyclohexane is expected to be readily biodegradable. The Level III Fugacity Model (MCI Method) predicts 66.1% will partition to water with a half-life of 15 days, 30.5% will partition to air with a half-life of 1.4 days, and 2.92% will partition to soil with a half-life of 30 days (Appendix I).
- Based on the weight of evidence, a score of Very Low was assigned. BIOWIN modeling predicts cyclohexane is readily biodegradable and Level III Fugacity modeling predicts cyclohexane will partition primarily to water. Cyclohexane was readily biodegradable and met the 10-day window in an OECD Guideline 301 F ready biodegradation test. The ECB (2004) authors concluded that cyclohexane is readily biodegradable in the aquatic environment and mainly partitions to the air based on Level I Fugacity modeling. The United States Environmental Protection Agency (U.S. EPA) "strongly recommends" the use of Level III Fugacity models for chemicals, including high production volume (HPV) chemicals, as the Level III model does not assume attainment of equilibrium distribution across environmental compartments, which is more realistic that models assuming equilibrium (such as level I) (U.S. EPA 2016). It is ToxServices internal policy to assign the hazard score for persistence based on the dominant environmental compartment(s) (ToxServices 2016). Therefore, when the major compartment is water based on Level II Fugacity modeling, GreenScreen® criteria specify a score of Very Low if the chemical meets the 10-day window in a ready biodegradation test.

Bioaccumulation (B) Score (vH, H, M, L, or vL): *L*
Cyclohexane was assigned a score of Low for bioaccumulation based on measured BCF values up to 129. GreenScreen® criteria classify chemicals as a Low hazard for bioaccumulation when BCF values are greater than 100 and less than 500 (CPA 2018b). The confidence in the score is reduced as it is based on a study with uncertain reliability and modeled data and measured log K_{ow} values suggest a Very Low score may be assigned.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists.
  - *Screening:* Not present on any screening lists.
- ECB 2004, ECHA 2019b
  - In a bioaccumulation test conducted according to OECD Guideline 305 C (GLP status unspecified), carp (*Cyprinus carpio*) were exposed to cyclohexane (purity not specified) at 10 or 100 µg/L for an unspecified amount of time. BCF values of 31-102 were measured at a water concentration of 100 µg/L and 37-129 at 10 µg/L. Authors of the REACH dossier assigned a Klimisch score of 4 (not assignable) for this study as the original study report was not available for review.
  - Log K_{ow} = 3.44 (measured).
- ECHA 2019b
  - Three additional records were included in the REACH dossier, but the dossier authors disregarded these records due to their major methodological deficiencies. Therefore, they are not included in this assessment.
- U.S. EPA 2017b
  - BCFBAF predicts a BCF of 69.98 using the Arnot-Gobas method based on a measured log K_{ow} of 3.44 (Appendix I).

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M, or L): *L*
Cyclohexane was assigned a score of Low for reactivity based on a structure indicating that it is not explosive or oxidizing. GreenScreen® criteria classify chemicals as a Low hazard for reactivity when no GHS classification is warranted (CPA 2017c). The confidence in the score is reduced as no experimental data are available.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists.
  - *Screening:* Not present on any screening lists.
- ECB 2004
  - Due to its lack of reactive functional groups, cyclohexane is not likely to have explosive or oxidizing properties.

Flammability (F) Score (vH, H, M, or L): H
Cyclohexane was assigned a score of High for flammability based on association with the authoritative H225 and being classified by DOT to Class 3 Group 2. In addition, based on its flash point and boiling point, ToxServices classified it to GHS Category 2 as a flammable liquid. GreenScreen® criteria classify chemicals as a High hazard for flammability when they are associated with H225, or GHS Category 2 as flammable liquids (CPA 2018b). The confidence in the score is high as it is based on authoritative lists and measured data.

- Authoritative and Screening Lists
  - *Authoritative:* EU - GHS (H-Statements) - H225 - Highly flammable liquid and vapor.

    - *Authoritative:* Québec CSST - WHMIS 1988 - Class B2 - Flammable liquids.
    - *Screening:* New Zealand - GHS - 3.1B - Flammable Liquids: high hazard
        - Based on a flash point of -18°C in a closed cup test and a boiling point of 80.7°C (CCID 2019).
    - *Screening:* Japan - GHS - Flammable liquids - Category 2
        - Based on a flash point of -20°C and a boiling point of 80.7°C (NITE 2006, 2013).
    - *Screening:* Australia - GHS - H225 - Highly flammable liquid and vapor.
- ECHA 2019b
    - Cyclohexane has a boiling point of 80.7°C.
    - Cyclohexane has a flash point of -20 to -18°C in closed cup tests.
- U.S. DOT 2008a
    - Hazard Class 3, Packaging Group 2.
- Based on the above information, ToxServices classified cyclohexane as a Category 2 flammable liquid under GHS criteria (UN 2017). GHS criteria define flammable liquids as chemicals with flash points less than 23°C and an initial boiling point greater than 35°C. Chemicals classified as GHS Category 2 flammable liquids are associated with H225, in agreement with the EU's classification for cyclohexane.

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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 Cyclohexane (CAS #110-82-7)

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
							S	R *	S	R *	*	*								
Table 2: Chemical Details																				
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	Cyclohexane	110-82-7	*L*	L	L	*M*	DG	*M*	H	L	M	L	L	*L*	H	L	vH	*vH*	vL	*L*	*L*	H

Table 3: Hazard Summary Table

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

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
Cyclohexane	2

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

Table 6

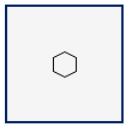
Chemical Name	Final GreenScreen® Benchmark Score
Cyclohexane	2

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 Cyclohexane (CAS #110-82-7)



110-82-7

CYCLOHEXANE

ALSO CALLED Benzene, hexahydro-, Benzenehexahydride, Cicloesano, Cicloesano [Italian], Ciclohexano, cyclo-hexane...

View all synonyms (31)

Share Profile

Hazards | Properties | Functional Uses | Process Chemistry | Resources

Pharos Hazards View

Download Lists

ENDPOINT	HAZARD LEVEL	HAZARD LIST	HAZARD DESCRIPTION	OTHER LISTS
Mammalian	High	EU - GHS (H-Statements)	H304 - May be fatal if swallowed and enters airways	+4
	Medium	GHS - New Zealand	6.1D (inhalation) - Acutely toxic	
	Medium	GHS - New Zealand	6.1D (oral) - Acutely toxic	
	High	GHS - Australia	H304 - May be fatal if swallowed and enters airways	
	Potential Concern	US EPA - OPP - Registered Pesticides	FIFRA Registered Pesticide	
Organ toxicant	High	GHS - Japan	Specific target organs/systemic toxicity following single exposure - Category 2 [H371]	+1
	Potential Concern	EU - Manufacturer REACH hazard submissions	H335 - May cause respiratory irritation (unverified)	
Acute aquatic	Very High	EU - GHS (H-Statements)	H400 - Very toxic to aquatic life	+1
	Very High	GHS - Japan	Hazardous to the aquatic environment (acute) - Category 1 [H400]	

Chron aquatic	High	EU - GHS (H-Statements)	H410 - Very toxic to aquatic life with long lasting effects	+4
	High	GHS - Australia	H410 - Very toxic to aquatic life with long lasting effects	
	Medium	GHS - Japan	Hazardous to the aquatic environment (chronic) - Category 3 [H412]	
	High	GHS - New Zealand	9.1B (crustacean) - Very ecotoxic in the aquatic environment	
	High	GHS - New Zealand	9.1B (fish) - Very ecotoxic in the aquatic environment	
Flammable	High	EU - GHS (H-Statements)	H225 - Highly flammable liquid and vapour	+4
	High	GHS - Australia	H225 - Highly flammable liquid and vapour	
	High	GHS - Japan	Flammable liquids - Category 2 [H225]	
	High	GHS - New Zealand	3.1B - Flammable Liquids: high hazard	
	Potential Concern	Québec CSST - WHMIS 1988	Class B2 - Flammable liquids	
Neurotoxicity	Medium	EU - GHS (H-Statements)	H336 - May cause drowsiness or dizziness	+2
	Medium	GHS - Australia	H336 - May cause drowsiness or dizziness	
	Potential Concern	G&L - Neurotoxic Chemicals	Neurotoxic	
Eye irritation	Medium	GHS - Japan	Serious eye damage / eye irritation - Category 2 [H319]	
Skin irritation	High	EU - GHS (H-Statements)	H315 - Causes skin irritation	+3
	High	GHS - Japan	Skin corrosion / irritation - Category 2 [H315]	
	High	GHS - Australia	H315 - Causes skin irritation	
	Medium	GHS - New Zealand	6.3B - Mildly irritating to the skin	
Terrestrial	Medium	GHS - New Zealand	9.3C - Harmful to terrestrial vertebrates	

Multiple	Medium	Québec CSST - WHMIS 1988	Class D2B - Toxic material causing other toxic effects	+2
	Potential Concern	EC - CEPA DSL	Inherently Toxic in the Environment (iTE)	
	Potential Concern	German FEA - Substances Hazardous to Waters	Class 2 - Hazard to Waters	
Restricted list	Potential Concern	C2C Certified™ - v4 RSL	Children's Products	+3
	Potential Concern	C2C Certified™ - v4 RSL	Formulated Consumer Products	
	Potential Concern	CA SCP - Candidate Chemicals	Candidate Chemical List	
	Potential Concern	EU - REACH Annex XVII non-CMRs	Substances restricted under REACH	
Cancer	Potential Concern	US EPA - IRIS Carcinogens	(1999) Data are inadequate for an assessment of human carcinogenic potential	
Developmental	Potential Concern	MAK	Pregnancy Risk Group D	

APPENDIX D: OncoLogic Evaluation of Cyclohexane (CAS #440-82-7)

OncoLogic Justification Report

CODE NUMBER: cyclohexane

SUBSTANCE ID:

User Inputs :

Select the Hydrocarbon type? : Saturated Hydrocarbons (Alkanes)

Select Saturated Hydrocarbon type? : All Other Alkanes

ALIPHATIC HYDROCARBONS, SATURATED (i.e., ALKANES)
Level of Concern: LOW

JUSTIFICATION:

With a few exceptions, there is no evidence that members of the saturated aliphatic hydrocarbon (i.e., alkane) class may be of any significant cancer concern.* Therefore, virtually ALL alkanes can be assigned a LOW concern with the following exceptions:

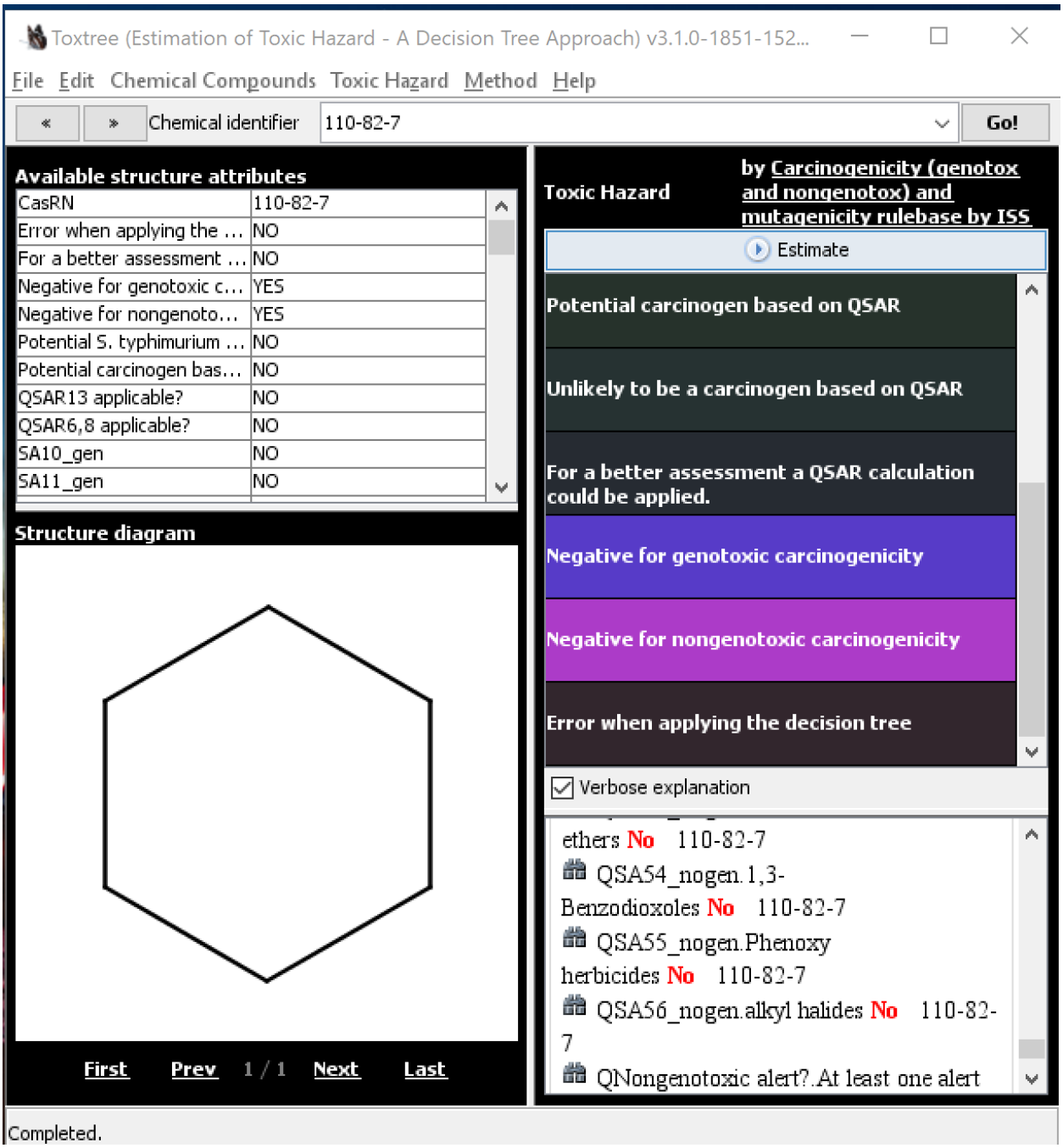
Marginal cancer concern may be assigned for the following cases:
[See other menu selections for additional information]

1. Medium sized (7 to 10 carbons) branched alkanes with at least one secondary carbon.

2. Medium sized (10 to 14 carbon) straight chain alkanes.

*It should be cautioned that the list of exceptional cases may not be exhaustive. If short-term predictive data are available, the Functional Arm of the OncoLogic system should also be used to assess the carcinogenic potential of your chemical.

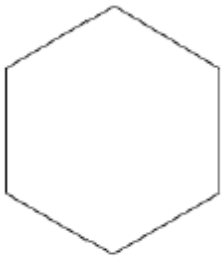
APPENDIX E: Toxtree Carcinogenicity Results for Cyclohexane (CAS #440-82-7)



APPENDIX F: VEGA Carcinogenicity Results for Cyclohexane (CAS #440-82-7)

1. Prediction Summary

Prediction for compound Molecule 0

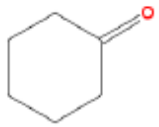


Prediction: Reliability:

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
- accuracy of prediction for similar molecules found in the training set is not adequate
- similar molecules found in the training set have experimental values that disagree with the predicted value

Compound: Molecule 0
Compound SMILES: C1CCCCC1
Experimental value: -
Predicted Carcinogen activity: Carcinogen
P(Carcinogen): 0.817
P(NON-Carcinogen): 0.183
Reliability: the predicted compound is outside the Applicability Domain of the model
Remarks:
none

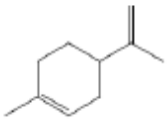
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 108-94-1
Dataset id: 187 (Test set)
SMILES: O=C1CCCCC1
Similarity: 0.795

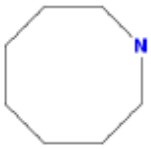
Experimental value: NON-Carcinogen
Predicted value: Carcinogen



Compound #2

CAS: 5989-27-5
Dataset id: 412 (Training set)
SMILES: C=C(C)C1CC=C(C)CC1
Similarity: 0.74

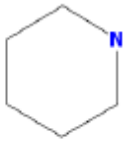
Experimental value: Carcinogen
Predicted value: NON-Carcinogen



Compound #3

CAS: 1121-92-2
Dataset id: 356 (Training set)
SMILES: N1CCCCCCC1
Similarity: 0.732

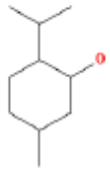
Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #4

CAS: 110-89-4
Dataset id: 659 (Training set)
SMILES: N1CCCCC1
Similarity: 0.725

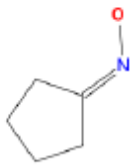
Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #5

CAS: 89-78-1
Dataset id: 427 (Training set)
SMILES: OC1CC(C)CCC1(C(C)C)
Similarity: 0.687

Experimental value: NON-Carcinogen
Predicted value: Carcinogen



Compound #6

CAS: 1192-28-5
Dataset id: 188 (Training set)
SMILES: ON=C1CCCC1
Similarity: 0.681

Experimental value: Carcinogen
Predicted value: Carcinogen

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 = 0.765
Explanation: only moderately similar compounds with known experimental value in the training set have been found.

Accuracy of prediction for similar molecules
Accuracy index = 0
Explanation: accuracy of prediction for similar molecules found in the training set is not adequate.

Concordance for similar molecules
Concordance index = 0.476
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 = 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 = 0.633
Explanation: model class assignment is well defined.

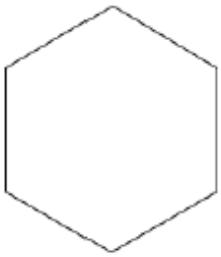
Neural map neurons concordance
Neurons concordance = 1
Explanation: predicted value agrees with experimental values of training set compounds laying in the same neuron.

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

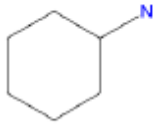


Prediction: Reliability:

Prediction is 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
- accuracy of prediction for similar molecules found in the training set is not optimal
- some similar molecules found in the training set have experimental values that disagree with the predicted value

Compound: Molecule 0
Compound SMILES: C1CCCCC1
Experimental value: -
Predicted Carcinogen activity: NON-Carcinogen
Structural alerts: -
Reliability: the predicted compound is outside the Applicability Domain of the model
Remarks:
none

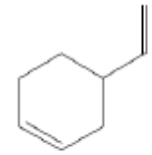
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 108-91-8
Dataset id: 834 (Training set)
SMILES: NC1CCCCC1
Similarity: 0.802

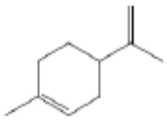
Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #2

CAS: 100-40-3
Dataset id: 238 (Training set)
SMILES: C=CC1CC=CCC1
Similarity: 0.781

Experimental value: Carcinogen
Predicted value: NON-Carcinogen



Compound #3

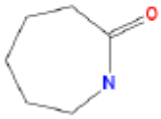
CAS: 5989-27-5
Dataset id: 267 (Training set)
SMILES: C=C(C)C1CC=C(C)CC1
Similarity: 0.74

Experimental value: Carcinogen
Predicted value: NON-Carcinogen

Compound #4

CAS: 109-99-9
Dataset id: 611 (Training set)
SMILES: O1CCCC1
Similarity: 0.674

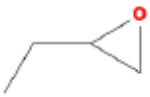
Experimental value: Carcinogen
Predicted value: NON-Carcinogen



Compound #5

CAS: 105-60-2
Dataset id: 78 (Training set)
SMILES: O=C1NCCCCC1
Similarity: 0.662

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



Compound #6

CAS: 106-88-7
Dataset id: 263 (Training set)
SMILES: O1CC1CC
Similarity: 0.65

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): SA7 Epoxides and aziridines

3.2 Applicability Domain:
Measured Applicability Domain Scores

Global AD Index
AD index = 0.634
Explanation: the predicted compound is outside the Applicability Domain of the model.

Similar molecules with known experimental value
Similarity index = 0.791
Explanation: only moderately similar compounds with known experimental value in the training set have been found.

Accuracy of prediction for similar molecules
Accuracy index = 0.509
Explanation: accuracy of prediction for similar molecules found in the training set is not optimal.

Concordance for similar molecules
Concordance index = 0.509
Explanation: some similar molecules found in the training set have experimental values that disagree with the predicted value.

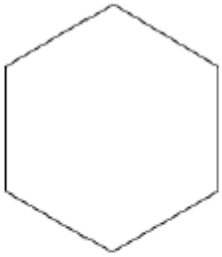
Atom Centered Fragments similarity check
ACF index = 1
Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

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

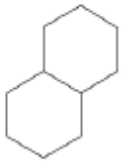


Prediction: Reliability:

Prediction is Possible NON-Carcinogen, but the result shows some critical aspects, which require to be checked:
- accuracy of prediction for similar molecules found in the training set is not optimal
- some similar molecules found in the training set have experimental values that disagree with the predicted value

Compound: Molecule 0
Compound SMILES: C1CCCCC1
Experimental value: -
Predicted Mutagen 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

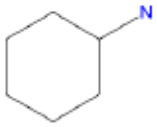
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: N.A.
Dataset id: 1202 (Training set)
SMILES: C1CCC2CCCCC2(C1)
Similarity: 0.86

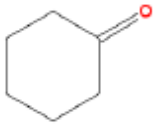
Experimental value: Carcinogen
Predicted value: Possible NON-Carcinogen



Compound #2

CAS: N.A.
Dataset id: 1343 (Training set)
SMILES: NC1CCCCC1
Similarity: 0.802

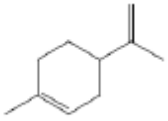
Experimental value: NON-Carcinogen
Predicted value: Possible NON-Carcinogen



Compound #3

CAS: N.A.
Dataset id: 187 (Training set)
SMILES: O=C1CCCCC1
Similarity: 0.795

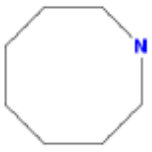
Experimental value: NON-Carcinogen
Predicted value: Possible NON-Carcinogen



Compound #4

CAS: N.A.
Dataset id: 412 (Training set)
SMILES: C=C(C)C1CC=C(C)CC1
Similarity: 0.74

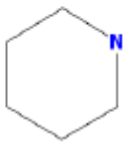
Experimental value: Carcinogen
Predicted value: Possible NON-Carcinogen



Compound #5

CAS: N.A.
Dataset id: 356 (Training set)
SMILES: N1CCCCCCC1
Similarity: 0.732

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



Compound #6

CAS: N.A.
Dataset id: 669 (Training set)
SMILES: N1CCCCC1
Similarity: 0.725

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

3.2 Applicability Domain:
Measured Applicability Domain Scores

Global AD Index
AD index = 0.726
Explanation: the predicted compound could be out of the Applicability Domain of the model.

Similar molecules with known experimental value
Similarity index = 0.816
Explanation: strongly similar compounds with known experimental value in the training set have been found.

Accuracy of prediction for similar molecules
Accuracy index = 0.646
Explanation: accuracy of prediction for similar molecules found in the training set is not optimal.

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.

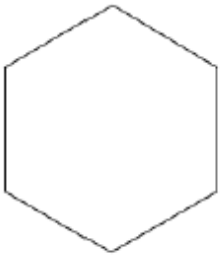
Atom Centered Fragments similarity check
ACF index = 1
Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

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

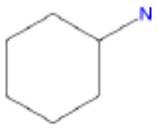


Prediction: Reliability:

Prediction is Possible NON-Carcinogen, the result appears reliable. Anyhow, you should check it through the evaluation of the information given in the following sections. Anyway some issues could be not optimal:
- only moderately similar compounds with known experimental value in the training set have been found

Compound: Molecule 0
Compound SMILES: C1CCCCC1
Experimental value: -
Predicted Mutagen activity: Possible NON-Carcinogen
No. alerts for carcinogenicity: 0
Structural alerts: -
Reliability: the predicted compound is into the Applicability Domain of the model
Remarks:
none

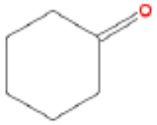
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 108-91-8
Dataset id: 748 (Training set)
SMILES: NC1CCCCC1
Similarity: 0.802

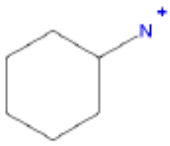
Experimental value: NON-Carcinogen
Predicted value: Possible NON-Carcinogen



Compound #2

CAS: 108-94-1
Dataset id: 934 (Training set)
SMILES: O=C1CCCCC1
Similarity: 0.795

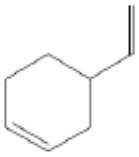
Experimental value: NON-Carcinogen
Predicted value: Possible NON-Carcinogen



Compound #3

CAS: 4998-76-9
Dataset id: 935 (Training set)
SMILES: [NH3+]C1CCCCC1
Similarity: 0.792

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

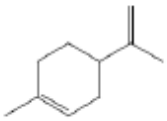


Compound #4

CAS: 100-40-3
Dataset id: 196 (Training set)
SMILES: C=CC1CC=CCC1
Similarity: 0.781

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): Carcinogenity alert no. 39

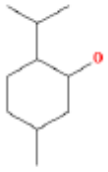


Compound #5

CAS: 5989-27-5
Dataset id: 218 (Training set)
SMILES: C=C(C)C1CC=C(C)CC1
Similarity: 0.74

Experimental value: Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): Carcinogenity alert no. 39



Compound #6

CAS: 15356-70-4
Dataset id: 654 (Training set)
SMILES: OC1CC(C)CCC1(C(C)C)
Similarity: 0.687

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

3.2 Applicability Domain:
Measured Applicability Domain Scores

✓	**Global AD Index** AD index = 0.892 Explanation: the predicted compound is into the Applicability Domain of the model.
⚠	**Similar molecules with known experimental value** Similarity index = 0.796 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:

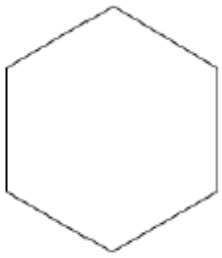
✓ The feature has a good assessment, model is reliable regarding this aspect.

⚠ 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.

1. Prediction Summary

Prediction for compound Molecule 0

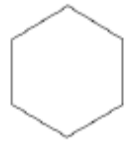
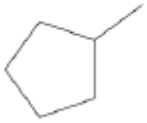
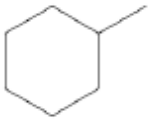
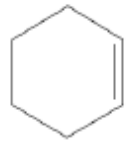
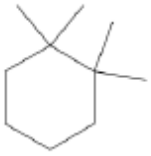
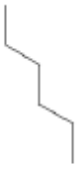


EXPERIMENTAL DATA

Experimental value is NON-Carcinogen. Model prediction is NON-Carcinogen (good reliability).

Compound: Molecule 0
Compound SMILES: C1CCCCC1
Experimental value: NON-Carcinogen
Predicted Oral Carcinogenic class: NON-Carcinogen
Reliability: the predicted compound is into the Applicability Domain of the model
Remarks:
none

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

	Compound #1 CAS: 110-82-7 Dataset id: 415 (Training set) SMILES: C1CCCCC1 Similarity: 1 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen
	Compound #2 CAS: 96-37-7 Dataset id: 588 (Training set) SMILES: CC1CCCC1 Similarity: 0.947 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen
	Compound #3 CAS: 108-87-2 Dataset id: 587 (Training set) SMILES: CC1CCCCC1 Similarity: 0.941 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen
	Compound #4 CAS: 110-83-8 Dataset id: 417 (Training set) SMILES: C1=CCCCC1 Similarity: 0.865 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen
	Compound #5 CAS: 30501-43-0 Dataset id: 692 (Training set) SMILES: CC1(C)(CCCCC1(C)(C)) Similarity: 0.848 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen
	Compound #6 CAS: 110-54-3 Dataset id: 540 (Training set) SMILES: CCCCCC Similarity: 0.847 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

Global AD Index
AD index = 1
Explanation: the predicted compound is into the Applicability Domain of the model.

Similar molecules with known experimental value
Similarity index = 1
Explanation: strongly similar compounds with known experimental value in the training set have been found.

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

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

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.

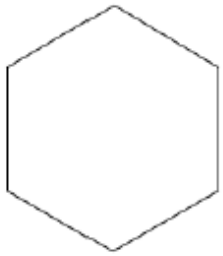
Atom Centered Fragments similarity check
ACF index = 1
Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

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

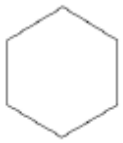
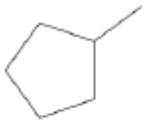
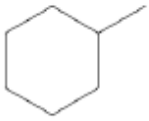
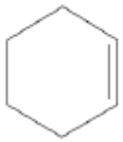
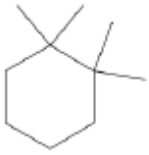
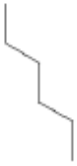


EXPERIMENTAL DATA

Experimental value is NON-Carcinogen. Model prediction is NON-Carcinogen (good reliability).

Compound: Molecule 0
Compound SMILES: C1CCCCC1
Experimental value: NON-Carcinogen
Predicted Inhalation Carcinogenic class: NON-Carcinogen
Reliability: the predicted compound is into the Applicability Domain of the model
Remarks:
none

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

	Compound #1 CAS: 110-82-7 Dataset id: 377 (Training set) SMILES: C1CCCCC1 Similarity: 1 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen
	Compound #2 CAS: 96-37-7 Dataset id: 568 (Training set) SMILES: CC1CCCC1 Similarity: 0.947 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen
	Compound #3 CAS: 108-87-2 Dataset id: 567 (Training set) SMILES: CC1CCCCC1 Similarity: 0.941 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen
	Compound #4 CAS: 110-83-8 Dataset id: 380 (Test set) SMILES: C1=CCCCC1 Similarity: 0.865 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen
	Compound #5 CAS: 30501-43-0 Dataset id: 687 (Training set) SMILES: CC1(C)(CCCCC1(C)(C)) Similarity: 0.848 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen
	Compound #6 CAS: 110-54-3 Dataset id: 516 (Training set) SMILES: CCCCCC Similarity: 0.847 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

✓	**Global AD Index** AD index = 1 Explanation: the predicted compound is into the Applicability Domain of the model.
✓	**Similar molecules with known experimental value** Similarity index = 1 Explanation: strongly similar compounds with known experimental value in the training set have been found.
✓	**Accuracy of prediction for similar molecules** Accuracy index = 1 Explanation: accuracy of prediction for similar molecules found in the training set is good.
✓	**Concordance for similar molecules** Concordance index = 1 Explanation: similar molecules found in the training set have experimental values that agree with the predicted value.
✓	**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.

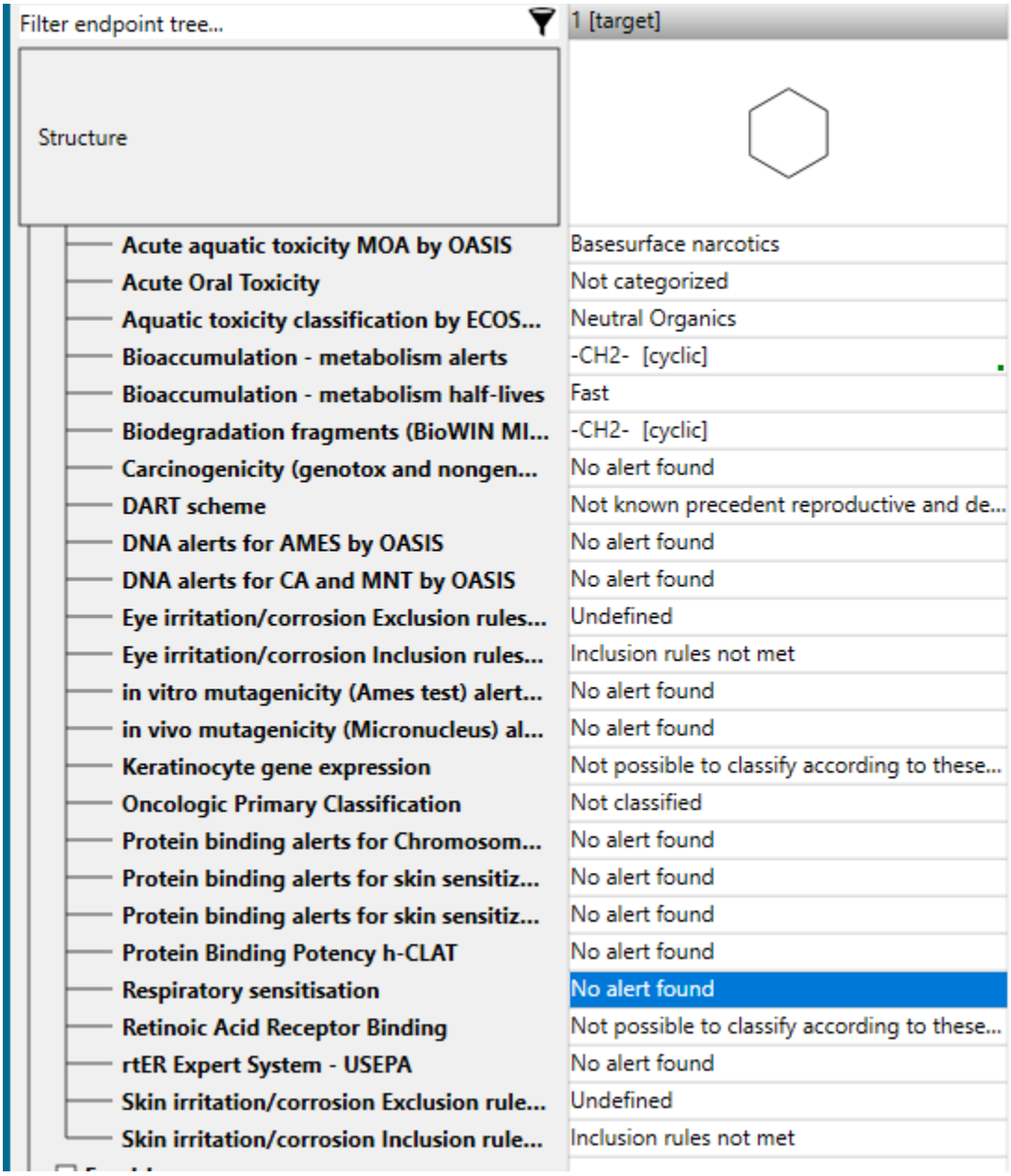
Symbols explanation:

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

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

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

APPENDIX G: OECD Toolbox Respiratory Sensitization Results for Cyclohexane (CAS #110-82-7)



Filter endpoint tree...	1 [target]
Structure	
Acute aquatic toxicity MOA by OASIS	Basesurface narcotics
Acute Oral Toxicity	Not categorized
Aquatic toxicity classification by ECOS...	Neutral Organics
Bioaccumulation - metabolism alerts	-CH2- [cyclic]
Bioaccumulation - metabolism half-lives	Fast
Biodegradation fragments (BioWIN MI...	-CH2- [cyclic]
Carcinogenicity (genotox and nongen...	No alert found
DART scheme	Not known precedent reproductive and de...
DNA alerts for AMES by OASIS	No alert found
DNA alerts for CA and MNT by OASIS	No alert found
Eye irritation/corrosion Exclusion rules...	Undefined
Eye irritation/corrosion Inclusion rules...	Inclusion rules not met
in vitro mutagenicity (Ames test) alert...	No alert found
in vivo mutagenicity (Micronucleus) al...	No alert found
Keratinocyte gene expression	Not possible to classify according to these...
Oncologic Primary Classification	Not classified
Protein binding alerts for Chromosom...	No alert found
Protein binding alerts for skin sensitiz...	No alert found
Protein binding alerts for skin sensitiz...	No alert found
Protein Binding Potency h-CLAT	No alert found
Respiratory sensitisation	No alert found
Retinoic Acid Receptor Binding	Not possible to classify according to these...
rtER Expert System - USEPA	No alert found
Skin irritation/corrosion Exclusion rule...	Undefined
Skin irritation/corrosion Inclusion rule...	Inclusion rules not met

APPENDIX H: ECOSAR Modeling Results for Cyclohexane (CAS #110-82-7)

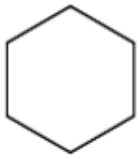
Created on Aug 22, 2019 1:04:30 PM

Organic Module Report

Results of Organic Module Evaluation

CAS	Name	SMILES
110827	Cyclohexane	C(CCCC1)C1

Structure



Details	
Mol Wt	84.16
Selected LogKow	3.18
Selected Water Solubility (mg/L)	55
Selected Melting Point (°C)	6.6
Estimated LogKow	3.18
Estimated Water Solubility (mg/L)	40.78
Measured LogKow	3.44
Measured Water Solubility (mg/L)	55
Measured Melting Point (°C)	6.6

Class Results:	

Neutral Organics

Organism	Duration	End Point	Concentration (mg/L)	Max Log Kow	Flags
Fish	96h	LC50	6.08	5	
Daphnid	48h	LC50	3.85	5	
Green Algae	96h	EC50	4.49	6.4	
Fish		ChV	0.68	8	
Daphnid		ChV	0.51	8	
Green Algae		ChV	1.5	8	
Fish (SW)	96h	LC50	7.71	5	
Mysid	96h	LC50	2.58	5	

1 / 2

Class Results:	

Organism	Duration	End Point	Concentration (mg/L)	Max Log Kow	Flags
Fish (SW)		ChV	1.72	8	
Mysid (SW)		ChV	0.16	8	
Earthworm	14d	LC50	110.51	6	• Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported

APPENDIX I: EPISuite Modeling Results for Cyclohexane (CAS #110-82-7)

CAS Number: 000110-82-7
SMILES : C(CCCC1)C1
CHEM : CYCLOHEXANE
MOL FOR: C6 H12
MOL WT : 84.16
---------- EPI SUMMARY (v4.11) ----------

Physical Property Inputs:
Log Kow (octanol-water): 3.44
Boiling Point (deg C) : 80.70
Melting Point (deg C) : 6.60
Vapor Pressure (mm Hg) : 96.9
Water Solubility (mg/L): 55
Henry LC (atm-m3/mole) : 0.15

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = 3.18
Log Kow (Exper. database match) = 3.44
Exper. Ref: HANSCH,C ET AL. (1995)

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 89.72 (Adapted Stein & Brown method)
Melting Pt (deg C): -82.72 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 93.9 (Mean VP of Antoine & Grain methods)
VP (Pa, 25 deg C) : 1.25E+004 (Mean VP of Antoine & Grain methods)
MP (exp database): 6.6 deg C
BP (exp database): 80.7 deg C
VP (exp database): 9.69E+01 mm Hg (1.29E+004 Pa) at 25 deg C

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 40.78
log Kow used: 3.44 (user entered)
melt pt used: 6.60 deg C
Water Sol (Exper. database match) = 55 mg/L (25 deg C)
Exper. Ref: MCAULIFFE,C (1966)

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 48.757 mg/L

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

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 2.55E-001 atm-m3/mole (2.59E+004 Pa-m3/mole)
Group Method: 1.94E-001 atm-m3/mole (1.97E+004 Pa-m3/mole)
Exper Database: 1.50E-01 atm-m3/mole (1.52E+004 Pa-m3/mole)

For Henry LC Comparison Purposes:
User-Entered Henry LC: 1.500E-001 atm-m3/mole (1.520E+004 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 1.951E-001 atm-m3/mole (1.977E+004 Pa-m3/mole)
VP: 96.9 mm Hg (source: User-Entered)
WS: 55 mg/L (source: User-Entered)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 3.44 (user entered)
Log Kaw used: 0.788 (user entered)
Log Koa (KOAWIN v1.10 estimate): 2.652
Log Koa (experimental database): 2.740

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.7075
Biowin2 (Non-Linear Model) : 0.8598
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 3.0132 (weeks)
Biowin4 (Primary Survey Model) : 3.7263 (days-weeks)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.5063
Biowin6 (MITI Non-Linear Model): 0.7170
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): 0.1160
Ready Biodegradability Prediction: YES

Hydrocarbon Biodegradation (BioHCwin v1.01):
LOG BioHC Half-Life (days) : 1.7434
BioHC Half-Life (days) : 55.3803

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled): 1.29E+004 Pa (96.9 mm Hg)
Log Koa (Exp database): 2.740
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 2.32E-010
Octanol/air (Koa) model: 1.35E-010
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 8.39E-009
Mackay model : 1.86E-008
Octanol/air (Koa) model: 1.08E-008

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 8.4783 E-12 cm3/molecule-sec
Half-Life = 1.262 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 15.139 Hrs
Ozone Reaction:
No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi):

1.35E-008 (Junge-Pankow, Mackay avg)
1.08E-008 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 145.8 L/kg (MCI method)
Log Koc: 2.164 (MCI method)
Koc : 966.4 L/kg (Kow method)
Log Koc: 2.985 (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 = 1.937 (BCF = 86.44 L/kg wet-wt)
Log Biotransformation Half-life (HL) = -0.1181 days (HL = 0.7619 days)
Log BCF Arnot-Gobas method (upper trophic) = 1.845 (BCF = 69.98)
Log BAF Arnot-Gobas method (upper trophic) = 1.845 (BAF = 69.98)
log Kow used: 3.44 (user entered)

Volatilization from Water:
Henry LC: 0.15 atm-m3/mole (entered by user)
Half-Life from Model River: 0.9397 hours (56.38 min)
Half-Life from Model Lake : 87.17 hours (3.632 days)

Removal In Wastewater Treatment (recommended maximum 95%):
Total removal: 98.38 percent
Total biodegradation: 0.04 percent
Total sludge adsorption: 6.20 percent
Total to Air: 92.14 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model: (MCI Method)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	30.5	36.8	1000
Water	66.1	360	1000
Soil	2.92	720	1000
Sediment	0.5	3.24e+003	0

Persistence Time: 93.1 hr

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

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	30.5	36.8	1000
Water	66.1	360	1000
water	(66.1)		
biota	(0.0091)		
suspended sediment	(0.0144)		

Soil	2.92	720	1000
Sediment	0.5	3.24e+003	0

Persistence Time: 93.1 hr

Level III Fugacity Model: (EQC Default)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	26.2	36.8	1000
Water	57.6	360	1000
water	(57.5)		
biota	(0.00791)		
suspended sediment	(0.0973)		
Soil	14	720	1000
Sediment	2.26	3.24e+003	0

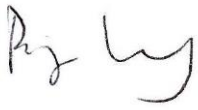
Persistence Time: 107 hr

Licensed GreenScreen® Profilers

Cyclohexane GreenScreen® Evaluation Prepared/Updated by:

Sara M. Ciotti, Ph.D.
Toxicologist
ToxServices LLC

Zach Guerrette, Ph.D., D.A.B.T.
Toxicologist
ToxServices LLC



Bingxuan Wang, Ph.D., D.A.B.T.
Senior Toxicologist
ToxServices LLC

Cyclohexane GreenScreen® Evaluation/Update QC'd by:

Bingxuan Wang, Ph.D., D.A.B.T.
Senior Toxicologist
ToxServices LLC

Cyclohexane GreenScreen® Evaluation QC'd by:

Jennifer Rutkiewicz, Ph.D.
Senior Toxicologist
ToxServices LLC