N-HEPTANE

(CAS #142-82-5)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: January 30, 2023

Expiration Date: January 30, 2028

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GreenScreen® Executive Summary for n-Heptane (CAS #142-82-5)

n-Heptane is a colorless liquid under standard temperature and pressure. It is highly volatile and flammable. It occurs in natural gas and crude oil at 0.1-1.9% and an n-heptane content of 2.5% has been reported in petroleum, 0.97% in gasoline, and 0.000107% in coal used by power plants. n-Heptane is also found as a volatile component of raw or cooked meat, seafood, and certain plants. n-Heptane functions as a solvent, anesthetic, standard for octane rating determinations, and chemical intermediate. n-Heptane does not have restrictions when used as a solvent in cosmetics/personal care products in Europe. The U.S. Food and Drug Administration (U.S. FDA) recognizes n-heptane as an indirect additive used in food contact substances.

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

The GreenScreen® Benchmark Score for n-heptane has not changed over time. The original GreenScreen® assessment was performed in 2014 under version 1.2 criteria and ToxServices assigned a Benchmark 2 (BM-2) score. The BM-2 score was maintained with version 1.4 updates in 2018, 2019, 2020, 2021, and in this 2023 report.

New Approach Methodologies (NAMs) used in this ChemFORWARD assessment include *in silico* modeling for carcinogenicity, skin and respiratory sensitization, aquatic toxicity, biodegradation, and bioaccumulation, and *in vitro* data for genotoxicity and endocrine activity. The quality, utility, and accuracy of NAM predictions are greatly influenced by two primary types of uncertainties:

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

Type I (input data) uncertainties in n-heptane's NAMs dataset include lack of, or insufficient *in vivo* experimental data for carcinogenicity, endocrine activity, skin and respiratory sensitization, ecotoxicity, and bioaccumulation, use of non-guideline or deleted (by OECD) test protocols, and lack of validated test methods for respiratory sensitization. n-Heptane's Type II (extrapolation output) uncertainties include lack of defined applicability domains of several models, uncertain *in vivo* relevance of *in vitro* testing and *in silico* testing of endocrine receptor binding due to lack of consideration of toxicokinetics, limitations of *in vitro* genotoxicity studies in mimicking *in vivo* metabolic conditions and that they each focused on only one or a few genotoxicity events, and the limitation respiratory sensitization structural alerts in evaluating non-immunologic mechanisms for respiratory sensitization. Some of n-heptane's type II uncertainties were alleviated by the use of *in vitro* test batteries and/or in combination of *in vivo* data.

GreenScreen® Hazard Summary Table for n-Heptane

Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
C	M	R	D	E	AT	ST		N		SnS	SnR	IrS	IrE	AA	CA	P	B	Rx	F
						s	r*	s	r*	*	*								
L	**L**	**L**	*L*	DG	**L**	**H**	**L**	**M**	**L**	**L**	*L*	**H**	*M*	**vH**	*vH*	**vL**	*M*	*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 n-Heptane (CAS #142-82-5)

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

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Date: June 17, 2014

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Date: July 31, 2018

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Organization: ToxServices LLC
Date: August 10, 2018

GreenScreen® Assessment (v.1.4) Prepared By:
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Date: August 23, 2019

Quality Control Performed By:
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Date: September 15, 2019

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Title: Toxicologist
Organization: ToxServices LLC
Date: August 23, 2019

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Date: September 15, 2019

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Date: November 2, 2020; December 17, 2020

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Organization: ToxServices LLC
Date: November 23, 2020, December 17, 2020, January 14, 2021

GreenScreen® Assessment (v.1.4) Prepared By:
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Date: December 12, 2022; January 26, 2023

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Organization: ToxServices LLC
Date: December 27, 2022, January 30, 2023

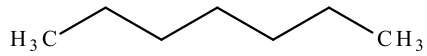
Expiration Date: January 30, 2028[2]

Chemical Name: n-Heptane

[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).

CAS Number: 142-82-5

Chemical Structure(s):



Also called: Heptane; Dipropyl methane; Dipropylmethane; EC 205-563-8; Heptyl hydride; Heptanes; UN1206 (PubChem 2023).

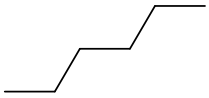
Suitable surrogates or moieties of chemicals used in this assessment (CAS #s):
For carcinogenicity, reproductive and developmental toxicity endpoints, commercial hexane containing ~50% n-hexane (no CAS number) is used to fill the data gaps in the REACH registration dossier for n-heptane. The exact composition of the specific commercial hexane tested was not reported. Typical components in commercial hexane include 2-methylpentane, 3-methylpentane, 2-3-dimethylbutene, cyclopentane, cyclohexane and small quantities of pentane and heptane isomers, acetone, methyl ethyl ketone, dichloromethane, trichloroethylene, toluene, and other petroleum hydrocarbons (U.S. EPA 2009, ATSDR 1999, PubChem 2023). Another hydrocarbon mixture, containing 25-35% n-hexane (C6 normal and iso paraffins (hexanes) and naphthenes (methyl-cyclohexane, dimethylcyclohexane), CAS #NA) is used to provide additional justification for the skin irritation endpoint. n-Hexane is known to be a highly toxic hydrocarbon due to the production of specific reproductively toxic metabolites, specifically γ-diketone 2,5-hexanedione (2,5-HD) (OEHHA 2017). n-Heptane may be metabolized via the same pathways (typical of n-alkane metabolism) and can also form a toxic γ-diketone metabolite, 2,5-heptanedione, similar to 2,5-HD, but this chemical is a very minor metabolite in both rats and humans for n-heptane, and is only detected as 0.01% of the total urinary metabolites following inhalational administration of n-heptane in humans (U.S. EPA 2016, Filser et al. 1996, see details in the toxicokinetic section below). In comparison, approximately 0.5% of all n-hexane metabolites are excreted as the toxic 2,5-HD compound following inhalational exposure in humans. According to Filser et al. (1996) the formation of 2,5-heptanedione from 500 ppm of n-heptane is approximately 4 times less than 2,5-HD from 50 ppm of n-hexane. That is, even if n-heptane is dosed at a concentration 10x greater than that of n-hexane, the formation of the corresponding toxic metabolite, 2,5-heptanedione, is significantly less than the formation of 2,5-HD over the same period. Therefore, using data on pure n-hexane would significantly overestimate the toxicity of n-heptane. The use of the commercial n-hexane mixture is considered more relevant than pure n-hexane to n-heptane, and yet still conservative, because of lower concentrations of n-hexane in the mixtures. content. Therefore, the use of commercial n-hexane (and other < 50% mixtures of n-hexane) as a surrogate to estimate skin irritation and reproductive and developmental toxicities of n-heptane is appropriate. ToxServices did not include reproductive or developmental toxicity data (or other systemic toxicity/neurotoxicity endpoints) on pure n-hexane, which would be overly conservative.

ToxServices also used data on United Nations Environment Programme (UNEP)'s C7-C9 aliphatic hydrocarbon solvents category as supportive evidence for developmental toxicity (OECD 2010). This category of hydrocarbons includes pure and multi-constituent aliphatic hydrocarbon solvent commercial chemicals containing < 3% (typically < 1%) aromatics and < 5% (typically < 0.1%) n-hexane. These hydrocarbons are straight chain (n-), branched (-iso), and/or cyclic. UNEP considers these chemicals to have similar toxicities due to similar structures and common modes of toxic action (OECD 2010).

For the sensitization endpoint, hydrocarbons, C7-C9, n-alkanes, isoalkanes, cyclics (no CAS number provided), was used as a surrogate in the REACH registration dossier for n-heptane. For skin and eye irritation, 2,4,4-trimethylpentane (CAS #540-84-1) was used as a surrogate for n-heptane in the REACH

registration dossier. As these surrogates also fit into to the UNEP C7-C9 aliphatic hydrocarbon solvents category, ToxServices considers them to be appropriate surrogates for n-heptane (ECHA 2023a).

Surrogate #1:



Commercial hexane, containing ~50% n-hexane (no CAS number); shown above is the structure of pure n-hexane (CAS #110-54-3) (PubChem 2023)

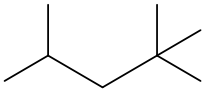
Surrogate #2: C7-C9 aliphatic hydrocarbon solvents category (multiple CAS and structures)

Multiple chemical structures

Surrogate #3: Hydrocarbons, C7-C9, n-alkanes, isoalkanes, cyclics (no CAS number provided)

No chemical structure available

Surrogate #4:



2,4,4-Trimethylpentane (CAS #540-84-1) (PubChem 2023)

Identify Applications/Functional Uses (ECB 2000, HSDB 2014)**:**
1. Standard for octane rating determinations of fuels
2. Anesthetic
3. Solvent
4. Organic synthesis/Chemical intermediate
5. Preparation of laboratory reagents
6. Research/laboratory Chemical
7. Cleaning/washing agent

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

GreenScreen® Summary Rating for n-Heptane[4,5 6,7]: n-Heptane 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:

[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.

- 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 (CPA 2018b) Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), n-heptane meets requirements for a GreenScreen Benchmark™ Score of 2 despite the hazard data gap. In a worst-case scenario, if n-heptane were assigned a High score for the data gap E, it would be categorized as a Benchmark 1 Chemical.

Figure 1: GreenScreen® Hazard Summary Table for n-Heptane

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

Per GreenScreen® guidance (CPA 2018b), transformation products of 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 products will not persist long enough to be encountered after use or release of the parent chemical. As n-heptane is readily biodegradable (see Persistence section below), it is not expected to have relevant transformation products. Furthermore, n-heptane exists solely as a vapor in the atmosphere and is readily degradable in water, therefore, hydrolysis or photolysis of n-heptane is not expected to be important environmental fate processes. Based on its molecular formula, possible combustion products of n-heptane are CO and CO_2, which are naturally occurring, ambient substances and not relevant with respect to the GreenScreen Benchmark™ Score for n-heptane.

Introduction

n-Heptane is a colorless volatile liquid with gasoline-like odor. It occurs in natural gas and crude oil (0.1-1.9%). A content of 2.5% has been reported in petroleum, and this results in the major proportion of heptane found in the atmosphere. n-Heptane has been detected at 9,700 ppm in gasoline, and 1.07 ppm in coal used by power plants. n-Heptane is also found as a volatile component of raw or cooked meat, seafood, and certain plants. n-Heptane is produced via fractional distillation of petroleum followed by purification via rectification or hydrogenation of 1-heptene (Ashford's Undated). n-Heptane is used as an industrial solvent (for adhesives, lacquers and inks in gravure printing), as an extraction solvent and in manufacture of plastic foams, and synthesis of toluene and alkylbenzenes (HSDB 2014). In Europe, n-heptane does not have restrictions when used as a solvent in cosmetics/ personal care products under EC No. 1223/2009 (EC 2023). The U.S. Food and Drug Administration

(U.S. FDA) authorizes n-heptane for indirect food additive applications when used in components of adhesives (21 CFR §175.105) and polycarbonate resins (21 CFR §177.1580)(U.S. FDA 2023).

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

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

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

n-Heptane is not listed on the SCP SCIL.

GreenScreen® List Translator Screening Results

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

- n-Heptane is an LT-P1 chemical when screened using Pharos, and therefore a full GreenScreen® is required.
- n-Heptane is listed on the U.S. DOT list as a Hazard Class 3 chemical, Packing Group II (UN1206).
- n-Heptane is on the following lists for multiple endpoints:
    - Québec 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.
    - GHS – Japan Hazardous to the aquatic environment (chronic) - Category 1 [H410].
    - GHS – Australia H410 - Very toxic to aquatic life with long lasting effects.
    - GHS – New Zealand – Hazardous to the aquatic environment – chronic category 1
- Specified lists for single endpoints are reported in individual hazard endpoints in the hazard assessment section below.

Hazard Statement and Occupational Control

A harmonized Globally Harmonized System of Classification and Labelling of Chemicals (GHS) classification is available for n-heptane as reported by the European Chemicals Agency (ECHA) (ECHA 2023b): it is classified as a GHS Category 2 flammable liquid (H225), a GHS Category 2 skin irritant (H315), a GHS Category 1 aspiration toxicant (H304), a GHS Category 3 specific target organ toxicant following single exposures for narcotic effects (H336), a GHS Category 1 acute aquatic toxicant (H400), and a GHS Category 1 chronic aquatic toxicant (H410). Details of the hazard statements are provided in Table 1 below. Recommended personal protective equipment and occupation exposure limits identified for n-heptane are presented in Table 2, below.

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

Table 1: GHS H Statements for n-Heptane (CAS #142-82-5) (ECHA 2023b)	
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 n-Heptane (CAS #142-82-5)			
Personal Protective Equipment (PPE)	**Reference**	**Occupational Exposure Limits (OEL)**	**Reference**
Appropriate personal protective clothing to prevent skin contact. Appropriate eye protection to prevent eye contact. Respirator	HSDB 2014	ACGIH 2019 TLV: 400 ppm (8-hour TWA), 500 ppm (STEL) Cal/OSHA PEL: 400 ppm (8-hour TWA), 500 ppm (STEL) EU OEL: 500 ppm, 2,085 mg/m^3 as TWA NIOSH REL: 85 ppm (350 mg/m^3) TWA, 440 ppm (1,800 mg/m^3) 15-minute STEL, 759 ppm (IDLH) OSHA PEL: 500 ppm (2,000 mg/m^3) TWA	ICSC 2015, OSHA 2023

ACGIH: American Conference of Governmental Industrial Hygienists
EU: European Union
IDLH: Immediately dangerous to life or health
NIOSH: National Institute for Occupational Safety and Health
OEL: Occupational exposure limit
OSHA: Occupational Safety and Health Administration
PEL: Permissible exposure limit
REL: Recommended exposure limit
STEL: Short term exposure limit
TLV: Threshold limit values
TWA: Time weighted average

Physicochemical Properties of n-Heptane

n-Heptane is a colorless volatile liquid with gasoline-like odor. It is slightly soluble in water.

Table 3: Physical and Chemical Properties of n-Heptane (CAS #142-82-5)		
Property	**Value**	**Reference**
Molecular formula	C_7H_{16}	PubChem 2023
SMILES Notation	C(CCC)CCC	PubChem 2023
Molecular weight	100.203 g/mol	PubChem 2023
Physical state	Liquid	ECHA 2023a
Appearance	Colorless	HSDB 2014
Melting point	-90.549°C	HSDB 2014
Boiling point	98.2-98.4°C (ASTM D 1078)	ECHA 2023a
Vapor pressure	48 hPa (36 mm Hg) at 20°C	ECB 2000

Table 3: Physical and Chemical Properties of n-Heptane (CAS #142-82-5)		
Property	**Value**	**Reference**
	6.09 kPa (45.7 mm Hg) at 25°C	ECHA 2023a
Water solubility	2.4 mg/L at 20°C	ECHA 2023a
Dissociation constant	Does not dissociate	ECHA 2023a
Density/specific gravity	0.69 g/cm^3 at 15°C	ECHA 2023a
Partition coefficient	Log K_{ow} = 4.66 Log K_{ow} = 4.5	HSDB 2014 ECHA 2023a

Toxicokinetics

- ECHA 2023a
  - *Absorption*: Male F344/N rats (n = 10) were administered nose-only inhalation exposures to escalating concentrations of heptane vapor (> 99% purity). The concentrations were 1, 10, 100, 1,000, and 5,000 ppm on days 1, 2, 3, 4, and 5, respectively. Each exposure lasted 80 minutes with the rat confined to the exposure tube connected to the second sampling loop of a dual column gas chromatograph. The amount of n-heptane absorbed "was calculated from the output of the gas chromatograph and the flow rate past the rat's nose. During the exposure to 100 ppm, the uptake of heptane was 4.5 ± 0.3 nmol/kg/min/ppm during minutes 60-70. No other data were provided. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) as only basic data were provided.
  - *Absorption*: The dermal absorption of n-heptane (purity not specified) was evaluated using an *in vitro* human skin model at three different laboratories. Human cadaver skin was exposed to 20 µL n-heptane for 10 or 60 minutes. After 10 and 60 minutes, the flux values were 113 µg/cm^2/h and 22.1 µg/cm^2/h, respectively. The barrier properties of the skin were 0.90 and 0.88 for the 10- and 60-minute exposures, indicating that the longer duration exposure compromised the barrier function of the skin.
  - *Distribution*: Male Sprague-Dawley rats (4/exposure duration) were administered whole body inhalation exposures to n-heptane vapor (> 99% purity) at 100 ppm (equivalent to 0.52 mg/L) 12 hours/day for one, two, or three consecutive days. The animals were sacrificed immediately after the end of the exposure period or 12 hours after exposure on day 3. The brain, blood, kidney, liver, and perirenal fat were collected and evaluated for n-heptane content. Perirenal fat had the highest concentration of n-heptane, followed by kidney, brain, blood, and liver. The concentration in perirenal fat decreased with increasing number of exposures. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) as it meets generally accepted scientific principles.
  - *Distribution*: Homogenized human tissues were exposed to 10-20 µg n-heptane (> 98% purity) for 12 hours. The mean partition coefficients were 1.9 for blood, 10.8 for liver, 8.9 for kidney, 12.4 for brain, 385 for fat, 12.5 for muscle, 6.1 for heart, and 2.5 for lung. In contrast, the mean olive oil partition coefficient was 452. The study authors suggest that the low partition coefficient of 1.9 in blood suggests that n-heptane has low respiratory absorption. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) as the original study was well documented and met basic scientific principles.
  - *Distribution and Metabolism*: Male Sprague-Dawley rats (n = 10) were administered whole body inhalation exposures to n-heptane (> 90% purity) at 1,800 ppm (equivalent to 7.4 mg/L) for 7 hours. Blood, urine, and tissues (liver, muscle, kidney, nervous tissue) were collected at the end of the exposure period and 24 hours after exposure. At the end of the exposure period, 2-heptanol, 3-heptanol, 2-heptanone, 3-heptanone, 4-heptanone, gamma-valerolactone, and 2,5-heptanedione were detected in the urine. Twenty-four hours after the

end of the exposure period, 2-heptanol (46.3%) and 3-heptanol (35.2%) accounted for approximately 80% of the urinary metabolites, with gamma-valerolactone accounting for an additional 11.5% of the metabolites. In addition, 4-heptanone (1.2%), 3-heptanone (1.5%), 2-heptanone (3.5%), and 2,5-heptanedione (0.8%) were detected. At the end of the exposure period, 2-heptanol was detected in kidney, liver, muscle, and nervous tissue at 0.2-2 mg/L, while the mean n-heptane concentrations were 25.6 mg/L in liver, 10.1 mg/L in kidney, 22.9 mg/L in muscle, 5.7 mg/L in blood, and 18.4 mg/L in nervous tissue. n-Heptane and 2-heptanol were not detected in blood or tissues 24 hours after the end of the exposure period (Perbellini et al. 1986). The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) as only basic data were provided.

- Bahima et al. 1984
  - *Metabolism:* In a similar metabolism study, 2,5-heptanedione was only detected at 0.1% in female rats exposed by inhalation to n-heptane at 2,000 ppm for 6 hours/day, 5 days/week, for 12 weeks.
- Csanády 2012
  - *Metabolism:* The metabolic pathways for n-heptane has been summarized in Figure 2 below.

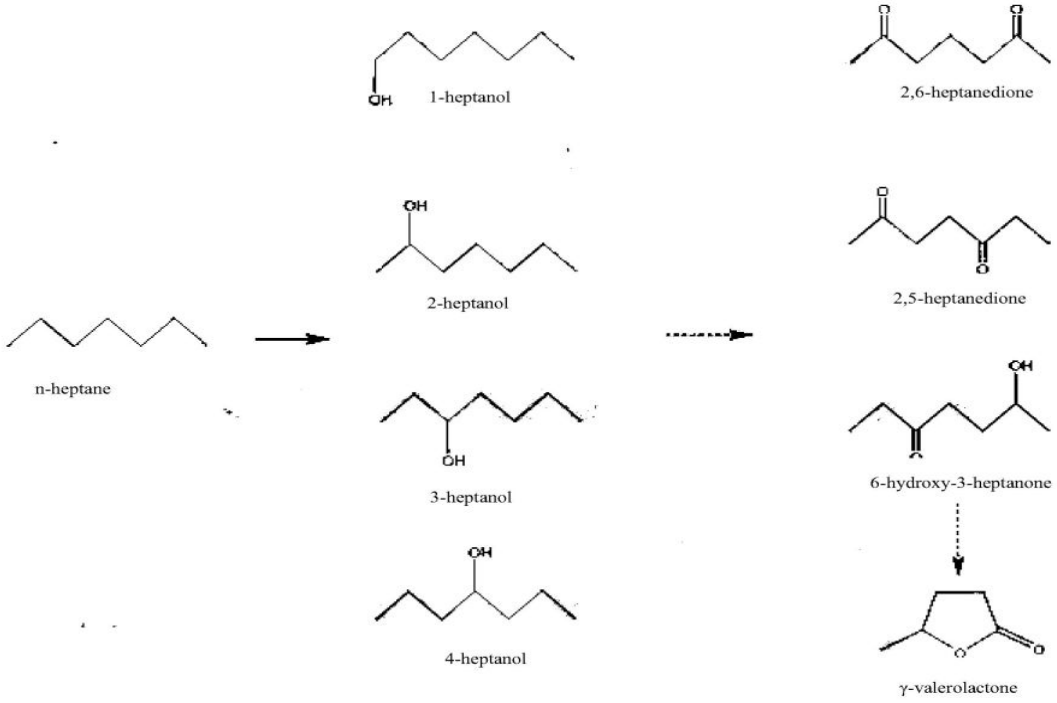


Figure 2: Primary metabolism of n-Heptane (Csanády 2012)

- U.S. EPA 2016
  - *Absorption*: Pulmonary retention after inhalation exposure is 25-29% in rats and humans, and the blood-air partition coefficients for n-heptane are 4.75-5.4 for rats and 1.9-2.85 for humans.
  - *Distribution*: Organ/air distribution coefficients identified *in vitro* for rats and humans indicate that n-heptane is distributed readily throughout the body, but accumulates the most in adipose tissue.
  - *Distribution and elimination*: Following inhalation exposures to concentrations < 35 ppm, the body clearance half-lives are 1.88 hours in humans and 0.174 hours in rats. At concentrations ≥ 100 ppm, n-heptane accumulated in the perirenal fat and brain of rats exposed for 1-2 weeks, but was no longer detectable after a 2-week recovery period.

- *Metabolism*: n-Heptane is metabolized via the cytochrome P450 enzymes to alcohols, mostly 2-heptanol and 3-heptanol, and then further metabolized via hydroxylation and dehydrogenation to produce monohydroxy, dihydroxy, diketo, and hydroxyketo metabolites. Following acute or prolonged inhalation exposure in rats, the most abundant urinary metabolites were 2-heptanol, 3-heptanol, γ-valerolactone, and 6-hydroxy-2-heptanone. Heptanol metabolites are conjugated by sulfates or glucuronates prior to excretion.

In summary, n-heptane is absorbed via inhalation and dermal routes of exposure. Following absorption, it distributes predominantly to fatty tissues. n-Heptane is primarily metabolized via cytochrome P-450-mediated oxidation reactions to 2-heptanol and 3-heptanol, which may undergo further metabolism via hydroxylation and dehydrogenation reactions. Metabolites are eliminated in the urine.

Hazard Classification Summary

Group I Human Health Effects (Group I Human)

Carcinogenicity (C) Score (H, M, or L): *L*
n-Heptane was assigned a score of Low for carcinogenicity based on negative modeling results. Limited evidence of carcinogenicity was reported in mice, but not rats, on surrogate commercial hexane. The observed tumors for commercial hexane were of borderline statistical significance and marginal human relevance. Additionally, the effects may be attributed to other components of commercial hexane. Therefore, ToxServices relied on modeling to score this endpoint. GreenScreen® criteria classify chemicals as a Low hazard for carcinogenicity when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is low as it is based on modeling.

- Authoritative and Screening Lists
  - *Authoritative:*
    - US EPA - IRIS Carcinogens - (1986) Group D - Not classifiable as to human carcinogenicity.
  - *Screening:* Not present on any screening lists for this endpoint.
- U.S. EPA 2005
  - *Surrogate: Commercial hexane:* In a GLP-compliant 2-year inhalation carcinogenicity study similar to OECD Guideline 451, male and female F344 rats (50/sex/dose) were administered 0, 900, 3,000, or 9,000 ppm of a commercial hexane blend containing 51.5% n-hexane for 6 hours/day, 5 days/week, for 2 years. There was no evidence of carcinogenicity at any dose and authors identified a NOAEC of 9,000 ppm (31.7 mg/L) for systemic toxicity effects. The chemical was concluded to be non-carcinogenic in this study.
  - *Surrogate: Commercial hexane:* In a GLP-compliant 2-year inhalation carcinogenicity study similar to OECD Guideline 451, male and female B6C3F1 mice (50/sex/dose) were administered 0, 900, 3,000, or 9,000 ppm of a commercial hexane blend containing 51.5% n-hexane for 6 hours/day, 5 days/week, for 2 years. At the high dose, there was an increase in hepatocellular neoplasms (not specified) in females at the high and an increased incidence of pituitary hyperplasia, adenomas, and adenocarcinomas in all treated females. Authors note that liver tumors are sensitive to body weight changes, especially in female B6C3F1 mice, and must be interpreted with care. In addition, these tumors were not observed in the male mice or either sex of F344 rats in the same study and toxicity contribution of the impurities was unknown. Thus, it was not possible to ascertain whether the tumors observed were direct carcinogenic effects from the chemical
- U.S. EPA 2019

    - The OncoLogic computer program, a structure-activity relationship program developed by the United States Environmental Protection Agency (U.S. EPA), was used to evaluate the carcinogenic potential of n-heptane. The compound is not supported in the newest version of OncoLogic (v.9.0), but can be modeled using an older version of OncoLogic (v.8.0) as an aliphatic hydrocarbon, and as a saturated alkane. With a few exceptions (to which n-heptane does not belong), there is no evidence that members of the saturated aliphatic hydrocarbons (i.e., alkanes) class may be of any significant cancer concern. Therefore, virtually all alkanes can be assigned a low concern (Appendix D).
- Toxtree 2018
    - n-Heptane does not have structural alerts for genotoxic or non-genotoxic carcinogenicity by Toxtree (Appendix E).
- VEGA 2021
    - The CAESAR model predicted n-heptane as carcinogen with low reliability as it is outside the model applicability domain (global applicability domain index (ADI) = 0) (Appendix F).
    - The ISS model predicted n-heptane as a non-carcinogen with low reliability as it is outside the model applicability domain (global ADI = 0) (Appendix F).
    - The IRFMN/Antares model predicted n-heptane as a possible non-carcinogen with moderate reliability as the compound could be out of the model applicability domain (global ADI = 0.716) (Appendix F).
    - The IRFMN/ISSCAN-CGX model predicted n-heptane as a possible non-carcinogen with low reliability as it is outside the model applicability domain (global ADI = 0) (Appendix F).
    - The IRFMN oral classification model predicted n-heptane as a non-carcinogen with high reliability (global ADI = 0.971) (Appendix F).
    - The IRFMN inhalation classification model predicted n-heptane as a non-carcinogen with high reliability (global ADI = 0.971) (Appendix F).
- DTU 2023
    - The Danish QSAR database reports negative predictions from Case Ultra for the FDA RCA Cancer male rat, female rat, rat, male mouse, female mouse, mouse and rodent models. The compound is within the applicability domain of these models; therefore, these results are considered reliable (Appendix G).
    - The Danish QSAR database reports a negative prediction from Leadscope for the FDA RCA Cancer mouse model. However, the compound is outside the applicability domain of this model; therefore, the results are not considered reliable. Inconclusive predictions from Leadscope were reported for the male rat, female rat, rat, male mouse, female mouse, and rodent models. The compound is outside the applicability domain of these models (Appendix G).
    - The Danish QSAR database reports negative predictions from the liver specific cancer in rat or mouse model battery consisting of Case Ultra, Leadscope and SciQSAR models. The compound is within the model applicability domain for the battery, as well as the Case Ultra and SciQSAR models. It is outside the domain of Leadscope model (Appendix G).
- Based on the weight of evidence, a score of Low was assigned. Commercial hexane produced equivocal carcinogenicity data that may or may not be relevant to n-heptane. OncoLogic predicted the compound to be of low concern for carcinogenicity, and Toxtree identified no structural alerts for genotoxic or non-genotoxic carcinogenicity. Only three of the six models in VEGA produced reliable predictions (i.e., global ADI > 0.7), and all three models predicted that n-heptane is a non-carcinogen. Finally, the Danish QSAR database reported reliable negative predictions from Case Ultra for the FDA RCA Cancer male rat, female rat, rat, male mouse, female mouse, mouse and

rodent models and from the liver specific cancer in rat or mouse model battery. The overall weight of evidence from modeling suggests a low carcinogenic concern.

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

n-Heptane was assigned a score of Low for mutagenicity/genotoxicity based on negative data for gene mutations and chromosome aberrations. GreenScreen® criteria classify chemicals as a Low hazard for mutagenicity/genotoxicity when adequate data are available and negative for both mutagenicity and clastogenicity and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on measured data from well-conducted studies on the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a
  - *In vitro:* n-Heptane was negative in a bacterial reverse gene mutation assay conducted in a manner similar to OECD Guideline 471. *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, and TA1538 and *Escherichia coli* tester strains WP_2 and WP_2 *uvr*A were exposed to heptane (100% commercial product) in Tween80/ethanol at 3.91-250 µg/mL with and without metabolic activation. There were no increases in the frequency of mutation observed in any strain at any concentration with or without metabolic activation. Cytotoxicity and the validity of the vehicle and negative controls were not specified. The positive control was valid. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) based on limited documentation.
  - *In vitro*: n-Heptane was negative in a mammalian chromosome aberration test similar to OECD Guideline 473. Rat liver RL4 hepatocytes were exposed to heptane (100% commercial product) in Tween 80/ethanol at concentrations of 2.5, 5, or 10 µg/mL. The addition of exogenous metabolic activation was not necessary as hepatocytes have intrinsic metabolic capacity in cell culture. A statistically significant increase in the frequency of chromatid gaps was detected at the highest concentration (0.027) compared to the solvent control (0.013). However, a dose response was not observed for chromatid gaps and there were no increases in the incidence of other aberrations (e.g., polyploidy or chromatid). Therefore, the study authors did not consider the increased frequency of chromatid gaps in the high concentration group to be treatment-related. No cytotoxicity was identified and the negative and positive controls were valid. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) based on deviations from the Guideline (only 100 cells from each culture analyzed instead of the 300 cells recommended[9]).
  - *In vitro:* n-Heptane was negative in a mitotic recombination assay conducted in a manner similar to OECD Guideline 481. *Saccharomyces cerevisiae* JD1 was exposed to heptane (100% pure commercial product) in Tween 80/ethanol at concentrations of 0.01, 0.1, 0.5, 1.0, or 5.0 mg/mL both with and without metabolic activation for 3 days. There was an increase in the ratio of prototrophs per plate in *S. cerevisiae* at the 5 mg/mL concentration. However, this reflected the decrease in cell viability at this concentration as there was no increase in the number of prototrophs per plate. No other significant increase in the ratio of mutations over controls was seen and under the conditions of this study the authors concluded that heptane was not mutagenic in either the presence or absence of metabolic activation. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) based on limited documentation.

[9] https://www.oecd-ilibrary.org/environment/test-no-473-in-vitro-mammalian-chromosomal-aberration-test_9789264264649-en.

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

n-Heptane was assigned a score of Low for reproductive toxicity based on lack of reproductive effects in a two-generation study in rats with commercial hexane. ToxServices notes that pure n-hexane is a known reproductive toxicant due to a specific 2,5-HD metabolite, which is not formed during the metabolism of n-heptane. Additionally, the commercial hexane mixture containing 52% n-hexane was not a reproductive toxicant in the two-generation toxicity study. GreenScreen® criteria classify chemicals as a Low hazard for reproductive toxicity when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on measured data from a conservative surrogate.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a, OECD 2010
  - *Inhalation (vapor): Surrogate: Commercial hexane*: In a GLP-compliant two-generation study in male and female Sprague-Dawley rats equivalent or similar to OECD Guideline 416, animals (25/sex/dose) were exposed to commercial hexane vapor at 0, 900, 3,000 or 9,000 ppm via whole body inhalation 6 hours/day 5 days/week. F0 rats were exposed for 10 weeks before breeding, 3 weeks during mating, and up through gestation day 19, then exposure was resumed on postnatal day 5 and continued through weaning. F1 were similarly exposed starting 8 weeks prior to mating. The F2 generation was standardized on postpartum day 4. For the F0 rats, there were no effects observed on clinical signs and mortality, food consumption, or gross pathology. Mid-dose males had reduced body weight gain during weeks 4-5, and reduced weight in weeks 9-10. High-dose males had reduced body weight gain in weeks 7 and 11-13, and reduced body weights during week 13. High dose females had reduced weight gain in weeks 5-6. In the high dose group, lactational food consumption was significantly reduced on days 7-11, and 19-21, and there were no differences in reproductive parameters compared to controls. In high dose males, there were observations of hyaline droplet nephropathy and tubular basophilia. For the F1 generation, there were no effects on clinical signs or gross pathology. One low dose female died on day 83 due to prolonged delivery. In males at 9,000 ppm, body weight gain was significantly reduced in weeks 9-11, and body weights were significantly reduced throughout the exposure period. High dose females had reduced body weight during the first 3 weeks of pre-breeding exposure. Food consumption was reduced at 9,000 ppm on gestation days 0-14, and during lactation days 21-24, and 26-28. At 3,000 ppm, food consumption was reduced during lactation days 22-23. At 900 ppm, food consumption was reduced on lactation days 21-22. In high dose F1 males, there were observations of hyaline droplet nephropathy and tubular basophilia. There were increased number of dead pups at 900 ppm, but not at higher concentrations, therefore the authors considered the effect not treatment related. In high dose pups, body weight was reduced beginning on lactation day 14, and body weight gain was reduced during lactation days 14-21 for females, and lactation days 7-14 for all pups. For F2 pups, there were no effects on gross pathology or viability, but there were decreased body weighs at 9,000 ppm on lactation days 7-28, and reduced body weight gain on lactation days 14-21 for females, and 7-14 for all ups. The NOAEC was assigned at 3,000 ppm, and the LOAEC of 9,000 ppm (reported in ECHA as 10,560 and 31,680 mg/m^3, respectively) based on reduced body weights in F1 and F2 animals. These values are equivalent to 10.56 and 31.86 mg/L, respectively.[10] There were no effects on reproductive parameters and the ECHA record established the NOAEC for reproduction at 9,000 ppm

[10] 10,560 mg/m^3 * 1 m^3/1,000 L = 10.56 mg/L; 31,680 mg/m^3 * 1 m^3/1,000 L = 30.68 mg/L

(reported in ECHA as 31,680 mg/m^3) The REACH dossier authors assigned a Klimisch score of 1 (reliable without restriction).

- ECHA 2017a
    - *Inhalation (vapor): Surrogate: Commercial hexane*: n-Hexane is classified by the Prop 65 authoritative list as a reproductive toxicant to males. The basis for this classification is likely due to its metabolites, methyl-n-butyl ketone (MnBK) and 2,5-hexanedione (2,5-HD), which are known to cause male reproductive toxicity (OEHHA 2017). n-Hexane is also associated with the EU-GHS H361f authoritative list (Category 2). It was included in the EU Community Rolling Action Plan (Corap) in 2017 and authors concluded the available experimental data for n-hexane are sufficient to support its current EU-GHS classification for reproductive toxicity Category 2 (ECHA 2017a). The authors stated that no further information needs to be requested under this substance evaluation. No adverse effects on reproductive parameters were seen in a GLP compliant 2-generation study (OECD Guideline 416) of commercial hexane (52% n-hexane) in rats at doses up to 5,000 ppm. However, in a non-guideline inhalation study in male rats with varying exposure durations of n-hexane, increases in testicular and epididymal lesions were observed with prolonging exposure time of male rats at a similar dose. The authors of the Corap document considered the results from this study as less reliable due to the limitation of the study design; a non-guideline with only one dose applied and low number of animals used. *ToxServices notes that n-heptane is metabolized via the same pathways (typical of n-alkane metabolism) to form the γ-diketone metabolite, 2,5-heptanedione, similar to the toxic n-hexane metabolite 2,5-HD, but this chemical is a very minor metabolite in both rats and humans, and is only detected as 0.01% of the total urinary metabolites following inhalational administration of n-heptane in humans (U.S. EPA 2016, Filser et al. 1996).*

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): *L*
n-Heptane was assigned a score of Low for developmental toxicity based on UNEP's expert judgment on the C7-C9 aliphatic hydrocarbons solvents category. GreenScreen® criteria classify chemicals as a Low hazard for developmental toxicity when adequate data are available and they are not classified under GHS (CPA 2018b). The confidence in the score is low as skeletal abnormalities were found in mice but not rats for the conservative surrogate commercial hexane, which might not be secondary to maternal toxicity.

- Authoritative and Screening Lists
    - *Authoritative:*
        - MAK - Pregnancy Risk Group D - "Either there are no data for an assessment of damage to the embryo or foetus or the currently available data are not sufficient for classification in one of the groups A–C".
    - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a
    - *Inhalation (vapor): Surrogate: Commercial hexane:* A GLP-compliant developmental study was conducted in a manner similar to OECD Guideline 414. Pregnant female CD-1 mice (30/group) were administered whole body inhalation exposures to commercial hexane (52.19% n-hexane) vapor at 0, 914, 3,026, or 9,107 ppm for 6 hours/day during gestational days (GD) 6-15. Maternal evaluations included clinical signs of toxicity, body weight, food and water consumption, and ovarian and uterine content. Fetal evaluations included examinations of external, visceral, and skeletal variations and malformations. Brown foci and tissues color changes were identified in the lungs of parental animals exposed to 3,000 and 9,000 ppm. A statistically significant increase in some skeletal abnormalities was

detected in fetuses at 9,000 ppm. Therefore, the ECHA record identified the maternal toxicity NOAEC and LOAEC at 900 and 3,000 ppm (reported in the REACH dossier as 3,168 and 10,560 mg/m^3, respectively, and are equivalent to 3.168 and 10.56 mg/L, respectively[11]) and the developmental NOAEC and LOAEC at 3,000 and 9,000 ppm (reported in the REACH dossier as 10,560 and 31,680 mg/m^3, respectively, and are equivalent to 10.56 and 31.68 mg/L, respectively). The REACH dossier authors assigned a Klimisch score of 1 (reliable without restriction).

- *Inhalation (vapor): Surrogate: Commercial hexane:* A GLP-compliant developmental study was conducted according to OECD Guideline 414. Pregnant female Sprague-Dawley rats (25/group) were administered whole body inhalation exposures to commercial hexane (52.19% n-hexane) at 0, 914, 3,026, or 9,107 ppm for 6 hours/day during gestational days 6-15. Maternal evaluations included clinical signs of toxicity, body weight, food and water consumption, and ovarian and uterine content. Fetal evaluations included examinations of external, visceral, and skeletal variations and malformations. There were color changes in the lungs of parental animals exposed to 9,000 ppm and these animals also showed reduced body weight gain and food consumption. No treatment-related abnormalities were found in the fetuses. Therefore, the ECHA record identified the NOAEC and LOAEC at 3,000 and 9,000 ppm, respectively, for maternal toxicity (reported in ECHA as 10,560 and 31,680 mg/m^3, respectively, and equivalent to 10.56 and 31.86 mg/L, respectively[12]) and developmental NOAEC at 9,000 ppm (reported in ECHA as 31,680 mg/m^3 which is equivalent to 31.68 mg/L). The REACH dossier authors assigned a Klimisch score of 1 (reliable without restriction).
- *Inhalation (vapor): Surrogate: Commercial hexane:* In the previously described GLP-compliant two-generation study in male and female Sprague-Dawley rats equivalent or similar to OECD Guideline 416, animals (25/sex/dose) were exposed to commercial hexane vapor at 0, 900, 3,000 or 9,000 ppm via whole body inhalation 6 hours/day 5 days/week. F0 rats were exposed for 10 weeks before breeding, 3 weeks during mating, and up through gestation day 19, then exposure was resumed on postnatal day 5 and continued through weaning. F1 were similarly exposed starting 8 weeks prior to mating. The F2 generation was standardized on postpartum day 4. For the F0 rats, there were no effects observed on clinical signs and mortality, food consumption, or gross pathology. Mid-dose males had reduced body weight gain during weeks 4-5, and reduced weight in weeks 9-10. High-dose males had reduced body weight gain in weeks 7 and 11-13, and reduced body weights during week 13. High dose females had reduced weight gain in weeks 5-6. In the high dose group, lactational food consumption was significantly reduced on days 7-11, and 19-21, and there were no differences in reproductive parameters compared to controls. In high dose males, there were observations of hyaline droplet nephropathy and tubular basophilia. For the F1 generation, there were no effects on clinical signs or gross pathology. One low dose female died on day 83 due to prolonged delivery. In males at 9,000 ppm, body weight gain was significantly reduced in weeks 9-11, and body weights were significantly reduced throughout the exposure period. High dose females had reduced body weight during the first 3 weeks of pre-breeding exposure. Food consumption was reduced at 9,000 ppm on gestation days 0-14, and during lactation days 21-24, and 26-28. At 3,000 ppm, food consumption was reduced during lactation days 22-23. At 900 ppm, food consumption was reduced on lactation days 21-22. In high dose F1 males, there were observations of hyaline droplet nephropathy and tubular basophilia. There were increased number of dead pups at

[11] 3,168 mg/m^3 * 1 m^3/1,000 L = 3.168 mg/L; 10,560 mg/m^3 * 1 m^3/1,000 L = 10.56 mg/L
[12] 10,560 mg/m^3 * 1 m^3/1,000 L = 10.56 mg/L; 31,680 mg/m^3 * 1 m^3/1,000 L = 30.68 mg/L

900 ppm, but not at higher concentrations, therefore the authors considered the effect not treatment related. In high dose pups, body weight was reduced beginning on lactation day 14, and body weight gain was reduced during lactation days 14-21 for females, and lactation days 7-14 for all pups. For F2 pups, there were no effects on gross pathology or viability, but there were decreased body weighs at 9,000 ppm on lactation days 7-28, and reduced body weight gain on lactation days 14-21 for females, and 7-14 for all ups. ToxServices identified a developmental toxicity NOAEC of 3,000 ppm, and a LOAEC of 9,000 ppm (reported in ECHA as 10,560 and 31,680 mg/m3, respectively) based on reduced body weights/body weight gains in F1 and F2 animals during lactation. The REACH dossier authors assigned a Klimisch score of 1 (reliable without restriction).

- OECD 2010
    - *Surrogate: C7-C9 aliphatic hydrocarbons:* No embryonic toxicity or teratogenicity were reported in a standard prenatal developmental toxicity study on C7-10 isoparaffins (CAS# 90622-56-3) up to the highest tested concentration of 1,200 ppm, and no developmental toxicities were found in reproductive/developmental toxicity screening studies performed according to OECD Guideline 421 with light alkylate naphtha (~97% C5-C9 isoparaffins, including ~40% C7-C9 isoparaffins, CAS #64741-66-8) and light catalytic reformed naphtha ~4% n-hexane, 1% n-heptane, 11-12% C7-C9 aliphatics and 12% aromatics (6% benzene and 6% toluene), CAS #64741-63-5) at the highest tested inhalation concentrations of 24.7 and 34.9 mg/L, respectively. The overall weight of evidence suggests that members of the C7-C9 aliphatic hydrocarbons solvents category are not developmental toxicants.
- Developmental effects included skeletal abnormalities observed in mice at maternally toxic doses in a prenatal developmental toxicity study on commercial hexane, and it is not known if these effects are secondary to maternal toxicity. No developmental effects were observed in rats in a prenatal developmental toxicity study, while reduced body weight/body weight gain were found in rats in a two-generation study on commercial hexane, also in the presence of maternal toxicity. While data are insufficient to discern if these developmental effects were secondary to maternal toxicity, ToxServices notes hexane is a conservative surrogate for n-heptane. Further, UNEP concluded that the C7-C9 aliphatic hydrocarbons solvents category, to which n-heptane belong, are not developmental toxicants. Based on UNEP's determination, ToxServices did not classify n-heptane for developmental toxicity under GHS.

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

n-Heptane was assigned a score of Data Gap for endocrine activity based on insufficient *in vivo* data to support a lack of effect on the estrogen and androgen pathways. Specifically, mechanistic *in vivo* assays for the three receptors (estrogen, androgen, and thyroid) and critical endocrine related endpoints such as estrous cyclicity and sperm parameters (count and mobility) were not measured in reproductive, developmental, and repeated dose toxicity studies identified. ToxServices did not assign a preliminary score of Moderate based on the TEDX listing, as the basis of this listing is insulin binding and degradation *in vitro*, and there are no consistent *in vivo* data indicating an effect on insulin or glucose levels.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:*
        - TEDX - Potential Endocrine Disruptors - Potential Endocrine Disruptor
- U.S. EPA 2023b
    - N-Heptane was predicted to be inactive for estrogen receptor agonism, antagonism and binding using the CERAPP Potency Level (Consensus and From literature) models. It was

also predicted to be inactive for androgen receptor agonism, antagonism and binding using the COMPARA (Consensus) model in ToxCast (Appendix H).
    - n-Heptane was active in 0/6 estrogen receptor (ER) assays, 1/8 androgen receptor (AR) assays, 0/2 steroidogenesis assays, and 0/8 thyroid receptor assays performed as part of the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) in the 21st Century (Appendix I).
- TEDX 2017
    - n-Heptane was placed on the TEDX list of potential endocrine disruptors in March 2017. The study was reviewed and is summarized below:
        - Zorad et al. (1987) investigated insulin binding and degradation in isolated human erythrocytes following the addition of n-heptane *in vitro*. The addition of increasing amounts of n-heptane to human erythrocyte suspension resulted in a dose-dependent enhancement of specific insulin binding; non-specific insulin binding remained unchanged over the range of the concentrations tested. Stimulation of insulin degradation as measured in the extracellular medium following the addition of n-heptane showed a similar dose-dependent pattern.
- DTU 2023
    - n-Heptane is predicted to be negative and is in domain by the Battery, CASE Ultra, and SciQSAR models for the estrogen receptor α binding, full and balanced training sets. It was also predicted to be negative and in domain by the CASE Ultra model and positive and in domain by the SciQSAR model for estrogen receptor α activation (Appendix J).
    - n-Heptane is predicted to be negative and is in domain for the Battery, CASE Ultra, Leadscope, and SciQSAR models for androgen receptor inhibition (Appendix J).
    - n-Heptane and its predicted metabolites are predicted to be non-binders for estrogen receptor binding (Appendix J).
    - n-Heptane has a predicted IC_{50} of 82,036 μM for thyroid receptor α binding, which is negative due to being greater than 100 μM, according to the model battery (in domain) consisted of only one in domain result by Case Ultra, with an IC_{50} of > 100 μM. n-Heptane has a predicted IC_{50} of 17,432 μM for thyroid receptor β binding, which is negative due to being greater than 100 μM, according to the model battery (in domain) consisted of only one in domain result by Case Ultra, with an IC_{50} of > 100 μM (Appendix J).
- VEGA 2021
    - The IRFMN estrogen receptor relative binding affinity model predicted n-heptane is inactive at the estrogen receptor with moderate reliability as no similar compounds with a known experimental value were identified in the training set (global ADI = 0.83) (Appendix K).
    - The IRFMN/CERAPP estrogen receptor-mediated effect model predicted that n-heptane has no estrogen receptor-mediated effects with low reliability as it is outside the applicability domain (global ADI = 0) (Appendix K).
    - The IRFMN/COMPARA androgen receptor-mediated effect model predicted that n-heptane was inactive with high reliability (global ADI = 0.958) (Appendix K).
- OECD 2010, API 1991
    - *Inhalation (vapor):* <u>*Surrogate: Commercial hexane*</u>: In the previously described GLP-compliant two-generation study in male and female Sprague-Dawley rats conducted in a manner similar to OECD Guideline 416 performed in 1989-1990, animals (25/sex/dose) were exposed to commercial hexane vapor (52% hexane, no details provided on remainder) at 0, 900, 3,000, or 9,000 ppm via whole body inhalation 6 hours/day, 5 days/week, for 10 weeks before breeding, 3 weeks during mating, and post-natal days (PND) 4-28. F2 generation animals were exposed for 8 weeks before breeding. Endpoints examined that are

related to endocrine activities include histopathology examination of liver, pituitary, vagina, uterus, ovaries, epididymides, seminal vesicles, testes and prostate. No effects were observed on these organs and tissues.

Table 4: Endocrine Activity Summary Table

Endocrine pathways		***In silico* mechanistic data**	***In vitro* mechanistic data**	***In vivo* effects**
Estrogen	Agonism	Negative by ToxCast, Danish (Q)SAR, VEGA	Negative in ToxCast EDSP assays	Conservative surrogate commercial hexane mixture has no effects on the histopathology of vagina, uterus, ovaries, epididymides, seminal vesicles, testes and prostate.
	Antagonism	Negative by ToxCast, Danish (Q)SAR, VEGA	Negative in ToxCast EDSP assays	
Androgen	Agonism	Negative by ToxCast,	Negative in ToxCast EDSP assays	
	Antagonism	Negative by ToxCast, Danish (Q)SAR	Negative in ToxCast EDSP assays	
Steroidogenesis	Activity	No models available	Negative in ToxCast EDSP assays	
Thyroid	Activity	Negative for thyroid receptor α and β binding by Danish (Q)SAR	Negative in ToxCast EDSP assays, negative for TPO inhibition.	Conservative surrogate commercial hexane mixture has no effects on the histopathology of thyroid.

- Based on the weight of evidence, a score of Data Gap was assigned. n-Heptane is present on the TEDX - Potential Endocrine Disruptors screening list, which corresponds to a score of Moderate to High. The reason provided for classification appears to be based on effects to insulin binding and degradation observed in an *in vitro* study. However, there was no *in vivo* measurement of insulin levels, and glucose levels measured routinely in repeated dose toxicity studies have not been consistently affected by n-heptane; therefore, ToxServices did not assign a hazard score for this endpoint based on the TEDX listing.

  As summarized in Table 4 above, the available *in vitro* high throughput data and modeling results indicate that n-heptane is not likely to interact with estrogen receptor, androgen receptor, or thyroid receptors or affect steroidogenesis. However, while *in vivo* data indicates no histopathological effects on reproductive organs and thyroid gland, no mechanistic *in vivo* assays for the three receptors (estrogen, androgen, and thyroid) were identified, such as measurement of hormone levels. Additionally, critical endocrine-sensitive parameters such as estrous cyclicity and sperm parameters (count and mobility) were not measured in reproductive, developmental, and repeated dose toxicity studies identified. Therefore, there is insufficient *in vivo* evidence to support a lack of effect on the estrogen and androgen pathways. According to GreenScreen® guidance, a chemical should be assigned a preliminary Moderate hazard classification if there is an indication of endocrine activity in the scientific literature, and it may remain a Moderate or be modified to a High score when there is a plausible related adverse effect corresponding to a Moderate or High, respectively. As the TEDX classification is based only on limited evidence of systemic effects, with no evidence of endocrine activity, the modeling results do not support endocrine activity for n-heptane, and

insufficient *in vivo* data were identified, ToxServices did not consider the available data to be sufficient to assign a preliminary score of Moderate for this endpoint.

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

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

Acute Mammalian Toxicity (AT) (Group II) Score (vH, H, M, or L): L

n-Heptane was assigned a score of Low for acute toxicity based on oral and dermal LD_{50} values > 2,000 mg/kg, and inhalation LC_{50} values > 20 mg/L. GreenScreen® criteria classify chemicals as a Low hazard for acute toxicity when oral and dermal LD_{50} are greater than 2,000 mg/kg and inhalation LC_{50} are greater than 20 mg/L for a gas or vapor (CPA 2018b). The confidence in the score is high as it is based on experimental data for all three routes of exposure.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a
  - *Inhalation:* 4-hour vapor LC_{50} (male and female Sprague-Dawley rats) > 29.29 mg/L (nominal), > 73.5 mg/L (analytical) (non-GLP-compliant, similar to OECD Guideline 403)
    - Test substance was 100% pure commercial product.
    - The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) based on limited documentation.
- HSDB 2014
  - *Inhalation:* 2-hour LC_{50} (mouse) = 75 g/m^3, or 75 mg/L.
  - *Inhalation:* 4-hour LC_{50} (rat) = 103 mg/m^3, or 0.103 mg/L.
    - According to PubChem (2023), this value is 103 g/m^3 (or 103 mg/L), not 103 mg/m^3.
- ECB 2000
  - *Oral:* LD_{50} (rats) = 17,000 mg/kg.
  - *Oral:* LD_{50} (mice) = 5,000 mg/kg.
  - *Dermal:* LD_{50} (rabbits) = 3,000 mg/kg.
  - *Inhalation:* 4-hour LC_{50} (species not specified) = 60 mg/L.

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

n-Heptane was assigned a score of High for systemic toxicity (single dose) based on the EU classifying it as a GHS Category 1 aspiration hazard (H304). GreenScreen® criteria classify chemicals as a High hazard for systemic toxicity (single dose) when they are classified as GHS Category 1 aspiration hazards (H304) by the EU (CPA 2018b). The confidence in the score is high as it is based on an authoritative listing.

- Authoritative and Screening Lists
  - *Authoritative:*
    - EU - GHS (H-Statements) - H304 - May be fatal if swallowed and enters airways.
  - *Screening:*
    - GHS - Japan - Specific target organs/systemic toxicity following single exposure - Category 3 (H335)
      - Based on irritation to the respiratory tract in mice and humans (NITE 2009, 2014).

        - GHS - Australia - H304 - May be fatal if swallowed and enters airways.
        - GHS - New Zealand - Aspiration hazard category 1
        - GHS - Japan - H304 - May be fatal if swallowed and enters airways.
            - Based on n-heptane being a hydrocarbon with a kinematic viscosity of 20.5 mm^2/s or less at 40°C (NITE 2009, 2014).
- ECHA 2023a
    - *Inhalation (vapor):* Commercial n-heptane was evaluated in an acute inhalation toxicity study equivalent or similar to OECD 403. Sprague-Dawley rats (10/sex) were exposed to the test substance as vapor for 4 hours at 29.29 mg/L (nominal), 17,937.5 +/- 5,665.92 ppm (analytical), or 73.5 +/- 2.32 mg/L as recalculated in the REACH dossier. There were no deaths and no clinical signs observed during the study period. There was a slight reduction in mean male body weight on day 2 post exposure, but recovery was complete by day 4. There were no significant observations at necropsy, except for one female with enlarged mandibular lymph nodes on the right side. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) based on limited documentation.
        - *ToxServices notes (17,937.5 ppm x MW of 100.203)/ 24.45 = 73.5 mg/L; however, it is unclear why the analytical concentration is nearly 2.5 times higher than nominal.*
    - The kinematic viscosity of n-heptane was measured at 0.641 mm^2/s at 20°C per EN ISO 3104. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions).
        - *GHS criteria classify chemicals as aspiration hazards Category 1 when they are hydrocarbons with a kinematic viscosity of < 20.5 mm^2/s at 40°C (UN 2021). n-Heptane is a hydrocarbon with an experimental kinematic viscosity of 0.641 mm^2/s at 20°C. The viscosity of this liquid is expected to decrease as temperatures increase. Therefore, the kinematic viscosity at 40°C is expected to be lower than 0.641 mm^2/s, and n-heptane is classified to GHS Category 1.*
- U.S. EPA 2016
    - Saturated air levels of n-heptane caused convulsions and death in rats within 20-26 minutes due to asphyxiation (displacement of oxygen due to high vapor pressure), however animals removed within 12 minutes survived and showed slight liver and kidney damage at autopsy.
        - *ToxServices notes there is no guideline specified, no mention of control animals, no discussion of the number of animals, and collectively this study is insufficiently detailed to determine its reliability. Therefore, this study is not included in the weight of evidence for assessing systemic toxicity following single inhalation exposure.*
- OECD 2010
    - <u>*Surrogate: C7-C9 aliphatic hydrocarbons:*</u> Members of the C7-C9 aliphatic hydrocarbon solvent category are respiratory tract irritants at high concentrations.
- HSDB 2014
    - Aspiration of n-heptane may cause chemical pneumonia.

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

n-Heptane was assigned a score of Low for systemic toxicity (repeated dose) based on a NOAEC of 8.82 mg/L in a 26-week inhalation (vapor) study in rats. GreenScreen® criteria classify chemicals as a Low hazard for systemic toxicity (repeated dose) when LOAEC values are greater than 1.0 mg/L for 90-day inhalation (vapor) studies in animals (CPA 2018b). The confidence in the score is high as it was based on a well-conducted study on the target chemical.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- U.S. EPA 2016, ECHA 2023a
    - *Inhalation (vapor):* Male Wistar rats (n=7) were administered whole body inhalation exposures to n-heptane vapor (> 99% purity) at 3,000 ppm (equivalent to 12,470 mg/m³ (12.47 mg/L) as calculated by authors of the ECHA dossier) n-heptane 12 hours/day, 7 days/week for 16 weeks. The animals were evaluated for clinical signs of toxicity and body weights (see the neurotoxicity section below for a discussion of the neurophysiologic and histopathological findings). No clinical signs of toxicity were identified following treatment. The mean body weight of animals exposed to heptane was not significantly different from those of control animals except at week 8. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) based on the inclusion of only one concentration in the study design and information lacking regarding statistical methods.
    - *Inhalation:* Male Sprague-Dawley rats (6-9/concentration) were administered inhalation exposures (type not specified) to n-heptane (form and purity not specified) at 1,500 ppm 9 hours/day, 5 days/week, for 7, 14, or 30 weeks. The animals were evaluated for urinary metabolites and the appearance of polyneuropathy. The study authors identified a NOAEC of 1,500 ppm based on the lack of adverse effects identified following exposure to the single concentration tests. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) based on the inclusion of only one concentration and only males in the study design.
    - *Inhalation (vapor):* In a non-GLP-compliant repeated inhalation exposure toxicity study conducted in a manner similar to OECD Guideline 413, male and female Sprague-Dawley rats (15/sex/concentration) were administered whole body inhalation exposures to n-heptane vapor (≥ 98.5% purity) at concentrations of 0, 1.65, or 12.35 mg/L, 6 hours/day, 5 days/week for 26 weeks. The equivalent concentrations for a 7-day/week exposure frequency were 0, 1.18, and 8.82 mg/L, respectively. Following the exposure period, the animals were maintained for a 2-week recovery period. Animals were evaluated for mortality, clinical signs, body weight, hematology, clinical chemistry, urinalysis, gross pathology and histopathology. Clinical signs included labored or rapid breathing and slight prostration during the first week of the study during exposure only, and anogenital fur and dry rales during weekly observations. There were no treatment-related effects to body weight, hematology, or urinalysis. Only female rats at 12.35 mg/L ppm showed a significant increase in serum alkaline phosphatase levels, but no liver, hematological, or renal abnormalities were found. The effects observed were consistent with acute central nervous system (CNS) depression and generally abated by the second week of study. The authors established a systemic NOAEC of 12.35 mg/L (equivalent to 8.82 mg/L for a 7 day/week exposure frequency) based on the lack of effects at the highest concentration tested. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) based on deviations from the OECD Guideline (26-week exposure period and only 15 animals/sex/group).
    - *Inhalation (vapor):* Male Long-Evans rats (10-11 in treatment group, 9 controls) were administered whole body inhalation exposures to n-heptane (99.5% purity) at 0, 3.3, or 16.6 mg/L n-heptane 6 hours/day, 7 days/week for 28 days. The animals were evaluated for clinical signs of toxicity and body weight. There were no differences in mean body weight gain until after cessation of exposure. From the termination of exposure over the remaining 8 weeks of the study, the body weight gain was statistically significantly lower in rats

exposed to 16.6 mg/L. No other visually observable indicators of toxicity were detected. The REACH dossier authors assigned a Klimisch score of 1 (reliable without restriction).

- U.S. EPA 2016
    - *Oral:* In a range-finding study, male Charles River CD cesarean-obtained barrier-sustained (COBS) rats (3/dose) were administered gavage doses of undiluted n-heptane (95.7% purity) at 1,000, 2,000, or 4,000 mg/kg/day, 5 days/week for 3 weeks (adjusted to daily doses of 714, 1,430, and 2,860 mg/kg/day, respectively). There were no treatment-related mortalities and no adverse clinical signs. There were no effects to body weight or food consumption. Treated rats had an increase in serum LDH, increased liver and kidney weights and hyperplasia of the gastric non-glandular epithelium. However, the authors concluded small group sizes and inadequate reporting of any measure of variability within treatment groups or statistical analyses preclude the determination of a critical effect or a LOAEL for this study.
    - *Oral:* In a subchronic study, male Charles River CD COBS rats (8/dose) were administered gavage doses of undiluted n-heptane (95.7% purity) at 4,000 mg/kg/day, 5 days/week for 13 weeks (adjusted to a daily dose of 2,860 mg/kg/day). Five of the eight rats died from acute chemically induced pneumonitis. Treated rats had significantly reduced food consumption in the first week only, and decreased body weight throughout most of the study. A slight, but statistically significant 20% reduction in serum glucose levels was observed in the three surviving rats. A statistically significant reduction in absolute heart weight and significant increase in relative liver weight, relative kidney weight and relative adrenal weight were reported. Grossly enlarged livers were found in all treated animals that died. Histopathological examination of exposed rats revealed local irritative effects on the forestomach mucosa. Low incidences of several hepatic (hepatocyte vacuolation, serosal adhesions, congestion) and renal (hyaline droplets, increased incidence of tubular dilation with casts, increased incidence of regenerating renal tubular epithelium, hemorrhage, congestion, focal nephritis) lesions were seen in treated rats. However, the authors concluded high mortality due to gavage error (5/8 treated rats) precludes the determination of a critical effect or LOAEL for this study. Potential treatment-related effects include persistent body-weight depression, gross liver enlargement, organ-weight changes, and histopathology of the forestomach, liver, kidney, and adrenal glands.
- HSDB 2014, ECHA 2023a
    - *Inhalation (vapor):* Biochemical effects were identified in male Wistar rats (15/group, 5 sacrificed after one week, 5 sacrificed after two weeks, and 5 sacrificed after a two-week recovery period) exposed to heptane vapors (purity not specified) at concentrations of 100, 500, or 1,500 ppm, 6 hours/day, 5 days/week for 2 weeks. These included increased brain RNA concentration, acid proteinase activity, decreased glutathione levels and increased NADPH-diaphorase activity. However, the effects were reversible and returned to normal 2 weeks after exposure was terminated. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) based on the inclusion of only males in the study design and limited statistical methods.
- Based on the weight of evidence, a score of Low was assigned. Oral studies in rats were not adequate for determining effect levels, due to deficiencies in study design and reporting. A majority of inhalation studies investigate neurotoxic effects of n-heptane and are discussed in the neurotoxicity section below. The 26-week study in rats provides the most adequate data for assigning a score for this endpoint. As the NOAEC in this study was 8.82 mg/L, which is greater than the GHS guidance value of 1.0 mg/L for a gas or vapor (UN 2021), a score of Low was assigned.

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

n-Heptane was assigned a score of Moderate for neurotoxicity (single dose) based on association with the hazard statement H336 and on being listed as neurotoxic by Boyes, supported by animal and human data. GreenScreen® criteria classify chemicals as a Moderate hazard for neurotoxicity (single dose) when associated with H336 and a Category 3 classification is warranted (CPA 2018b). The confidence in the score is high as it is based on an authoritative list with support from animal and human data.

- *Authoritative:*
  - EU - GHS (H-Statements) - H336 - May cause drowsiness or dizziness.
- *Screening:*
  - GHS - Australia - H336 - May cause drowsiness or dizziness.
  - Boyes - Neurotoxicants – Neurotoxic.
  - GHS - Japan - Specific target organs/systemic toxicity following single exposure - Category 3 (H336)
    - Based on acute central nervous system depression in human volunteers and narcosis in exposed mice (NITE 2009, 2014).

- HSDB 2014
  - Inhalation exposures of 10,000-15,000 ppm heptane in mice produced CNS depression within 30-60 min. At higher concentrations (<20,000 ppm), 30-60 min exposures elicited convulsions and death in mice. Inhalation exposures to 48,000 ppm produced respiratory arrest in 3/4 exposed mice in 3 min. Righting reflexes of mice were affected at an inhalation concentration of 40 mg/L, and inhalation exposures to 70 mg/L were lethal.
  - Inhalation exposure of mice to heptane (form not specified) at 13,000-19,000 ppm elicited convulsions and deaths within 15-40 min. Prior to death, the mice typically exhibited prostration and loss of reflexes.
  - Human volunteers exposed to 0.1% heptane experienced slight vertigo within 6 min; those exposed to 0.2% heptane experienced vertigo in 4 min; and those exposed to 0.5% heptane exhibited clinical signs of CNS depression in 7 min.
  - In humans, concentrations of 4.8% heptane produced respiratory arrest within 3 minutes. Survivors exhibited marked vertigo and incoordination requiring 30 min for recovery as well as mucous membrane irritation, slight nausea, and lassitude.
  - Workers exposed to solvent mixtures containing pentane, hexane, and heptane (concentration not specified) exhibited polyneuropathy characterized as asthenia (decreased muscle strength), signs of anorexia, paresthesia (tingling or prickling feeling in extremities), fatigue, and bilateral symmetrical muscle paralysis of the legs.
- ANSES 2013; NEG 2010
  - n-Heptane is a known ototoxin, and the effect is related to its neurotoxicity.
- U.S. EPA 2016
  - CNS depression and cyanosis were observed in mice exposed to 800-2,500 ppm aerosols containing n-heptane for 2-3 minutes, but animals recovered once removed from the chamber.
  - Male and female volunteers (number not specified) were exposed to n-heptane at up to 5,000 ppm (equivalent to 20,000 mg/m^3) in air for up to 15 minutes. Subjects reported slight vertigo after 6 minutes at 1,000 ppm, after 4 minutes at 2,000 ppm, moderate vertigo after 4 minutes at 3,500 ppm, and marked vertigo at 4 minutes at 5,000 ppm. Additional effects observed after 4-15 minutes of exposure to 5,000 ppm included hilarity (i.e., amusement), incoordination, and inability to walk straight, with effects lasting up to 30 minutes post exposure. A LOAEC of 4,000 ppm was assigned based on vertigo.

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

n-Heptane was assigned a score of Low for neurotoxicity (repeated dose) based on an inhalation LOAEC of 16.6 mg/L and NOAEC of 3.3 mg/L for a 28-day study in rats. GreenScreen® criteria classify chemicals as a Low hazard for neurotoxicity (repeated dose) when inhalation LOAEC values for a gas or vapor are greater than 1.0 mg/L for 90 days studies (3.25 mg/L for a 28-day study) (CPA 2018b). The confidence in the score is high as it is based on reliable measured data on the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - Boyes - Neurotoxicants – Neurotoxic.
    - GHS - Japan - Specific target organs/systemic toxicity following repeated exposure - Category 1 (H372 – nervous system).
      - No rationale provided (NITE 2014). Not classified with H372 during previous assessments (NITE 2006, 2009).
- RTECS 2013
  - Hallucinations and distorted perceptions have been reported in humans following inhalation exposure at 1,000 ppm / 6M (no further details provided). Convulsions or effect on seizure threshold is reported at 59 g/m^3 / 41M (no further details provided).
- HSDB 2014
  - Biochemical effects were seen in rats exposed to n-heptane vapors at concentrations of 100, 500, and 1,500 ppm for 2 weeks. These included increased brain RNA concentration, acid proteinase activity, decreased glutathione levels and increased NADPH-diaphorase activity. However, the effects were reversible and returned to normal 2 weeks after exposure was terminated.
- U.S. EPA 2016
  - In a case report of a 32-year female exposed to n-heptane, along with other solvents (i.e., ethyl acetate and cyclohexane), exposure to approximately 153 mg/m^3 n-heptane during working hours over a 6-month period resulted in symptoms of vertigo, leg and arm paresthesia, leg pain, abnormal electroencephalogram (EEG), and altered peripheral nerve conduction velocity. Complete recovery was achieved within 7 months following cessation of exposure. Due to exposure to several solvents, it is unknown if exposure to n-heptane caused or contributed to the peripheral nervous system deficits.
- HSDB 2014, U.S. EPA 2016, ECHA 2023a
  - *Inhalation*: In a subchronic study, male Wistar rats (n=7) were administered whole body inhalation exposures to n-heptane vapor (> 99% purity) at 3,000 ppm (equivalent to 12,470 mg/m³ (12.47 mg/L) as calculated in the REACH dossier) 12 hours/day, 7 days/week for 16 weeks. No abnormal behavioral changes were observed. Peripheral nerves, muscles and neuromass junctions, examined microscopically, were normal. There were no statistically significant differences in motor nerve conduction velocity, distal latency or mixed nerve conduction velocity in any region of the tail. Authors concluded n-heptane was not a neurotoxicant in this study and established a NOAEC of 12,470 mg/m^3 (12.47 mg/L). The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) based on the inclusion of only one concentration in the study design and information lacking regarding statistical methods.
  - *Inhalation*: Male Sprague-Dawley rats (6-9/concentration) were administered inhalation exposures (type not specified) to n-heptane (form and purity not specified) at a concentration of 1,500 ppm 9 hours/day, 5 days/week for 7, 14, or 30 weeks, did not develop any signs of

neuropathy. Histopathological examination of nerve tissues obtained from n-heptane dosed rats showed no signs of morphological "giant axonal degeneration" in the peripheral and central nervous systems. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) based on the inclusion of only one concentration and only males in the study design.

- *Inhalation*: In a non-GLP-compliant repeated inhalation exposures study conducted in a manner similar to OECD Guideline 413, 26-week study, male and female Sprague-Dawley rats (15/sex/concentration) were administered whole body inhalation exposures to n-heptane vapor (≥ 98.5% purity) at 0, 1.65 and 12.35 mg/L, 6 hours/day, 5 days/week for 26 weeks. The equivalent concentrations for a 7-day/week exposure frequency were 0, 1.18, and 8.82 mg/L, respectively. Following the exposure period, the animals were maintained for a 2-week recovery period. Acute CNS depression was observed and generally abated by the second week of the study. No additional evidence of neurological disturbances or organ toxicity was reported. A LOAEC of 1,650 mg/m^3 (1.65 mg/L) for acute CNS depression was reported. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) based on deviations from the OECD Guideline (26-week exposure period and only 15 animals/sex/group).
- *Inhalation*: The effects of whole body inhalation exposures to n-heptane vapor (99.5% purity) at 800 or 4,000 ppm for 6 hours/day, 7 days/week for 28 days on the function of the auditory system were examined by measurements of auditory brain stem response (ABR) in male Long Evans rats (9 controls, 10-11 in treatment groups). Decreased auditory sensitivity was seen in rats at 4,000 ppm (16.6 mg/L), but not at 800 ppm (3.3 mg/L). ToxServices identified a NOAEC of 3.3 mg/L and a LOAEC of 16.6 mg/L for this study. The REACH dossier authors assigned a Klimisch score of 1 (reliable without restriction).

- HSDB 2014, ECHA 2023a
  - *Inhalation*: Male Wistar rats (15/groups) were exposed to n-heptane via inhalation at 4.2, 21 and 62 µM, 6 hours/day, 5 days/week for two weeks. Levels of n-heptane increased in brain and perirenal fat during the 2 weeks of exposure. None of the rats showed clinical signs of neurotoxicity. Neurochemical changes reported at week 2, including increased proteolysis and higher brain RNA content, were at control levels after 2 weeks recovery. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) based on the inclusion of only males in the study design and limited statistical methods.
- HSDB 2014, U.S. EPA 2016
  - The neurotoxicity of n-pentane, n-hexane, and n-heptane has been studied in Wistar strain male rats after exposure to 3,000 ppm for 12 hours a day for 16 weeks. The experiment showed that n-hexane disturbed the conduction velocity of the motor nerve and the mixed nerve and prolonged the distal latency in the rat's tail, but that n-pentane and n-heptane did not. The light and electron microscopic examination showed that the peripheral nerve, the neuromuscular junction, and the muscle fiber of the rats exposed to n-hexane were severely impaired, but those of the rats exposed to n-pentane or n-heptane showed no particular changes even after 16 weeks of exposure.
- Based on the above data, repeated exposure of animals to n-heptane vapor has been shown to depress the CNS. Accordingly, Japan classified n-heptane as a GHS Category 1 specific target organ toxicant following repeated exposures based on effects to the nervous system (no justification provided). However, the effects are transient. Transient narcotic effects were reported in the single dose neurotoxicity section above. No other adverse neurological effects were reported in repeated dose toxicity studies that warrant GHS classification. Therefore, a score of Low was assigned.

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

n-Heptane was assigned a score of Low for skin sensitization based on negative results identified in a guinea pig maximization test with a surrogate, supported by negative modeled results. GreenScreen® criteria classify chemicals as a Low hazard for skin sensitization when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is high based on reliable data from a strong surrogate with support from consistent negative modeled results.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- Payne and Walsh 1994
  - n-Heptane has no structural alerts for skin sensitization (Appendix L).
- Toxtree 2018
  - Toxtree did not identify any structural alerts for skin sensitization on n-heptane (Appendix M).
- OECD 2022
  - OECD Toolbox did not identify any structural alerts for skin sensitization on n-heptane (Appendix N).
- LabMol 2020
  - The LabMol Pred-Skin platform predicts n-heptane is not a skin sensitizer based on the Bayesian outcome prediction with high confiability. Pred-Skin estimates confiability (or degree of confidence in the prediction) by calculating the ratio of predictions made by internal models (trees in the random forest statistical model), the applicability domain (AD), and maps for the predicted fragment contribution of the structure (Borba et al. 2021). The Bayesian outcome is based on positive predictions from DPRA and h-CLAT models, with confiability scores of 59.8% and 57.7%, respectively, and negative predictions with the KeratinoSens, LLNA, and HRIPT/HMT models, with confiability scores of 60.7%, 92.9%, and 97.1%, respectively. It may be noted n-heptane was in domain for all models except HRIPT/HMT (Appendix O).
- ECHA 2023a
  - *Surrogate: Hydrocarbons, C7-C9, n-alkanes, isoalkanes, cyclics (CAS #N/A):* The skin sensitization potential of hydrocarbons, C7-C9, n-alkanes, isoalkanes, cyclics (100% pure commercial product[13]) was evaluated in a non-GLP-compliant guinea pig maximization test conducted in a manner similar to OECD Guideline 406. p-Strain guinea pigs (10/sex in treatment group, 5/sex in control group) were administered induction doses as intradermal injections of the test material at 1% w/v in corn oil and topical applications of 50% w/v test material in corn oil (coverage not specified). The challenge dose was applied as a topical application of 25% w/v test material in corn oil under occlusive dressing. No positive reactions were identified at readings 24 or 48 hours after the challenge dose. The study authors concluded that the test substance was not sensitizing to the skin under the tested conditions. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) as only basic data were provided.

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

n-Heptane 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

[13] Consisted of 35% C7, 50% C8, 15% C9 saturated hydrocarbon isomers, 65% n and isoparaffins, and 35% naphthenes. The percentage of n-heptane in this mixture is not characterized.

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 for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- OECD 2022
  - n-Heptane does not contain any structural alerts for respiratory sensitization (Appendix P).
- 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 2017b). 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 2017b). As n-heptane 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 n-heptane, and as n-heptane does not contain any structural alerts for respiratory sensitization (OECD 2022), n-heptane is not expected to be a respiratory sensitizer.

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

n-Heptane was assigned a score of High for skin irritation/corrosivity based on the EU classifying it as a GHS Category 2 skin irritant (H315). A study on the surrogate 2,2,4-trimethylpentane indicated minimal skin irritation, which was insufficiently detailed to discern if data meet the criteria for GHS Category 3 or not classified. On the other hand, UNEP concluded that C7-C9 hydrocarbon solvents, including n-heptane, are moderate skin irritants. GreenScreen® criteria classify chemicals as a High hazard for skin irritation/corrosivity when they are classified as GHS Category 2 skin irritants (H315) by the EU (CPA 2018b). The confidence in the score is high as it is based on an authoritative A list.

- Authoritative and Screening Lists
  - *Authoritative:*
    - EU - GHS (H-Statements) - H315 - Causes skin irritation.
  - *Screening:*
    - GHS - Japan - Skin corrosion / irritation - Category 2
      - Based on irritation and dermatitis in humans after 1-hour exposure and the EU harmonized classification (NITE 2009, 2014).
    - GHS - Australia - H315 - Causes skin irritation.
- HSDB 2014
  - n-Heptane causes dry skin and irritates the skin.
- OECD 2010
  - *Surrogate: C7-C9 aliphatic hydrocarbons:* Members of the C7-C9 aliphatic hydrocarbon solvent category are moderate skin irritants.
- ECHA 2023a
  - *Surrogate: C7-C9 aliphatic hydrocarbons:* 2,2,4-Trimethylpentane was evaluated for skin irritation in a GLP-compliant acute dermal irritation / corrosion study equivalent or similar to OECD 404 and EPA OPPTS 870.2500. New Zealand White rabbits were exposed to the test substance (commercial purity) on intact and abraded skin for 24 hours at 0.5 mL (no vehicle, occlusion not specified). At 72 hours post exposure, 3 animals had erythema scores of 1 out of 4, and 3 animals had erythema scores of 0 out of 4, thus the mean score was 0.5.

The mean edema score at 72 hours was 0, and the primary irritation index was calculated at 0.25. Authors concluded the test substance was irritating to the skin under the conditions of the test. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions).

- *ToxServices notes the exposure duration was significantly longer than the 4 hours specified in OECD 404, and the scores are below the GHS guidance values (UN 2021); however, effects were not fully reversed within 72 hours, scores at 24 hours and 48 hours were not reported, and authors concluded the test substance was minimally irritating.*

- ECHA 2023c
  - *Surrogate: C-6 normal and iso paraffins (hexanes) and naphthenes (methyl-cyclohexane, dimethylcyclohexane), 25-35% n-hexane:* In a non GLP-compliant skin irritation study conducted similar to OECD Guideline 404, the n-hexane mixture was applied to intact and abraded skin of New Zealand white rabbits (n=6) under semi-occlusive conditions for 24 hours. The primary dermal irritation index (PDII) was 1.92 which is calculated from the average erythema score and the average edema score. The authors classified the n-hexane mixture as irritating to the skin based on this study. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions).

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

Two sources in the public literature indicate n-heptane causes redness and pain, and is slightly irritating to the eyes. In the absence of additional details regarding the study design (i.e., number of animals, duration of exposure, and period of observation), "slightly irritating" could equate to GHS Category 2B or not classified. Accordingly, ToxServices conservatively classified n-heptane to Category 2B with low confidence. Japan classified n-heptane to GHS Category 2, but did not differentiate between 2A and 2B classifications. GreenScreen® criteria classify chemicals as a Moderate hazard for eye irritation/corrosivity when a GHS Category 2B (mildly irritating) classification is warranted (CPA 2018b). The confidence in the score is low due to limited and conflicting animal and human data.

- *Authoritative:* Not present on any authoritative lists for this endpoint.
- *Screening:*
  - GHS - Japan - Serious eye damage / eye irritation - Category 2
    - Based on mild irritation detected in rabbit studies and irritation reported in humans (NITE 2006, 2014).

- HSDB 2014
  - n-Heptane causes redness and pain in the eyes.
- ECB 2000
  - n-Heptane was reported to be slightly irritating to the rabbit eye. No further details were provided.
- OECD 2010
  - *Surrogate: C7-C9 aliphatic hydrocarbons:* Members of the C7-C9 aliphatic hydrocarbon solvent category are not eye irritants in rabbits.
- ECHA 2023a
  - *Surrogate: C7-C9 aliphatic hydrocarbons:* 2,2,4-Trimethylpentane was evaluated for eye irritation in a GLP-compliant acute eye irritation / corrosion study equivalent or similar to OECD 405 and EPA OPPTS 870.2400. New Zealand White rabbits were exposed to the test substance (commercial purity) instilled in one eye each at 0.1 mL (no vehicle, rinsing not specified). The conjunctivae score in 1 animal was 0.67 at 24, 48 and 72 hours, and was fully reversible by 48 hours. Conjunctivae scores in all other animals were 0 at 24, 48, and

72 hours. The mean scores for cornea opacity, iris, and chemosis was 0 at 24, 48 and 72 hours. Authors concluded the test substance meets the criteria for GHS not classified. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions).

Ecotoxicity (Ecotox)

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

n-Heptane was assigned a score of Very High for acute aquatic toxicity based on the EU classifying it as a GHS Category 1 acute aquatic toxicant (H400) and measured acute aquatic toxicity values as low as 0.1 mg/L in aquatic invertebrates. GreenScreen® criteria classify chemicals as a Very High hazard for acute aquatic toxicity when acute aquatic toxicity values are no greater than 1 mg/L and they are classified as GHS Category 1 acute aquatic toxicants (H400) by the EU (CPA 2018b). The confidence in the score is high as it is based on experimental data and an authoritative listing.

- Authoritative and Screening Lists
  - *Authoritative:*
    - EU - GHS (H-Statements) - H400 - Very toxic to aquatic life.
  - *Screening:*
    - GHS - Japan - Hazardous to the aquatic environment (acute) - Category 1
      - Based on a 96-hour LC_{50} of 0.1 mg/L in mysid shrimp (NITE 2009, 2014).
    - EC - CEPA DSL - Inherently Toxic in the Environment (iTE)
      - Based on a 96-hour LC_{50} of 0.1 mg/L in mysid shrimp (OECD 2023).
- HSDB 2014
  - LC_{50} = 4 mg/L in *Carassius auratus* (fish, 24 hours).
  - LC_{50} = 2,940 mg/L in *Leuciscus idus melanotus* (fish, 48 hours)
    - Assigned a Klimisch score of 3 (not reliable) in ECHA (2023a) due to significant methodological deficiencies.
  - LC_{50} = 4,924 mg/L in *Gambusia affinis* (fish, 24, 48 and 96 hours)
    - Assigned a Klimisch score of 3 (not reliable) in ECHA (2023a) due to significant methodological deficiencies.
  - LC_{50} = 375 mg/L in *Tilapia mossambica* (fish, 96 hours).
  - EC_{50} = 82.5 mg/L in *Daphnia magna* (invertebrates, 96 hours).
  - EC_{50} > 10 mg/L in *D. magna* (invertebrates, 24 hours)
    - Assigned a Klimisch score of 3 (not reliable) in ECHA (2023a) due to insufficient documentation of testing procedure test concentrations.
- HSDB 2014, ECHA 2023a
  - LC_{50} = 0.2 mg/L in C*haetogammarus marinus* (invertebrates, 96 hours)
    - The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) as a non-standard organism was used and the LC_{50} was derived from a 96-hour exposure rather than a 48-hour exposure typically performed for invertebrates.
  - LC_{50} = 0.1 mg/L in *Americamysis bahia* (invertebrates, 96 hours)
    - The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) as a non-standard organism was used and the LC_{50} was derived from a 96-hour exposure rather than a 48-hour exposure typically performed for invertebrates.
- U.S. EPA 2017a

- n-Heptane belongs to the neutral organics ECOSAR chemical class. The most conservative predicted acute E/LC_{50} values are 2.08 mg/L in fish (96h), 1.39 mg/L in daphnia (48h), and 2.04 mg/L in green algae (96h) (Appendix Q).

Chronic Aquatic Toxicity (CA) Score (vH, H, M, or L): *vH*
n-Heptane was assigned a score of Very High for chronic aquatic toxicity based on predicted ChV values of 0.04 mg/L in fish and 0.05 mg/L in daphnia. GreenScreen® criteria classify chemicals as a Very High hazard for chronic aquatic toxicity when chronic aquatic toxicity values are less than 0.1 mg/L (CPA 2018b). The confidence in the score is low as it is based on modeled data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint
  - *Other*
    - EU - GHS (H-Statements) - H410 - Very toxic to aquatic life with long lasting effects.
    - GHS - Australia - H410 - Very toxic to aquatic life with long lasting effects.
    - GHS - Japan - Hazardous to the aquatic environment (chronic) - Category 1
      - Based on the acute aquatic toxicity classification and a high bioaccumulative potential based on a log K_{ow} = 4.66 (NITE 2009, 2014).
    - GHS - New Zealand – Hazardous to the aquatic environment – chronic category 1.
- U.S. EPA 2017a
  - n-Heptane was assigned to the neutral organics ECOSAR chemical class. The most conservative chronic aquatic toxicity values were 0.04 mg/L in fish, 0.05 mg/L in daphnia, and 0.23 mg/L in green algae (Appendix Q).

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): vL
n-Heptane was assigned a score of Very Low for persistence based on experimental data that indicate this chemical is readily biodegradable and meets the 10-day window. GreenScreen® criteria classify chemicals as a Very Low hazard for persistence when they are readily biodegradable and meet the 10-day window when the predominant environmental compartment is not air (CPA 2018b). The confidence in the score is high as it is based on reliable data from experimental studies on the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a
  - In a non-GLP-compliant biodegradation test conducted according to an American Public Health Association guideline, aerobic, natural soil was exposed to heptane (> 99% purity) at 3.3 mg/L for 20 days. The test substance degraded 28.2% in 2 days, 63.2% in 5 days, and 70% in 10 days. Although the test was not a ready biodegradation test, the overall results suggest that it would meet the criteria for ready biodegradation. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) as the method used was comparable to an acceptable guideline.
  - In a biodegradation test, 400 mL of gasoline containing 150 mg/L of n-heptane was exposed to non-adapted, domestic activated sludge for 25 days. The activated sludge used as the test inoculum is prepared as a microbial suspension containing 3 g/L dry weight of sludge

collected from an urban wastewater treatment plant that is resuspended in a nutrient solution (composed of 80% water) (OECD 1992). About 20% degradation was reached on day 2 and more than 60% degradation was reached prior to day 10; 100% degradation was measured after 25 days. Although the test was not a ready biodegradation test, the overall results suggest that it would meet the criteria for ready biodegradation. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) as the method used was comparable to an acceptable guideline.

- In a biodegradation test, adapted soil was exposed to n-heptane (99% purity) at an unspecified concentration for 4 days. At the end of the exposure period, 100% degradation was measured. The n-heptane degradation rate was reported as 87 µg/day. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) as the original source was not available and was not reviewed.
- n-Heptane (99% purity) biodegraded to an extent of 23.4% after 3 days incubation with an inoculum acclimated to gasoline, which contains n-heptane. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) as the original source was not available and was not reviewed.
- *Surrogate: C7-C9 aliphatic hydrocarbons (hydrocarbons, C6-C7, n-alkanes, isoalkanes, cyclics, < 5% hexane):* In a GLP-compliant biodegradation test conducted according to OECD 301F (Ready Biodegradability: Manometric Respirometry Test), 100 mg/L of a C6-C7 hydrocarbon mixture (< 5% hexane) was exposed to domestic activated sludge (containing 80% water) for 28 days. The test substance was degraded by 98% at the end of 28 days and by 83% at the end of the 10-day window. The study authors concluded that the hydrocarbon mixture was readily biodegradable under the conditions of the test. The REACH dossier authors assigned a Klimisch score of 1 (reliable without restriction) as the method used was an acceptable guideline.

- U.S. EPA 2017b
  - The BIOWIN modeling Ready Biodegradable Predictor indicates that n-heptane is expected to be readily biodegradable (Appendix S). The Level III Fugacity Model (MCI Method) predicts 62.6% will partition to water with a half-life of 208 hours (8.67 days), 35.7% will partition to air with a half-life of 38 hours (1.58 days), and 1.17% will partition to soil with a half-life of 416 hours (17.3 days) (Appendix R).
- Based on the weight of evidence, a score of Very Low was assigned. n-Heptane met the 10-day window in several non-standard biodegradation tests. The Level III Fugacity Model (MCI Method) predicts n-heptane will mainly partition to water. It is ToxServices internal policy to assign the hazard score for persistence based on the dominant environmental compartment(s) identified via fugacity modeling (ToxServices 2021). Therefore, ToxServices assigned a Very Low score for this endpoint as it met the 10-day window in biodegradation tests.

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

n-Heptane was assigned a score of Moderate for bioaccumulation based on estimated BCF values of 551.7-681.8. GreenScreen® criteria classify chemicals as a Moderate hazard for bioaccumulation when BCF values are > 500-1,000 (CPA 2018b). The confidence in the score is low as it is based on modeling.

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

- U.S. EPA 2017b
    - BCFBAF predicts a BCF of 551.7 using the regression-based model based on a measured log K_{ow} of 4.66, and a BCF of 681.8 using the Arnot-Gobas model for the upper trophic level, taking metabolism into consideration (Appendix R).

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M, or L): *L*
n-Heptane was assigned a score of Low for reactivity based on ToxServices not classifying it as a reactive chemical under GHS criteria. GreenScreen® criteria classify chemicals as a Low hazard for reactivity when no GHS classification is available (CPA 2018b). The confidence in the score was low as it is not based on measured data or authoritative lists.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023a
    - n-Heptane does not contain any chemical groups associated with explosive properties.
    - Based on chemical structure, n-heptane is not capable of reacting exothermically with combustible materials.
- No measured data were identified. Therefore, screening procedures for explosivity were used here to estimate the reactivity property of n-heptane. These procedures are listed in the GHS (UN 2021).
    - Based on its structure, n-heptane is not considered explosive or self-reactive due to lack of functional groups associated with explosive or self-reactive properties (See Appendix S).
    - Based on its structure, n-heptane is not considered to have oxidizing properties as it does not contain any structural groups known to be correlated with a tendency to react exothermally with combustible materials.
- Based on the above information and assessment, ToxServices did not classify n-heptane as a reactive chemical under GHS criteria (UN 2021).

Flammability (F) Score (vH, H, M, or L): H
n-Heptane was assigned a score of High for flammability based on the EU classifying it as a GHS Category 2 flammable liquid (H225). GreenScreen® criteria classify chemicals as a High hazard for flammability when they are classified as GHS Category 2 flammable liquids (H225) by the EU (CPA 2018b). The confidence in the score was high as it is based on authoritative lists supported by measured data.

- Authoritative and Screening Lists
    - *Authoritative:*
        - EU - GHS (H-Statements) - H225 - Highly flammable liquid and vapour.
        - Québec CSST - WHMIS 1988 - Class B2 - Flammable liquids.
    - *Screening:*
        - GHS - Australia - H225 - Highly flammable liquid and vapour.
        - GHS - Japan - Flammable liquids - Category 2
            - Based on a flash point of -4°C and an initial boiling point of 98.4°C (NITE 2009, 2014).
        - GHS - New Zealand - 3.1B - Flammable Liquids: high hazard
            - Based on a flash point of -3.9°C and an initial boiling point of 98°C (CCID 2023).

- ECHA 2023a
    - n-Heptane has a flash point of -4°C as identified in a non-GLP-compliant test of unspecified type. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) as the study was peer reviewed with limited details.
    - n-Heptane has a boiling point of 98.2-98.4°C as identified in a non-GLP-complaint ASTM D 1078 test. The REACH dossier authors assigned a Klimisch score of 2 (reliable with restrictions) as the study was not performed according to GLP but a standard test procedure was followed.
- U.S. DOT 2008a
    - n-Heptane is classified to hazard class or division 3 (flammable liquid) and packaging group II.
- Based on the available data, n-heptane is classified to GHS Category 2 for flammable liquids (liquids which have a flash point < 23°C and initial boiling point > 35°C) (UN 2021).

Use of New Approach Methodologies (NAMs)[14] in the Assessment, Including Uncertainty Analyses of Input and Output

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

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

As shown in Table 5, Type I (input data) uncertainties in n-heptane's NAMs dataset include lack of, or insufficient *in vivo* experimental data for carcinogenicity, endocrine activity, skin and respiratory sensitization, ecotoxicity, and bioaccumulation, use of non-guideline or deleted (by OECD) test protocols, and lack of validated test methods for respiratory sensitization. n-Heptane's Type II (extrapolation output) uncertainties include lack of defined applicability domains of several models, uncertain *in vivo* relevance of *in vitro* testing and *in silico* testing of endocrine receptor binding due to lack of consideration of toxicokinetics, limitations of *in vitro* genotoxicity studies in mimicking *in vivo* metabolic conditions and that they each focused on only one or a few genotoxicity events, and the limitation respiratory sensitization structural alerts in evaluating non-immunologic mechanisms for respiratory sensitization. Some of n-heptane's type II uncertainties were alleviated by the use of *in vitro* test batteries and/or in combination of *in vivo* data.

Table 5: Summary of NAMs Used in the GreenScreen® Assessment, Including Uncertainty Analyses	
Uncertainty Analyses (OECD 2020)	
Type I Uncertainty: Data/Model Input	**Carcinogenicity**: No experimental data are available. **Mutagenicity**: Only three *in vitro* studies are available (OECD 471, 439, and 481). The yeast mitotic recombination assay test guideline (OECD Guideline 481) has been deleted from the recommended OECD test guidelines due to lack of use under regulatory settings, and inferior performance compared to other tests.[15] **Endocrine activity**: Data are limited to *in vitro* studies. Zorad et al. (1987) is a mechanistic study and does not follow GLP or any guidelines. **Skin sensitization:** Limited experimental data are available only on a mixture.

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

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

	Respiratory sensitization: No experimental data are available and there are no validated test methods. **Ecotoxicity:** Limited acute aquatic toxicity data and no chronic aquatic toxicity data are available. **Bioaccumulation**: No experimentally derived bioconcentration factors are available.
Type II Uncertainty: Extrapolation Output	**Carcinogenicity**: Toxtree was only used to check for structural alerts, and no applicability domains were defined for these structural alerts. **Genotoxicity:** The bacterial reverse mutation assay (as defined in OECD Guideline 471) only tests point-mutation inducing activity in non-mammalian cells, and the exogenous metabolic activation system does not entirely mimic *in vivo* conditions[16]. The *in vitro* chromosome aberration assay (OECD Guideline 473) does not measure aneuploidy and it only measures structural chromosomal aberrations. The exogenous metabolic activation system does not entirely mirror *in vivo* metabolism[17]. The *in vitro* mitotic recombination assay detects repair in non-mammalian cells, which is only an indirect measure, and non-specific, of gene mutation events. **Endocrine activity:** The *in vivo* relevance of EDSP screening assays and the Zorad et al. (1987) *in vitro* study on insulin binding and degradation, and the *in silico* modeling for receptor binding activities, are unknown due to lack of consideration of metabolism and other toxicokinetic factors. **Skin sensitization:** The Toxtree tool only evaluates the presence of structural alerts, and does not define applicability domains. **Respiratory sensitization**: The OECD Toolbox only identifies structural alerts and does not define applicability domains. Additionally, the ECHA guidance (2017), on which the use of OECD Toolbox structural alerts is based, does not evaluate non-immunologic mechanisms for respiratory sensitization.

Endpoint	NAMs Data Available and Evaluated? (Y/N)	Types of NAMs Data (*in silico* modeling/*in vitro* biological profiling/frameworks)
Carcinogenicity	Y	*In silico* modeling: OncoLogic/ VEGA/Danish QSAR database/Toxtree
Mutagenicity	Y	*In vitro* data: Bacterial reverse mutation assay/ *in vitro* chromosome aberration assay / yeast mitotic recombination assay
Reproductive toxicity	N	
Developmental toxicity	N	

[16] https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1614097593&id=id&accname=guest&checksum=89925F80B9F4BD2FFC6E90F94A0EE427
[17] https://www.oecd-ilibrary.org/docserver/9789264264649-en.pdf?expires=1614098015&id=id&accname=guest&checksum=6A4F9CE52EA974F5A74793DD54D54352

Endocrine activity	Y	*In vitro* data: insulin binding assay in human erythrocytes/ high throughput screening data from ToxCast EDSP screening assays; *in silico* modeling: ToxCast models / VEGA/ Danish (Q)SAR
Acute mammalian toxicity	N	
Single exposure systemic toxicity	N	
Repeated exposure systemic toxicity	N	
Single exposure neurotoxicity	N	
Repeated exposure neurotoxicity	N	
Skin sensitization	Y	*In silico* modeling: Payne and Walsh (1994) structural alerts/Toxtree/OECD Toolbox/LabMol
Respiratory sensitization	Y	*In silico* modeling: OECD Toolbox structural alerts
Skin irritation	N	
Eye irritation	N	
Acute aquatic toxicity	Y	*In silico* modeling: ECOSAR
Chronic aquatic toxicity	Y	*In silico* modeling: ECOSAR
Persistence	Y	*In silico* modeling: EPI Suite™ Non-animal testing: Biodegradation tests
Bioaccumulation	Y	*In silico* modeling: EPI Suite™

References

Agence Nationale de Sécurité Sanitaire de l'Alimentation (ANSES) (French Agency for Food, Environmental and Occupational Health & Safety). 2013. Occupational exposure limits. Occupational co-exposure to noise and chemicals. Available: https://www.anses.fr/en/system/files/VLEP2012sa0047RaEN.pdf

Agency for Toxic Substances and Disease Registry (ATSDR). 1999. Toxicological profile for n-hexane. 3. Chemical and physical information. Available: https://www.atsdr.cdc.gov/toxprofiles/tp113-c3.pdf

American Petroleum Institute (API). 1991. TSCA submission of the final report for commercial hexane – Two generation reproduction study of inhaled commercial hexane in CD (Sprague-Dawley) rats. Available: www.toxplanet.com

Ashford's Dictionary of Industrial Chemicals (Ashford's). Undated. Entry for heptane, CAS 142-82-5. Third Edition, ISBN 978-0-9522674-3-0. Wavelength Publications. Available: http://www.industrialchemistry.org/PDF/r11145.pdf

Bahima, J., A. Cert, and M. Menéndez-Gallego. 1984. Identification of volatile metabolites of inhaled n-heptane in rat urine. *Toxicol. Appl. Pharmacol,* 76(3): 473–482. Available: https://pubmed.ncbi.nlm.nih.gov/6095491/

Borba, J.V.B., R.C. Braga, V.M. Alves, E.N. Muratov, N. Kleinstreur, A. Tropsha, and C.H. Andrade. 2021. *Chem. Res. Toxicol.* 34:258-267.

Chemical Classification and Information Database (CCID). 2023. Entry for heptane (CAS #142-82-5). Approval number HSR001164. Environmental Protection Authority, Government of New Zealand. Available: https://www.epa.govt.nz/database-search/chemical-classification-and-information-database-ccid/view/21CC863E-2530-4B74-8542-5B599143FC82

Clean Production Action (CPA). 2018a. GreenScreen® Assessment Expiration Policy. October 2, 2018.

Clean Production Action (CPA). 2018b. The GreenScreen® for Safer Chemicals Guidance. Version 1.4 Guidance. Dated January, 2018. Available: https://www.greenscreenchemicals.org/static/ee_images/uploads/resources/GreenScreen_Guidance_v1_4_2018_01_Final.pdf

Csanády, G. 2012. n-Heptane Metabolism. The MAK-Collection for Occupational Health and Safety: Annual Thresholds and Classifications for the Workplace. Volume 5. 64–70. Available: https://onlinelibrary.wiley.com/doi/full/10.1002/3527600418.bb14282e0005

Danish Technical University (DTU). 2023. National Food Institute. Danish QSAR Database. Available: http://qsar.food.dtu.dk/.

European Chemicals Agency (ECHA). 2017a. Substance Evaluation Conclusion as required by REACH Article 48 and Evaluation Report for n-Hexane. Dated: May 2017. Available: https://echa.europa.eu/documents/10162/167cb34b-2110-c1e4-b50d-45f676bc5280

European Chemicals Agency (ECHA). 2017b. Guidance on information requirements and Chemical Safety Assessment. Chapter R.7a: Endpoint specific guidance. Version 6.0. Dated: July 2017. Available: https://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf

European Chemicals Agency (ECHA). 2023a. REACH registration dossier for n-heptane (CAS #142-82-5). Available: https://echa.europa.eu/information-on-chemicals

European Chemicals Agency (ECHA). 2023b. Harmonised GHS Classification for n-heptane (CAS #142-82-5). Available: https://echa.europa.eu/information-on-chemicals

European Chemicals Agency (ECHA). 2023c. REACH Dossier for n-Hexane (CAS #110-54-3). Available: https://echa.europa.eu/registration-dossier/-/registered-dossier/15741/1

European Chemicals Bureau (ECB). 2000. IUCLID Chemical Data Sheet for n-Heptane (CAS#142-82-5). European Commission Joint Research Center. Available: https://www.toxplanet.com/

European Commission (EC). 2023. CosIng database. Available: https://ec.europa.eu/growth/tools-databases/cosing/index.cfm?fuseaction=search.details_v2&id=76388

European Food Safety Authority (EFSA). 2012. Scientific opinion on the evaluation of the substances currently on the list in the annex to Commission Directive 96/3/EC as acceptable previous cargoes for edible fats and oils – Part II of III. *EFSA J.* 10(5): 2703. Available: https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/j.efsa.2012.2703

European Food Safety Authority (EFSA). 2018. Guidance on uncertainty analysis in scientific assessments. *EFSA J.* 16(1): e05123. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7009727/

Filser, J.G, G.A. Csanady, W. Dietz, W. Kessler, P.E. Kreuzer, M. Richter, and A. Stomer. 1996. Comparative estimation of the neurotoxic risks of n-hexane and n-heptane in rats and humans based on the formation of the metabolites 2,5-hexanedione and 2,5-heptanedione. In Snyder R. et al. (eds) Biological Reactive Intermediates V. Advances in Experimental Medicine and Biology. Vol 387. Springer, Boston, M.A. Abstract available: https://link.springer.com/chapter/10.1007/978-1-4757-9480-9_50#citeas

Hazardous Substances Data Bank (HSDB). 2014. Online record for n-heptane. United States National Library of Medicine. Available at: https://pubchem.ncbi.nlm.nih.gov/source/hsdb/90

International Chemical Safety Cards (ICSC). 2015. n-Heptane. ICSC # 0657. Validated June 2015. Available: https://www.ilo.org/dyn/icsc/showcard.display?p_lang=en&p_card_id=0657&p_version=2

LabMol. 2020. Pred-Skin Web App 3.0. Available: http://predskin.labmol.com.br/

Madden, J.C., S.J. Enoch, A. Paini, and M.T.D. Cronin. 2020. A Review of In Silico Tools as Alternatives to Animal Testing: Principles, Resources, and Applications. *Alt. Lab. Animals.* 1-27. Available: https://journals.sagepub.com/doi/pdf/10.1177/0261192920965977

National Institute of Technology and Evaluation (NITE). 2006. FY2006 GHS classification result for n-heptane (CAS #142-82-5). ID 946. Incorporated Administrative Agency, Government of Japan. Available: https://www.nite.go.jp/chem/english/ghs/06-imcg-0936e.html

National Institute of Technology and Evaluation (NITE). 2009. FY2009 GHS classification result for n-heptane (CAS #142-82-5). ID 21B3055. Incorporated Administrative Agency, Government of Japan. Available: https://www.nite.go.jp/chem/english/ghs/09-mhlw-2049e.html

National Institute of Technology and Evaluation (NITE). 2014. FY2014 GHS classification result for n-heptane (CAS #142-82-5). ID H26-B-088 / R-067. Incorporated Administrative Agency, Government of Japan. Available: https://www.nite.go.jp/chem/english/ghs/14-mhlw-2088e.html

Nordic Expert Group (NEG). 2010. Criteria Documentation of Health Risks from Chemicals. 142. Occupational exposure to chemicals and hearing impairment. Available: https://www.norskoljeoggass.no/globalassets/dokumenter/drift/arbeidsmiljo/kjemisk-arbeidsmiljo/fagtema/horselsskadelige-kjemikalier/occupational-exposure-to-chemicals-and-hearing-impairment.pdf

Occupational Safety and Health Administration (OSHA). 2023. Permissible exposure limits – annotated tables. United States Department of Labor. Available: https://www.osha.gov/dsg/annotated-pels/

Office of Environmental Health Hazard Assessment (OEHHA). 2017. Proposition 65. Consideration of n-Hexane for Listing under Proposition 65 as Known to Cause Reproductive Toxicity. Dated; September 2017. Available: https://oehha.ca.gov/media/downloads/proposition-65/chemicals/n-hexanehid090117.pdf

Organisation for Economic Co-operation and Development (OECD). 1992. OECD Guideline for Testing Chemicals: 301A-F Ready Biodegradability. Available: https://www.oecd.org/chemicalsafety/risk-assessment/1948209.pdf

Organisation for Economic Co-operation and Development (OECD). 2010. SIDS Initial Assessment Profile. C7-C9 Aliphatic Hydrocarbon Solvents Category. Available: http://webnet.oecd.org/Hpv/UI/handler.axd?id=a918c6e7-6c70-4987-a7b5-3120655d8a58

Organisation for Economic Co-operation and Development (OECD). 2020. Overview of Concepts and Available Guidance related to Integrated Approaches to Testing and Assessment (IATA), Series on Testing and Assessment, No. 329, Environment, Health and Safety, Environment Directorate. Available: http://www.oecd.org/chemicalsafety/risk-assessment/concepts-and-available-guidance-related-to-integrated-approaches-to-testing-and-assessment.pdf

Organisation for Economic Co-operation and Development (OECD). 2022. OECD QSAR Toolbox for Grouping Chemicals into Categories Version 4.5. SP1. Available: http://toolbox.oasis-lmc.org/?section=download&version=latest.

Organisation for Economic Co-operation and Development (OECD). 2023. Categorization results from the Canadian Domestic Substance List (DSL) for heptane (CAS #142-82-5). Available: https://canadachemicals.oecd.org/Search.aspx

Payne, M.P., and P.T. Walsh. 1994. Structure-activity relationships for skin sensitization potential: development of structural alerts for use in knowledge-based toxicity prediction systems. *J Chem Inf Comput Sci*. 34:154-161.

Pharos. 2023. Pharos Chemical and Material Library Entry for n-Heptane (CAS #142-82-5). Available: http://www.pharosproject.net/material/.

PubChem. 2023. Heptane (CAS #142-82-5). United States National Library of Medicine. Available: https://pubchem.ncbi.nlm.nih.gov/compound/8900

Perbellini, L., F. Brugnone,, V. Cocheo,, E. De Rosa, and G.B. Bartolucci. 1986. Identification of the n-heptane metabolites in rat and human urine. *Arch Toxicol*, 58(4): s. 229–234. Available: https://link.springer.com/article/10.1007/BF00297111

Registry of Toxic Effects of Chemical Substances (RTECS). 2013. National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention. Entry for Heptane, CAS 142-82-5. Available: https://www.cdc.gov/niosh-rtecs/MI757E20.html

TEDX. 2017. n-Heptane (CAS #142-82-5). Available: https://endocrinedisruption.org/interactive-tools/tedx-list-of-potential-endocrine-disruptors/search-the-tedx-list#sname=142-82-5&searchfor=any&sortby=chemname&action=search&searchcats=all&sortby=chemname

ToxServices. 2021. SOP 1.37: GreenScreen® Hazard Assessments. Dated: March 24, 2021.

Toxtree. 2018. Estimation of Toxic Hazard- A Decision Tree Approach v3.1.0. Available: http://toxtree.sourceforge.net.

United Nations (UN). 2021. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Ninth revised edition.

United States Department of Transportation (U.S. DOT). 2008a. Chemicals Listed with Classification. 49 CFR § 172.101. Available: http://www.gpo.gov/fdsys/pkg/CFR-2008-title49-vol2/pdf/CFR-2008-title49-vol2-sec172-101.pdf.

United States Department of Transportation (U.S. DOT). 2008b. Classification Criteria. 49 CFR § 173. Available: http://www.ecfr.gov/cgi-bin/text-idx?c=ecfr&tpl=/ecfrbrowse/Title49/49cfr173_main_02.tpl

United States Environmental Protection Agency (U.S. EPA). 2005. Toxicological Review of n-Hexane (CAS #110-54-3). Available: http://www.epa.gov/iris/toxreviews/0486tr.pdf.

United States Environmental Protection Agency (U.S. EPA). 2009. Provisional Peer-Reviewed Toxicity Values for Commercial or Practical Grade Hexane. Available: https://cfpub.epa.gov/ncea/pprtv/documents/HexaneCommercial.pdf.

United States Environmental Protection Agency (U.S. EPA). 2013. Interpretive assistance document for assessment of discrete organic chemicals. Sustainable Futures Summary Assessment. Updated June 2013. Available at: https://www.epa.gov/sites/production/files/2015-05/documents/05-iad_discretes_june2013.pdf

United States Environmental Protection Agency (U.S. EPA). 2015. Safer Choice Standard. Available: https://www.epa.gov/saferchoice/standard

United States Environmental Protection Agency (U.S. EPA). 2016. Provisional Peer-Reviewed Toxicity Values for n-Heptane (CAS #142-82-5). Available: https://cfpub.epa.gov/ncea/pprtv/recordisplay.cfm?deid=340083

United States Environmental Protection Agency (U.S. EPA). 2017a. ECOSAR 2.0. Washington, DC, USA. Available: http://www.epa.gov/oppt/newchems/tools/21ecosar.htm/.

United States Environmental Protection Agency (U.S. EPA). 2017b. Estimation Programs Interface (EPI) Suite™ Web, v4.11, Washington, DC, USA. Available: http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm.

United States Environmental Protection Agency (U.S. EPA). 2019. Oncologic™. Version 8.0. Available: https://www.epa.gov/tsca-screening-tools/oncologictm-expert-system-evaluate-carcinogenic-potential-chemicals

United States Environmental Protection Agency (U.S. EPA). 2020. New Approach Methods Workplan. Office of Research and Development. Office of Chemical Safety and Pollution Prevention. EPA 615B20001. June 2020. Available: https://www.epa.gov/sites/production/files/2020-06/documents/epa_nam_work_plan.pdf

United States Environmental Protection Agency (U.S. EPA). 2023a. Safer Chemical Ingredients List (SCIL). Available: https://www.epa.gov/saferchoice/safer-ingredients

United States Environmental Protection Agency (U.S. EPA). 2023b. Bioactivity – ToxCast Models – n-Heptane (CAS #142-82-5). Available: https://comptox.epa.gov/dashboard/chemical/bioactivity-toxcast-models/DTXSID6024127

United States Food and Drug Administration (U.S. FDA). 2023. Indirect additives used in food contact substances entry for heptane (CAS #142-82-5). Available: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=IndirectAdditives&id=HEPTANE

Virtual Models for Evaluating the Properties of Chemicals within a Global Architecture (VEGA). 2021. Predictive Model Platform version 1.1.5. Available: http://www.vega-qsar.eu/index.php.

Zorad, S., I. Klimes, E. Svabova, A. Mitkova, and L. Macho. 1987. n-Heptane and n-hexane enhance in a dose-dependent manner insulin binding to erythrocytes and its degradation. *Gen Physiol Biophys*. 6: 197-201. Available: http://www.gpb.sav.sk/1987/1987_02_197.pdf

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 n-Heptane (CAS #142-82-5)

TOXSERVICES
TOXICOLOGY RISK ASSESSMENT CONSULTING

GreenScreen® Score Inspector

Table 1: Hazard Table

	Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
	Carcinogenicity	Mutagenicity/Genotoxicity	Reproductive Toxicity	Developmental Toxicity	Endocrine Activity	Acute Toxicity	Systemic Toxicity		Neurotoxicity		Skin Sensitization*	Respiratory Sensitization*	Skin Irritation	Eye Irritation	Acute Aquatic Toxicity	Chronic Aquatic Toxicity	Persistence	Bioaccumulation	Reactivity	Flammability
Table 2: Chemical Details							S	R *	S	R *	*	*								
Inorganic Chemical? / Chemical Name / CAS#	C	M	R	D	E	AT	STs	STr	Ns	Nr	SNS*	SNR*	IrS	IrE	AA	CA	P	B	Rx	F
No / n-Heptane / 142-82-5	*L*	L	L	*L*	DG	L	H	L	M	L	L	*L*	H	*M*	vH	*vH*	vL	*M*	*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	No	Yes	Yes
3	STOP						
4	STOP						

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
n-Heptane	2

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

Table 6

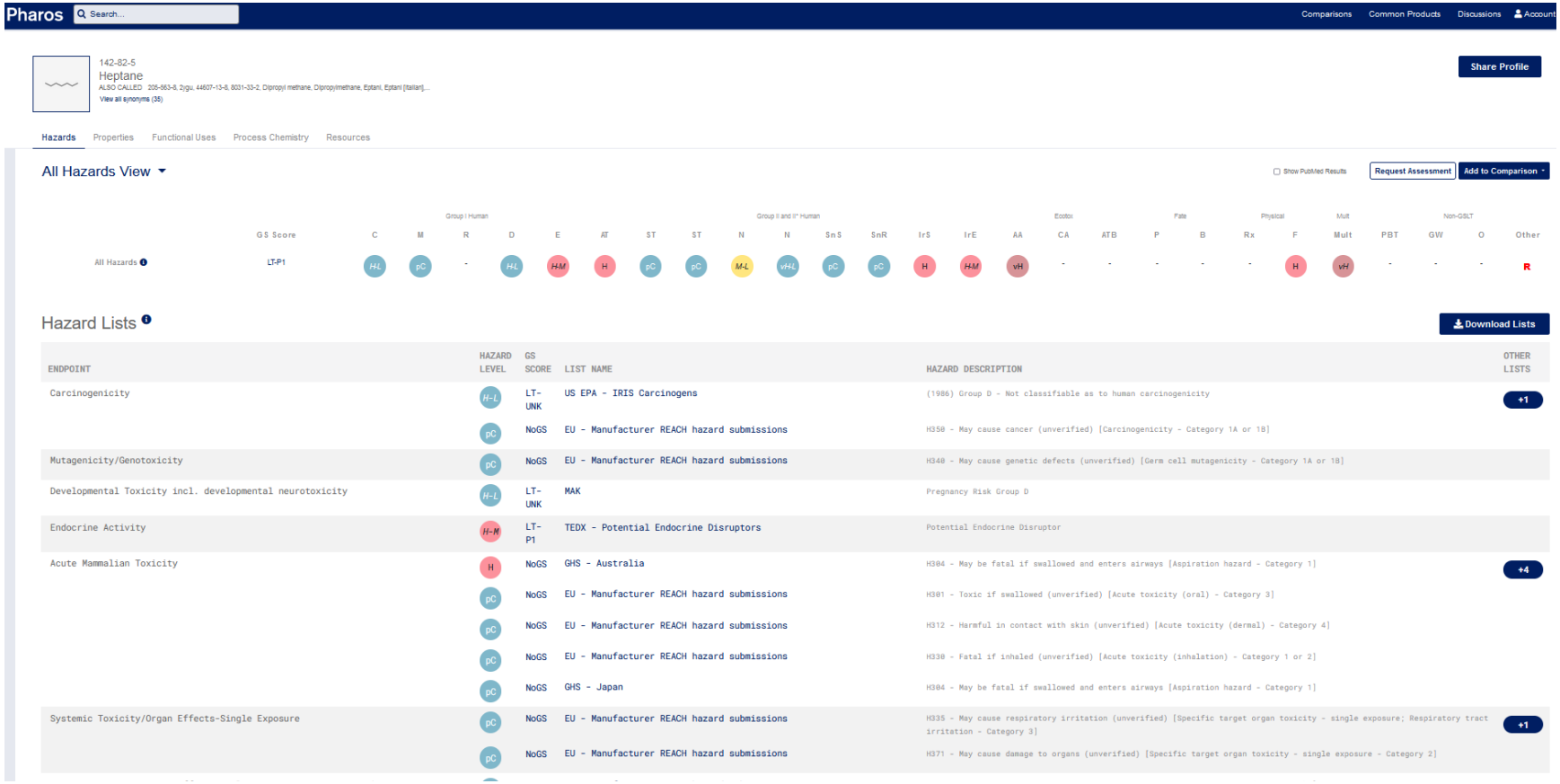
Chemical Name	Final GreenScreen® Benchmark Score
n-Heptane	2

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 n-Heptane (CAS #142-82-5)



Pharos Search... Comparisons Common Products Discussions Account

142-82-5
Heptane
ALSO CALLED 205-563-8, 2jigu, 44607-13-8, 8031-33-2, Dipropyl methane, Dipropylmethane, Eptani, Eptani [Italian]...
View all synonyms (35)

Share Profile

Hazards | Properties | Functional Uses | Process Chemistry | Resources

All Hazards View

Show PubMed Results | Request Assessment | Add to Comparison

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

Hazard Lists

Download Lists

ENDPOINT	HAZARD LEVEL	GS SCORE	LIST NAME	HAZARD DESCRIPTION	OTHER LISTS
Carcinogenicity	H-L	LT-UNK	US EPA - IRIS Carcinogens	(1986) Group D - Not classifiable as to human carcinogenicity	+1
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H350 - May cause cancer (unverified) [Carcinogenicity - Category 1A or 1B]	
Mutagenicity/Genotoxicity	pC	NoGS	EU - Manufacturer REACH hazard submissions	H340 - May cause genetic defects (unverified) [Germ cell mutagenicity - Category 1A or 1B]	
Developmental Toxicity incl. developmental neurotoxicity	H-L	LT-UNK	MAK	Pregnancy Risk Group D	
Endocrine Activity	H-M	LT-P1	TEDX - Potential Endocrine Disruptors	Potential Endocrine Disruptor	
Acute Mammalian Toxicity	H	NoGS	GHS - Australia	H304 - May be fatal if swallowed and enters airways [Aspiration hazard - Category 1]	+4
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H301 - Toxic if swallowed (unverified) [Acute toxicity (oral) - Category 3]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H312 - Harmful in contact with skin (unverified) [Acute toxicity (dermal) - Category 4]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H330 - Fatal if inhaled (unverified) [Acute toxicity (inhalation) - Category 1 or 2]	
	pC	NoGS	GHS - Japan	H304 - May be fatal if swallowed and enters airways [Aspiration hazard - Category 1]	
Systemic Toxicity/Organ Effects-Single Exposure	pC	NoGS	EU - Manufacturer REACH hazard submissions	H335 - May cause respiratory irritation (unverified) [Specific target organ toxicity - single exposure; Respiratory tract irritation - Category 3]	+1
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H371 - May cause damage to organs (unverified) [Specific target organ toxicity - single exposure - Category 2]	

Systemic Toxicity/Organ Effects incl. immunotoxicity-Repeated Exposure	pC	NoGS	EU - Manufacturer REACH hazard submissions	H372 - Causes damage to organs through prolonged or repeated exposure (unverified) [Specific target organ toxicity - repeated exposure - Category 1]	
Neurotoxicity-Single Exposure	M-L	LT-UNK	EU - GHS (H-Statements) Annex 6 Table 3-1	H336 - May cause drowsiness or dizziness [Specific target organ toxicity - single exposure; Narcotic effects - Category 3]	+2
	M-L	LT-UNK	GHS - Australia	H336 - May cause drowsiness or dizziness [Specific target organ toxicity - single exposure; Narcotic effects - Category 3]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H336 - May cause drowsiness or dizziness (unverified) [Specific target organ toxicity - single exposure; Narcotic effects - Category 3]	
Neurotoxicity-Repeated Exposure	vH-L	LT-UNK	Boyes - Neurotoxicants	Neurotoxic	
Skin Sensitization	pC	NoGS	EU - Manufacturer REACH hazard submissions	H317 - May cause an allergic skin reaction (unverified) [Skin sensitization - Category 1]	
Respiratory Sensitization	pC	NoGS	EU - Manufacturer REACH hazard submissions	H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled (unverified) [Respiratory sensitization - Category 1]	
Skin Irritation/Corrosivity	H	LT-UNK	EU - GHS (H-Statements) Annex 6 Table 3-1	H315 - Causes skin irritation [Skin corrosion/irritation - Category 2]	+4
	H	LT-UNK	GHS - Japan	H315 - Causes skin irritation [Skin corrosion / irritation - Category 2]	
	H	LT-UNK	GHS - Australia	H315 - Causes skin irritation [Skin corrosion/irritation - Category 2]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H314 - Causes severe skin burns and eye damage (unverified) [Skin corrosion/irritation - Category 1A or 1B or 1C]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H315 - Causes skin irritation (unverified) [Skin corrosion/irritation - Category 2]	
Eye Irritation/Corrosivity	H-M	LT-UNK	GHS - Japan	H319 - Causes serious eye irritation [Serious eye damage / eye irritation - Category 2]	
Acute Aquatic Toxicity	vH	LT-UNK	EU - GHS (H-Statements) Annex 6 Table 3-1	H400 - Very toxic to aquatic life [Hazardous to the aquatic environment (acute) - Category 1]	+2
	vH	LT-UNK	GHS - Japan	H400 - Very toxic to aquatic life [Hazardous to the aquatic environment (acute) - Category 1]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H400 - Very toxic to aquatic life (unverified) [Hazardous to the aquatic environment (acute) - Category 1]	
Flammability	H	LT-UNK	EU - GHS (H-Statements) Annex 6 Table 3-1	H225 - Highly flammable liquid and vapour [Flammable liquids - Category 2]	+5
	H	LT-UNK	GHS - Australia	H225 - Highly flammable liquid and vapour [Flammable liquids - Category 2]	
	H	LT-UNK	GHS - Japan	H225 - Highly flammable liquid and vapour [Flammable liquids - Category 2]	
	H	LT-UNK	GHS - New Zealand	Flammable liquids category 2	
	vH-M	LT-UNK	Québec CSST - WHMIS 1988	Class B2 - Flammable liquids	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H225 - Highly flammable liquid and vapour (unverified) [Flammable liquids - Category 2]	

Reproductive and/or Developmental Toxicity	pC	NoGS	EU - Manufacturer REACH hazard submissions	H360 - Suspected of / May damage fertility and/or the unborn child (unverified) [Reproductive toxicity - Category 1A or 1B]	
T & P and/or B [(Chronic Aquatic Toxicity and Persistence) or (Acute Aquatic Toxicity and Persistence and/or Bioaccumulation)]	vH	LT-P1	GHS - New Zealand	Hazardous to the aquatic environment - chronic category 1	+3
	U	LT-P1	EU - GHS (H-Statements) Annex 6 Table 3-1	H410 - Very toxic to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) - Category 1]	
	U	LT-P1	GHS - Australia	H410 - Very toxic to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) - Category 1]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H410 - Very toxic to aquatic life with long lasting effects (unverified) [Hazardous to the aquatic environment (chronic) - Category 1]	
Systemic Toxicity/Organ Effects [Repeated Exposure] and/or Neurotoxicity [Repeated Exposure]	H	LT-UNK	GHS - Japan	H372 - Causes damage to organs through prolonged or repeated exposure [Specific target organs/systemic toxicity following repeated exposure - Category 1]	
Acute aquatic toxicity; Chronic aquatic toxicity	U	LT-UNK	EC - CEPA DSL	Inherently Toxic in the Environment (iTE)	
Systemic Toxicity/Organ Effects [Single Exposure] and/or Neurotoxicity [Single Exposure]	M	LT-UNK	GHS - Japan	H335 or H336 [Specific target organs/systemic toxicity following single exposure - Category 3]	
Human and/or Aquatic toxicity and/or Persistence and/or Bioaccumulation	U	LT-P1	German FEA - Substances Hazardous to Waters	Class 2 - Hazard to Waters	
Systemic Toxicity/Organ Effects (Single Exposure - Aspiration Hazard)	H	LT-UNK	EU - GHS (H-Statements) Annex 6 Table 3-1	H304 - May be fatal if swallowed and enters airways [Aspiration hazard - Category 1]	+2
	H	NoGS	GHS - New Zealand	Aspiration hazard category 1	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H304 - May be fatal if swallowed and enters airways (unverified) [Aspiration hazard - Category 1]	
Carcinogenicity,Mutagenicity/Genotoxicity Reproductive Toxicity, Developmental Toxicity, Acute Mammalian Toxicity, or System Toxicity/Organ Effects.	U	LT-UNK	Québec CSST - WHMIS 1988	Class D2B - Toxic material causing other toxic effects	
T & P and/or B [(Chronic Aquatic Toxicity and sometimes Persistence) or (Acute Aquatic Toxicity and Persistence and/or Bioaccumulation)]	U	LT-P1	GHS - Japan	H410 - Very toxic to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) - Category 1]	

Restricted Substance Lists (9)

- CA SCP - Candidate Chemicals: Candidate Chemical List
- EU - PACT-RMOA Substances: Substances selected for RMOA or hazard assessment
- Food Contact Chemicals Database (FCCdb): Food Contact Chemicals Database Version 5.0
- Food Contact Chemicals of Concern (FCCoCL): Food Contact Chemicals of Concern List (FCCoCL)
- Food Contact Chemicals of Concern (FCCoCL): Food Contact Chemicals of Concern List (FCCoCL) - TIER 3
- FPF Priority Substance List from FCCdb: FPF Priority Substance List from FCCdb
- GreenScreen Certified Standard for Food Service Ware RSL: Food Packaging Forum Priority Substances List
- GSPI - Six Classes of Problematic Chemicals: Some Solvents
- TSCA Chemical Substance Inventory (Active-Inactive): TSCA Chemical Substance Inventory - Active

Positive Lists (2)

- Inventory of Existing Cosmetic Ingredients in China (IECIC 2015): Cosmetic Ingredients
- TCO Certified Accepted Substance List: Benchmark-2 Accepted Substance

APPENDIX D: Oncologic Carcinogenicity Modeling Results for n-Heptane (CAS #142-82-5)

OncoLogic Justification Report

CODE NUMBER: 1428255
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:

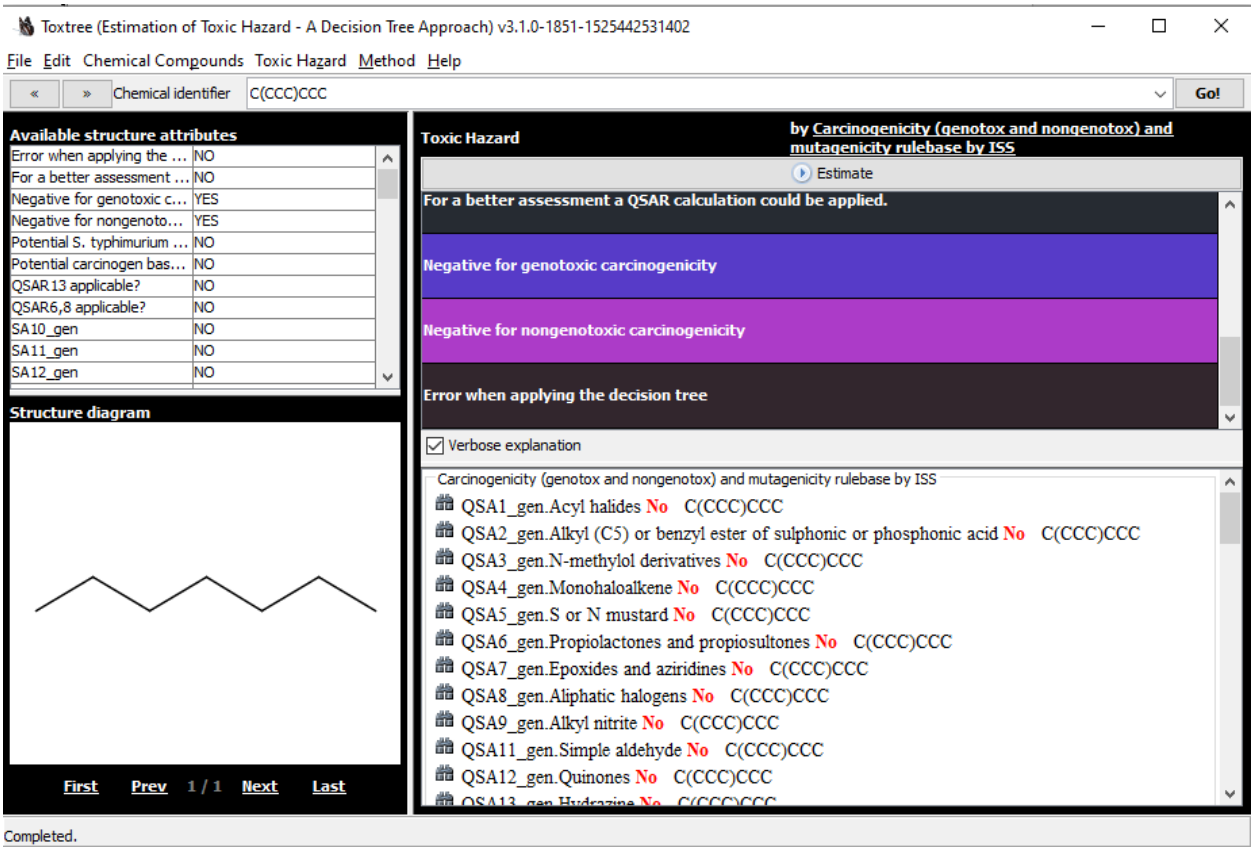
With a few exceptions, there is no evidence that members of the saturated aliphatic hydrocarbon (i.e., alkane) class may be of any significant cancer concern.* Therefore, virtually ALL alkanes can be assigned a LOW concern with the following exceptions:

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.

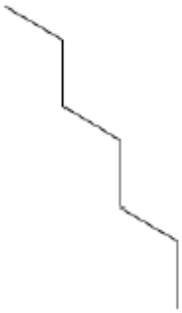
APPENDIX E: Toxtree Carcinogenicity Modeling Results for n-Heptane (CAS #142-82-5)



APPENDIX F: VEGA Carcinogenicity Modeling Results for n-Heptane (CAS #142-82-5)

1. Prediction Summary

Prediction for compound Molecule 0

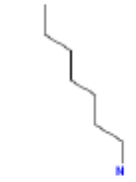


Prediction: Reliability:

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: CCCCCCC
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

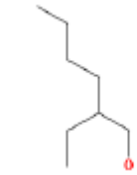
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 111-68-2
Dataset id: 357 (Test set)
SMILES: NCCCCCCC
Similarity: 0.782

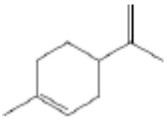
Experimental value: NON-Carcinogen
Predicted value: Carcinogen



Compound #2

CAS: 104-76-7
Dataset id: 314 (Training set)
SMILES: OCC(CC)CCCC
Similarity: 0.757

Experimental value: NON-Carcinogen
Predicted value: Carcinogen



Compound #3

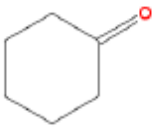
CAS: 5989-27-5
Dataset id: 412 (Training set)
SMILES: C=C(C)C1CC=C(C)CC1
Similarity: 0.748

Experimental value: Carcinogen
Predicted value: NON-Carcinogen

Compound #4

CAS: 115-11-7
Dataset id: 399 (Test set)
SMILES: C=C(C)C
Similarity: 0.715

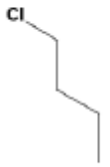
Experimental value: Carcinogen
Predicted value: Carcinogen



Compound #5

CAS: 108-94-1
Dataset id: 187 (Test set)
SMILES: O=C1CCCCC1
Similarity: 0.7

Experimental value: NON-Carcinogen
Predicted value: Carcinogen



Compound #6

CAS: 109-69-3
Dataset id: 110 (Training set)
SMILES: CCCCCl
Similarity: 0.687

Experimental value: NON-Carcinogen
Predicted value: NON-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 = 0.769 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 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:

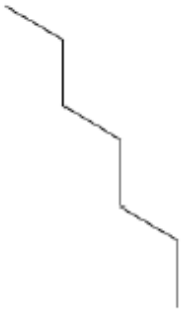
✔ The feature has a good assessment, model is reliable regarding this aspect.

⚠ 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

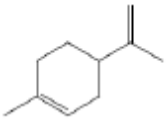


Prediction: Reliability:

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 adequate
- similar molecules found in the training set have experimental values that disagree with the predicted value

Compound: Molecule 0
Compound SMILES: CCCCCCC
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

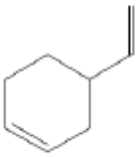
CAS: 5989-27-5
Dataset id: 267 (Training set)
SMILES: C=C(C)C1CC=C(C)CC1
Similarity: 0.748

Experimental value: Carcinogen
Predicted value: NON-Carcinogen

Compound #2

CAS: 115-11-7
Dataset id: 597 (Training set)
SMILES: C=C(C)C
Similarity: 0.715

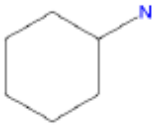
Experimental value: Carcinogen
Predicted value: NON-Carcinogen



Compound #3

CAS: 100-40-3
Dataset id: 238 (Training set)
SMILES: C=CC1CC=CCC1
Similarity: 0.704

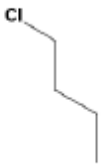
Experimental value: Carcinogen
Predicted value: NON-Carcinogen



Compound #4

CAS: 108-91-8
Dataset id: 834 (Training set)
SMILES: NC1CCCCC1
Similarity: 0.697

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

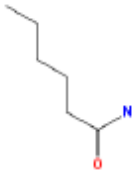


Compound #5

CAS: 109-69-3
Dataset id: 157 (Training set)
SMILES: CCCCCl
Similarity: 0.687

Experimental value: NON-Carcinogen
Predicted value: Carcinogen

Alerts (not found in the target): SA8 Aliphatic halogens



Compound #6

CAS: 628-02-4
Dataset id: 387 (Training set)
SMILES: O=C(N)CCCCC
Similarity: 0.665

Experimental value: Carcinogen
Predicted value: NON-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 = 0.731 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 Explanation: similar molecules found in the training set have experimental values that disagree with the predicted value.
✔	**Atom Centered Fragments similarity check** ACF index = 1 Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

Symbols explanation:

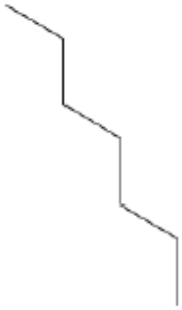
✔ The feature has a good assessment, model is reliable regarding this aspect.

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

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

1. Prediction Summary

Prediction for compound Molecule 0

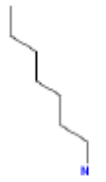


Prediction: Reliability:

Prediction is Possible NON-Carcinogen, but the result shows some critical aspects, which require to be checked:
- only moderately similar compounds with known experimental value in the training set have been found
- 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: CCCCCCC
Experimental value: -
Predicted Carcinogenic activity: Possible NON-Carcinogen
No. alerts for carcinogenicity: 0
Structural alerts: -
Reliability: the predicted compound could be out of the Applicability Domain of the model
Remarks:
none

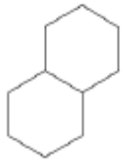
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: N.A.
Dataset id: 357 (Training set)
SMILES: NCCCCCCC
Similarity: 0.782

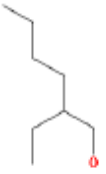
Experimental value: NON-Carcinogen
Predicted value: Possible NON-Carcinogen



Compound #2

CAS: N.A.
Dataset id: 1202 (Training set)
SMILES: C1CCC2CCCCC2(C1)
Similarity: 0.776

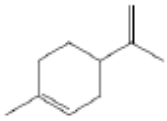
Experimental value: Carcinogen
Predicted value: Possible NON-Carcinogen



Compound #3

CAS: N.A.
Dataset id: 314 (Training set)
SMILES: OCC(CC)CCCC
Similarity: 0.757

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



Compound #4

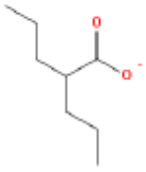
CAS: N.A.
Dataset id: 412 (Training set)
SMILES: C=C(C)C1CC=C(C)CC1
Similarity: 0.748

Experimental value: Carcinogen
Predicted value: Possible NON-Carcinogen

Compound #5

CAS: N.A.
Dataset id: 381 (Training set)
SMILES: C=C(C)C
Similarity: 0.715

Experimental value: Carcinogen
Predicted value: Possible NON-Carcinogen



Compound #6

CAS: N.A.
Dataset id: 1216 (Training set)
SMILES: O=C([O-])C(CCC)CCC
Similarity: 0.707

Experimental value: Carcinogen
Predicted value: Possible NON-Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

⚠	**Global AD Index** AD index = 0.716 Explanation: the predicted compound could be out of the Applicability Domain of the model.
⚠	**Similar molecules with known experimental value** Similarity index = 0.771 Explanation: only moderately similar compounds with known experimental value in the training set have been found.
⚠	**Accuracy of prediction for similar molecules** Accuracy index = 0.664 Explanation: accuracy of prediction for similar molecules found in the training set is not optimal.
⚠	**Concordance for similar molecules** Concordance index = 0.664 Explanation: some similar molecules found in the training set have experimental values that disagree with the predicted value.
✓	**Atom Centered Fragments similarity check** ACF index = 1 Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

Symbols explanation:

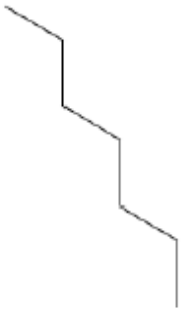
✓ The feature has a good assessment, model is reliable regarding this aspect.

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

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

1. Prediction Summary

Prediction for compound Molecule 0

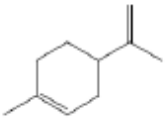


Prediction: Reliability:

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

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

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



Compound #1

CAS: 5989-27-5
Dataset id: 218 (Training set)
SMILES: C=C(C)C1CC=C(C)CC1
Similarity: 0.748

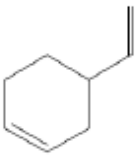
Experimental value: Carcinogen
Predicted value: Carcinogen

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

Compound #2

CAS: 115-11-7
Dataset id: 682 (Training set)
SMILES: C=C(C)C
Similarity: 0.715

Experimental value: Carcinogen
Predicted value: Possible NON-Carcinogen

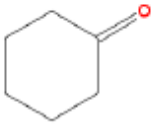


Compound #3

CAS: 100-40-3
Dataset id: 196 (Training set)
SMILES: C=CC1CC=CCC1
Similarity: 0.704

Experimental value: Carcinogen
Predicted value: Carcinogen

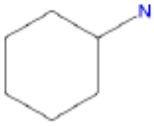
Alerts (not found in the target): Carcinogenity alert no. 39



Compound #4

CAS: 108-94-1
Dataset id: 934 (Training set)
SMILES: O=C1CCCCC1
Similarity: 0.7

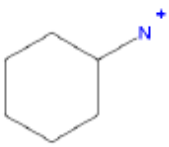
Experimental value: NON-Carcinogen
Predicted value: Possible NON-Carcinogen



Compound #5

CAS: 108-91-8
Dataset id: 748 (Training set)
SMILES: NC1CCCCC1
Similarity: 0.697

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



Compound #6

CAS: 4998-76-9
Dataset id: 935 (Training set)
SMILES: [NH3+]C1CCCCC1
Similarity: 0.691

Experimental value: NON-Carcinogen
Predicted value: Possible NON-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 = 0.721 Explanation: only moderately similar compounds with known experimental value in the training set have been found.
⚠	**Accuracy of prediction for similar molecules** Accuracy index = 0.672 Explanation: accuracy of prediction for similar molecules found in the training set is not optimal.
✖	**Concordance for similar molecules** Concordance index = 0 Explanation: similar molecules found in the training set have experimental values that disagree with the predicted value.
✔	**Atom Centered Fragments similarity check** ACF index = 1 Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

Symbols explanation:

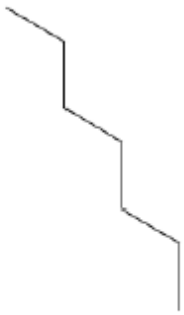
✔ The feature has a good assessment, model is reliable regarding this aspect.

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

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

1. Prediction Summary

Prediction for compound Molecule 0

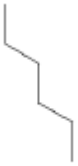


Prediction: Reliability:

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

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

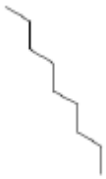
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 110-54-3
Dataset id: 540 (Training set)
SMILES: CCCCCC
Similarity: 0.957

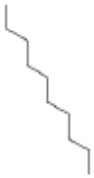
Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #2

CAS: 111-84-2
Dataset id: 610 (Training set)
SMILES: CCCCCCCCC
Similarity: 0.929

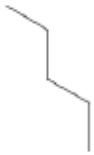
Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #3

CAS: 124-18-5
Dataset id: 425 (Training set)
SMILES: CCCCCCCCCC
Similarity: 0.908

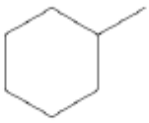
Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #4

CAS: 109-66-0
Dataset id: 626 (Training set)
SMILES: CCCCC
Similarity: 0.908

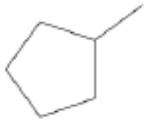
Experimental value: NON-Carcinogen
Predicted value: NON-Carcinogen



Compound #5

CAS: 108-87-2
Dataset id: 587 (Training set)
SMILES: CC1CCCCC1
Similarity: 0.891

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



Compound #6

CAS: 96-37-7
Dataset id: 588 (Training set)
SMILES: CC1CCCC1
Similarity: 0.856

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

3.2 Applicability Domain:
Measured Applicability Domain Scores

Global AD Index
AD index = 0.971
Explanation: the predicted compound is into the Applicability Domain of the model.

Similar molecules with known experimental value
Similarity index = 0.942
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.

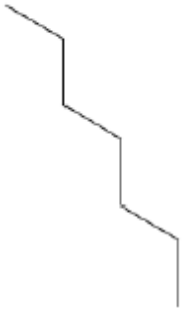
Atom Centered Fragments similarity check
ACF index = 1
Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

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

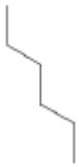
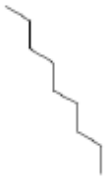
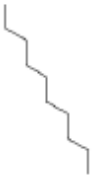
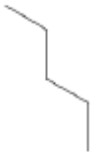
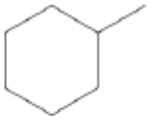
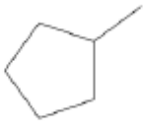


Prediction: Reliability:

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

Compound: Molecule 0
Compound SMILES: CCCCCCC
Experimental value: -
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: 110-54-3 Dataset id: 516 (Training set) SMILES: CCCCCC Similarity: 0.957 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen
	Compound #2 CAS: 111-84-2 Dataset id: 593 (Training set) SMILES: CCCCCCCCC Similarity: 0.929 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen
	Compound #3 CAS: 124-18-5 Dataset id: 389 (Training set) SMILES: CCCCCCCCCC Similarity: 0.908 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen
	Compound #4 CAS: 109-66-0 Dataset id: 612 (Training set) SMILES: CCCCC Similarity: 0.908 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen
	Compound #5 CAS: 108-87-2 Dataset id: 567 (Training set) SMILES: CC1CCCCC1 Similarity: 0.891 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen
	Compound #6 CAS: 96-37-7 Dataset id: 568 (Training set) SMILES: CC1CCCC1 Similarity: 0.856 Experimental value: NON-Carcinogen Predicted value: NON-Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

✓	**Global AD Index** AD index = 0.971 Explanation: the predicted compound is into the Applicability Domain of the model.
✓	**Similar molecules with known experimental value** Similarity index = 0.942 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: Danish (Q)SAR Carcinogenicity Modeling Results for n-Heptane (CAS #142-82-5)

Carcinogenicity

	E Ultra	Leadscope
FDA RCA Cancer Male Rat	NEG_IN	INC_OUT
FDA RCA Cancer Female Rat	NEG_IN	INC_OUT
FDA RCA Cancer Rat	NEG_IN	INC_OUT
FDA RCA Cancer Male Mouse	NEG_IN	INC_OUT
FDA RCA Cancer Female Mouse	NEG_IN	INC_OUT
FDA RCA Cancer Mouse	NEG_IN	NEG_OUT
FDA RCA Cancer Rodent	NEG_IN	INC_OUT

Commercial models from CASE Ultra and Leadscope

FDA RCA: Data from US Food and Drug Administration as part of Research Cooperation Agreement

Carcinogenicity (genotox and nongenotox) alerts by ISS, alerts in:	
- parent only	No alert found
Oncologic Primary Classification, alerts in:	
- parent only	Not classified

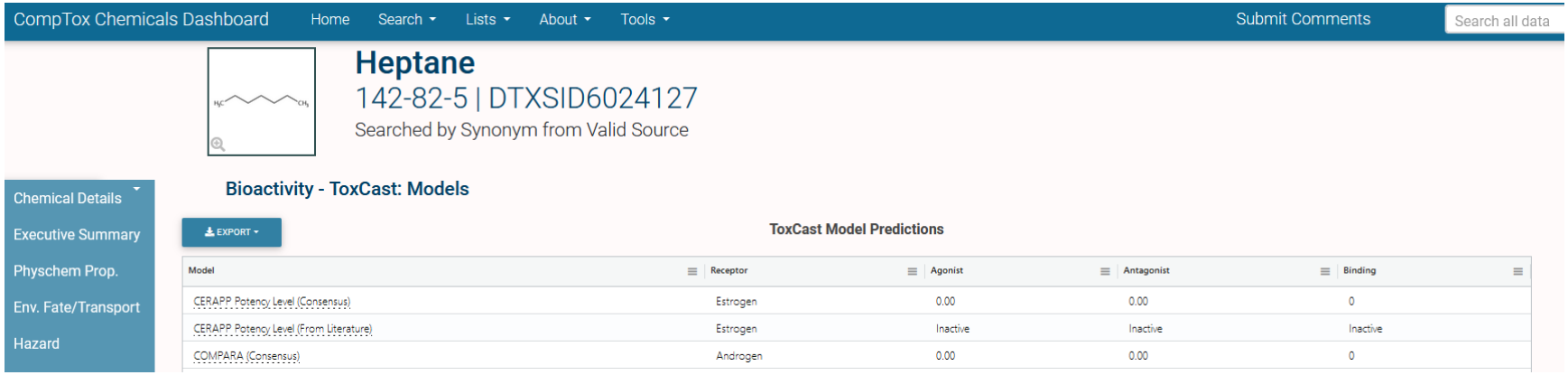
OECD QSAR Toolbox v.4.2 profilers

Profiler predictions are supporting information to be used together with the relevant QSAR predictions

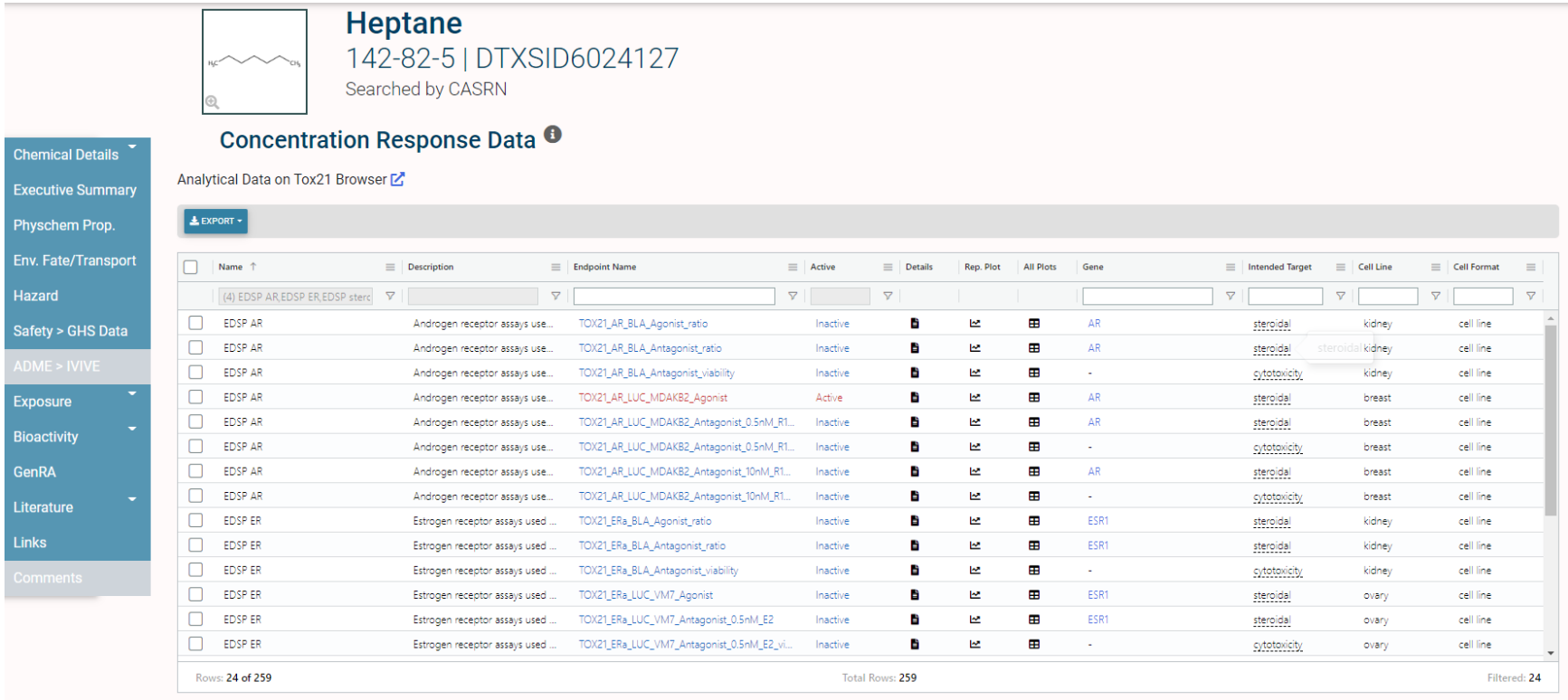
	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Liver Specific Cancer in Rat or Mouse		NEG_IN	NEG_IN	NEG_OUT	NEG_IN

DTU-developed models

APPENDIX H: ToxCast Endocrine Activity Modeling Results for n-Heptane (CAS #142-82-5)



APPENDIX I: Tox21 EDSP Endocrine Activity High Throughput Screening Assay Results for n-Heptane (CAS #142-82-5)



Heptane

142-82-5 | DTXSID6024127

Searched by CASRN

Chemical Details
Executive Summary
Physchem Prop.
Env. Fate/Transport
Hazard
Safety > GHS Data
ADME > IVIVE
Exposure
Bioactivity
GenRA
Literature
Links
Comments

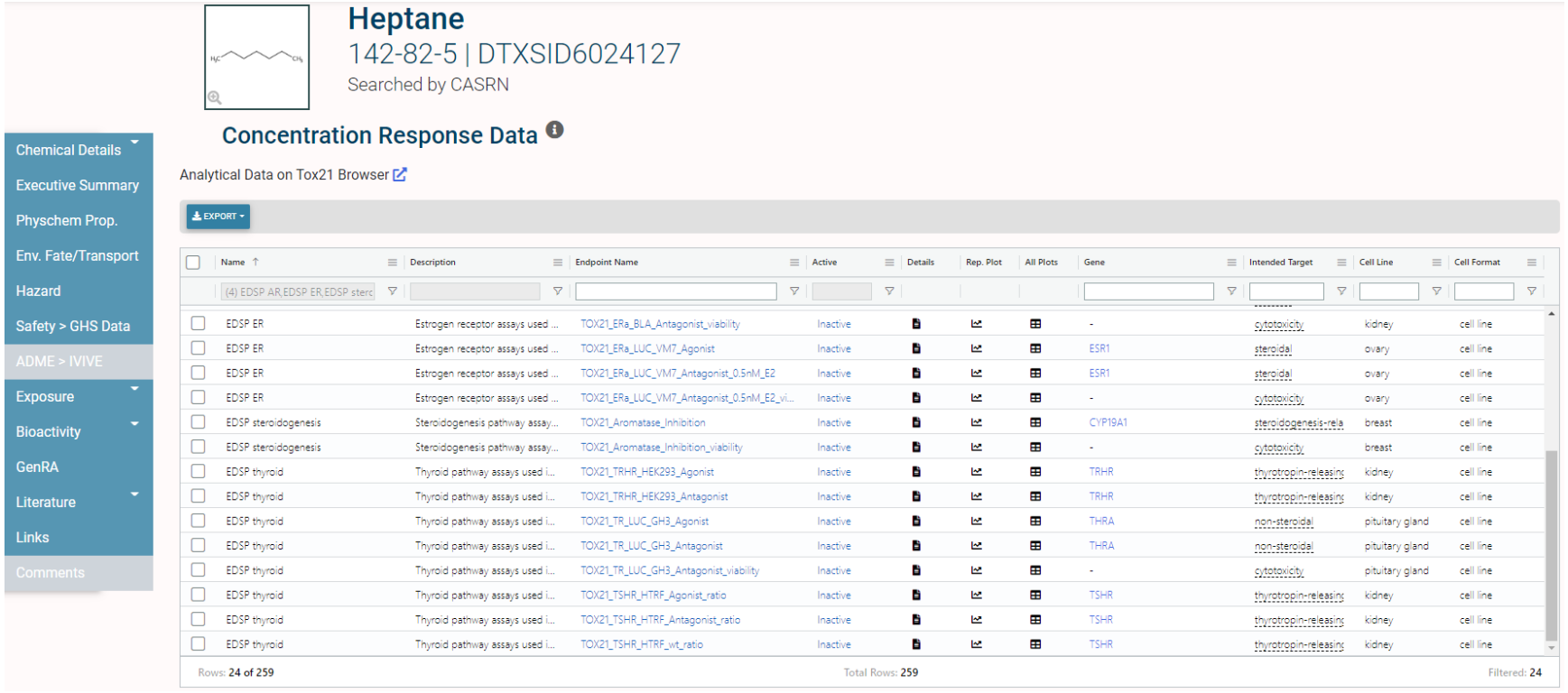
Concentration Response Data

Analytical Data on Tox21 Browser

EXPORT

Name	Description	Endpoint Name	Active	Details	Rep. Plot	All Plots	Gene	Intended Target	Cell Line	Cell Format
(4) EDSP AR,EDSP ER,EDSP steroi										
EDSP AR	Androgen receptor assays use...	TOX21_AR_BLA_Agonist_ratio	Inactive				AR	steroidal	kidney	cell line
EDSP AR	Androgen receptor assays use...	TOX21_AR_BLA_Antagonist_ratio	Inactive				AR	steroidal	kidney	cell line
EDSP AR	Androgen receptor assays use...	TOX21_AR_BLA_Antagonist_viability	Inactive				-	cytotoxicity	kidney	cell line
EDSP AR	Androgen receptor assays use...	TOX21_AR_LUC_MDAKB2_Agonist	Active				AR	steroidal	breast	cell line
EDSP AR	Androgen receptor assays use...	TOX21_AR_LUC_MDAKB2_Antagonist_0.5nM_R1...	Inactive				AR	steroidal	breast	cell line
EDSP AR	Androgen receptor assays use...	TOX21_AR_LUC_MDAKB2_Antagonist_0.5nM_R1...	Inactive				-	cytotoxicity	breast	cell line
EDSP AR	Androgen receptor assays use...	TOX21_AR_LUC_MDAKB2_Antagonist_10nM_R1...	Inactive				AR	steroidal	breast	cell line
EDSP AR	Androgen receptor assays use...	TOX21_AR_LUC_MDAKB2_Antagonist_10nM_R1...	Inactive				-	cytotoxicity	breast	cell line
EDSP ER	Estrogen receptor assays used ...	TOX21_ERa_BLA_Agonist_ratio	Inactive				ESR1	steroidal	kidney	cell line
EDSP ER	Estrogen receptor assays used ...	TOX21_ERa_BLA_Antagonist_ratio	Inactive				ESR1	steroidal	kidney	cell line
EDSP ER	Estrogen receptor assays used ...	TOX21_ERa_BLA_Antagonist_viability	Inactive				-	cytotoxicity	kidney	cell line
EDSP ER	Estrogen receptor assays used ...	TOX21_ERa_LUC_VM7_Agonist	Inactive				ESR1	steroidal	ovary	cell line
EDSP ER	Estrogen receptor assays used ...	TOX21_ERa_LUC_VM7_Antagonist_0.5nM_E2	Inactive				ESR1	steroidal	ovary	cell line
EDSP ER	Estrogen receptor assays used ...	TOX21_ERa_LUC_VM7_Antagonist_0.5nM_E2_vi...	Inactive				-	cytotoxicity	ovary	cell line

Rows: 24 of 259 Total Rows: 259 Filtered: 24



Heptane

142-82-5 | DTXSID6024127

Searched by CASRN

Concentration Response Data

Analytical Data on Tox21 Browser

Chemical Details
Executive Summary
Physchem Prop.
Env. Fate/Transport
Hazard
Safety > GHS Data
ADME > IVIVE
Exposure
Bioactivity
GenRA
Literature
Links
Comments

EXPORT

Name	Description	Endpoint Name	Active	Details	Rep. Plot	All Plots	Gene	Intended Target	Cell Line	Cell Format
(4) EDSP AR,EDSP ER,EDSP sterc										
EDSP ER	Estrogen receptor assays used ...	TOX21_ERa_BLA_Antagonist_viability	Inactive				-	cytotoxicity	kidney	cell line
EDSP ER	Estrogen receptor assays used ...	TOX21_ERa_LUC_VM7_Agonist	Inactive				ESR1	steroidal	ovary	cell line
EDSP ER	Estrogen receptor assays used ...	TOX21_ERa_LUC_VM7_Antagonist_0.5nM_E2	Inactive				ESR1	steroidal	ovary	cell line
EDSP ER	Estrogen receptor assays used ...	TOX21_ERa_LUC_VM7_Antagonist_0.5nM_E2_vi...	Inactive				-	cytotoxicity	ovary	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	TOX21_Aromatase_Inhibition	Inactive				CYP19A1	steroidogenesis-rela	breast	cell line
EDSP steroidogenesis	Steroidogenesis pathway assay...	TOX21_Aromatase_Inhibition_viability	Inactive				-	cytotoxicity	breast	cell line
EDSP thyroid	Thyroid pathway assays used i...	TOX21_TRHR_HEK293_Agonist	Inactive				TRHR	thyrotropin-releasing	kidney	cell line
EDSP thyroid	Thyroid pathway assays used i...	TOX21_TRHR_HEK293_Antagonist	Inactive				TRHR	thyrotropin-releasing	kidney	cell line
EDSP thyroid	Thyroid pathway assays used i...	TOX21_TR_LUC_GH3_Agonist	Inactive				THRA	non-steroidal	pituitary gland	cell line
EDSP thyroid	Thyroid pathway assays used i...	TOX21_TR_LUC_GH3_Antagonist	Inactive				THRA	non-steroidal	pituitary gland	cell line
EDSP thyroid	Thyroid pathway assays used i...	TOX21_TR_LUC_GH3_Antagonist_viability	Inactive				-	cytotoxicity	pituitary gland	cell line
EDSP thyroid	Thyroid pathway assays used i...	TOX21_TSHR_HTRF_Agonist_ratio	Inactive				TSHR	thyrotropin-releasing	kidney	cell line
EDSP thyroid	Thyroid pathway assays used i...	TOX21_TSHR_HTRF_Antagonist_ratio	Inactive				TSHR	thyrotropin-releasing	kidney	cell line
EDSP thyroid	Thyroid pathway assays used i...	TOX21_TSHR_HTRF_wt_ratio	Inactive				TSHR	thyrotropin-releasing	kidney	cell line

Rows: 24 of 259 Total Rows: 259 Filtered: 24

APPENDIX J: Danish (Q)SAR Endocrine and Molecular Endpoints Modeling Results for n-Heptane (CAS #142-82-5)

Endocrine and Molecular Endpoints

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Estrogen Receptor α Binding, Full training set (Human *in vitro*)		NEG_IN	NEG_IN	NEG_OUT	NEG_IN
Estrogen Receptor α Binding, Balanced Training Set (Human *in vitro*)		NEG_IN	NEG_IN	NEG_OUT	NEG_IN
Estrogen Receptor α Activation (Human *in vitro*)		INC_OUT	NEG_IN	NEG_OUT	POS_IN
Estrogen Receptor Activation, CERAPP data (*in vitro*)		N/A	N/A	INC_OUT	N/A
Androgen Receptor Inhibition (Human *in vitro*)		NEG_IN	NEG_IN	NEG_IN	NEG_IN
Androgen Receptor Binding, CoMPARA data (*in vitro*)		N/A	N/A	INC_OUT	N/A
Androgen Receptor Inhibition, CoMPARA data (*in vitro*)		N/A	N/A	INC_OUT	N/A
Androgen Receptor Activation, CoMPARA data (*in vitro*)		N/A	N/A	INC_OUT	N/A
Thyroperoxidase (TPO) inhibition QSAR1 (Rat *in vitro*)		N/A	N/A	INC_OUT	N/A
Thyroperoxidase (TPO) inhibition QSAR2 (Rat *in vitro*)		N/A	N/A	INC_OUT	N/A
Thyroid Receptor α Binding (Human *in vitro*)					
- mg/L		8220.782	16029.17	662.1266	412.3927
- µM		82035.55	159955.8	6607.391	4115.285
- Positive for IC_{50} ≤ 10 µM					
- Positive for IC_{50} ≤ 100 µM					
- Domain		IN	IN	OUT	OUT
Thyroid Receptor β Binding (Human *in vitro*)					
- mg/L		1746.906	3242.734	19.21282	251.0794
- µM		17432.46	32359.38	191.7256	2505.532
- Positive for IC_{50} ≤ 10 µM					
- Positive for IC_{50} ≤ 100 µM					
- Domain		IN	IN	OUT	OUT
Arylhydrocarbon (AhR) Activation – Rational final model (Human *in vitro*)		N/A	N/A	INC_OUT	N/A
Arylhydrocarbon (AhR) Activation –		N/A	N/A	INC_OUT	N/A

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

DTU-developed models

Estrogen Receptor Binding, alerts in:	
- parent only	Non binder, non cyclic structure
- metabolites from *in vivo* Rat metabolism simulator only	Non binder, non cyclic structure
- metabolites from Rat liver S9 metabolism simulator only	Non binder, non cyclic structure
rtER Expert System - USEPA, alerts in:	
- parent only	No alert found
- metabolites from *in vivo* Rat metabolism simulator only	No alert found
- metabolites from Rat liver S9 metabolism simulator only	No alert found

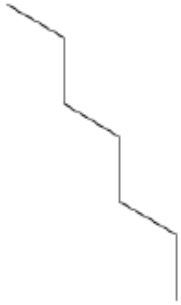
OECD QSAR Toolbox v.4.2 profilers

Profiler predictions are supporting information to be used together with the relevant QSAR predictions

APPENDIX K: VEGA Endocrine Activity Modeling Results for n-Heptane (CAS #142-82-5)

1. Prediction Summary

Prediction for compound Molecule 0

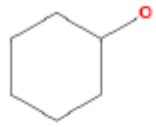


Prediction: Reliability:

Prediction is Inactive, but the result shows some critical aspects, which require to be checked:
- no similar compounds with known experimental value in the training set have been found

Compound: Molecule 0
Compound SMILES: CCCCCCC
Experimental value: -
Predicted activity: Inactive
Classification tree final node: 4
Reliability: the predicted compound could be out of the Applicability Domain of the model
Remarks:
none

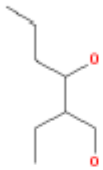
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 108-93-0
Dataset id: 876 (Test set)
SMILES: OC1CCCCC1
Similarity: 0.693

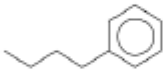
Experimental value: Inactive
Predicted value: Inactive



Compound #2

CAS: 94-96-2
Dataset id: 17 (Training set)
SMILES: OCC(CC)C(O)CCC
Similarity: 0.685

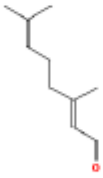
Experimental value: Inactive
Predicted value: Inactive



Compound #3

CAS: 104-51-8
Dataset id: 357 (Training set)
SMILES: c1ccc(cc1)CCCC
Similarity: 0.677

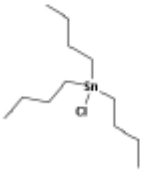
Experimental value: Inactive
Predicted value: Inactive



Compound #4

CAS: 5392-40-5
Dataset id: 15 (Training set)
SMILES: O=CC=C(C)CCC=C(C)C
Similarity: 0.621

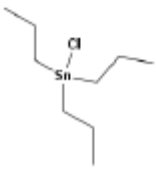
Experimental value: Inactive
Predicted value: Inactive



Compound #5

CAS: 1461-22-9
Dataset id: 33 (Test set)
SMILES: CCCC[Sn](CCCC)(CCCC)Cl
Similarity: 0.621

Experimental value: Active
Predicted value: Active



Compound #6

CAS: 2279-76-7
Dataset id: 26 (Training set)
SMILES: CCC[Sn](CCC)(CCC)Cl
Similarity: 0.62

Experimental value: Active
Predicted value: Active

3.2 Applicability Domain:
Measured Applicability Domain Scores

Global AD Index
AD index = 0.83
Explanation: the predicted compound could be out of the Applicability Domain of the model.

Similar molecules with known experimental value
Similarity index = 0.689
Explanation: no similar compounds with known experimental value in the training set have been found.

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

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

Model's descriptors range check
Descriptors range check = True
Explanation: descriptors for this compound have values inside the descriptor range of the compounds of the training set.

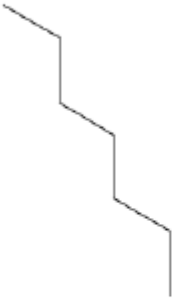
Atom Centered Fragments similarity check
ACF index = 1
Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

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



Prediction: Reliability:

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

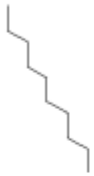
Compound: Molecule 0
Compound SMILES: CCCCCCC
Experimental value: -
Predicted ER-mediated effect: Not predicted
No. alerts for activity: 0
No. alerts for possible activity: 0
No. alerts for non-activity: 0
No. alerts for possible non-activity: 0
Structural alerts: -
Reliability: the predicted compound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

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

Compound #1

CAS: N.A.
Dataset id: 494 (Training set)
SMILES: CCCCCCCCC
Similarity: 0.929

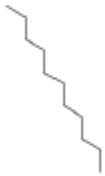
Experimental value: NON-active
Predicted value: Not predicted



Compound #2

CAS: N.A.
Dataset id: 601 (Training set)
SMILES: CCCCCCCCCC
Similarity: 0.908

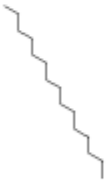
Experimental value: NON-active
Predicted value: Not predicted



Compound #3

CAS: N.A.
Dataset id: 882 (Training set)
SMILES: CCCCCCCCCCC
Similarity: 0.889

Experimental value: NON-active
Predicted value: Not predicted



Compound #4

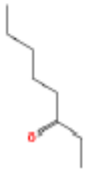
CAS: N.A.
Dataset id: 817 (Training set)
SMILES: CCCCCCCCCCCCCCC
Similarity: 0.825

Experimental value: NON-active
Predicted value: Not predicted

Compound #5

CAS: N.A.
Dataset id: 776 (Training set)
SMILES: CCCCCCCCCCCCCCCC
Similarity: 0.81

Experimental value: NON-active
Predicted value: Not predicted



Compound #6

CAS: N.A.
Dataset id: 414 (Training set)
SMILES: O=C(CC)CCCCC
Similarity: 0.79

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

Alerts (not found in the target): ER possible non-activity alert no. 9

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 = 0.907
Explanation: strongly 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
Explanation: similar molecules found in the training set have experimental values that disagree with the predicted value.

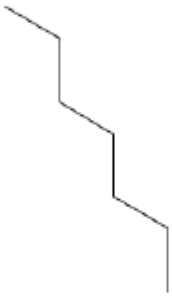
Atom Centered Fragments similarity check
ACF index = 1
Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

Symbols explanation:

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

1. Prediction Summary

Prediction for compound Molecule 0

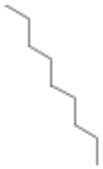


Prediction: Reliability:

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

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

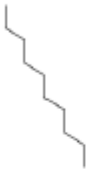
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 111-84-2
Dataset id: 840 (Training set)
SMILES: CCCCCCCCC
Similarity: 0.929

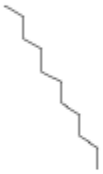
Experimental value: NON-active
Predicted value: NON-active



Compound #2

CAS: 124-18-5
Dataset id: 773 (Training set)
SMILES: CCCCCCCCCC
Similarity: 0.908

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



Compound #3

CAS: 1120-21-4
Dataset id: 506 (Training set)
SMILES: CCCCCCCCCCC
Similarity: 0.889

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

Compound #4

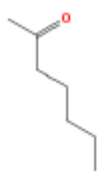
CAS: 629-62-9
Dataset id: 953 (Training set)
SMILES: CCCCCCCCCCCCCCC
Similarity: 0.825

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

Compound #5

CAS: 544-76-3
Dataset id: 942 (Training set)
SMILES: CCCCCCCCCCCCCCCC
Similarity: 0.81

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



Compound #6

CAS: 110-43-0
Dataset id: 550 (Training set)
SMILES: CCCCCC(C)=O
Similarity: 0.79

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

3.2 Applicability Domain:
Measured Applicability Domain Scores

Global AD Index
AD index = 0.958
Explanation: the predicted compound is into the Applicability Domain of the model.

Similar molecules with known experimental value
Similarity index = 0.918
Explanation: strongly similar compounds with known experimental value in the training set have been found.

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

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

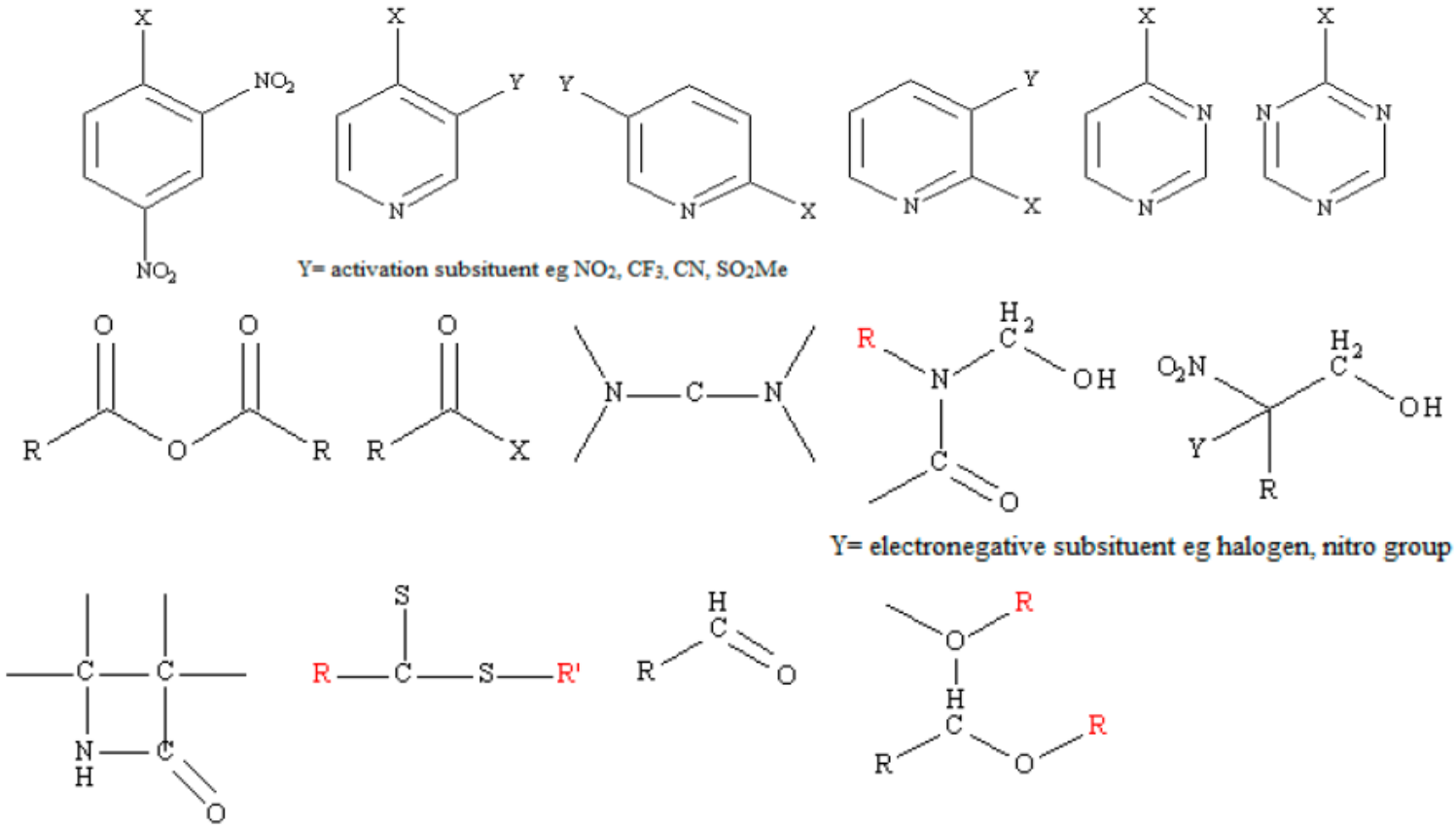
Atom Centered Fragments similarity check
ACF index = 1
Explanation: all atom centered fragment of the compound have been found in the compounds of the training set.

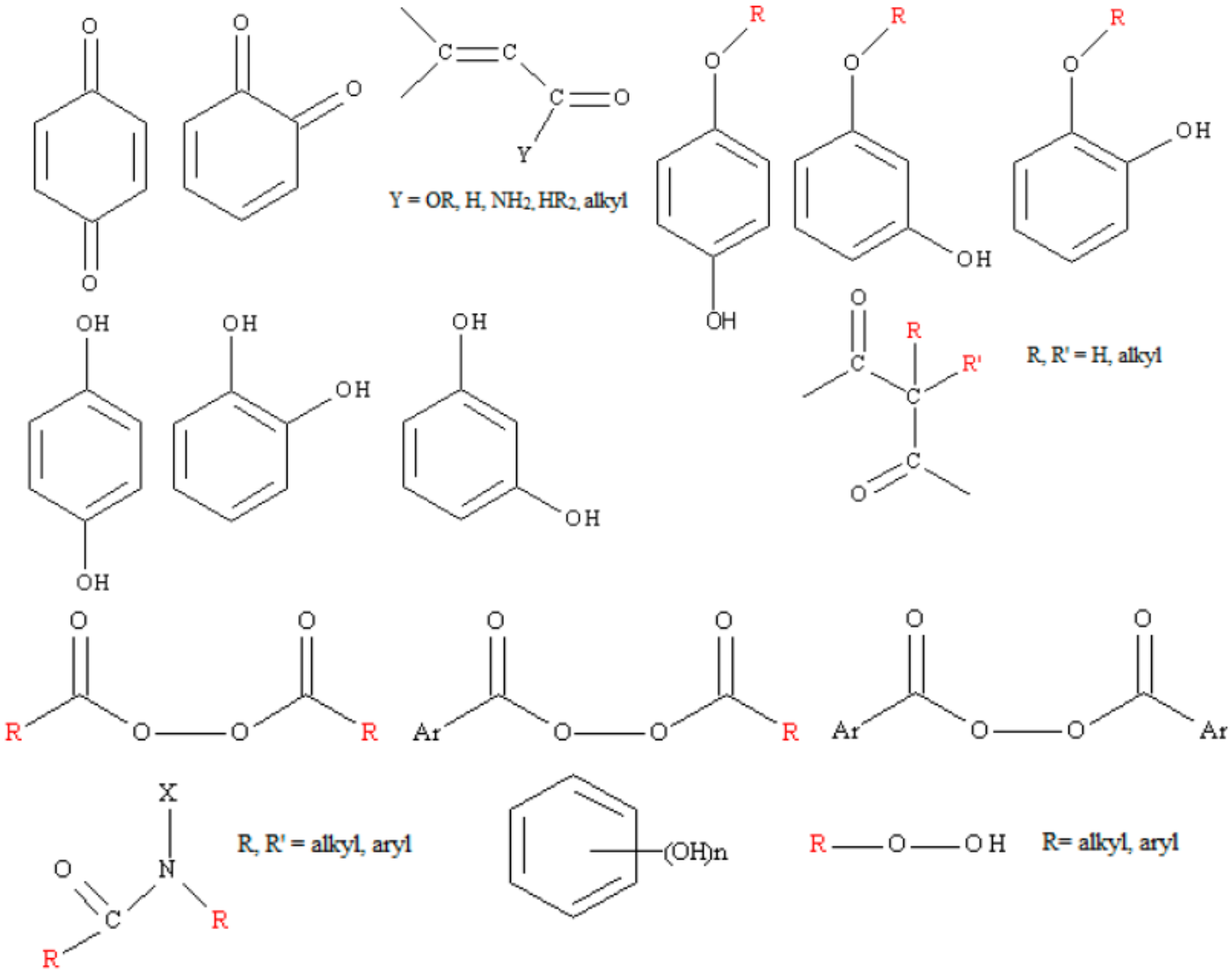
Symbols explanation:

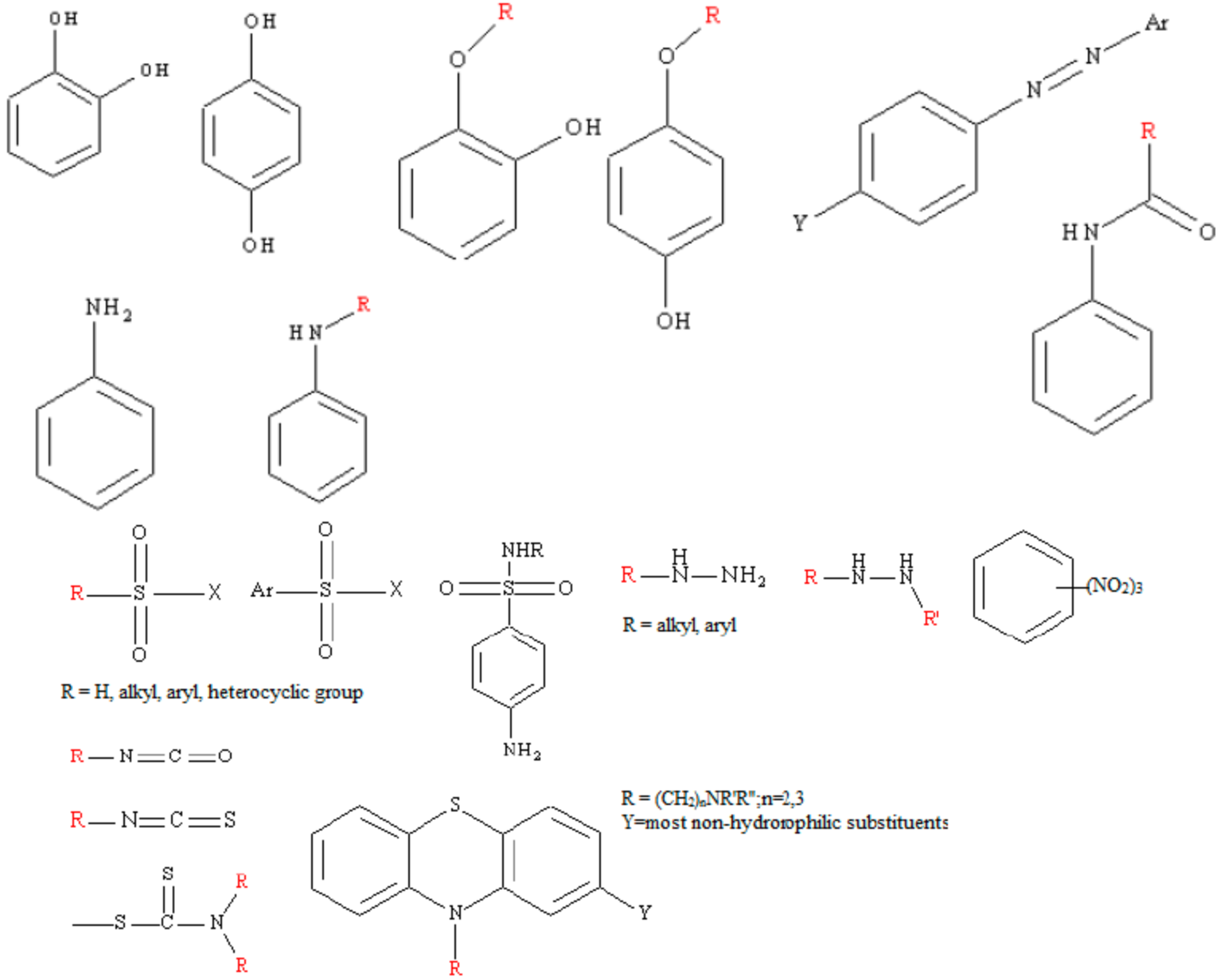
The feature has a good assessment, model is reliable regarding this aspect.
The feature has a non optimal assessment, this aspect should be reviewed by an expert.
The feature has a bad assessment, model is not reliable regarding this aspect.

APPENDIX L: Known Structural Alerts for Skin Sensitization

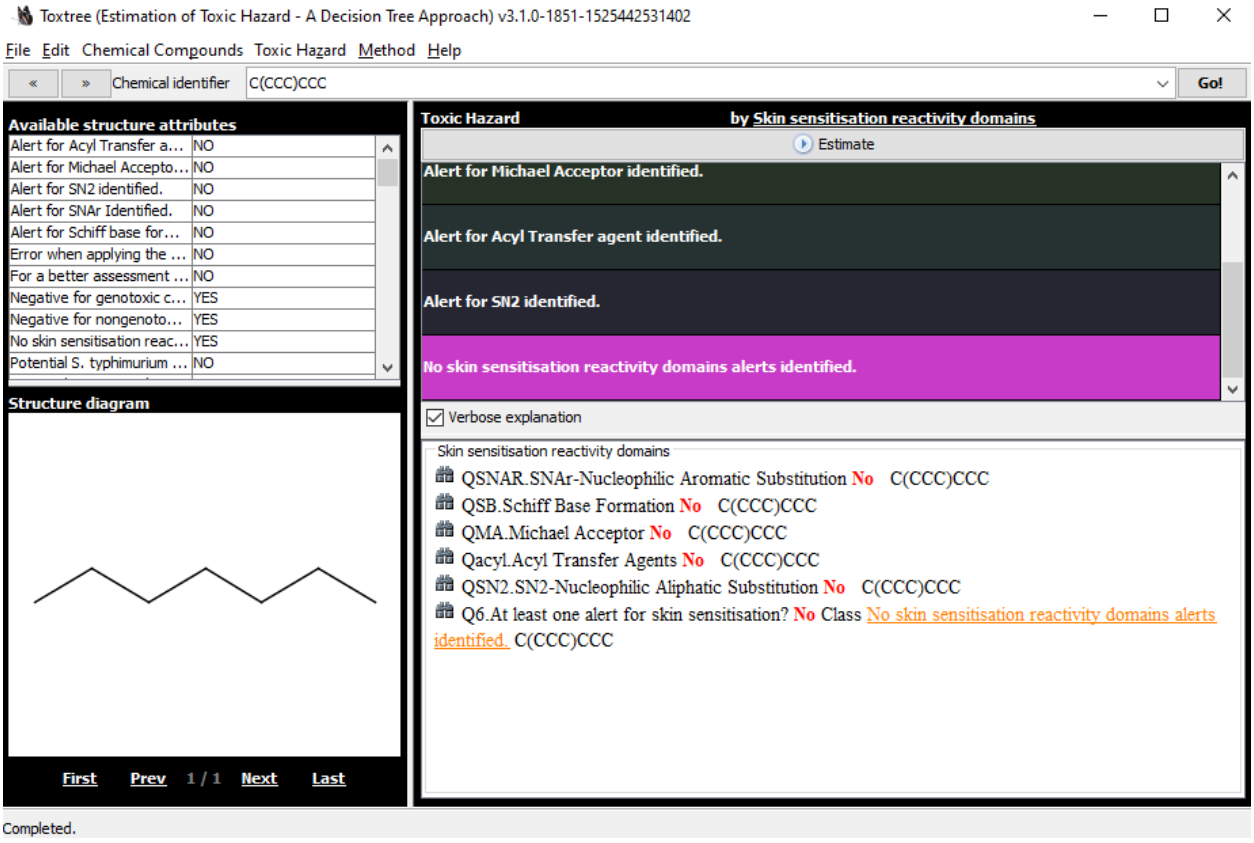
Below are known structural alerts for skin sensitizers (Payne and Walsh 1994). n-Heptane possesses no known structural alerts for skin sensitization.



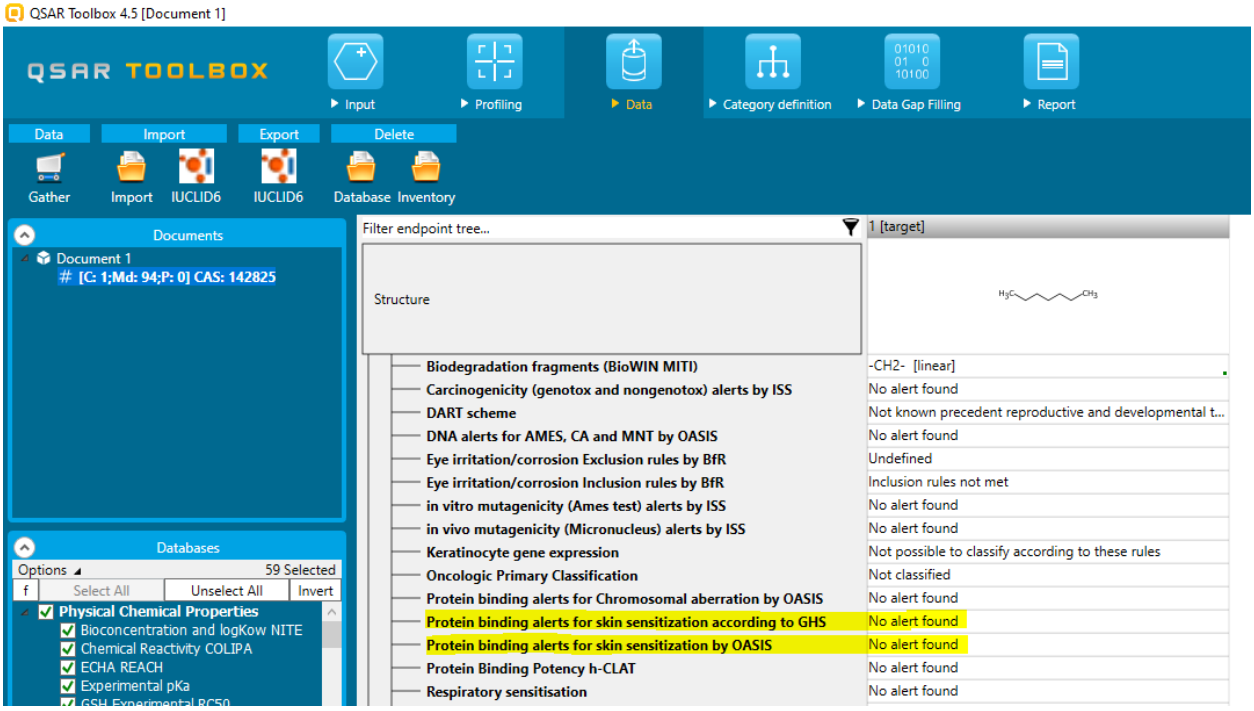




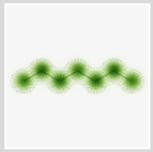
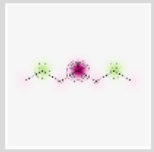
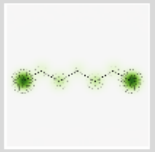
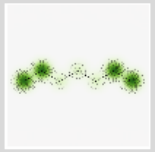
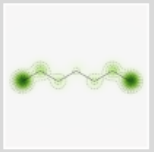
APPENDIX M: Toxtree Skin Sensitization Modeling Results for n-Heptane (CAS #142-82-5)



APPENDIX N: OECD Toolbox Skin Sensitization Results for n-Heptane (CAS #142-82-5)

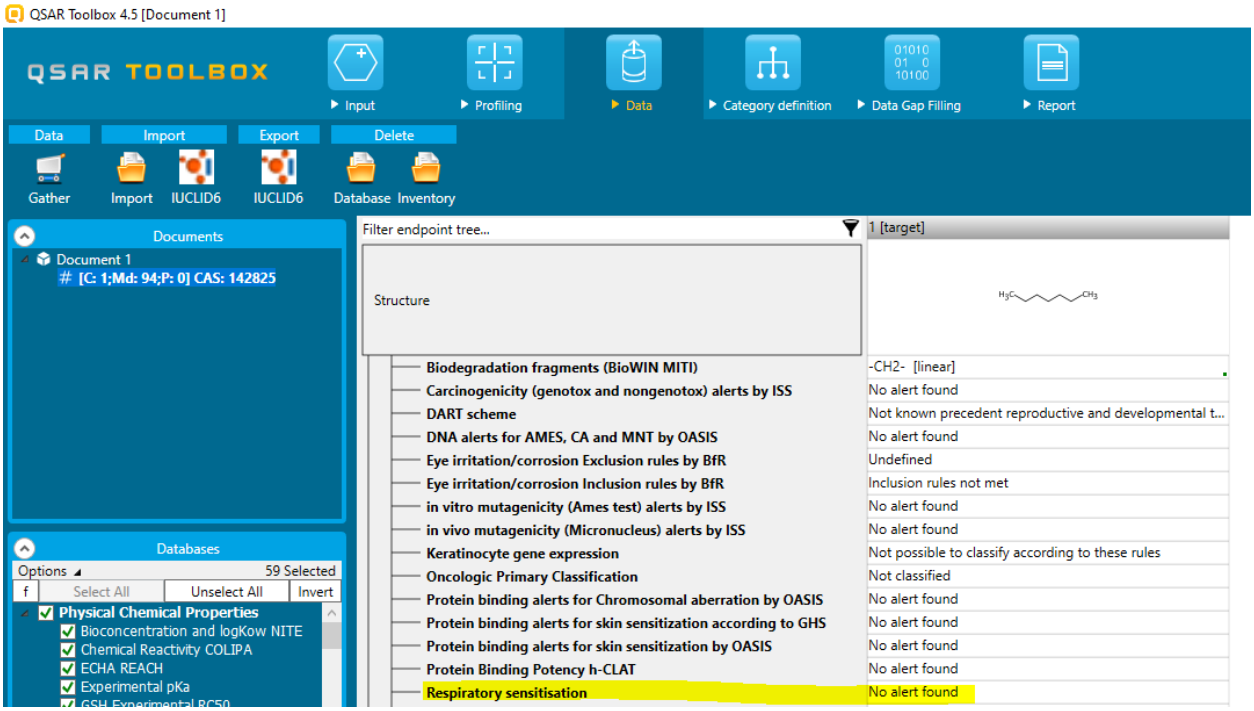


APPENDIX O: LabMol Skin Sensitization Results for n-Heptane (CAS #142-82-5)

Chemical Exposure	**Molecular initiating event** *in chemico*	**Cellular response** *in vitro*		**Tissue / Organ response** *in vivo*	**Organism response** *in vivo*	**Pred-Skin 3.0 Outcome** *in silico*
•Skin Penetration •Electrophilic substance: directly or via auto-oxidation or metabolism	Covalent interaction with proteins in the skin (OECD442C) Haptenation: covalent modification of epidermal proteins	Keratinocyte responses (OECD442D) • Activation of inflammatory cytokines •Induce cytoprotective genes	Dendritic cells (DCs) (OECD442E) • Induction of inflammatory cytokines •Mobilization of DCs	Proliferation of antigen-specific T cells (OECD429) •Histocompatibility complex representation by DCs •Activation of T cells •Proliferation of activated T cells	Inflammation upon challenge allergen To maximise the use of existing knowledge, we also incorporate historical HRIPT (human repeated insult patch test) and HMT (human maximization test)	The Bayesian model is a consensus model integrating predictions from all the other assays for an integrative qualitative risk assessment (QRA) of skin sensitization based on the weight of evidence (WoE).
•Exposure consideration ? •Physicochemical and Biopharmaceutical properties ? •Skin Penetration ? •Skin Metabolism ? 	**Prediction DPRA** Sensitizer (+) (AD, Confiability) (Inside , 59.8%) Probability map 	**Prediction KeratinoSens** Non-Sensitizer (-) (AD, Confiability) (Inside, 60.7%) Probability map 	**Prediction h-CLAT** Sensitizer (+) (AD, Confiability) (Inside, 57.7%) Probability map 	**Prediction LLNA** Non-Sensitizer (-) (AD, Confiability) (Inside, 92.9%) Probability map 	**Prediction HRIPT/HMT** Non-Sensitizer (-) (AD, Confiability) (Outside, 97.1%) Probability map 	**Bayesian Outcome** Non-sensitizer (-) (Confiability) (High)

Low (-) confidence prediction for the Bayesian model means two or more individual predictions are in disagreement with Bayesian Outcome.

APPENDIX P: OECD Toolbox Respiratory Sensitization Modeling Results for n-Heptane (CAS #142-82-5)



APPENDIX Q: ECOSAR Modeling Results for n-Heptane (CAS #142-82-5)

Created on Dec 6, 2022 1:05:18 PM

Organic Module Report

Results of Organic Module Evaluation

CAS	Name	SMILES
142825	Heptane	C(CCCCC)C

Structure

Details	
Mol Wt	100.21
Selected LogKow	3.78
Selected Water Solubility (mg/L)	3.4
Selected Melting Point (°C)	-90.6
Estimated LogKow	3.78
Estimated Water Solubility (mg/L)	2.92
Measured LogKow	4.66
Measured Water Solubility (mg/L)	3.4
Measured Melting Point (°C)	-90.6

Class Results:	

Neutral Organics

Organism	Duration	End Point	Concentration (mg/L)	Max Log Kow	Flags
Fish	96h	LC50	2.08	5	
Daphnid	48h	LC50	1.39	5	
Green Algae	96h	EC50	2.04	6.4	
Fish		ChV	0.25	8	
Daphnid		ChV	0.21	8	
Green Algae		ChV	0.77	8	
Fish (SW)	96h	LC50	2.64	5	
Mysid	96h	LC50	0.59	5	

1 / 2

Class Results:	

Organism	Duration	End Point	Concentration (mg/L)	Max Log Kow	Flags
Fish (SW)		ChV	0.85	8	
Mysid (SW)		ChV	0.03	8	
Earthworm	14d	LC50	113.92	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 R: EPI Suite™ Modeling Results for n-Heptane (CAS #142-82-5)

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

CAS Number: 142-82-5
SMILES: C(CCCCC)C
CHEM: Heptane
MOL FOR: C7 H16
MOL WT: 100.21
---------- EPI SUMMARY (v4.11) ----------
Physical Property Inputs:
Log Kow (octanol-water): 4.66
Boiling Point (deg C): 98.10
Melting Point (deg C): -90.55
Vapor Pressure (mm Hg): 46
Water Solubility (mg/L): 2.4
Henry LC (atm-m3/mole): ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = 3.78
Log Kow (Exper. database match) = 4.66
Exper. Ref: MILLER,MM ET AL. (1985)

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 96.16 (Adapted Stein & Brown method)
Melting Pt (deg C): -81.01 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 46.7 (Mean VP of Antoine & Grain methods)
VP (Pa, 25 deg C) : 6.22E+003 (Mean VP of Antoine & Grain methods)
MP (exp database): -90.6 deg C
BP (exp database): 98.5 deg C
VP (exp database): 4.60E+01 mm Hg (6.13E+003 Pa) at 25 deg C

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 2.915
log Kow used: 4.66 (user entered)
melt pt used: -90.55 deg C
Water Sol (Exper. database match) = 3.4 mg/L (25 deg C)
Exper. Ref: YALKOWSKY,SH & DANNENFELSER,RM (1992)

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 2.6374 mg/L

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

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method: 2.27E+000 atm-m3/mole (2.30E+005 Pa-m3/mole)
Group Method: 2.39E+000 atm-m3/mole (2.42E+005 Pa-m3/mole)

Exper Database: 2.00E+00 atm-m3/mole (2.03E+005 Pa-m3/mole)
For Henry LC Comparison Purposes:
User-Entered Henry LC: not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 2.527E+000 atm-m3/mole (2.561E+005 Pa-m3/mole)
VP: 46 mm Hg (source: User-Entered)
WS: 2.4 mg/L (source: User-Entered)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 4.66 (user entered)
Log Kaw used: 1.913 (exp database)
Log Koa (KOAWIN v1.10 estimate): 2.747
Log Koa (experimental database): 2.950

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model): 0.8083
Biowin2 (Non-Linear Model): 0.9686
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 3.2761 (days-weeks)
Biowin4 (Primary Survey Model): 3.9722 (days)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model): 0.6039
Biowin6 (MITI Non-Linear Model): 0.8280
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): 0.4892
Ready Biodegradability Prediction: YES

Hydrocarbon Biodegradation (BioHCwin v1.01):
LOG BioHC Half-Life (days): 0.7385
BioHC Half-Life (days): 5.4758

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled): 6.13E+003 Pa (46 mm Hg)
Log Koa (Exp database): 2.950
Kp (particle/gas partition coef. (m3/ug)):
Mackay model: 4.89E-010
Octanol/air (Koa) model: 2.19E-010
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model: 1.77E-008
Mackay model: 3.91E-008
Octanol/air (Koa) model: 1.75E-008

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 6.8713 E-12 cm3/molecule-sec
Half-Life = 1.557 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 18.679 Hrs
Ozone Reaction:
No Ozone Reaction Estimation

Fraction sorbed to airborne particulates (phi):
2.84E-008 (Junge-Pankow, Mackay avg)
1.75E-008 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc: 239.7 L/kg (MCI method)
Log Koc: 2.380 (MCI method)
Koc: 1.107E+004 L/kg (Kow method)
Log Koc: 4.044 (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 = 2.742 (BCF = 551.7 L/kg wet-wt)
Log Biotransformation Half-life (HL) = 0.2835 days (HL = 1.921 days)
Log BCF Arnot-Gobas method (upper trophic) = 2.834 (BCF = 681.8)
Log BAF Arnot-Gobas method (upper trophic) = 2.837 (BAF = 687.7)
log Kow used: 4.66 (user entered)

Volatilization from Water:
Henry LC: 2 atm-m3/mole (Henry experimental database)
Half-Life from Model River: 1.022 hours
Half-Life from Model Lake : 95.09 hours (3.962 days)

Removal In Wastewater Treatment (recommended maximum 95%):
Total removal: 99.91 percent
Total biodegradation: 0.12 percent
Total sludge adsorption: 38.71 percent
Total to Air: 61.08 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model: (MCI Method)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	**35.7**	**38**	**1000**
Water	**62.6**	**208**	**1000**
Soil	**1.17**	**416**	**1000**
Sediment	**0.546**	**1.87e+003**	**0**

Persistence Time: 78 hr

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

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	35.7	38	1000
Water	62.6	208	1000
water	(62.4)		
biota	(0.143)		

suspended sediment (0.0224)
Soil 1.17 416 1000
Sediment 0.546 1.87e+003 0
Persistence Time: 78 hr

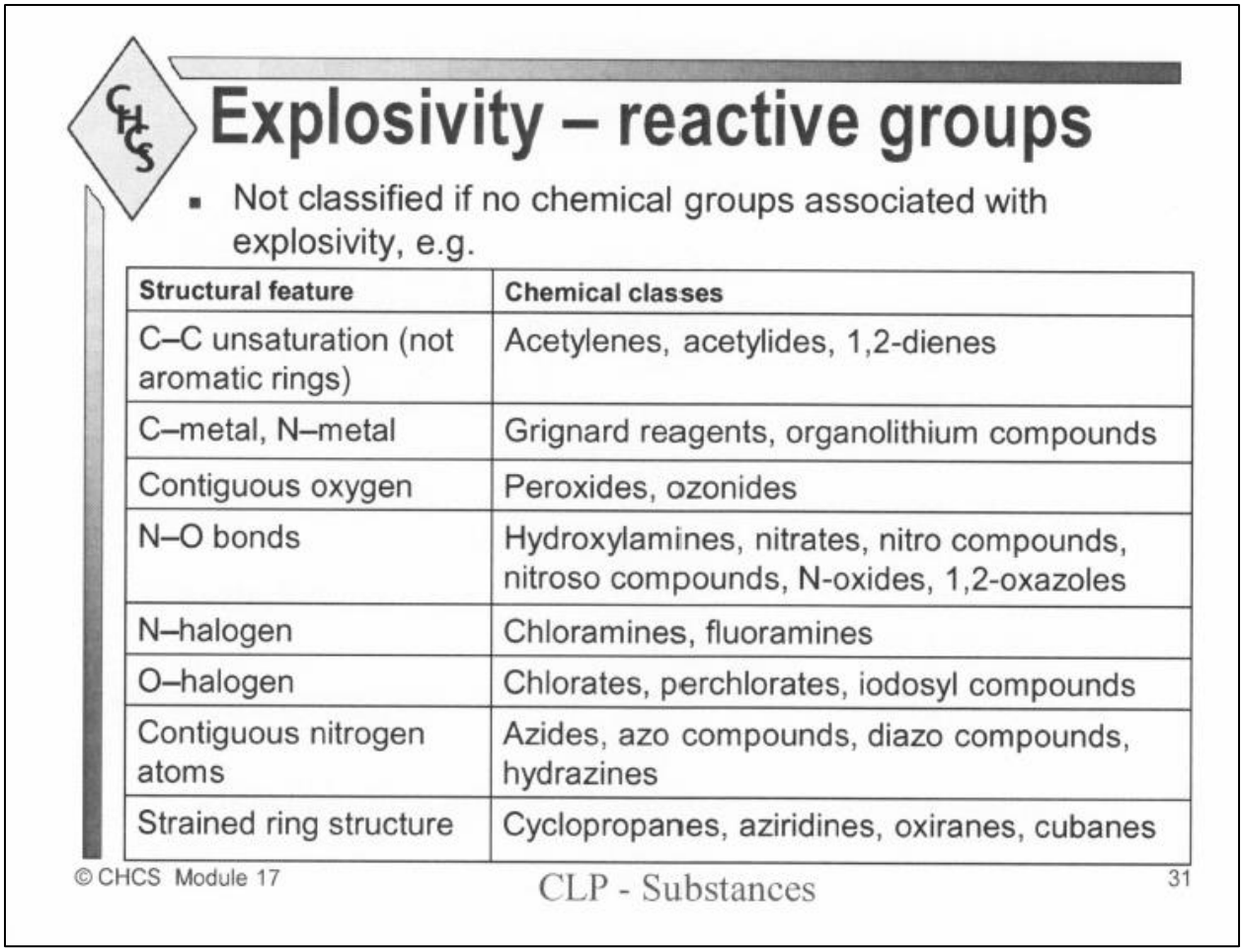
Level III Fugacity Model: (EQC Default)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	24.5	38	1000
Water	44.4	208	1000
water	(43.1)		
biota	(0.0985)		
suspended sediment	(1.21)		
Soil	16	416	1000
Sediment	15.1	1.87e+003	0

Persistence Time: 109 hr

APPENDIX S: Known Structural Alerts for Reactivity

Explosivity – Abbreviated List



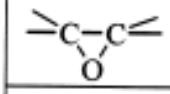
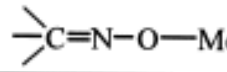
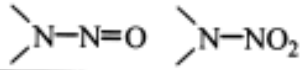
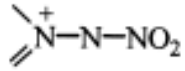
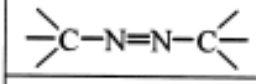
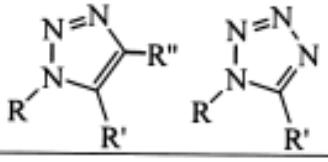
Explosivity – reactive groups

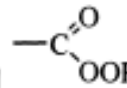
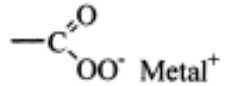
- Not classified if no chemical groups associated with explosivity, e.g.

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

Explosivity – Full List

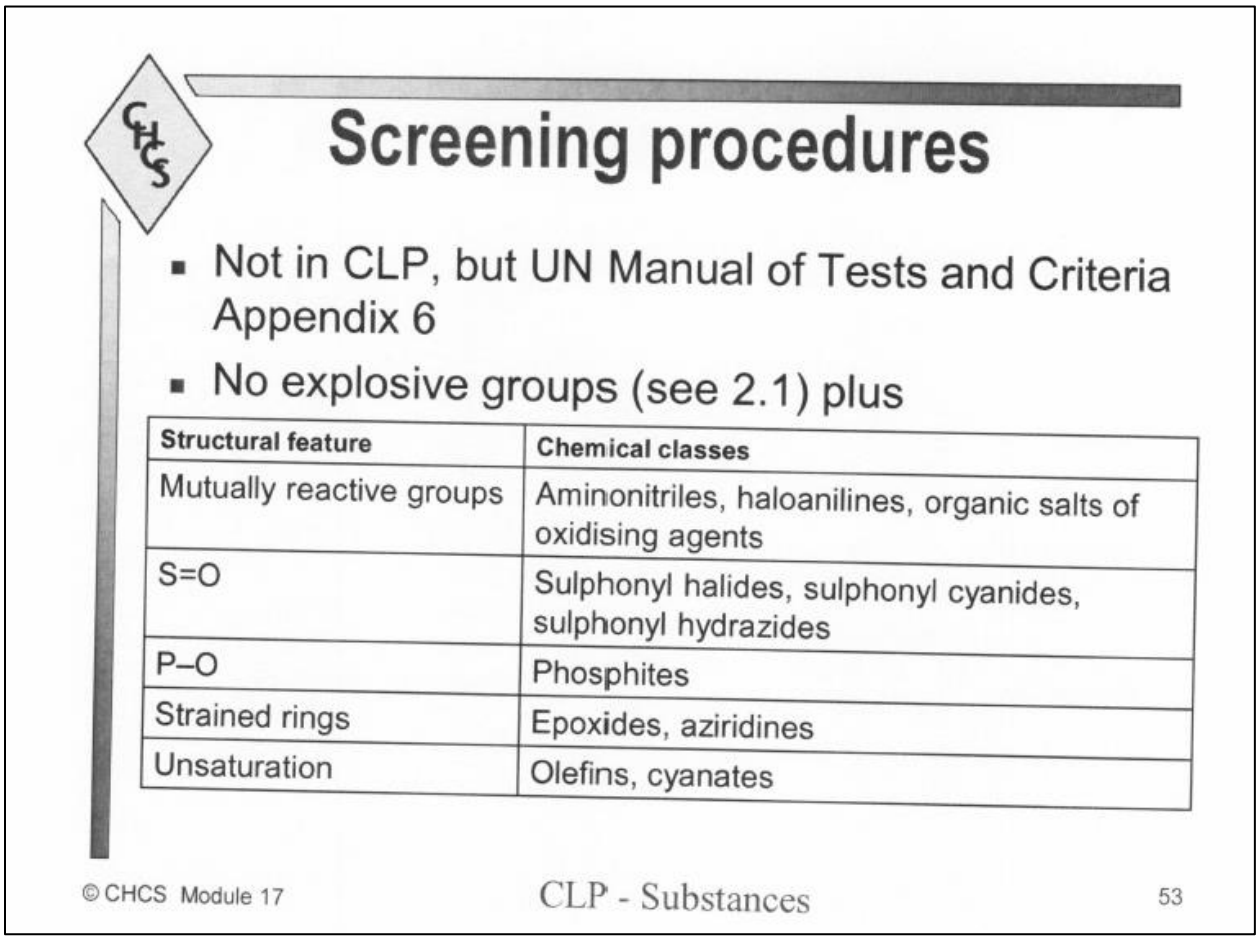
Table R.7.1-28 Chemical groups associated with explosive properties

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

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

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

Self-Reactive Substances



APPENDIX T: Change in Benchmark Score

Table 6 provides a summary of changes to the GreenScreen® Benchmark™ for n-heptane. The GreenScreen® Benchmark Score for n-heptane has not changed over time. The original GreenScreen® assessment was performed in 2014 under version 1.2 criteria and ToxServices assigned a Benchmark 2 (BM-2) score. The BM-2 score was maintained with version 1.4 updates in 2018, 2019, 2020, 2021 and in this 2023 report.

Table 6: Change in GreenScreen® Benchmark™ for n-Heptane

Date	GreenScreen® Benchmark™	GreenScreen® Version	Comment
June 17, 2014	BM-2	v. 1.2	New GreenScreen® assessment.
August 10, 2018	BM-2	v. 1.4	No change in BM score. The GreenScreen® assessment was updated with a v.1.4 template.
September 15, 2019	BM-2	v. 1.4	No change in BM score. The GreenScreen® assessment was updated with a v.1.4 template.
November 23, 2020	BM-2	v. 1.4	No change in BM score. The GreenScreen® assessment was updated with a v.1.4 template.
January 14, 2021	BM-2	v. 1.4	No change in BM score. The GreenScreen® assessment was updated with a v.1.4 template.
December 12, 2022	BM-2	v. 1.4	No change in BM score. The GreenScreen® assessment was updated with a v.1.4 template.
January 30, 2023	BM-2	v. 1.4	No changes in BM score. Revised to address Washington Department of Ecology's comments.

Licensed GreenScreen® Profilers

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