N-BUTYL ALCOHOL

(CAS #71-36-3)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: May 23, 2023

Expiration Date: June 2, 2028

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TABLE OF CONTENTS

TABLE OF FIGURES

TABLE OF TABLES

GreenScreen® Executive Summary for n-Butyl Alcohol (CAS #71-36-3)

n-Butyl alcohol is a clear, colorless liquid that is flammable. It is classified as a primary alcohol and is used primarily as a chemical intermediate, as a denaturant and perfuming agent in cosmetics, and as a solvent. N-Butyl alcohol is a volatile organic compound (VOC). It is hydrophilic and flammable.

n-Butyl alcohol 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 Group II Human Toxicity (eye irritation-IrE)
- Benchmark 2g
  - High Flammability-F

A data gap exists for endocrine activity-E. As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), n-butyl alcohol meets requirements for a GreenScreen® Benchmark Score of 2 despite the hazard data gap. In a worst-case scenario, if n-butyl alcohol 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-butyl alcohol 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 a version 1.3 update in 2016, and a version 1.4 update in 2022. Most recently, ToxServices maintained the GreenScreen® benchmark score with a version 1.4 update in 2023.

New Approach Methodologies (NAMs) used in this GreenScreen® include use of *in silico* modeling for carcinogenicity, endocrine activity, respiratory sensitization, persistence, and bioaccumulation, and *in vitro* testing for genotoxicity, endocrine activity, and skin sensitization. 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-butyl alcohol's NAMs dataset include lack or insufficient experimental data to assess carcinogenicity, endocrine activity, and respiratory sensitization, and lack of validated test methods for respiratory sensitization. n-Butyl alcohol's Type II (extrapolation output) uncertainties include reliance on structural alerts or models with undefined applicability domains, reliance on *in vitro* genotoxicity studies that do not fully mimic *in vivo* metabolism and focusing on only a few types of genotoxic events, uncertain *in vivo* relevance of *in silico* receptor binding activity predictions and *in vitro* high throughput screening assays of receptor activities, inability of *in vitro* skin sensitization assays to identify pro- and pre-haptens, and lack of consideration of non-immunological mechanisms of respiratory sensitization.

GreenScreen® Hazard Summary Table for n-Butyl Alcohol

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	M	M	L	M	L	L	*L*	H	vH	L	M	vL	vL	*L*	H

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect estimated values, authoritative B lists, screening lists, weak analogues, and lower confidence. Hazard levels in **BOLD** font are used with good quality data, authoritative A lists, or strong analogues. 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. Please see Appendix A for a glossary of hazard acronyms.

GreenScreen® Chemical Assessment for n-Butyl Alcohol (CAS #71-36-3)

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

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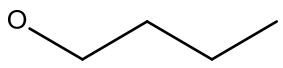
Organization: ToxServices LLC
Date: June 2, 2023

Expiration Date: June 2, 2028[2]

Chemical Name: n-Butyl alcohol

CAS Number: 71-36-3

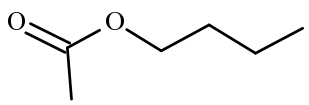
Chemical Structure(s):



[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).

Also called: 1-Butanol; Butyl alcohol; n-Butyl alcohol; Butyl alcohol; Propyl carbinol; Butanol, 1-; n-Butan-1-ol; 1-Hydroxybutane; Butyl hydroxide; Propylmethanol; Butanolen; Butyric alcohol; Methylolpropane, EC 200-751-6 (PubChem 2023).

Suitable analogs or moieties of chemicals used in this assessment (CAS #'s):
For some endpoints, insufficient reliable data are available for n-butyl alcohol. Therefore, n-butyl acetate (CAS #123-86-4) is used as a surrogate to fill data gaps where feasible. This surrogate has been used to support the safety of n-butyl alcohol by the United States Environmental Protection Agency (U.S. EPA), Organisation for Economic and Community Development (OECD), European Centre for Ecotoxicity and Toxicology of Chemicals (ECETOC), Cosmetic Ingredient Review (CIR), Australian Industrial Chemicals Introduction Scheme (AICIS), and the authors of the European Chemicals Agency (ECHA) dossier for n-butyl alcohol (U.S. EPA 2011, OECD 2001, ECETOC 2003, CIR 2008, AICIS 2013, ECHA, CAS #71-36-3, 2023). A good laboratory practices (GLP)-compliant *in vivo* toxicokinetic study in rats using radiolabeled butyl acetate demonstrated that 99% of the intravenously administered butyl acetate was hydrolyzed in the blood and brain within 2.7 minutes to form n-butyl alcohol (OECD 2001). Therefore, toxicity studies on butyl acetate provide information on the toxicity of butanol for endpoints involving systemic exposure. It should be noted that data on butyl acetate may not be relevant for n-butyl alcohol for site-of-contact effects and other physical-chemical property-dependent endpoints (OECD 2001).



Surrogate: n-Butyl acetate (CAS #123-86-4)

Identify Applications/Functional Uses
1. Denaturant (PubChem 2023, AICIS 2013).
2. Solvent for anti-freeze, paints, lacquers, varnishes, resins, gums, vegetable oils, dyes and alkaloids (PubChem 2023, AICIS 2013).
3. An intermediate in the manufacture of semiconductors, pharmaceuticals, and chemicals (PubChem 2023, AICIS 2013).
4. Employed in industries producing artificial leather, textiles, safety glass, rubber cement, shellac, raincoats, photographic films and perfumes (PubChem 2023, AICIS 2013).
5. Denaturant, solvent, and perfuming agent in cosmetic formulations (EC 2023).
6. Additives in beverages and foods as a flavoring agent, solvent, or vehicle (U.S. FDA 2023).
7. Inactive ingredient in prescription drug products (HSDB 2015).

Known Impurities[3]:
Commercially available grades of n-butyl alcohol are 99.9% pure. Isobutanol has been reported as an impurity that is present at 0.1% (OECD 2001). The screen is performed on the theoretical pure substance.

[3] Impurities of the chemical will be assessed at the product level instead of in this GreenScreen®.

GreenScreen® Summary Rating for n-Butyl Alcohol[4,5 6,7]: n-Butyl alcohol was assigned a **GreenScreen Benchmark™ Score of 2** ("Use but Search for Safer Substitutes") (CPA 2016b). This score is based on the following hazard score combinations:

- Benchmark 2f
  - Very High Group II Human Toxicity (eye irritation-IrE)
- Benchmark 2g
  - High Flammability-F

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

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	**M**	**M**	**L**	**M**	**L**	**L**	*L*	**H**	**vH**	**L**	*M*	**vL**	**vL**	*L*	**H**

Note: Hazard levels (Very High (vH), High (H), Moderate (M), Low (L), Very Low (vL)) in *italics* reflect estimated (modeled) values, authoritative B lists, screening lists, weak analogues and lower confidence. Hazard levels in **BOLD** font are used with good quality data, authoritative A lists, or strong analogues. 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. Please see Appendix A for a glossary of hazard acronyms.

Environmental Transformation Products

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

Introduction

n-Butyl alcohol (IUPAC name: butan-1-ol) is a four-carbon primary aliphatic alcohol (PubChem 2023, Djoumbou et al. 2016). It is produced via the catalyzed hydrogenation of butyraldehyde followed by distillation (OECD 2001). Butyl alcohol functions as a denaturant, as a solvent in the manufacturing of anti-freeze, paints, resins, vegetable oils, and dye, and as an intermediate in semiconductor manufacturing and in the textile, coatings, and pharmaceutical industries (PubChem 2023, AICIS 2013). It also functions as a denaturant, solvent, and perfuming agent in cosmetic formulations (EC 2023).

[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.

Furthermore, butyl alcohol is approved by the United States Food and Drug Administration (U.S. FDA) as generally recognized as safe (GRAS), as a direct food additive and as an indirect food additive when used as a flavoring agent or adjuvant, solvent, or vehicle under 21 CFR § 172.515, § 172.560, § 175.105, § 175.320, § 176.180, § 176.200, § 176.210, § 177.1200, § 177.1440, § 177.1650, and § 177.2800 (U.S. FDA 2023). In addition, it is approved in prescription drug products as an inactive ingredient (HSDB 2015).

ToxServices assessed n-butyl alcohol 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-Butyl alcohol is not listed on the U.S. EPA 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-butyl alcohol can be found in Appendix C.

- n-Butyl alcohol is listed in Pharos as a Benchmark 2 chemical based on an outdated GreenScreen® performed by ToxServices in 2016. However, this GreenScreen® is now outdated and the current assessment brings it up to date.
- n-Butyl alcohol (UN1120) is listed on the U.S. DOT list as a Hazard Class 3 chemical, Packing Group II (5L), and III (10L) (U.S. DOT 2008a).
- n-Butyl alcohol is on the following lists for multiple endpoints. Specified lists for single endpoints are reported in individual hazard endpoints in the hazard assessment section below.
    - German FEA – Substances Hazardous to Waters – Class 1 – Low Hazard to Waters
    - Québec CSST – WHMIS 1998 – Class D2B – Toxic material causing other toxic effects
    - EC – CEPA DSL – Inherently Toxic to Humans (iTH)

Hazard Statement and Occupational Control

n-Butyl alcohol is associated with several Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard statements reported by ECHA as well as other countries as shown in Table 1. General personal protective equipment (PPE) recommendations and occupational exposure limits (OELs) 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-Butyl Alcohol (CAS #71-36-3) (Pharos 2023, ECHA, CAS #71-36-3, 2023)	
H Statement	**H Statement Details**
H226	Flammable liquid and vapor
H302	Harmful if swallowed
H315	Causes skin irritation
H318	Causes serious eye damage
H335	May cause respiratory irritation
H336	May cause drowsiness or dizziness

Table 2: Occupational Exposure Limits and Recommended Personal Protective Equipment of n-Butyl Alcohol (CAS #71-36-3)			
Personal Protective Equipment (PPE)	**Reference**	**Occupational Exposure Limits (OEL)**	**Reference**
Use protective gloves, safety goggles, and ensure there is proper ventilation or use breathing protection during use	PubChem 2023	TLV: 20 ppm as TWA	PubChem 2023
		MAK: 100 ppm, 310 mg/m^3	PubChem 2023
		OSHA PEL: TWA 100 ppm (300 mg/m^3)	PubChem 2023
		NIOSH REL: C 50 ppm (150 mg/m^3) skin	PubChem 2023
		NIOSH IDLH: 1400 ppm 10% LEL	PubChem 2023

IDLH: Immediately Dangerous to Life or Health
LEL: Lower explosive limit
MAK: Maximum Workplace Concentration
NIOSH: National Institute for Occupational Safety and Health
OEL: Occupational Exposure Limit
OSHA: Occupational Safety and Health Administration
PEL: Permissible Exposure Limit
REL: Recommended Exposure Limits
TLV: Threshold Limit Value
TWA: Time Weighted Average

Physicochemical Properties of n-Butyl Alcohol

The physicochemical properties of n-butyl alcohol are summarized in Table 3. n-Butyl alcohol is a colorless liquid that is partially soluble in water and volatile at room temperature. Its partition coefficients (log K_{ow}) of 0.88-1 indicate that it is hydrophilic.

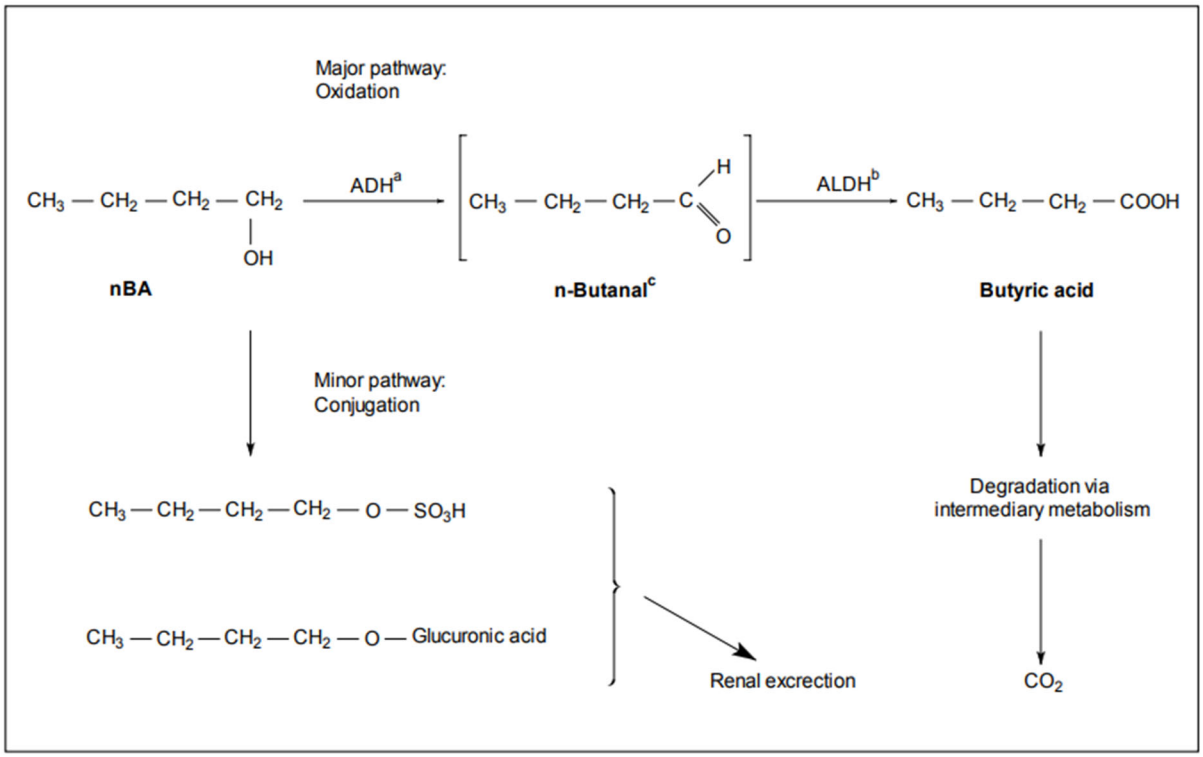
Table 3: Physical and Chemical Properties of n-Butyl Alcohol (CAS #71-36-3)		
Property	**Value**	**Reference**
Molecular formula	$C_4H_{10}O$	PubChem 2023, ECHA, CAS #71-36-3, 2023
SMILES Notation	C(CC)CO	PubChem 2023, ECHA, CAS #71-36-3, 2023
Molecular weight	74.12 g/mol	PubChem 2023, ECHA, CAS #71-36-3, 2023
Physical state	Liquid	PubChem 2023, ECHA, CAS #71-36-3, 2023
Appearance	Colorless, clear	PubChem 2023, ECHA, CAS #71-36-3, 2023

Table 3: Physical and Chemical Properties of n-Butyl Alcohol (CAS #71-36-3)		
Property	**Value**	**Reference**
Melting point	< 90°C (exp, DIN ISO 3016 / ASTM D97); -89.80°C (exp)	PubChem 2023, ECHA, CAS #71-36-3, 2023; U.S. EPA 2017a
Boiling point	119°C at 101.3 kPa (exp, equiv. to OECD Guideline 103); 117.70°C (exp)	ECHA, CAS #71-36-3, 2023; U.S. EPA 2017a
Vapor pressure	6.7 mm Hg at 25°C; 10 hPa (7.5 mm Hg) at 20°C (exp, DIN-EN 13016-2)	U.S. EPA 2017a, PubChem 2023; ECHA, CAS #71-36-3, 2023
Water solubility	63.2 g/L (63,200 mg/L) at 25°C (exp); 66 g/L (66,000 mg/L) at 20°C and pH 7	U.S. EPA 2017a, PubChem 2023; ECHA, CAS #71-36-3, 2023
Dissociation constant	pKa = 16.1 at 25°C	PubChem 2023
Density/specific gravity	0.81 g/cm^3 at 20ºC (exp, DIN 51757 / ASTM D4052)	ECHA, CAS #71-36-3, 2023
Partition coefficient	Log K_{ow} = 0.88 (exp); Log K_{ow} = 1 at 25°C (exp, OECD Guideline 117)	U.S. EPA 2017a, PubChem 2023; ECHA, CAS #71-36-3, 2023

Toxicokinetics

The pharmacokinetics of n-butyl alcohol has been studied extensively in animals.

- *Absorption:* n-Butyl alcohol is rapidly and well absorbed by all routes (oral, dermal, and inhalation) with 100%, 50%, and 60% absorption rates, respectively (ECHA, CAS #71-36-3, 2023).
    - *Oral:* Single oral doses of 2000 mg/kg n-butyl alcohol were administered to fasted Wistar rats and maximum blood concentrations were 500 and 150 mg/L after two and four hours, respectively (ECHA, CAS #71-36-3, 2023, OECD 2001, ECETOC 2003).
    - *Dermal: In vitro* studies with isolated human epidermis reported absorption rats in the range of 0.048 – 2.30 mg/cm^2/h and permeability constants in the range of 2.84 x 10^{-3} – 30 x 10^{-3} cm/h (ECETOC 2003). A dermal absorption study with Beagle dogs exposed to n-butyl alcohol for 60 minutes reported a dermal absorption rate of 8.8 mg/min/cm^2 (OECD 2001).
    - *Inhalation:* In a clinical study with male volunteers (2 groups of 6) exposed to concentrations of 100 or 200 ppm n-butyl alcohol vapor, 47% and up to 39% of the dose applied, respectively, was absorbed (ECETOC 2003). Additionally, a study on Beagle dogs exposed to 50 ppm n-butyl alcohol vapor for 6 hours reported that 55% of the administered dose was absorbed (OECD 2001).
- *Distribution:* n-Butyl alcohol is rapidly and widely distributed throughout the body via the blood stream. Pharmacokinetic studies in animals found the highest concentrations, regardless of exposure route, in the liver; however, bioaccumulation was not observed as it was readily released and not detectable 20 minutes after exposure (ECHA, CAS #71-36-3, 2023, AICIS 2013, ECETOC 2003). Sprague-Dawley rats administered radioactive doses of 450 mg/kg n-butyl alcohol in corn oil via gavage reported maximum plasma concentrations of 70.9 µg/mL and below the limit of detection at 1 hour and 4 hours post exposure, respectively.
- *Metabolism:* The majority of the n-butyl alcohol undergoes metabolism (ECHA, CAS #71-36-3, 2023, OECD 2001). n-Butyl alcohol is rapidly metabolized by alcohol and aldehyde dehydrogenase in the liver to butyric acid and ultimately to carbon dioxide. n-Butyl alcohol may also be metabolized via a minor conjugation pathway into n-butyl alcohol-O-glucuronide or n-butyl alcohol-O-sulfate (ECETOC 2003) (Figure 2).



[a] ADH = alcohol dehydrogenase
[b] ALDH = aldehyde dehydrogenase
[c] *n*-Butanal = butyric aldehyde

Figure 2: Proposed Metabolic Pathways for n-Butyl Alcohol (ECETOC 2003)

- *Excretion:* n-butyl alcohol is excreted as its major metabolite carbon dioxide (83%) via the expired air, excreted unchanged via the urine or expired air, or as its minor metabolites, n-butyl alcohol-O-glucuronide or n-butyl alcohol-O-sulfate, in the urine (4%) (ECHA, CAS #71-36-3, 2023, ECETOC 2003, AICIS 2013).

In summary, n-butyl alcohol is readily and extensively absorbed via oral, dermal, and inhalation exposure, widely distributed (although primarily to the liver), and completely metabolized and/or excreted without bioaccumulation.

Hazard Classification Summary

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

Carcinogenicity (C) Score (H, M, or L): *L*
n-Butyl alcohol was assigned a score of Low for carcinogenicity based on expert judgment by the authoritative body ECHA supported by negative and in domain predictions from the statistical-based model Ultra FDA in the Danish (Q)SAR database and negative and reliable predictions by two VEGA models (rule-based and statistically-based models). GreenScreen® criteria classify a chemical as a Low

hazard for carcinogenicity when adequate and negative data are available, and they are not GHS-classified (CPA 2018b). The confidence in the score is low due to lack of measured data.

- Authoritative and Screening Lists
  - *Authoritative:* U.S. EPA – IRIS Carcinogen – (1986) Group D – Not classifiable as to human carcinogenicity.
  - *Screening:* Not listed on any screening lists for this endpoint.
- OECD 2001
  - There were no reliable data on n-butyl alcohol regarding carcinogenicity. However, based on n-butyl alcohol's negative mutagenicity and clastogenicity, it presents a very small potential for carcinogenicity.
- ECHA 2018
  - There were no experimental data available on the carcinogenic potential of n-butyl alcohol. However, based the lack of genotoxic potential of n-butyl alcohol, the lack of "specific repeat-dose target organ" toxicity, the lack of pre-neoplastic lesions in the repeated dose toxicity studies as well as absence of (Q)SAR alerts for non-genotoxic mode of action, and considering that no human cancer data available in the scientific literate - no concern for a carcinogenic potential of n-butyl alcohol has been raised.
- VEGA 2023
  - ToxServices predicted the carcinogenicity potential of n-butyl alcohol using the following six VEGA v1.3.18 models: CAESAR v2.1.10, ISS v.1.0.3, IRFMN/Antares v1.0.2, IRFMN/ISSCAN-CGX v1.0.2, and IRFMN Oral and Inhalation Classification 1.0.1 models. If an external compound is beyond the defined scope of a given model, it is considered outside that model's applicability domain (AD) and cannot be associated with a reliable prediction (Sahigara 2007). Values for AD range from 0 (worst case) to 1 (best case). Generally, AD values of > 0.70 indicate that the prediction has moderate or better predictivity (Gad 2016).
  - n-Butyl alcohol was predicted to be non-carcinogenic in all six models, with acceptable reliability in three models (i.e., the AD index (ADI) > 0.7). These are the statistically based model, CAESAR v2.1.10 (ADI = 0.799), IRFMN Oral Classification 1.0.1 (ADI = 1.0), and IRFMN Inhalation Classification 1.0.1 (ADI = 1.0); therefore, results of these models are suitable for a weight of the evidence evaluation. n-Butyl alcohol was also identified as non-carcinogenic in the IRFMN Oral and Inhalation Classification 1.0.1 models based on experimental data; however, ToxServices could not identify any experimental carcinogenicity data for n-butyl alcohol (Appendix E).
- U.S. EPA 2019, 2021
  - ToxServices attempted to evaluate n-butyl alcohol using OncoLogic™ (v9.0). However, n-butyl alcohol belongs to a class of chemicals not supported by OncoLogic™ 9.0; therefore, ToxServices evaluated the carcinogenic potential of n-butyl alcohol as an aliphatic alcohol using OncoLogic™ (v8.0). According to OncoLogic™, low molecular weight alcohols (C < 6, especially methanol and ethanol) are of carcinogenic concern because of possible oxidation to reactive aldehydes, especially in individuals deficient in aldehyde dehydrogenase that converts aldehydes to carboxylic acids. Low molecular weight aliphatic alcohols with a terminal double bond or Cl/Br/I, α,β-unsaturation, or monosubstitution with Cl/Br/I at α carbon have genotoxic carcinogenicity concerns. n-Butyl alcohol has no structures of concern, and therefore the carcinogenicity concern is negligible (Appendix F).
- DTU 2023
  - ToxServices evaluated n-butyl alcohol with the Danish (Q)SAR Database for carcinogenicity. The QSAR modeling reports that n-butyl alcohol is in the domains of all

seven E Ultra FDA RCA cancer models and is predicted to be negative in all models (i.e., male rat, female rat, rat, male mouse, female mouse, mouse, and rodent). n-Butyl alcohol is in the domain of one out of seven Leadscope FDA RCA cancer models and predicted to be negative by that model (i.e., female rat). Regarding the liver-specific cancer in rat or mouse models, the CaseUltra model prediction is negative and the compound is in its applicability domains; it is outside the applicability domains of the Leadscope, SciQSAR, and overall battery models (Appendix G).

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

n-Butyl alcohol was assigned a score of Low for mutagenicity/genotoxicity based on negative results obtained from *in vitro* and *in vivo* mutagenicity/genotoxicity assays. GreenScreen® criteria classify chemicals as a Low hazard for mutagenicity/genotoxicity when adequate data are available and negative for both gene mutation and chromosomal aberration, and they are not GHS-classified (CPA 2018b). The confidence in the score is high as it was based on the results of well-conducted studies.

- Authoritative and Screening Lists
  - *Authoritative:* Not listed on any authoritative lists for this endpoint.
  - *Screening:* Not listed on any screening lists for this endpoint.
- ECHA, CAS# 71-36-3, 2023
  - *In vitro:* In a bacterial reverse mutation assay conducted according to National Toxicology Program (NTP) protocol (GLP unspecified), *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 were exposed to n-butyl alcohol (purity and vehicle unspecified) at concentrations up to 10,000 µg/plate with and without metabolic activation (10% and 30% hamster and/or rat liver). Negative and positive controls were reported as valid. No precipitation was reported; however, cytotoxicity was reported at the highest dose tested in tester strains TA98 and TA100 in the presence of metabolic activation. No increase in the mutation frequency was reported in the presence or absence of metabolic activation (Klimisch 2, reliable with restrictions).
  - *In vitro:* In an Ames test bacterial reverse mutation assay conducted according to the methods by Maron and Ames (1983) and D. Levin et al. 1982 (GLP unspecified), *S. typhimurium* tester strain TA102 was exposed to n-butyl alcohol (purity and vehicle unspecified) at concentrations up to 5,000 µg/plate with and without metabolic activation (S9 mix from livers of Aroclor 1254-induced Sprague-Dawley rats). Negative and positive controls were reported as valid. No information on precipitation or cytotoxicity was reported; however, the compound was tested up to guideline limit concentration or precipitating/cytotoxic concentrations. No increase in the mutation frequency was reported in the presence or absence of metabolic activation (Klimisch 2, reliable with restrictions).
  - *In vitro:* In a GLP-compliant *in vitro* mammalian cell hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene mutation assay conducted according to OECD Guideline 476, CHL fibroblasts (V79) were exposed to concentrations of 23.1 to 740 µg/mL (equivalent to 10 mM as identified by the authors of the ECHA dossier) of n-butyl alcohol (purity unspecified) for 4 hours with and without metabolic activation in Experiment 1, and in Experiment II for 4 hours with metabolic activation and 24 hours without metabolic activation. The metabolic activation system was S-9 mix from phenobarbital/β-naphthoflavone induced rat liver. The positive and vehicle controls were valid. No precipitation or cytotoxicity was reported; however, the compound was tested up to the guideline limit concentration. There were no statistically significant increases in the mutant frequency at any of the concentrations tested with or without metabolic activation (Klimisch 1, reliable without restriction).

- *In vitro:* In a non-GLP-compliant *in vitro* mammalian cell thymidine kinase (TK) gene mutation assay conducted according to the modified Olive and Spector (1975) method, mouse lymphoma L5178Y cells were exposed to concentrations of 0.39 - 12.5 µg/mL and 0.1 - 5.00 µg/mL of n-butyl alcohol (purity unspecified) for 24 hours without and with metabolic activation, respectively. The metabolic activation system was S-9 mix from Aroclor 1254 induced rat livers. The positive and vehicle controls were valid. No precipitation was reported; however, the compound was tested up to cytotoxic doses. There were no statistically significant increases in the mutant frequency at any of the concentrations tested with or without metabolic activation (Klimisch 2, reliable with restrictions).
- *In vitro:* In an *in vitro* mammalian cell micronucleus test (GLP unspecified), CHL fibroblasts (V79) were exposed to concentrations of 50 µg/mL of n-butyl alcohol (purity and vehicle unspecified) for a 1 hour exposure with and without metabolic activation (not specified). The positive and vehicle controls were valid. No information on precipitation or cytotoxicity was reported. There were no statistically significant increases in the mutant frequency at any of the concentrations tested with or without metabolic activation (Klimisch 2, reliable with restrictions).
- *In vitro:* In a non-GLP-compliant *in vitro* mammalian cell SCE assay, CHO cells were exposed to final concentrations of 0.1% (v/v) n-butyl alcohol (purity and vehicle unspecified) in cell cultures without metabolic activation. The positive, negative, and vehicle controls were valid. No information on precipitation was reported, and the lowest cytotoxic concentration was reported to be > 0.1% (v/v). There were no significant increases in the SCE frequency reported compared to controls (Klimisch 2, reliable with restrictions.
- *In vivo*: In a GLP-compliant *in vivo* micronucleus assay conducted according to OECD Guideline 474, male and female NMRI mice (5/sex/dose) were administered a single dose of 500, 1,000, or 2,000 mg/kg via gavage and were sacrificed after 24h (all doses) or 48h (control and high dose). Positive, negative, and vehicle controls were valid. Clinical signs of toxicity were reported at the mid and high doses. There were no increases in micronuclei in the bone marrow at any dose (Klimisch 1, reliable without restriction).
- The authors of the ECHA dossier identified a few more studies for *in vitro* genotoxicity; however, only GLP or guideline studies were evaluated for this endpoint due to their higher reliability.

- ECETOC 2003
  - The ECETOC evaluated n-butyl alcohol and reported n-butyl alcohol was positive in inducing aneuploidy in *Aspergillus nidulans*, it was negative in an *umu* test using *S. typhimurium* strain TA1535/pSK1002 exposed to concentrations of up to 27,000 µg/mL, and it did not induce chromosome aberrations in mammalian cells *in vitro* and *in vivo*, indicating a lack of potential to induce chromosomal aberrations in humans. Therefore, based on this weight of evidence, ECETOC concluded that n-butyl alcohol does not have a genotoxic potential.
- The weight of evidence for all genotoxicity toxicity testing indicates that n-butyl alcohol does not have a genotoxic potential (ECETOC 2003, ECHA 2018). Although n-butyl alcohol was positive in inducing aneuploidy in *Aspergillus nidulans*, it did not induce chromosome aberration in mammalian cells *in vitro* or *in vivo* or micronuclei formation *in vivo*, indicating a lack of potential to induce clastogenicity in humans.

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

n-Butyl alcohol was assigned a score of Low for reproductive toxicity based on no reproductive effects detected in animal studies performed with the target chemical and the surrogate. GreenScreen® criteria classify chemicals as a Low hazard for reproductive toxicity when adequate data are available and are negative for reproductive toxicity, and they are not GHS-classified (CPA 2018b). The confidence in the score is high as it is based on the results of well-conducted studies for the target chemical and a strong surrogate.

- Authoritative and Screening Lists
  - *Authoritative:* Not listed on any authoritative lists for this endpoint.
  - *Screening:* Not listed on any screening lists for this endpoint.
- OECD 2001, ECETOC 2003, U.S. EPA 2011, ECHA, CAS #71-36-3, 2023
  - *Oral:* In a non-GLP drinking study, female Wistar Imp:DAK rats (11-17/group) were provided drinking water containing n-butyl alcohol (purity not specified) at 0, 0.24, 0.8, or 4% (contributing doses of 0, 300, 1,000, and 5,000 mg/kg/day, respectively according to ECHA record) for eight weeks prior to mating, for three weeks during mating period to untreated males, and through gestation. No data were provided for gross pathology or histopathology. Treatment did not affect maternal survival, food or water consumption, body weight, hemoglobin concentration, hematocrit, or organ weights (specific organ evaluated were not reported by ECHA or ECETOC). There were no differences in estrous cycle duration or duration of individual status of the cycle. No treatment related effects on pregnancy rate, number of corpora lutea or total implants, number of litters with resorptions, and number of pre- or post-implantation losses/litter were reported. The developmental toxicity results for this study are discussed under the oral developmental toxicity section. As treatment did not adversely affect maternal reproductive parameters, ToxServices identified a no observed adverse effect level (NOAEL) of 5,000 mg/kg/day, the highest dose tested for maternal and reproductive toxicity (Klimisch 2, reliable with restrictions).
- OECD 2001, ECETOC 2003, ECHA, CAS #71-36-3, 2023
  - *Oral:* In a GLP-compliant oral repeated dose toxicity study, male and female CD rats (30/sex/groups) were exposed to doses of 0, 30, 125, or 500 mg/kg/day n-butyl alcohol (purity not specified) via gavage for either 6 or 13 weeks. No treatment related effects were reported for body weight or mortality, body weight changes, food consumption, and ophthalmic evaluation, in addition to organ weights, gross pathology and histology of the testes with epididymis and ovaries. Clinical signs of ataxia and hypoactivity lasting less than an hour post-exposure were reported for both males and females of the high dose group. ECETOC notes these clinical signs are common with exposure to high doses of alcohols. The authors of the ECHA dossier identified a NOAEL of 500 mg/kg/day, the highest dose tested, based on no effects reported on reproductive organs (Klimisch 1, reliable without restriction). *Note: OECD (2001) and ECETOC (2003) report a NOAEL and low observed adverse effect level (LOAEL) of 30 mg/kg/day and 125 mg/kg/day, respectively, based on clinical signs of neurotoxicity as this study was reported under the repeated-dose endpoint and not the reproductive toxicity endpoint.*
- OECD 2001
  - *Oral:* No testicular toxicity was found in male Sprague-Dawley rats (n=6) administered gavage doses of n-butyl alcohol (analar reagent grade) in corn oil at 533 mg/kg/day for six days. In contrast, an equimolar dose of dibutyl phthalate at 2,000 mg/kg/day produced decreased testes weights, testicular atrophy, and altered zinc metabolism. As n-butyl alcohol did not cause effects on testes weight or histology, an evaluation for zinc metabolism was not performed.

  - *Inhalation:* In the OECD's evaluation of n-butyl alcohol, no changes in reproductive performance were reported in female rats exposed to 1,500 ppm of n-butyl alcohol for three weeks prior to mating and during gestation. However, the males were unexposed (Klimisch 1, reliable without restriction).
  - *Inhalation:* There was no evidence of testicular toxicity in male Sprague-Dawley rats exposed via inhalation to 0, 500, 1,500, or 3,000 ppm of n-butyl alcohol, 6 hrs/day for at least 65 exposures over 14 weeks. Therefore, the NOAEL of 3,000 ppm, the highest dose tested, for male reproductive toxicity following repeated inhalation exposure was established (Klimisch 1, reliable without restriction).
- U.S. EPA 2011
  - *Inhalation:* In a testicular toxicity study, male Sprague-Dawley rats (5/group) received inhalation doses of 150 mg/m^3 n-butyl alcohol for 6 hours/day for 1 day or 1 week. Rats exposed for 1 day had statistically significant reductions in testosterone concentrations, but the decrease was not statistically significant in rats exposed for 1 week. Rats treated for 1 day had statistically significant increase in serum corticosterone, but those treated for 1 week had lower levels, which were not statistically significant. *It is not clear if the temporary changes in hormone levels are of toxicological significance. Therefore, ToxServices did not assign an effect concentration for this study.*
- OECD 2001, ECETOC 2003, ECHA, CAS #71-36-3, 2023
  - *Inhalation:* In a non-GLP behavioural peri-, post-natal developmental neurotoxicity study, male and female Sprague-Dawley rats (15 females/group, 18/males/group) were exposed to inhalation doses of 3,000 or 6,000 ppm (equivalent to 9.2 mg/L or 18.5 mg/L as identified by ECETOC) n-butyl alcohol (> 99% purity as reported by OECD). Females were exposed throughout gestation and males were exposed for six weeks prior to mating. No detectable treatment-related effect on parental toxicity or pregnancy rate was found up to 6,000 ppm. The study authors identified a NOAEC of 6,000 ppm, the highest dose tested (equivalent to 18.5 mg/L as identified by the authors of the ECHA dossier) for reproductive toxicity (Klimisch 2, reliable with restrictions).
- ECHA, CAS #71-36-3, 2023
  - *Inhalation: <u>Surrogate: n-Butyl acetate (CAS #123-86-4):</u>* A GLP-compliant two-generation reproduction toxicity study conducted in 2007 according to the current OECD Guideline 416/EPA OPPTS 870.3800 from 2001 was performed with Sprague-Dawley rats (30/sex/dose group) administered whole body inhalation exposures of surrogate butyl acetate (99.8% purity) vapor at 0, 750, 1,500, or 2,000 ppm for 6 hours/day, 7 days/week. Based on the conversion factor of 1 ppm = 4.75 mg/m^3 butyl acetate, ToxServices converted the ppm concentrations to 0, 3.6, 7.1, and 9.5 mg/L, respectively (CDC 2019). The parental animals were exposed for at least 70 consecutive days prior to mating. The F0 and F1 females were continuously exposed throughout mating and gestation through GD 20. No inhalation exposures were administered on GD 21 through lactation day 4, but oral gavage doses of 0, 1,125, 2,250, or 3,000 mg/kg/day were administered to the parental females on lactational days 1-4. Inhalation exposures were continued on lactation day 5 through the day prior to sacrifice. Inhalation exposures of the F1 generation began on post-natal day (PND) 22. The parental animals were evaluated for clinical signs of toxicity, body weight, food consumption, estrous cyclicity, sperm parameters, and reproductive indices. The offspring were evaluated for viability indices, number and sex, stillbirths, live births, postnatal mortality, presence of gross anomalies, body weights, and physical or behavioral abnormalities. No treatment-related effects were reported on the reproduction endpoints including estrous cycles, mating and fertility indices, number of days between pairing and

coitus, spermatogenic endpoints and gestation length in the treated groups of the F0 or F1 generations. Treatment-related effects on the offspring of treated dams included decreased pup body weights in the mid and high concentration groups and delayed attainment of post-weaning developmental landmarks in the mid and high concentration groups, which were considered to be secondary to lower body weights. The survival of the F1and F2 pups was not affected by treatment. The study authors identified a fertility NOAEC of 2,000 ppm, the highest dose tested (equivalent to 9.7 mg/L butyl acetate or 6.178 mg/L n-butyl alcohol as identified by the authors of the ECHA dossier) based on the lack of treatment-related effects on reproductive parameters detected at up to the highest concentration tested (Klimisch 1, reliable without restriction).

- The weight of evidence for the inhalation and oral studies in animals indicates that n-butyl alcohol is not a reproductive toxicant. n-Butyl alcohol did not produce testicular toxicity in male rats or reproductive toxicity in female rats exposed prior to mating through gestation. No effects on reproductive organs were reported for male and female rats exposed to n-butyl alcohol in an OECD Guideline 408 repeated dose toxicity study. Testicular toxicity was only seen in one inhalation study in rats of short duration where the changes were not clearly explained. However, there were no adverse effects on reproductive organs in rats in subacute and subchronic studies. Additionally, a lack of adverse effects on reproduction was reported in Sprague-Dawley rats following inhalation exposures to vapor concentrations ≤ 3,000 ppm in an OECD Guideline 416 two-generation reproduction toxicity study in rats exposed to surrogate butyl acetate. Furthermore, numerous authoritative bodies concluded n-butyl alcohol was not a concern for reproductive toxicity. Therefore, a score of Low is assigned.

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): L
n-Butyl alcohol was assigned a score of Low for developmental toxicity based on a conclusion from an authoritative body (ECHA) that it is not a specific developmental toxicant since the effects detected in animal studies were secondary to maternal toxicity. GreenScreen® criteria classify as a Low hazard for developmental 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 a conclusion from an authoritative body.

- Authoritative and Screening Lists
  - *Authoritative:* MAK – Pregnancy Risk Group C.
  - *Screening:* Not listed on any screening lists for this endpoint.
- OECD 2001, ECETOC 2003, U.S. EPA 2011, ECHA 2018, ECHA, CAS #71-36-3, 2023
  - *Oral:* In the previously described non-GLP drinking study, female Wistar Imp:DAK rats (11-17/group) were provided drinking water containing n-butyl alcohol (purity not specified) at 0, 0.24, 0.8, or 4% (contributing doses of 0, 300, 1,000, and 5,000 mg/kg/day, respectively, according to ECHA record) for eight weeks prior to mating, for three weeks during mating period to untreated males, and through gestation. No data were provided for gross pathology or histopathology. Treatment did not affect maternal survival, food or water consumption, body weight, hemoglobin concentration, hematocrit, or organ weights (specific organ evaluated were not reported by ECHA or ECETOC). Pregnancy rate, number of corpora lutea or total implants, number of litters with resorptions, and number of pre- or post-implantation losses/litter were not affected. Mean fetal crown-rump length was statistically significantly reduced in the high dose group. Statistically significant and dose-related increases in the litter incidences with any dilation (i.e., subarachnoid space and cerebral ventricles) or renal pelvis were found at all doses. The percentages of litters with internal hydrocephalus were statistically significantly increased at the mid and high doses,

and the percentage of litters with external hydrocephalus was increased at the mid dose only. In addition, the percentages of litters with extra rib were statistically significantly higher at the high dose. The U.S. EPA assigned a 300 mg/kg/day LOAEL (the lowest dose tested), based on increased incidences of dilation of the subarachnoid space and of the lateral and/or third ventricle of the brain in the offspring (Klimisch 2, reliable with restrictions). *This study was selected as the key study by the U.S. EPA and the developmental effects were considered critical effects in the derivation of a reference dose for n-butyl alcohol. It should be noted this draft report was never finalized. However, in a recent evaluation report, ECHA considered the study unreliable for the assessment due to several limitations: 1) the author reported structural deformations (wavy 13th pair and extra 14th pair of the ribs) and central nervous system (CNS) defects without historical control data, 2) these "congenital defects" are commonly found variations or delayed development in historical databases, 3) an in-house bred species was used and fed a non-standard diet which made interpretation of results difficult, and 4) no dose response relationship was identified for these effects. Further, the NOAEL for maternal and developmental (ToxServices notes that this might be a typo and should be reproductive instead of developmental, because developmental LOAEL appears to be 300 mg/kg/day) toxicity was 5,000 mg/kg/day, which is much higher than the ECHA-determined oral LD_{50} value of 2,290 mg/kg in rats. Therefore, ECHA concluded that data from this study are questionable and a clear indication for developmental toxicity or developmental neurotoxicity cannot be deduced from this study (ECHA 2018). Based on ECHA's conclusion on this study and that ECHA's evaluations is more recent than the U.S EPA's draft assessment which was a draft report, ToxServices did not heavily weigh the study in the weight of evidence.*

- OECD 2001, U.S. EPA 2011, ECHA, CAS #71-36-3, 2023
    - *Oral:* In a GLP-compliant drinking water study conducted according to Ministry of Health and Welfare, Japan and in a manner similar to OECD Guideline 414, pregnant Sprague-Dawley rats (20/group) were given n-butyl alcohol (purity unspecified) in drinking water at daily concentrations of 0.2%, 1.0%, or 5.0%, (equivalent doses of 316, 1,454, or 5,654 mg/kg/day as identified by the authors of the ECHA dossier) on gestational days (GD) 0 – 20. Maternal body weight gain was significantly ($p<0.05$) decreased at the high dose. Food and water consumption were statistically significantly decreased at mid and high doses. Fetal body weight was statistically significantly decreased at the high dose. Statistically significant increase in skeletal variations (primarily short supernumerary ribs) and decreased degree of ossification were reported at the high dose, which were attributed to growth retardation by the authors. The incidence of litters with thymic remnant in the neck increased dose-dependently and the incidence at the high dose was twice that of the controls. The U.S. EPA identified a LOAEL and NOAEL of 5,654 mg/kg/day and 1,454 mg/kg/day for maternal and development toxicity based on decreased maternal body weight gain, decreased fetal body weight, and increased incidence of skeletal variations for developmental toxicity (Klimisch 1, reliable without restrictions).
- OECD 2001, U.S. EPA 2011
    - *Oral:* White rats (10-16/group) were administered n-butyl alcohol by gavage at approximately 1,300 mg/kg/day during GD 1 – 15, and sacrificed on GD 20. There were statistically significant increases in the percentage of pre- and post-implantation losses and in total fetal deaths. In addition, fertility index was reduced. Antidiuretic hormone (ADH) activity in fetal livers was reduced. Due to lack of study details, U.S. EPA did not identify an effect level for this study.
- OECD 2001, U.S. EPA 2011, ECHA 2018, ECHA, CAS #71-36-3, 2023

- *Inhalation:* Pregnant Sprague-Dawley rats (15-20/group) were exposed to n-butyl alcohol at concentrations of 0, 3,500, 6,000, 8,000 ppm (equivalent to 0, 11, 18 or 24 mg/L as identified by U.S. EPA 2011 and 10.8, 18.5, and 24.7 mg/L by the authors of the ECHA dossier) for 7 hours/day on GD 1 – 19. Two dams died prior to sacrifice at the highest concentration. Food consumption was reduced in dams at the mid and high concentrations ($p<0.05$). No treatment related effects were reported for mean corpora lutea, resorptions, and live fetuses per litter, and sex ratio. There was a statistically significant concentration related decrease in fetus body weight at the mid and high concentrations. At the high dose, the percentages of fetuses with normal skeletal development were statistically significantly reduced, with the primary skeletal malformation found to be rudimentary cervical ribs. The incidences of litters with skeletal malformations were statistically significantly increased at all concentrations and the incidence of litters with visceral malformations was statistically significantly increased at the highest concentration. The U.S. EPA identified the LOAEC at 11 mg/L/7h/day based on increased incidence of litters with skeletal variations. However, the authors of the ECHA dossier established a NOAEL of 10.8 mg/L/7h/day for maternal and developmental toxicity based on a lack of selective developmental effects in the presence of maternal toxicity and delayed ossification reported in the presence of maternal toxicity (Klimisch 2, reliable with restrictions).

- ECHA, CAS #71-36-3, 2023
  - *Inhalation: Surrogate: n-Butyl acetate (CAS #123-86-4):* In the previously described GLP-compliant two-generation reproduction toxicity study conducted according to OECD Guideline 416/EPA OPPTS 870.3800 with Sprague-Dawley rats (30/sex/dose group) administered whole body inhalation exposures of butyl acetate (99.8% purity) vapor at 0, 750, 1,500, or 2,000 ppm for 6 hours/day, 7 days/week. Based on the conversion factor of 1 ppm = 4.75 mg/m^3 butyl acetate, ToxServices converted the ppm concentrations to 0, 3.6, 7.1, and 9.5 mg/L, respectively (CDC 2019). The parental animals were exposed for at least 70 consecutive days prior to mating. The F0 and F1 females were continuously exposed throughout mating and gestation through GD 20. No inhalation exposures were administered on GD 21 through lactation day 4, but oral gavage doses of 0, 1,125, 2,250, or 3,000 mg/kg/day were administered to the parental females on lactational days 1-4. Inhalation exposures were continued on lactation day 5 through the day prior to sacrifice. Inhalation exposures of the F1 generation began on PND 22. The parental animals were evaluated for clinical signs of toxicity, body weight, food consumption, estrous cyclicity, sperm parameters, and reproductive indices. The offspring were evaluated for viability indices, number and sex, stillbirths, live births, postnatal mortality, presence of gross anomalies, body weights, and physical or behavioral abnormalities. Treatment-related effects on the offspring of treated dams included decreased pup body weights in the mid and high concentration groups and delayed attainment of post-weaning developmental landmarks in the mid and high concentration groups, which were considered to be secondary to lower body weights. The survival of the F1 and F2 pups was not affected by treatment. The study authors identified a developmental toxicity NOAEC of 750 ppm (3.6 mg/L) and LOAEC of 1,500 ppm (7.1 mg/L) based on decreases of pup body weights measured in the mid and high concentration groups (Klimisch 1, reliable without restriction).
  - *Inhalation: Surrogate: n-Butyl acetate (CAS #123-86-4):* A GLP-compliant prenatal developmental toxicity study conducted according to OECD Guideline 414 was performed with female Sprague-Dawley rats (37-43/concentration group) administered whole body inhalation exposures of butyl acetate (99.1% purity) vapor at 1,500 ppm (equivalent to 7.23 mg/L) for 7 hours/day. Four different groups were included in this study: the controls were

administered filtered air (group 1), one treatment group was exposed on GD 7-16 (group 2), another group was exposed on GD 1-16 (group 3), and the last group was exposed for 5 days/week for 3 weeks prior to mating and then on GD 1-16 (group 4). All exposures were for 7 hours/day. The females were evaluated for clinical signs of toxicity, body weight, food consumption, and ovarian and uterine content. The fetuses were evaluated for external, visceral, skeletal, and head malformations. Food consumption was decreased during the first week in females exposed prior to gestation. At sacrifice, extra-gestational weights and liver weights decreased in exposed rats. Relative lung and kidney weights were increased in treated rats. The body weights and crown-rump lengths of male and female fetuses were lower in the treatment groups relative to controls. The duration of exposure and period of gestation in which the treatment was administered did not affect the developmental outcomes. Reductions in placental weight were measured with treatment. Major malformations consisting of multiple facial defects, eye defects, diaphragmatic hernias, and generalized brain dysmorphology were reported in 2 fetuses in group 2, one fetus in group 3, and 3 fetuses in group 4. The generalized brain dysmorphology consisted of massive distortion of the external and internal architecture of the brain; inequalities in size of the olfactory lobes, and abnormalities in shape and size of the cerebral hemispheres. Hemorrhages were apparent around the exterior brain surfaces. The incidence of rib dysmorphology was increased in fetuses in groups treated during gestation. The incidence of reduced pelvic ossification was also measured in fetuses of groups 2 and 3. The study authors identified a maternal toxicity and developmental toxicity LOAEC of 7.23 mg/L based on the changes to food consumption and body weight in the dams and reduced fetal size and increased frequency of malformations with treatment (Klimisch 1, reliable without restrictions).

- *Inhalation: Surrogate: n-Butyl acetate (CAS #123-86-4):* In a second prenatal developmental toxicity study in Sprague-Dawley rats that was conducted in a manner similar to OECD Guideline 414, dams (19-21/dose) were exposed to 0, 500, 1,000, 2,000, or 3,000 ppm butyl acetate (≥ 99.0% purity) vapor (equivalent to 0, 2.38, 4.75, 9.50, or 14.25 mg/L according to ECHA records) via whole body inhalation for 6 hours/day on GD 6-20 and were sacrificed on GD 21. Dams were evaluated for clinical signs of toxicity, body weight and food consumption, and ovaries uterine content. Fetuses were evaluated for external, skeletal, and soft tissue malformations. Maternal weight gain was significantly reduced at 2,000 and 3,000 ppm, and food consumption was significantly decreased at 1,000 ppm and above. Fetal weights were slightly reduced at 2,000 and 3,000 ppm (by 3% and 12-13%, respectively; statistically significant at the high dose). Malformation was only found in single fetuses in these dose groups. Authors reported a NOAEC and LOAEC of 2.38 and 4.75 mg/L, respectively, for maternal toxicity, and a NOAEC of 14.25 mg/L for developmental toxicity, as effects on fetal weight were measured only in the presence of maternal toxicity (Klimisch 2, reliable with restrictions).
- *Inhalation: Surrogate: n-Butyl acetate (CAS #123-86-4):* A GLP-compliant prenatal developmental toxicity study conducted in a manner similar to OECD Guideline 414 (additional exposure during pre-gestation period) was performed with female New Zealand White rabbits (21-25/dose group) administered whole body inhalation exposures of butyl acetate (99.1% purity) vapor at 0 or 1,500 ppm (equivalent to 7.23 mg/L). Three groups of animals were exposed in the following manner: the control were provided filtered air, one treatment group received treatment on GD 7-19, and the second treatment group received treatment on GD 1-19. The animals were sacrificed and necropsied on GD 30. The dams were evaluated for clinical signs of toxicity, body weight, food consumption, and ovarian and uterine content. The fetuses were evaluated for the incidence of external, visceral,

skeletal, and head malformations. No treatment-related effects were measured on body weight or reproductive performance for the dams and no evidence of developmental toxicity was reported in the fetuses. The study authors identified a maternal toxicity and developmental toxicity NOAEC of 1,500 ppm (equivalent to 7.23 mg/L) based on the lack of treatment-related effects reported (Klimisch 1, reliable without restrictions).

- n-Butyl alcohol increased the incidence of subarachnoid space and of the lateral and/or third ventricle of the brain in the offspring of female rats exposed via drinking water prior to mating through gestation, without maternal toxicity present. Of all the studies identified, the lowest LOAEL is 300 mg/kg/day in rats based on statistically significant increases in the incidence of litters with subarachnoid space dilation and lateral and/or third brain ventricle dilation reported in all groups. This change was dose-related, and was consistent with effects in rats exposed prenatally to ethanol, a known neurodevelopmental toxicant. In a draft report, U.S. EPA considered this effect relevant for humans and derived a benchmark lower dose lower bound ($BMDL_{10}$) of 26.1 mg/kg/day for this study. This value was used to derive the oral reference dose (RfD) for n-butyl alcohol (U.S. EPA 2011). However, in a more recent report ECHA concluded n-butyl alcohol was not a developmental toxicant and all the effects observed should be considered "variations" rather than "congenital defects" (ECHA 2018). Therefore, ToxServices did not weigh the study heavily. For the inhalation route, a LOAEL of 3,500 ppm (equivalent to 11 mg/L/7h/day) for maternal and developmental toxicity in rats exposed to n-butyl alcohol was identified by U.S. EPA in a draft report, based on increased incidences of litters with skeletal variations in rats exposed throughout gestation. MAK classified n-butyl alcohol as Pregnancy Group C and concluded a low potential for developmental toxicity when the MAK value of 100 mL/m^3 was observed (MAK 2003). Additional studies with rats exposed to surrogate butyl acetate, which metabolizes into the target chemical, reported reduced fetal or pup body weights and/or increased malformations in reproductive/developmental rat studies in the presence of parental systemic toxicity. ECHA (2018) concluded n-butyl alcohol was a low concern for specific developmental toxicity and developmental neurotoxicity and that reported effects on offspring were not direct effects by the test substance, but a result of maternal toxicity. Classification as Pregnancy Group C by MAK corresponds to a score of Low to Moderate for this endpoint. Based on the conclusion by ECHA, ToxServices assigned a Low score for this endpoint.

Endocrine Activity (E) Score (H, M, or L): DG
n-Butyl alcohol was assigned a score of data gap for endocrine disruption based on insufficient data identified for this endpoint. Although *in vitro* data and *in silico* modeling do not indicate a concern for endocrine effects on four pathways (estrogen, androgen, thyroid and steroidogenesis), no *in vivo* data are available.

- Authoritative and Screening Lists
  - *Authoritative:* Not listed on any authoritative lists for this endpoint.
  - *Screening:* Not listed on any screening lists for this endpoint.
- U.S. EPA 2023b
  - n-Butyl alcohol was active in 2/10 estrogen receptor (ER) assays, 0/11 androgen receptor (AR) assays, 0/2 steroidogenesis assays, and 0/9 thyroid receptor assays performed as part of the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) in the 21st Century (Appendix H).
- OECD 2001, ECETOC 2003, U.S. EPA 2011, ECHA, CAS #71-36-3, 2023
  - *Oral:* In the previously described non-GLP drinking water study, female Wistar Imp:DAK rats (11-17/group) were provided drinking water containing n-butyl alcohol (purity not specified) at 0, 0.24, 0.8, or 4% (contributing doses of 0, 300, 1,000, and 5,000 mg/kg/day,

respectively according to ECHA record) for eight weeks prior to mating, for three weeks during mating period to untreated males, and through gestation. There were no differences in estrous cycle duration or duration of individual status of the cycle. No treatment related effects on pregnancy rate, number of corpora lutea or total implants, number of litters with resorptions, and number of pre- or post-implantation losses/litter were reported. Mean fetal crown-rump length was statistically significantly reduced in the high dose group. Statistically significant and dose-related increases in the litter incidences with any dilation (i.e., subarachnoid space and cerebral ventricles) or renal pelvis were found at all doses. The percentages of litters with internal hydrocephalus were statistically significantly increased at the mid and high doses, and the percentage of litters with external hydrocephalus was increased at the mid dose only. In addition, the percentages of litters with extra rib were statistically significantly higher at the high dose. The U.S. EPA assigned a 300 mg/kg/day LOAEL (the lowest dose tested) for developmental toxicity, based on increased incidences of dilation of the subarachnoid space and of the lateral and/or third ventricle of the brain in the offspring (Klimisch 2, reliable with restrictions).

- OECD 2001, ECETOC 2003, ECHA, CAS #71-36-3, 2023
    - *Oral:* In a GLP-compliant subchronic toxicity study, Sprague-Dawley rats (30/sex/dose) received gavage doses of n-butyl alcohol (purity not specified) in water at 0, 30, 125, or 500 mg/kg/day for 13 weeks. There were no treatment-related effects on body weight or organ weight changes, food consumption, mortality, gross pathology, histopathology and ophthalmic evaluations. Sporadic changes in thyroid weight were found at one evaluation time only, and without a dose-response relationship. U.S. EPA and REACH dossier authors assigned the NOAEL and LOAEL at 125 and 500 mg/kg/day, respectively, based on reduced hematology findings in females and transient clinical signs of CNS depression, respectively (Klimisch 1, reliable without restriction).
- OECD 2001, U.S. EPA 2011, ECHA, CAS #71-36-3, 2023
    - *Inhalation:* In a previously described developmental study, pregnant Sprague-Dawley rats (15-20/group) were exposed to n-butyl alcohol at inhalation doses of 3,500, 6,000, 8,000 ppm (equivalent to 0, 11, 18 or 24 mg/L as identified by U.S. EPA 2011 and 10.8, 18.5, and 24.7 mg/L by the authors of the ECHA dossier) for 7 hours/day on GD 1 – 19. Two dams died prior to sacrifice at the highest concentration. Food consumption was reduced in dams at the mid and high concentrations ($p<0.05$). No treatment related effects were reported for mean corpora lutea, resorptions, and live fetuses per litter, and sex ratio. There was a statistically significant concentration related decrease in fetus body weight at the mid and high concentrations. At the high dose, the percentages of fetuses with normal skeletal development were statistically significantly reduced, with rudimentary cervical ribs found to be the primary skeletal malformation. The incidences of litters with skeletal malformations were statistically significantly increased at all concentrations and the incidence of litters with visceral malformations was statistically significantly increased at the highest concentration. The U.S. EPA identified the LOAEC at 11 mg/L/7h/day based on increased incidence of litters with skeletal variations. However, the authors of the ECHA dossier established a NOAEL of 10.8 mg/L/7h/day for maternal and developmental toxicity based on a lack of selective developmental effects in the presence of maternal toxicity and delayed ossification reported in the presence of maternal toxicity (Klimisch 2, reliable with restrictions).
- U.S. EPA 2011
    - *Inhalation:* In a 4-month study, rats and mice were exposed to n-butyl alcohol at 0, 0.8, 6.6 or 40 mg/m^3 for 4 months (exposure duration and frequency not specified). Increased thyroid gland activity occurred in all groups. Pituitary-adrenal system was disrupted,

eosinophilic response following administration of adrenocorticotropic hormone was reduced, and oxygen demand was reduced in the "cold test" at 6.6 and 40 mg/m^3. The authors concluded that a NOAEC and LOAEC of 0.8 and 6.6 mg/m^3 (equivalent to 0.0008 and 0.04 mg/L as calculated by ToxServices: 0.8 mg/m^3 x 1 m^3/1000 L = 0.0008 mg/L and 40 mg/m^3 x 1 m^3/1000 L = 0.04 mg/L) could be established for this study. However, the U.S. EPA considered information in this publication inadequate for the purpose of identifying effect levels.

- ECHA, CAS #71-36-3, 2023
  - *Inhalation: Surrogate: n-Butyl acetate (CAS #123-86-4):* In the previously described GLP-compliant two-generation reproduction toxicity study conducted according to OECD Guideline 416/EPA OPPTS 870.3800 with Sprague-Dawley rats (30/sex/dose group) administered whole body inhalation exposures of butyl acetate (99.8% purity) vapor at 0, 750, 1,500, or 2,000 ppm for 6 hours/day, 7 days/week. Based on the conversion factor of 1 ppm = 4.75 mg/m^3 butyl acetate, ToxServices converted the ppm concentrations to 0, 3.6, 7.1, and 9.5 mg/L, respectively (CDC 2019). The parental animals were exposed for at least 70 consecutive days prior to mating. The F0 and F1 females were continuously exposed throughout mating and gestation through GD 20. No inhalation exposures were administered on GD 21 through lactation day 4, but gavage doses of 0, 1,125, 2,250, or 3,000 mg/kg/day were administered to the parental females on lactational days 1-4. Inhalation exposures were continued on lactation day 5 through the day prior to sacrifice. Inhalation exposures of the F1 generation began on PND 22. The parental animals were evaluated for endocrine related parameters including estrous cyclicity, sperm parameters (count, motility, and morphology), and histopathology of adrenals, prostate, coagulating lands, seminal vesicles, liver, testis, epididymis, thyroid, uterus, oviducts, cervix, ovaries, vagina, oviducts, and pituitary. The offspring were evaluated for endocrine related parameters including preputial separation and other (unspecified) measures of sextual maturation. No treatment-related effects were reported on the reproduction endpoints including estrous cycles, mating and fertility indices, number of days between pairing and coitus, spermatogenic endpoints and gestation length in the treated groups of the F0 or F1 generations. Treatment-related effects on the offspring of treated dams included decreased pup body weights in the mid and high concentration groups and delayed attainment of post-weaning developmental landmarks in the mid and high concentration groups, which were considered to be secondary to lower body weights. The study authors identified a fertility NOAEC of 2,000 ppm based on the lack of treatment-related effects on reproductive parameters and a developmental toxicity NOAEC of 750 ppm based on decreases of pup body weights measured in the mid and high concentration groups (Klimisch 1, reliable without restriction).
  - *Inhalation: Surrogate: n-Butyl acetate (CAS #123-86-4):* A GLP-compliant repeated exposure toxicity test conducted according to EPA OTS 798.2450 was performed with Sprague-Dawley rats (15/sex/concentration group) administered whole-body inhalation exposures of butyl acetate (at least 99.9% purity) vapor at 0, 500, 1,500, or 3,000 ppm (equivalent to 0, 2.4, 7.2, and 14.4 mg/L, respectively according to study record) for 6 hours/day, 5 days/week for 13 weeks. The equivalent concentrations for a 7-day/week exposure frequency were 0, 357, 1,071, and 2,142 mg/L, respectively. Organ weights and histopathological examination was performed on endocrine related organs such as pituitary gland, thymus, thyroid gland, parathyroid gland, ovaries, vagina, uterus, fallopian tubes, adrenals, prostate, testes, epididymides, and seminal vesicles. Mean relative testes weight was increased for mid and high concentration males and mean relative adrenal gland weight

was increased for mid concentration females and high concentration males and females. No gross pathological lesions in endocrine related organs were reported in any treatment animals. The study authors identified a NOAEC of 2.4 mg/L based on epithelial necrosis reported at 7.2, and 14.4 mg/L (Klimisch 1, reliable without restriction).

- DTU 2023
    - Modeling in the Danish QSAR database provides the following results that are within the applicability domains of the models (Appendix I):
        - n-Butanol is predicted to be negative for estrogen receptor α binding (full training set and balanced training set, human in vitro) by the model battery consisting of negative and in domain predictions by the CaseUltra and SciQSAR models and overall model battery;
        - n-Butanol is predicted to be negative for estrogen receptor α activation (human *in vitro*) by the model battery consisting of negative and in domain predictions by the CaseUltra model;
        - n-Butanol is predicted to be negative for androgen receptor inhibition (human *in vitro*) by the model battery consisting of negative and in domain predictions by CaseUltra, Leadscope, and SciQSAR models and the overall model battery;
        - n-Butanol is predicted to be negative for estrogen receptor activation (CERAPP data in vitro) and for androgen receptor binding, inhibition and activation (CoMPARA data *in vitro*) and thyroperoxidase (TPO) inhibition (QSAR1 and QSAR2, rat *in vitro*) by the Leadscope models.
- While there are negative results in *in vitro* mechanistic assays for four pathways (estrogen, androgen, thyroid and steroidogenesis) for n-butyl alcohol and negative results in *in vivo* reproductive studies in rats exposed to n-butyl alcohol, effects on reduced fetal or pup body weights and/or increased malformation were reported *in vivo* in reproductive/developmental inhalation rat studies in the presence of parental systemic toxicity for surrogate butyl acetate and the target chemical, which is the metabolite of butyl acetate. These are parameters sensitive, but not diagnostic, of endocrine disruption (EFSA 2018b). It is not clear if these effects reported *in vivo* are attributable to endocrine mechanisms. n-Butyl alcohol was predicted negative based on QSAR models for estrogen receptor binding and activation, androgen receptor binding, inhibition, and activation, and thyroperoxidase inhibition. However, ToxServices identified no data available for *in vivo* endocrine hormone signaling or reporting hormone levels. Therefore, insufficient data are available to draw a conclusion 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): M

n-Butyl alcohol was assigned a score of Moderate for acute toxicity based on oral LD_{50} values being between 300-2,000 mg/kg and association with the EU-GHS authoritative list of H302. GreenScreen® criteria classify chemicals as a Moderate hazard for acute toxicity when oral LD_{50} values are between 300-2,000 mg/kg and when associated with EU GHS-H Statement of H302 (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) Annex 6 Table 3-1 – H302 – Harmful if swallowed [Acute toxicity (oral) – Category 4].
  - *Screening:* GHS – New Zealand: Acutely oral toxicity, Category 4.
  - *Screening:* Malaysia – GHS – H302 – Harmful if swallowed.
  - *Screening:* Australia – GHS – H302 – Harmful if swallowed.
- OECD 2001, ECHA 2018
  - *Oral:* LD_{50} of 790 – 4,360 mg/kg (rats)
  - *Oral:* LD_{50} of 2,680 mg/kg (mice)
  - *Oral:* LD_{50} of 3,500 mg/kg (rabbits)
  - *Oral:* LD_{50} of 1,200 mg/kg (hamsters)
  - *Oral:* LD_{50} of 1,782 mg/kg (dogs)
  - *Inhalation:* LC_{50} of 8,000 ppm, equivalent to 24.2 mg/L[9] (4hr, rats)
- OECD 2001, ECHA 2018, ECHA, CAS #71-36-3, 2023
  - *Oral:* LD_{50} of 4,360 mg/kg (female Sherman rats) (Klimisch 2, reliable with restrictions) (non-GLP-compliant, similar to OECD Guideline 401)
- ECHA, CAS #71-36-3, 2023
  - *Oral:* LD_{50} of 2.83 mL/kg (equivalent to 2,292 mg/kg as identified by the authors of the ECHA dossier) (female Harlan rats) (Klimisch 2, reliable with restrictions) (non-GLP-compliant, similar to OECD Guideline 401)
  - *Oral:* LD_{50} of 2,510 mg/kg (male and female Osborne-Mendel rats) (Klimisch 2, reliable with restrictions)
  - *Dermal:* LD_{50} was calculated to be 3,430 mg/kg (male New Zealand White rabbits) (GLP-compliant, similar to OECD Guide 402) (Klimisch 2, reliable with restrictions)
  - *Inhalation:* LC_0 of > 17.76 mg/L (4h, vapor, male and female Sprague-Dawley rats) (Klimisch 2, reliable with restrictions) (non-GLP-compliant, similar to OECD Guideline 403)
  - *Inhalation:* LC_0 of > 21.48 mg/L (7h, vapor, male and female Sprague-Dawley rats) (Klimisch 2, reliable with restrictions) (non-GLP-compliant)
  - *Inhalation:* LC_0 of > 17.76 mg/L (4h, vapor, male and female Sherman rats) (Klimisch 2, reliable with restrictions) (non-GLP-compliant, similar to OECD Guideline 403)
- OECD 2001, ECHA 2018, AICIS 2013
  - *Dermal:* LD_{50} of 3,402 – 7,500 mg/kg (rabbits)

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

n-Butyl alcohol was assigned a score of Moderate for systemic toxicity (single dose) based on association with the EU-GHS authoritative list of H335 supported by measured data showing respiratory irritation following inhalation exposure. GreenScreen® criteria classify chemicals as a Moderate hazard for systemic toxicity (single dose) when they are associated with H-Statement of H335 and when they are classified to GHS category 3 (single exposure) (CPA 2018b). The confidence in the score is high as it is based on human and animal data as well as an authoritative listing.

- Authoritative and Screening Lists

[9] To convert concentrations in air (at 25°C) from ppm to mg/m^3: mg/m^3 = (ppm) × (molecular weight of the compound)/(24.45). For n-butanol: 1 ppm = 3.03 mg/m^3. Then to convert from mg/m^3 to mg/L: (mg/m^3) × 0.001

  - *Authoritative:* EU – GHS (H-Statements) Annex 6 Table 3-1 – H335 – May cause respiratory irritation [Specific target organ toxicity – single exposure; Respiratory tract irritation – Category 3].
  - *Screening:* GHS – Australia – H335 – May cause respiratory irritation [Specific target organ toxicity – single exposure; Respiratory tract irritation – Category 3].
  - *Screening:* GHS – Malaysia – H335 – May cause respiratory irritation [Specific target organ toxicity – single exposure; Respiratory tract irritation – Category 3].
- ECHA, CAS #71-36-3, 2023
  - *Oral:* In a non-GLP-compliant oral toxicity study, conducted in a manner similar to OECD Guideline 401, female Harlan Wistar rats (number reported by the REACH dossier authors to likely be 6/dose) were exposed to n-butyl alcohol (purity unspecified) via gavage and observed for 14 days. The study authors established an oral LD_{50} of 2.83 mL/kg, equivalent to 2,292 mg/kg as calculated by the authors of the ECHA dossier based on a density of 0.81 g/mL. No further details were provided (Klimisch 2, reliable with restrictions).
  - *Oral:* In an acute oral toxicity study (GLP-unspecified), male and female Osborne-Mendel rats (5/sex/dose) were exposed to n-butyl alcohol (purity unspecified) via gavage and observed for 14 days. The study authors established an oral LD_{50} of 2,510 mg/kg. Limited details were provided. An unknown number of deaths occurred within 4-18 hours after exposure, clinical signs included depression and coma (unknown dose levels), and no data were provided on gross pathology (Klimisch 2, reliable with restrictions).
  - *Oral:* In a non-GLP-compliant oral acute toxicity study conducted in a manner similar to OECD Guideline 401, female Sherman rats (10/dose) were exposed to a single dose of 3,160, 3,980, 5,000, or 6,300 n-butyl alcohol (purity unspecified) via gavage and observed for 14 days. The study authors established an oral LD_{50} of 4,360 mg/kg. Mortalities were reported as 0/10, 3/10, 8/10, and 10/10 at 3,160, 3,980, 5,000, and 6,300 mg/kg, respectively. Clinical signs of narcosis and prostration occurring before death were reported; however, specific doses at which these clinical signs occurred were not specified. Furthermore, gross pathology findings of higher dose animals included hemorrhage of the stomach, intestinal irritation, congested livers, and pale kidneys (Klimisch 2, reliable with restrictions).
  - *Dermal:* In a GLP-compliant dermal acute toxicity study conducted in a manner similar to OECD Guideline 402 in male New Zealand White rabbits (4/dose), a dermal LD_{50} was calculated to be 3,430 mg/kg. Topical applications of 1.26, 2.52, 5.0, or 10 mL/kg (equivalent to up to 8,100 mg/kg based on a density of 0.81 g/mL) n-butyl alcohol (purity unspecified) were applied undiluted to the clipped skin of rats under occlusion for 24 hours and observations occurred for 14 days. No mortalities were reported for the 1.26 and 2.52 mL/kg dose groups; however, 1/4 and 4/4 deaths were reported for the top doses 5 and 10 mL/kg, respectively. No additional information was provided (Klimisch 2, reliable with restrictions).
  - *Inhalation:* In a non-GLP-compliant inhalation study conducted in a manner similar to OECD Guideline 403 in male and female Sprague-Dawley rats exposed whole-body to n-butyl alcohol vapors via inhalation, a 4h LC_0 of > 17.76 mg/L (vapor) was identified. Animals (10/sex/dose) were exposed to 6.58 and 17.76 mg/L and observed for 14 days. There were no treatment-related mortalities, clinical signs, and gross pathology findings. Slightly reduced weight gain was reported for both treatment groups when compared to the control groups; however, no dose-dependent relationship was found (Klimisch 2, reliable with restrictions).

- *Inhalation:* In a non-GLP-compliant inhalation study in male and female Sprague-Dawley rats exposed to n-butyl alcohol vapors via inhalation, a 7-hour LC_0 of > 21.48 mg/L (vapor) was identified. Animals (6/sex/dose) were exposed to 21.48 mg/L by whole body inhalation and observed for 14 days. There were no treatment-related mortalities or gross pathology findings. Clinical signs were reported including accelerated and intermittent breathing and irritating effects during the exposure. No information was provided on body weights (Klimisch 2, reliable with restrictions).
- *Inhalation:* In a non-GLP-compliant inhalation study conducted in a manner similar to OECD Guideline 403 in male and female Sherman rats exposed to n-butyl alcohol vapors via inhalation, a 4h LC_0 of > 8,000 ppm (vapor) (calculated as > 24 mg/L by the authors of the ECHA dossier) was identified. Animals (6/exposure, females for the first and males for the second) were exposed to a substantially saturated vapor in the first exposure and 8,000 ppm in the second exposure for 4 to 8 hours by whole body inhalation and observed for 14 days. There were no treatment-related mortalities. Clinical signs were reported for both exposure groups including poor coordination and prostration at the end of exposure. No information was provided for body weights or gross pathology (Klimisch 2, reliable with restrictions).
- *Inhalation:* In an older study performed with volunteers, the sensatory limits of certain solvent vapors was estimated and 25 ppm (equivalent to 0.075 mg/L as identified by the authors of the ECHA dossier) n-butyl alcohol caused signs of sensory irritation in nose and throat in the majority of the test subjects after 3 -5 min (Klimisch 2, reliable with restrictions).
- *Inhalation:* In another older study, the long term effects of n-butyl alcohol exposure to workers of a baryta coating operation was performed in a longitudinal study over 10 years in which exposure levels of up to 99 workers were measured by clinical signs, lung X-ray and clinical chemistry. There were no exposure related eye injuries, eye symptoms, and no systemic effects in any individual at concentrations averaging 310 mg/m^3 (equivalent to 100 ppm as identified by the authors of the ECHA dossier). However, concentrations of 620 mg/m^3 (equivalent to 200 ppm as identified by the authors of the ECHA dossier) caused transient corneal inflammation, with associated burning sensation, lacrimation, and photophobia (Klimisch 2, reliable with restrictions).

- ECHA, CAS #123-86-4, 2023
  - *Oral: Surrogate: n-Butyl acetate (CAS #123-86-4):* In a non-GLP-compliant oral acute toxicity study conducted in a manner similar to OECD Guideline 423 with male and female Sprague-Dawley rats exposed to surrogate n-butyl acetate, the study authors identified LD_{50} values of 10,760 mg/kg and 12,789 mg/kg for females and males, respectively (calculated by the authors of the ECHA dossier). Clinical signs of sluggishness and prostration were reported at all doses. No treatment-related effects were measured on body weight. At necropsy, no dose-related effects were found in those animals that survived to the scheduled sacrifice but those that died during the course of the observation period exhibited gas-filled stomachs that were tan to red and livers that were pale tan in color on the ventral surface. The lowest dose tested in this study was 9,966 mg/kg (Klimisch 2, reliable with restrictions).
- OECD 2001
  - The irritating effect of n-butyl alcohol on the respiratory system was studied in mice and it was predicted that 40 mg/m^3 (13 ppm) in air would have a minimal or no effect on humans, 390.9 mg/m^3 (127 ppm) would be uncomfortable, and 3,909 mg/m^3 (1,268 ppm) would be intolerable.

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

n-Butyl alcohol was assigned a score of Low for systemic toxicity (repeated dose) based on an oral NOAEL of 125 mg/kg/day for n-butyl alcohol in a 13-week study, and an inhalation LOAEC/LOEC of 1.7 mg/L/6h/day for the surrogate for n-butyl acetate in a 90-day study; which are above the GHS cut-offs for classification. GreenScreen® criteria classify chemicals as a Low hazard for systemic toxicity (repeated dose) when animal studies identify oral LOAEL values greater than 100 mg/kg/day, dermal LOAEL values greater than 200 mg/kg/day and inhalation LOAEC values greater than 1.0 mg/L/6h/day for vapor, and they are not GHS-classified(CPA 2018b). The confidence in the score is high as it is based on well-conducted studies for the target chemical and a strong surrogate.

- Authoritative and Screening Lists
  - *Authoritative:* Not listed on any authoritative for this endpoint.
  - *Screening:* Not listed on any screening lists for this endpoint.
- U.S. EPA 2011, ECHA 2018, ECHA, CAS #71-36-3, 2023
  - *Oral:* In a GLP-compliant subchronic toxicity study, Sprague-Dawley rats (30/sex/dose) received gavage doses of n-butyl alcohol (purity not specified) in water at 0, 30, 125, or 500 mg/kg/day for 13 weeks. There were no treatment-related effects on body weight or organ weight changes, food consumption, mortality, gross pathology, histopathology and ophthalmic evaluations. Transient ataxia and hypoactivity were detected 2-3 minutes after dosing of males and females in the high dose group during the last 6 weeks of treatment. At week 6, there were reduced hematocrit, erythrocyte count and hemoglobin content (5% lower) in high dose females, but no hematological changes were found in males or in either sex at terminal sacrifice. Sporadic changes in neutrophil count, lymphocyte count, cholesterol, urine pH and thyroid weight were found at one evaluation time only, and without a dose-response relationship. U.S. EPA and ECHA assigned the NOAEL and LOAEL at 125 and 500 mg/kg/day, respectively, based on reduced hematocrit, erythrocyte count, and hemoglobin content in females and transient clinical signs of CNS depression (evaluated under the neurotoxicity section below) (Klimisch 1, reliable without restriction).
- U.S. EPA 2011
  - *Oral*: In a subchronic toxicity study male Wistar rats (15/group) received n-butyl alcohol in drinking water at 0 or 8,200 mg/kg/day for 3 months. Toxicological evaluation was limited to electron microscopy of liver sections. Exposed animals had poor appetite, weakness and loss of body weight (no data were provided). Hepatic mitochondria were smaller with poorly developed cristae after 2 months of exposure. After 3 months, mitochondria were enlarged with little or no cristae, and some were cup-shaped or elongated. In addition, proliferation of smooth ER and increased numbers of lysosomes and microbodies were detected in the liver. U.S. EPA did not identify an effect level for this study due to limited parameters examined.
  - *Oral:* In another drinking water study focused on effects on protein synthesis in the brain, male Wistar rats (3/group) received n-butyl alcohol at 1% during the first week, 2% during the second week and 4% for up to 4 months, corresponding to a time-weighed concentration of 3.7% (4,400 mg/kg/day calculated by U.S. EPA). Only mortality, body weight, selected serum chemistry parameters, and *in vitro* protein synthesis of brain extract were examined. There were no mortalities, and body weight gain of treated animals was 46% of controls after 3 months. Signs of severe pathology were identified in several tissues, especially liver and kidney (no additional details provided) in treated animals, and they had statistically significantly higher total protein and albumin levels. U.S. EPA concluded that the information provided was inadequate to establish an effect level.

- ECETOC 2003, ECHA, CAS #71-36-3, 2023
    - *Dermal:* In a 21-day short-term repeated dose dermal toxicity study, undiluted n-butyl alcohol was applied to the unabraded skin of rabbits (n=2) under occlusion for 5 hours per exposure with 12 exposures over the study period. There were no treatment related effects reported on mortality, clinical signs of toxicity, and gross pathology. Local irritation was reported as slight transient erythema. Histopathology findings reported drying of the skin, cracking, furrowing and exfoliation of the epidermis. No further details were reported. Reported data are insufficient to establish effect levels (Klimisch 2, reliable with restrictions).
- OECD 2001
    - *Dermal:* In another dermal study, application of 42 to 55 ml/kg n-butyl alcohol to the skin of rabbits, each day for 1 to 4 consecutive days, resulted in 100% mortality. However, 30 applications of 20 ml/kg (equivalent to 16,196 mg/kg[10]) over a period of six weeks did not produce any fatalities.
- OECD 2001, ECETOC 2003, ECHA, CAS #71-36-3, 2023
    - *Inhalation:* In the previously described non-GLP behavioural peri-, post-natal developmental neurotoxicity study, male and female Sprague-Dawley rats (15 females/group, 18/males/group) were exposed to inhalation doses of 3,000 or 6,000 ppm (equivalent to 9.2 mg/L or 18.5 mg/L as identified by ECETOC) n-butyl alcohol (> 99% purity as reported by OECD). Females were exposed throughout gestation and males were exposed for six weeks prior to mating. No detectable treatment-related effect on parental toxicity was found up to 6,000 ppm. The study authors identified a NOAEC of 6,000 ppm, the highest dose tested (equivalent to 18.5 mg/L as identified by the authors of the ECHA dossier) for systemic toxicity (Klimisch 2, reliable with restrictions).
- ECHA, CAS #71-36-3, 2023
    - *Inhalation:* In a non-GLP compliant sub-chronic inhalation toxicity study, male Wistar rats were exposed to n-butyl alcohol at a vapor concentration of 320 mg/m^3 (equivalent to 0.320 mg/L as calculated by ToxServices) for 5 h/day, 5 days/week for 3 months. Rats were evaluated for body weight and weight changes, food consumption and intake, clinical biochemistry, and organ weights. No significant treatment related effects were reported for body weight and body weight gain, protein content of liver microsomes and glutathione sulfhydryls (GSH) levels, and liver organ weight. Cytochrome P450 was induced in liver microsomes with prolonged exposure. No further information was provided (Klimisch 2, reliable with restrictions). *As it is not clear if liver cytochrome P450 induction is toxicologically significant, a NOAEC/LOAEC could not be assigned for this study.*
- U.S. EPA 2011
    - *Inhalation:* In a subchronic toxicity study, male Wistar rats (12/exposure group, 24 controls) were exposed to n-butyl alcohol at vapor concentrations of 0, 50, or 100 ppm (equivalent to 0, 154, or 308 mg/m^3, respectively, as calculated by the U.S. EPA) for 6 h/day, 5 days/week for 3 months. All rats survived to the end of the study without clinical abnormalities. There were no significant changes in mean body weight and absolute and relative organ weights. There was a statistically significant decrease in erythrocyte counts (16%) at the highest dose and in hemoglobin level (10%) in both exposure groups, but hematocrit was not changed. There were statistically significantly increased leukocyte counts and in the percentage of eosinophils at the highest dose. In addition, lipid peroxidation (16 and 30%) was statistically significantly increased in the liver in both exposure groups. Although decreased

[10] To convert ml/kg to mg/kg = (ml/kg) × (density of the compound (g/cm^3) × (1000). For n-butanol: density of the compound = 0.8098 g/cm^3.

hemoglobin and increased lipid peroxidation were detected at 154 mg/m^3, the U.S. EPA did not consider the changes to be biologically relevant. U.S. EPA established the NOAEC and LOAEC of 154 and 308 mg/m^3, respectively, based on increased leukocyte count. The authors of REACH dossier considered this study as unreliable due to major methodological deficiencies and assigned a Klimisch score of 3 (ECHA, CAS #71-36-3, 2023).

- *Inhalation:* Guinea pigs (> 3/group, sex and number not specified) were exposed to n-butyl alcohol vapor at 300 mg/m^3 every day for 2 weeks (exposure time not specified) and then 4h/day , 6 days/week for 1 – 2.5 months. Two control groups were also used, including one sham-treated and one untreated group. In animals treated for 64 exposures, decreased erythrocyte and lymphocyte counts were detected. Two of the three animals in this group had hemorrhagic areas in the lungs and transient albuminuria. A second group tested at this concentration developed severe skin infections after 30th exposure and two died during the 38th exposure. These animals had decreased erythrocytes and hemoglobin and increased total leukocytes. The surviving animal gained weight with improved blood parameters at sacrifice. The authors indicated that all three animals in this group had toxic degeneration of the livers and kidneys. The third group was maintained for 28 days, and similar changes in hematology were detected, along with central liver and marked renal degeneration. In the two control groups, one animal each died of skin infection. The U.S. EPA established a LOAEC of 300 mg/m^3 (equivalent to 0.3 mg/L/day x 6 days/7 days = 0.26 mg/L) based on decreased erythrocyte count and hemoglobin, and histopathological changes in the liver and kidney. *ToxServices notes that many study details are missing, which preclude a determination of the actual daily exposure concentration.*
- *Inhalation:* In a 4-month study, rats and mice were exposed to n-butyl alcohol at 0, 0.8, 6.6 or 40 mg/m^3 for 4 months (exposure duration and frequency not specified). Increased thyroid gland activity occurred in all groups. The pituitary-adrenal system was disrupted, eosinophilic response following administration of adrenocorticotropic hormone was reduced, and oxygen demand was reduced in the "cold test" at 6.6 and 40 mg/m^3. The authors concluded that a NOAEC and LOAEC of 0.8 and 6.6 mg/m^3, respectively could be established for this study.[11] However, the U.S. EPA considered information in this publication inadequate for the purpose of identifying effect levels.

- ECHA 2018, ECHA, CAS #71-36-3, 2023
  - *Inhalation: Surrogate: n-Butyl acetate (CAS #123-86-4):* A GLP-compliant repeated exposure toxicity test conducted according to EPA OTS 798.2450 was performed with Sprague-Dawley rats (15/sex/concentration group) administered whole-body inhalation exposures of butyl acetate vapor (at least 99.9% purity) at nominal concentrations of 0, 500, 1,500, or 3,000 ppm (equivalent to 0, 2.4, 7.2, and 14.4 mg/L, respectively, as identified by the authors of the ECHA dossier) for 6 hours/day, 5 days/week for 13 weeks. The equivalent nominal concentrations for a 7-day/week exposure frequency were 0, 1.7, 5.1, and 10.3 mg/L, respectively. The animals were evaluated for clinical signs of toxicity, body weight, food consumption, hematology, clinical chemistry, gross pathology, and histopathology. Decreased activity was reported in the mid and high concentration groups. High dose animals also exhibited decreased alertness and slower response to stimuli. Body weights and food consumption were significantly reduced in the mid and high concentration groups. No significant treatment-related effects were measured on hematology or clinical chemistry parameters. Mean absolute liver and spleen weights were significantly decreased in mid and high concentration males and females, and decreased mean absolute kidney

[11] 0.8 mg/m^3 x 1 m^3/1000 L = 0.0008 mg/L
40 mg/m^3 x 1 m^3/1000 L = 0.04 mg/L

weights were measured in mid concentration females and high concentration males and females. Relative spleen weights were decreased for high concentration males, mean relative testes weight was increased for mid and high concentration males, and mean relative lung weight was increased for high concentration males. Mean relative adrenal gland weight was increased for mid concentration females and high concentration males and females. No gross pathological lesions were found in males with treatment. Two high concentration females exhibited minimal hemorrhage of the glandular stomach and white discoloration in the non-glandular stomach. All high concentration males and females and 4/10 males and 6/10 females in the mid concentration group exhibited necrosis of the olfactory epithelium that was mild for the mid concentration group and mild to moderate for the high concentration group. The study authors identified a NOEC of 500 ppm (equivalent to 1.7 mg/L/day) based on epithelial necrosis reported at 5.1 and 10.3 mg/L (Klimisch 1, reliable without restriction).

- *Inhalation: Surrogate: n-Butyl acetate (CAS #123-86-4):* In a GLP-compliant subchronic inhalation neurotoxicity study in rats, Sprague-Dawley rats (10/sex/dose) were exposed to n-butyl acetate vapor at 0, 500, 1,500, or 3,000 ppm for 6 hours/day, 5 days/week for 13 weeks. Animals at each concentration were separated into two groups, one group was fed food ad libitum and another group received restricted amount of food. Mean body weight of animals at the high dose were 15-19% lower than that of control animals, and the body weight gain was 65% and 59% of males and females in control group. The mean body weight in the mid dose group was 9% lower than that of the control group. The body weight of the low dose group was comparable to controls. No treatment-related effects on mortality were found and there were no effects on gross pathology or histology [including brain, spinal cord (cervical and lumbar regions), dorsal and ventral spinal roots, dorsal root ganglia, sciatic nerve, and tibial nerve]. A neurobehavioral exam was performed, the results of which are discussed below under repeated exposure neurotoxicity. The study authors established NOECs of 500 ppm and 3,000 ppm for systemic toxicity and subchronic neurotoxicity, respectively, based on effects on body weight and weight gain and clinical signs, and based on a lack of neurotoxicity reported (Klimisch 1, reliable without restriction). *Based on decreased body weight, ToxServices established the NOAEC and LOAEC at 500 and 1,500 ppm, which are equivalent to 1.7 and 5.1 mg/L/6h/day n-butyl acetate, respectively, as calculated by ToxServices in the study summary above.*

- David et al. 1998, 2001
  - *Inhalation: Surrogate: n-Butyl acetate (CAS #123-86-4):* In a GLP-compliant two-week inhalation probe study in rats, Sprague-Dawley rats (10/sex/dose) were exposed to n-butyl acetate vapor (99.9% pure) at 0, 750, 1,500 or 3,000 ppm for 6 hours/day, 6 days/week. Half of the animals at each dose were given food ad libitum while the other half were feed-restricted. Clinical observation, feed consumption and body weight were monitored periodically. Neurological examinations were performed, which are discussed below under neurotoxicity endpoint. At study termination, lungs, liver, and kidneys were weighed and gross pathology was performed. There was a statistically significant decrease in mean body weight in feed-restricted males at the 3,000 ppm dose on Day 14, compared to control rats. No other statistically significant, treatment-related (non-neurological) changes were reported. *Based on reduced body weight in males, ToxServices established the systemic toxicity LOAEC and NOAEC at 3,000 and 1,500 ppm, respectively, which are equivalent to 10.3 and 5.1 mg/L/6h/day n-butyl acetate, respectively, after adjustment for the less than daily exposure frequency.*

- n-Butyl alcohol produced a NOAEL of 125 mg/kg/day based on reduced hematocrit, erythrocyte count and hemoglobin content in females and transient neurotoxicity effects reported for the high-dose group in a subchronic repeated oral toxicity test in rats. Two additional studies were identified for n-butyl alcohol but the U.S. EPA did not consider them to be reliable due to inadequate study details provided or limited parameters evaluated. Based on the NOAEL of 125 mg/kg/day for n-butyl alcohol in a 13-week study, ToxServices did not classify it as a specific target organ toxicant following repeated oral doses under GHS criteria (UN 2021). Insufficient dermal data are available to assign a GHS classification for this route. The available studies on inhalation exposure, which is a relevant route of concern for this chemical, were performed decades ago and/or were non-GLP compliant. They were judged not appropriate to identify effect levels for risk assessments by U.S. EPA (2011) and ECHA (2018), assigned Klimisch score of 3 (not reliable) by REACH dossier authors, or missing critical information to determine the NOAEC/LOAEC. Therefore, data on the surrogate chemical n-butyl acetate were considered. These studies were all conducted under GLP and are therefore more robust than the n-butyl alcohol studies. The lowest LOAEC/LOEC identified for n-butyl acetate was 500 ppm in 90-day studies, which is equivalent to 1.7 mg/L/6h/day n-butyl acetate and above the GHS Category 2 cut-off value of 1 mg/L/6h/day for vapor. Transient CNS effects were identified in most of the studies and evaluated further under repeated dose neurotoxicity (below). Because adequate data on the surrogate n-butyl acetate were available, the GHS - Japan Specific target organs/systemic toxicity Category 1 classification was not used for hazard assignment.

Neurotoxicity (single dose, N-single) (Group II) Score (vH, H, M, or L): M
n-Butyl alcohol was assigned a score of Moderate for neurotoxicity (single dose) based on association with the EU-GHS authoritative list of H336 (transient narcotic effect) which corresponds to GHS Category 3 supported by measured data that showed acute transient narcotic effects following oral and inhalation exposures. GreenScreen® criteria classify chemicals as a Moderate hazard for neurotoxicity (single dose) when they are classified to GHS Category 3 for neurotoxicity single exposure. The authoritative EU-GHS authoritative list of H336 correspond to a score of Low to Moderate (CPA 2018b). The confidence in the score is high as it is based on experimental data supported by an authoritative listing.

- Authoritative and Screening Lists
  - *Authoritative:* 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].
  - *Screening:* Malaysia – H336 – May cause drowsiness or dizziness [Specific target organ toxicity – single exposure; Narcotic effects – Category 3].
  - *Screening:* GHS – Australia – H336 – May cause drowsiness or dizziness [Specific target organ toxicity – single exposure; Narcotic effects – Category 3].
  - *Screening:* GHS – Japan – H335 or H336 [Specific target organ toxicity – single exposure; Category 3].
  - *Screening:* GHS – New Zealand – Specific target organ toxicity – single exposure; Narcotic effects – Category 3].
- OECD 2001
  - *Oral*: The oral dose that caused narcosis in 50% of the animals was 800 mg/kg in rabbits.
  - *Inhalation*: A 4-hour inhalation EC_{50} of 6,530 ppm for decreased rotarod performance was reported in Wistar rats
  - *Inhalation:* Signs of CNS depression were evident after 2 hours of exposure to 6,600 ppm n-butyl alcohol (species not reported).
- ECHA, CAS #71-36-3, 2023

- *Oral*: In the previously described oral toxicity study (GLP-unspecified), male and female Osborne-Mendel rats (5/sex/dose) were exposed to n-butyl alcohol (purity unspecified) via gavage and observed for 14 days. The study authors established an LD_{50} of 2,510 mg/kg. Limited details were provided. An unknown number of deaths and clinical signs of depression and coma (unknown dose levels) were reported within 4-18 hours after exposure, and no data were provided on gross pathology (Klimisch 2, reliable with restrictions).
- *Oral*: In the previously described non-GLP-compliant oral toxicity study conducted in a manner similar to OECD Guideline 401, female Sherman rats (10/dose) were exposed to a single dose of 3,160, 3,980, 5,000, or 6,300 n-butyl alcohol (purity unspecified) via gavage and observed for 14 days. The study authors established an LD_{50} of 4,360 mg/kg. Mortalities were reported as 0/10, 3/10, 8/10, and 10/10 at 3,160, 3,980, 5,000, and 6,300 mg/kg, respectively. Clinical signs of narcosis and prostration occurring before death were reported; however, specific doses in which these clinical signs occurred were not specified. Furthermore, gross pathology findings of higher dose animals included hemorrhage of the stomach, intestinal irritation, congested livers, and pale kidneys (Klimisch 2, reliable with restrictions).
- *Inhalation:* In the previously described non-GLP-compliant inhalation study conducted in a manner similar to OECD Guideline 403 in male and female Sprague-Dawley rats exposed to n-butyl alcohol vapors via inhalation, a 4h LC_0 of > 17.76 mg/L (vapor) was identified. Animals (10/sex/dose) were exposed to 6.58 and 17.76 mg/L by whole body inhalation and observed for 14 days. There were no treatment related mortalities, clinical signs, and gross pathology findings (Klimisch 2, reliable with restrictions).
- *Inhalation*: In the previously described non-GLP-compliant inhalation study in male and female Sprague-Dawley rats exposed to n-butyl alcohol vapors via inhalation, a 7h LC_0 of > 21.48 mg/L (vapor) was identified. Animals (6/sex/dose) were exposed to 21.48 mg/L by whole body inhalation and observed for 14 days. There were no treatment related mortalities or gross pathology findings. Clinical signs were reported including accelerated and intermittent breathing and irritating effects during the exposure. No information was provided on body weights (Klimisch 2, reliable with restrictions).
- *Inhalation*: In the previously described non-GLP-compliant inhalation study conducted in a manner similar to OECD Guideline 403 in male and female Sherman rats exposed to n-butyl alcohol vapors via inhalation, a 4h LC_0 of > 8,000 ppm (vapor) (calculated as > 24 mg/L by the authors of the ECHA dossier) was identified. Animals (6/exposure, females for the first and males for the second) were exposed to a substantially saturated vapor in the first exposure and 8,000 ppm in the second exposure for 4 to 8 hours by whole body inhalation and observed for 14 days. There were no treatment related mortalities. Clinical signs were reported for both exposure groups including poor coordination and prostration at the end of exposure. No information was provided for gross pathology (unidentified) (Klimisch 2, reliable with restrictions).

- ECHA, CAS #123-86-4, 2023
  - *Oral: <u>Surrogate: n-Butyl acetate (CAS #123-86-4):</u>* In a non-GLP-compliant oral acute toxicity study conducted in a manner similar to OECD Guideline 423 with male and female Sprague-Dawley rats exposed to surrogate n-butyl acetate, the study authors identified LD_{50} values of 10,760 mg/kg and 12,789 mg/kg for females and males, respectively (calculated by the authors of the ECHA dossier). Clinical signs of sluggishness and prostration were reported at all doses. No treatment-related effects were measured on body weight. At necropsy, no dose-related effects were found in those animals that survived to the scheduled sacrifice but those that died during the course of the observation period exhibited gas-filled

stomachs that were tan to red and livers that were pale tan in color on the ventral surface. The lowest dose tested in this study was 9,966 mg/kg (Klimisch 2, reliable with restrictions).

- ECETOC 2003
  - CNS depression was reported with n-butyl alcohol administration and it is frequently recorded with most organic solvents at high levels. Additional data from well-designed neurotoxicity studies with n-butyl acetate which is rapidly cleaved into n-butyl alcohol do not indicate selective neuro- or CNS-related toxicity, but there were transient signs of reduced general activity at airborne levels of 1,500 and 3,000 ppm. In a well-designed developmental study following prenatal exposure of rats to n-butyl alcohol, no behavioral effects on the offspring were found. Therefore, it was concluded that n-butyl alcohol does not show selective or cumulative neurotoxicity in experimental animals.
- ECHA 2018
  - Due to the transient effects seen on the CNS (drowsiness and dizziness) in the above studies, ECHA concluded that the current EU-GHS harmonized classification of n-butyl alcohol as STOT Single Exposure Category 3 with a hazard statement of H336 "May cause drowsiness or dizziness" is appropriate.

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

n-Butyl alcohol was assigned a score of Low for neurotoxicity (repeated dose) based on inhalation NOAECs at up to 3,000 ppm (equivalent to 10.3 mg/L/6h/day) in subchronic animal studies on the surrogate, n-butyl acetate. GreenScreen® criteria classify chemicals as a Low hazard for neurotoxicity (repeated dose) when adequate and negative data are available, and they are not GHS-classified (i.e., subchronic inhalation LOAEC values greater than 1.0 mg/L/6h/day for vapor) (CPA 2018b). The confidence in the score is high as it is based on well-conducted studies on a strong analog.

- Authoritative and Screening Lists
  - *Authoritative:* Not listed on any authoritative lists for this endpoint.
  - *Screening:* GHS – Japan: Specific target organs/systemic toxicity following repeated exposure – Category 1.
    - Based on dizziness, headache, and hearing loss identified under occupational exposure settings (target organ: CNS and auditory organ) (NITE 2013).
- U.S. EPA 2011, RTI 1985, OECD 2001, ECETOC 2003, ECHA, CAS #71-36-3, 2023
  - *Oral:* In the previously described GLP-compliant subchronic toxicity study, Sprague-Dawley rats (30/sex/dose) received gavage doses of n-butyl alcohol (purity not specified) in water at 0, 30, 125, or 500 mg/kg/day for 13 weeks. There were no treatment-related effects on body weight or organ weight changes, food consumption, mortality, gross pathology, histopathology and ophthalmic evaluations. Transient ataxia and hypoactivity were detected 2-3 minutes after dosing of males and females in the high dose group during the last 6 weeks of treatment. U.S. EPA and REACH dossier authors assigned the NOAEL and LOAEL at 125 and 500 mg/kg/day, respectively, based on reduced hematocrit, erythrocyte count and hemoglobin content in females and transient clinical signs of CNS depression, respectively (Klimisch 1, reliable without restriction).
- U.S. EPA 2011
  - *Inhalation:* In the previously described 4-month study, rats and mice were exposed to n-butyl alcohol at 0, 0.8, 6.6 or 40 mg/m^3 for 4 months (exposure duration and frequency not specified). At 6.6 and 40 mg/m^3, narcosis induced by n-butyl alcohol was shortened, and conditioned reflex activity increased. Other changes included a loss of CNS summation capacity (an electrophysiological measurement of nerve response) and an increase in blood cholinesterase activity in rats. No further details were provided. The study authors

concluded that a NOAEC and LOAEC of 0.8 and 6.6 mg/m^3 (equivalent to 0.0008 and 0.0067 mg/L as calculated by ToxServices) could be established for this study. However, the U.S. EPA considered information in this publication inadequate for the purpose of identifying effect levels.

- ECETOC 2003
  - The CNS depression detected with n-butyl alcohol administration is frequently recorded with most organic solvents at high levels. Additional data from well-designed neurotoxicity studies with n-butyl acetate which is rapidly cleaved into n-butyl alcohol do not indicate selective neuro- or CNS-related toxicity, but there were transient signs of reduced general activity at airborne levels of 1,500 and 3,000 ppm. In a well-designed developmental study following prenatal exposure of rats to n-butyl alcohol, no behavioral effects on the offspring were identified. Therefore, it was concluded that n-butyl alcohol does not show selective or cumulative neurotoxicity in experimental animals.
  - In the occupational setting, chronic exposure of workers to high concentrations of n-butyl alcohol vapor was associated with CNS effects and hearing losses. However, these results must be interpreted with caution due to deficiencies in documentation and methodology of the studies. Therefore, no definitive conclusion could be drawn based on available data.
- ECHA, CAS #71-36-3, 2023, U.S EPA 2011, ECETOC 2003, OECD 2001
  - In the previously described subchronic toxicity study, male Wistar rats (12/exposure group, 24 controls) were exposed to n-butyl alcohol at vapor concentrations of 0, 50 or 100 ppm for 6 h/day, 5 days/week for 3 months. U.S. EPA calculated the equivalent doses of 0, 154 and 308 mg/m^3, respectively. Neuromuscular function (rotarod performance tests), learned avoidance behavior, and latency of the paw-lick response (hot-plate behavior) were examined. There were dose- and duration-related increases in the percentage of rotarod test failures but no effects on pain sensitivity (assessed by hot-plate behavior), and only the increased failure rates at the high dose were statistically significant. U.S. EPA identified the NOAEC and LOAEC at 154 and 308 mg/m^3, respectively, based on increased percentage of rotarod test failures. After adjustment for treatment frequency (i.e., 5 days/week to 7 days/week), ToxServices calculated the NOAEC and LOAEC to be 110 and 220 mg/m^3 (equivalent to 0.11 and 0.22 mg/L), respectively. The rotarod test is a test of motor function rather than memory or leaning that incorporates both central and peripheral nervous system components. The animal's performance on a rotarod was evaluated prior to and at monthly intervals during the study. The U.S. EPA indicated that there are insufficient details presented by the authors on the rotarod test, such as number of rotarod trials per animal. ECHA dossier authors and OECD assigned a Klimisch score of 3 (not reliable) for this study based on documentation insufficiency. ECETOC concluded from this study that n-butyl alcohol caused a moderate disturbance to co-ordination performance, which is frequently reported as an unspecific solvent-related CNS effect. As such, these results did not indicate neurotoxicity in the sense of irreversible CNS or peripheral nervous system impacts. Further, the poorer performance on rotarod test in this study is questionable as the same authors identified the EC50 of more than 7,500 ppm for this test with a 4-h exposure to n-butyl alcohol (Korsak and Rydzynski 1994), and it could be deduced from this publication that exposure to concentrations of 2,500 ppm did not produce effects (MAK 2003). In the Korsak et al. publication (1994), the study authors claimed to have trained the rats on the rotarod using the procedure of Kaplan and Murphy (1972), which described the challenge with this method including the constant daily training of the animals on the device. Korsak and colleagues did not maintain the training of the animals, but simply tested them every month without re-acclimating them to the rotarod method, which was inconsistent with the test

implementation method recommended by the inventors of this method (i.e., Kaplan and Murphy). For this reason (improper use of the method), this study was considered invalid by the neurotoxicologists at the U.S. EPA Health Effects Research Laboratory. As the validity of this study is questionable, along with the other identified studies, data on the surrogate n-butyl acetate were therefore evaluated to support the safety of n-butyl alcohol.

- ECHA, CAS #71-36-3, 2023
    - *Inhalation: Surrogate: n-Butyl acetate (CAS #123-86-4)*: A GLP-compliant repeated exposure toxicity test conducted according to EPA OTS 798.2450 was performed with Sprague-Dawley rats (15/sex/concentration group) administered whole-body inhalation exposures of butyl acetate (at least 99.9% purity) vapor at 0, 500, 1,500, or 3,000 ppm (equivalent to 0, 2.4, 7.2, and 14.4 mg/L, respectively, as identified by the authors of the ECHA dossier) for 6 hours/day, 5 days/week for 13 weeks. The equivalent concentrations for a 7-day/week exposure frequency were 0, 1.7, 5.1, and 10.3 mg/L, respectively. Acute but transient signs of reduced activity were reported at mid (minimal severity) and high doses (minor severity), demonstrated by less movement, decreased alertness and slower response to tapping on the chamber wall. These effects were also reported in the 13-week inhalation neurotoxicity study conducted by the same laboratory below, and the results are discussed in more details (David et al. 2001) (Klimisch 1, reliable without restriction).
    - *Inhalation: Surrogate: n-Butyl acetate (CAS #123-86-4)*: In a GLP-compliant subchronic inhalation neurotoxicity study in rats, Sprague-Dawley rats (10/sex/dose) were exposed to n-butyl acetate vapor at 0, 500, 1,500 or 3,000 ppm for 6 hours/day, 5 days/week for 13 weeks. Animals at each concentration were separated into two groups, one group was fed food ad libitum and designated for functional observation battery, motor activity and neuropathology endpoints (FOB/MA/NP), and another group received restricted amount of food and was designated for schedule-controlled operant behavior (SCOB). At study termination, neurohistopathology was evaluated on randomly selected animals of control and high dose groups for the examination of brain (including forebrain, cerebrum, midbrain, cerebellum, pons, medulla oblongata), spinal cord swellings with dorsal and ventral roots (cervical and lumbar), dorsal root ganglia (cervical and lumbar), sciatic nerve (both hind limbs at mid-thigh and sciatic notch), and tibial nerve (both hind limbs including branches to the calf musculature). Acute but transient signs of reduced activity were reported at mid (minimal severity) and high doses (minor severity), demonstrated by less movement, decreased alertness and slower response to tapping on the chamber wall. No treatment-related effects were reported in FOB/MA/PA or SCOB. The study authors concluded that repeated exposure to n-butyl acetate vapors led to an acute, transient reduction of activity level on a daily basis at the mid and high doses, but no cumulative effects on activity were reported. Therefore, the authors established the NOEC for subchronic neurotoxicity at 3,000 ppm based on lack of cumulative neurotoxicity following repeated exposure. This is equivalent to 10.3 mg/L/6h/day n-butyl acetate as described above (David et al. 1998, 2001) (Klimisch 1, reliable without restriction).
- Adequate data (i.e., inclusion of a functional observation battery) are only available for the inhalation route of exposure for the surrogate n-butyl acetate. While narcotic effects were reported in an oral OECD Guideline 402 study in rats for n-butyl alcohol, they were transient and consistent with those reported in acute toxicity studies, which have been used to classify n-butyl alcohol to GHS Category 3 for single exposure neurotoxicity. The David et al. (1998) inhalation neurotoxicity study comprehensively evaluated the neurotoxicity of n-butyl acetate and did not identify any adverse effects at the highest dose of 3,000 ppm (equivalent to 6.48 mg/L/6h/day n-butyl alcohol). The David et al. (1998, 2001) inhalation neurotoxicity studies comprehensively evaluated the

neurotoxicity of n-butyl acetate and did not identify any cumulative effects at the highest dose of 3,000 ppm (equivalent to 10.3 mg/L/6h/day n-butyl acetate). Because adequate data on the surrogate n-butyl acetate were available, the GHS-Japan Specific target organs/systemic toxicity Category 1 classification was not used for hazard assignment.

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

n-Butyl alcohol was assigned a score of Low for skin sensitization based on negative results in an OECD Guideline 429 assay in mice, and in *in vitro* assays (OECD Guideline 442C equivalent *in chemico* Skin sensitization: Direct peptide reactivity assay (DPRA) and an OECD Guideline 422D equivalent *in vitro* Skin sensitization: Antioxidant response element (ARE)-Nrf2 luciferase test) for the target chemical. GreenScreen® criteria classify chemicals as a Low hazard for skin sensitization when adequate data are available and negative, and they are not GHS-classified (CPA 2018b). The confidence in the score is high as it is based on reliable measured data on the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not listed on any authoritative lists for this endpoint.
  - *Screening:* Not listed on any screening lists for this endpoint.
- OECD 2001
  - Although there are no animal data available, human studies and experience with n-butyl alcohol indicate that it is not likely to be a skin sensitizer.
- ECHA, CAS #71-36-3, 2023
  - In a GLP-unspecified local lymph node assay (LLNA) conducted in a manner similar to OECD Guideline 429, female CBA mice (4/Labs B and C, 5/Lab A) were administered 25 µL of 5, 10 or 20 % of n-butyl alcohol (purity unspecified) in distilled water to the dorsal skin of the ears for three consecutive days. The study was performed in triplicate with individual mouse samples harvested at Lab A and experimental group samples harvested together at Labs B and C. Stimulation indices (SI) based on the increase in 3H-methylthymidine (3H-TdR) compared to controls were reported to be 1.6, 1.2, and 1.4 for the 5%, 10%, and 20% dose groups, respectively. The study authors concluded n-butyl alcohol is a non-sensitizer based on SI values < 3 (Klimisch 1, reliable without restriction).
  - n-Butyl alcohol was evaluated in a GLP-unspecified *in chemico* skin sensitization: DPRA conducted in a manner similar to OECD Guideline 442C. n-Butyl alcohol (purity unspecified) in acetonitrile was evaluated with both lysine and cysteine and resulted in an overall mean peptide depletion of -0.3%. Under OECD Guideline 442C,[12] chemicals with ≤ 6.38% mean cysteine and lysine depletion are considered to have no or minimal reactivity and are not sensitizing. Study authors concluded that n-butyl alcohol was not a dermal sensitizer under the tested conditions due to "no to minimal" reactivity (Klimisch 2, reliable with restrictions).
  - n-Butyl alcohol was evaluated in a GLP-unspecified *in vitro* Skin sensitization: ARE-Nrf2 luciferase test conducted in a manner similar to OECD Guideline 442D. The KeratinoSens™ test system was exposed to a single application of 12 concentrations of 0.1 mM to 200 mM of n-butyl alcohol (purity unspecified) in DMSO and performed in triplicates. The negative and positive controls were reported as valid. No concentration-induced luciferase activity above the threshold of EC1.5 in any replicate was reported. Under OECD Guideline 442D,[13] chemicals that do not produce a 1.5-fold increase in luciferase activity over the solvent control are not sensitizing. Study authors concluded that

[12] https://www.oecd-ilibrary.org/environment/test-no-442c-in-chemico-skin-sensitisation_9789264229709-en
[13] https://www.oecd-ilibrary.org/environment/test-no-442d-in-vitro-skin-sensitisation_9789264229822-en

n-butyl alcohol was not a dermal sensitizer under the tested conditions (Klimisch 2, reliable with restrictions).

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

n-Butyl alcohol was assigned a score of Low for respiratory sensitization based on lack of structural alerts for respiratory sensitization and negative skin sensitization data, according to ECHA (2017)'s recommended strategy on evaluation of respiratory sensitization. GreenScreen® criteria classify chemicals as a Low hazard for respiratory sensitization when adequate data are available and negative studies, and 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 listed on any authoritative lists for this endpoint.
  - *Screening:* Not listed on any screening lists for this endpoint.
- OECD 2001
  - Although there are no animal data available, human studies and experience with n-butyl alcohol indicate that it is not likely to be a skin sensitizer.
- OECD 2022
  - n-Butyl alcohol does not contain any structural alerts for respiratory sensitization as identified by OECD QSAR Toolbox v 4.5 SP1 (Appendix J).
- Based on the weight of evidence and guidance from ECHA regarding assessment of respiratory sensitization potential, a score of Low was assigned. The guidance from ECHA states that the mechanisms leading to respiratory sensitization are essentially similar to those leading to skin sensitization (ECHA 2017). ECHA recommended that if a chemical is not a dermal sensitizer based on high quality data, it is unlikely to be a respiratory sensitizer. ECHA also noted that this rationale does not cover respiratory hypersensitivity caused by non-immunological mechanisms, for which human experience is the main evidence of activity (ECHA 2017). As n-butyl alcohol 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-butyl alcohol, and as n-butyl alcohol does not contain any structural alerts for respiratory sensitization (OECD 2022, see Appendix J), n-butyl alcohol is not expected to be a respiratory sensitizer.

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

n-Butyl alcohol was assigned a score of High for skin irritation/ corrosivity based on association with the EU-GHS authoritative list of H315 supported by experimental data (severe irritation after 1 hour up to 24 hours in two non-guideline dermal irritation assays in rabbits) and screening lists. GreenScreen® criteria classify chemicals as a High hazard for skin irritation when associated with EU-GHS hazard statement of H315 and when they are classified to GHS Category 2 (CPA 2018b). The confidence in the score is high as it is based on an authoritative listing and measured data.

- Authoritative and Screening Lists
  - *Authoritative:* EU – GHS (H-Statements) – Annex 6 Table 3-1: H315: Causes skin irritation – Category 2.
  - *Screening:* GHS -– Japan: H315: Causes skin irritation (Skin corrosion/irritation – Category 2).
  - *Screening:* GHS – New Zealand: Skin irritation Category 2.
  - *Screening:* GHS – Malaysia – GHS – H315 – Causes skin irritation (Skin corrosion/irritation - Category 2).

  - *Screening:* GHS – Australia – GHS – H315 – Causes skin irritation (Skin corrosion/irritation – Category 2).
- OECD 2001
  - Available animal data showed that n-butyl alcohol was non-irritating to moderately irritating to skin. Moderate skin irritation was detected in a 24-hour patch test where 405 or 500 mg of n-butyl alcohol was applied to the skin of rabbits (no further details provided).
- ECHA, CAS #71-36-3, 2023
  - In a non-GLP-compliant dermal irritation assay, Vienna White rabbits (2/exposure, sex unspecified) were exposed to 0.5 mL undiluted n-butyl alcohol (purity unspecified) to the shaved skin under occlusive conditions for 5 minutes, 1 hour and 2 hours with an observation period of 8 days. There were no signs of toxicity reported. The mean erythema scores for animals 1 and 2 exposed for 2 hours were 2.7 and 4, respectively, with signs of superficial necrosis reported that persisted to day 8 (no information was provided for reversal after day 8). The mean edema scores for animals 1 and 2 exposed for 2 hours were both 2, with irritative effects persisting past day 8. The mean erythema scores for animals 1 and 2 exposed for 1 hour were 2.5 and 3, respectively, with signs of scaling, superficial necrosis and bloody crust which persisted to day 8 (no information was provided for reversal after day 8). The mean edema scores for animals 1 and 2 exposed for 1 hour were both 2, with irritative effects persisting to day 8 (no information was provided for reversal after day 8). Lastly, the mean erythema scores for animals 1 and 2 exposed for 5 minutes were both 1.7 with full reversal reported within 8 days. The mean edema scores for animals 1 and 2 exposed for 5 minutes were 0.7 and 1, respectively, with irritative effects cleared up by day 8. Furthermore, the study authors reported that no corrosion occurred for the full thickness of the skin (Klimisch 2, reliable with restrictions).
  - In a GLP-unspecified dermal irritation assay conducted according to Code of Federal Regulations, Title 16, Section 1500.41, New Zealand White rabbits (n=6, sex unspecified) were exposed to 0.5 mL undiluted n-butyl alcohol (purity unspecified) to the shaved or abraded skin under occlusive conditions for 24 hours with an observation period of 8 days. There were no signs of toxicity reported. The mean erythema score for all animals was 4. The mean edema score for all animals was 3.17. All irritative effects persisted to day 8 (no information was provided for reversal after day 8), and readings were only reported for shaved skin. Furthermore, the study authors reported that no corrosion occurred for the full thickness of the skin (Klimisch 2, reliable with restrictions).
  - In a GLP-compliant dermal irritation assay conducted according to OECD Guideline 404, three female New Zealand White rabbits were exposed to 0.5 mL undiluted n-butyl alcohol (purity unspecified), to the shaved, intact skin under semi-occlusive conditions for 4 hours with an observation period of 14 days. In the summary for the irritation/corrosion endpoints, the authors of the ECHA dossier indicate the test substance was a "pure substance". There were no signs of toxicity reported. One animal died during the study without symptoms or irritative effects and necropsy findings were unremarkable. The mean erythema and edema scores for all animals at 24 hours, 48 hours, and 72 hours were 0.56 with all irritative effects fully reversed by 14 days. The mean erythema and edema scores for 24, 48, and 72 hours, for animals 1, 2, 3 was 0, 0, and 1.67. No corrosion of skin tissue was reported (Klimisch 1, reliable without restriction). *Based on the results of this study, ToxServices did not classify the n-butyl alcohol as a skin irritant under GHS criteria. GHS criteria classify a chemical as Category 3 skin irritant when mean scores are ≥ 1.5 and < 2.3 for erythema/eschar in 2/3 animals following gradings at 24, 48, and 72 hours (UN 2021). Although GHS criteria also classify a chemical as a Category 2 chemical when there is inflammation that persists to the*

end of the observation period in 2/3 animals, particularly taking into account alopecia, hyperkeratosis, and scaling, ToxServices did not consider slight erythema, edema, with dry, rough, hardened skin in one animal sufficient to meet this criterion.

  - Additional studies in the REACH dossier did not add to, or provide inconsistent findings in terms of GHS classification for, the studies listed above. Therefore, only the key and supporting studies are included in this report.
- AICIS 2013
  - Available human data showed that n-butyl alcohol was irritating (edema) to the skin in a 20-minute patch test where 20 µL undiluted n-butyl alcohol was applied to the back under occlusion.
- ECHA 2018
  - Various experimental data were available for the skin irritation potential of n-butyl alcohol with four studies were performed in rabbits with different exposure conditions such as the duration of treatment, amount of applied test item, occlusive or semi-occlusive conditions. Based on the results from these studies, ECHA concluded that the current harmonized EU-GHS classification of Category 2 for n-butyl alcohol is appropriate.

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

n-Butyl alcohol was assigned a score of Very High for eye irritation based on association with the EU-GHS authoritative list of H318 supported by experimental data and screening lists. Although experimental data (two OECD Guideline 405 studies) in rabbits only support Category 2A classification, which is equivalent to a High score. GreenScreen® criteria classify chemicals as a Very High hazard for eye irritation when associated with EU -GHS hazard statement of H318, and when they are classified to GHS Category 1 (CPA 2018b). The confidence in the score is high as it is based on an authoritative listing and measured data.

- Authoritative and Screening Lists
  - *Authoritative:* EU – GHS (H-Statements) – Annex 6 Table 3-1 – H318 - Causes serious eye damage [Serious eye damage/eye irritation – Category 1].
  - *Screening:* GHS - Japan: H319 - Causes serious eye irritation (Serious eye damage/eye irritation – Category 2A).
  - *Screening:* GHS - New Zealand: Serious eye damage category 1.
  - *Screening:* GHS – Malaysia – H318 – Causes serious eye damage – Category 1.
  - *Screening:* GHS – Australia – H318 – Causes serious eye damage – Category 1.
- OECD 2001
  - The eye irritation potential of n-butyl alcohol was evaluated in rabbits. In studies in which 1.62 or 20 mg n-butyl alcohol was instilled into rabbit eyes, severe eye irritation occurred after 72 and 24 hours, respectively. In another study, 0.005 ml n-butyl alcohol instilled in rabbit eyes resulted in severe corneal irritation (no further details provided).
- ECHA, CAS #71-36-3, 2023
  - In a GLP-compliant eye irritation study conducted according to OECD Guideline 405, three New Zealand White rabbits (sex unspecified) were exposed to undiluted 0.1 mL undiluted n-butyl alcohol (purity unspecified) in the eye for 24 hours and observed for up to 7 days without rinsing. No clinical signs of toxicity were identified with treatment. The mean scores reported were 2.11 for cornea opacity, 1 for iritis, 2.89 for conjunctivae, and 3 for chemosis in all animals at 24, 48, and 72 hours. All irritative effects were not fully reversible within 7 days. At least 2 out of 3 animals had individual mean scores of at least 2 for corneal opacity, and greater than 1 for iritis in 2/3 animals following gradings at 24, 48, and 72 hours (Klimisch 1, reliable without restriction). *ToxServices notes a lack of*

information on the reversal of irritation up to 21 days. Irritation effects were not fully reversed by Day 7. However, based on the GHS criteria which classifies a chemical as an ocular irritant when mean scores are at least 1 for corneal opacity and/or iritis, and/or 2 for conjunctival redness and/or chemosis in 2/3 animals following gradings at 24, 48, and 72 hours, n-butyl alcohol meets classification criteria for Categories 2A/2B (UN 2021).

- In a GLP-compliant eye irritation study conducted according to OECD Guideline 405, four New Zealand White rabbits (sex unspecified) were exposed to undiluted 0.1 mL n-butyl alcohol (purity unspecified) in the eye for 24 hours and observed for up to 21 days without rinsing. No clinical signs of toxicity were reported. The mean scores reported were 2 for cornea opacity, 0.75 for iritis, 2.42 for conjunctivae, and 2.08 for chemosis in all animals at 24 hours, 48 hours, and 72 hours. All irritative effects were fully reversible within 21 days; however, only iris irritation was cleared by Day 7. Additionally, all four animals had individual mean cornea scores greater than 1, conjunctiva redness scores greater than 2, and chemosis scores greater than 2 (Klimisch 1, reliable without restriction). *Based on the results of this study, ToxServices classified n-butyl alcohol as a GHS Category 2A ocular irritant under GHS criteria. GHS criteria classify a chemical as a GHS Category 2 ocular irritant when mean scores are at least 1 for corneal opacity, at least 1 for iritis, at least 2 for conjunctival redness, and/or at least 2 for chemosis in 2/3 animals following gradings at 24, 48, and 72 hours, and the effects are fully reversible within 7 days (UN 2021).*
- Additional studies in the REACH dossier did not add to or provide inconsistent findings in terms of GHS classification for, the studies listed above. Therefore, only the GLP-compliant, OECD Guideline 405 studies are included in this report.

- ECHA 2018
  - In another ocular irritation test with limited details, the rabbit's eye was severely injured by the undiluted n-butyl alcohol (0.5 ml), moderately damaged by a 15% dilution of the substance in propylene glycol and only slightly affected by a 5% dilution applied in excess.
  - Due to irreversible and severe effects on corneal opacity, iritis, conjunctivae redness and chemosis in the 7-day study described above, the ECHA considered the current EU-GHS harmonised classification of Category 1 for n-butyl alcohol is appropriate.

Ecotoxicity (Ecotox)

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

n-Butyl alcohol was assigned a score of Low for acute aquatic toxicity based on L/EC_{50} values being greater than 100 mg/L in fish, daphnia, and algae. GreenScreen® criteria classify chemicals as a Low hazard for acute aquatic toxicity when acute aquatic toxicity values are higher than 100 mg/L (CPA 2018b). The confidence in the score was high as it was based on well-conducted studies in all three trophic levels.

- Authoritative and Screening Lists
  - *Authoritative:* Not listed on any authoritative lists for this endpoint.
  - *Screening:* Not listed on any screening lists for this endpoint.
- OECD 2001, ECETOC 2003, ECHA, CAS #71-36-3, 2023
  - 96-hour LC_{50} (*Pimephales promela*s, fish) = 1,376 mg/L (GLP-compliant, OECD Guideline 203 test) (Klimisch 1, reliable without restriction).
  - 96-hour LC_{50} (*P. promela*s, fish) = 1,730 mg/L (U.S. EPA Committee on Methods for Toxicity (1975)) (Klimisch 2, reliable with restrictions).
  - 96-hour LC_{50} (*P. promelas,* fish) = 1,400 mg/L (nominal) (non-GLP, acute aquatic toxicity test conducted according to Standard Methods for the Examination of Water and

Wastewater, 13th Edition, 1971, published by APHA, AWWA, and WPCF) (Klimisch 2, reliable with restrictions).
  - 96-hour LC_{50} (*P. promelas,* fish) > 100 mg/L (nominal) (non-GLP, acute aquatic toxicity test conducted according to Standard Methods for the Examination of Water and Wastewater, 13th Edition, 1971, published by American Public Health Assn., NY, NY 10019 (Klimisch 2, reliable with restrictions).
  - 48-hour LC_{50} (*Leuciscus idus,* golden orfe) = 1,834 mg/L (nominal) (non-GLP, non-guideline acute aquatic toxicity test (Klimisch 2, reliable with restrictions).
  - 48-hour EC_{50} (*Daphnia magn*a, invertebrates) = 1,328 mg/L for mobility (GLP-compliant, OECD Guideline 202 test) (Klimisch 1, reliable without restriction).
  - The authors of the ECHA dossier of n-butyl alcohol identified three additional reliable studies for the aquatic invertebrate trophic level, which all reported 48-hour EC_{50} values of > 100 mg/L; therefore, only the GLP-compliant and OECD Guideline study was evaluated above.
  - 96-hour EC_{50} (*Raphidocelis subcapitata*, algae) = 225 mg/L for growth rate (GLP-compliant, OECD Guideline 201 test) (Klimisch 1, reliable without restriction).

Chronic Aquatic Toxicity (CA) Score (vH, H, M, or L): M
n-Butyl alcohol was assigned a score of Moderate for chronic aquatic toxicity based on a 21-day NOEC of 4.1 mg/L in daphnia. GreenScreen® criteria classify chemicals as a Moderate hazard for chronic aquatic toxicity when chronic values are between 1 and 10 mg/L (CPA 2018b). The confidence in the score is high based on experimental data for the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not listed on any authoritative lists for this endpoint.
  - *Screening:* Not listed on any screening lists for this endpoint.
- ECHA, CAS #71-36-3, 2023
  - 21-day NOEC (*D. magn*a, invertebrates) = 4.1 mg/L (reproduction) (GLP compliant, OECD Guideline 211) (Klimisch 2, reliable with restrictions).
  - 96-hour NOEC (*R. subcapitata,* algae) = 129 mg/L (GLP-compliant, OECD Guideline 201 test) (Klimisch 1, reliable without restriction).
- U.S. EPA 2017b
  - n-Butyl alcohol belongs to the neutral organics ECOSAR chemical class. The most conservative predicted chronic value (ChV) in fish is 53.4 mg/L (Appendix K).

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): vL
n-Butyl alcohol was assigned a score of Very Low for persistence based on experimental data indicating this chemical is readily biodegradable within a 10-day window, and based on modeled data indicating that soil is the major partitioning compartment. GreenScreen® criteria classify chemicals as a Very Low hazard for persistence when they mainly partition to water, soil or sediment, and are readily biodegradable within a 10-day window (CPA 2018b). The confidence in the score is high as it is based on experimental data of good quality on the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not listed on any authoritative lists for this endpoint.
  - *Screening:* Not listed on any screening lists for this endpoint.
- OECD 2001, ECETOC 2003, ECHA, CAS #71-36-3, 2023

  - In a biological oxygen demand (BOD) biodegradability test conducting according to the Standard Methods for the Examination of Water and Wastewater (1971, 13th Ed. American Public Health Association, New York, NY), domestic, non-adapted, unacclimated wastewater was exposed to n-butyl alcohol (purity not specified) at an initial concentration of 3 mg/L for 20 days. A biodegradation rate, based on ThOD (percent of theoretical oxygen demand), at 5, 10, 15, and 20 days was 68%, 87%, 92%, and 92%, respectively. It was concluded that n-butyl alcohol was readily biodegradable. The authors of REACH dossier stated that n-butyl alcohol was biodegradable, meeting the 10-day window in this test (Klimisch 2, reliable with restrictions). *According to OECD criteria for biodegradation tests that apply ThOD as a parameter to measure biodegradation such as 301 C, 301 D and TG 301 F tests, the pass level is 60%. As n-butyl alcohol reached the pass level (68%) within 5 days, ToxServices concluded that the 10-day window was met.*
- ECETOC 2003
  - Several studies have been conducted to assess the biodegradation of n-butyl alcohol in water and treated sewage under aerobic conditions. In these studies, n-butyl alcohol was considered readily biodegradable; 88% degradation within 30 days in a closed bottle test (OECD Guideline 301D); 98% degradation within 14 days in an OECD-screening test; 100% degraded in 5 days in a Zahn-Wellens test (assumed to be similar to OECD Guideline 302B); 98.5% degradation in a Coupled Units test; and rapid biodegradation within a few days in a number of other non-standard studies. Additional details were not provided
- U.S. EPA 2017a
  - The BIOWIN modeling Ready Biodegradable Predictor indicates that n-butyl alcohol is expected to be readily biodegradable. Fugacity modeling (MCI method) predicts 55.2% will partition to soil with a half-life of 416 hours (17.3 days), 40.1% will partition to water with a half-life of 208 hours (8.7 days), 4.58% will partition to air with a half-life of 30.2 hours (1.3 days), and less than 0.1% will partition to sediment (Appendix D).
- Based on the weight of evidence, a score of Very Low was assigned. Fugacity modeling predicts that n-butyl alcohol will partition primarily to soil. n-Butyl alcohol was readily biodegradable and met the 10-day window. When the major compartment is soil, GreenScreen® criteria specify a score of Very Low if the chemical meets the 10-day window in a ready biodegradation test.

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

n-Butyl alcohol was assigned a score of Very Low for bioaccumulation based on its experimental partition coefficient of 0.88 and its estimated BCF of 3.162. GreenScreen® criteria classify chemicals as a Very Low hazard for bioaccumulation when log K_{ow} values are no greater than 4 or BCFs/BAFs are less than 100 (CPA 2018b). The confidence in the score is high as it is based on an experimentally-derived partition coefficient.

- Authoritative and Screening Lists
  - *Authoritative:* Not listed on any authoritative lists for this endpoint.
  - *Screening:* Not listed on any screening lists for this endpoint.
  - OECD 2001.
- ECHA, CAS #71-36-3, 2023
  - n-Butyl alcohol has a log K_{ow} value of 1 at 25ºC as identified in a GLP-compliant, OECD Guideline 117/EU Method A.8 (HPLC Method) test (Klimisch 1, reliable without restriction).
- U.S. EPA 2017a
  - BCFBAF model predicted a BCF of 3.162, using the regression based model and a BCF of 1.475 using the Arnot-Gobas model for the upper trophic level, taking metabolism into

consideration based on an experimental partition coefficient of 0.88. See Appendix D for EPI Suite™ output.

Physical Hazards (Physical)

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

n-Butyl alcohol was assigned a score of Low for reactivity based on NFPA reactivity rating supported by lack of structural alerts for oxidizing and explosive endpoints. GreenScreen® criteria classify chemicals as a Low hazard for reactivity when available data indicate that the chemical does not warrant GHS classification for any of the reactivity sub-endpoints and the chemical is not present on authoritative or screening lists (CPA 2018b). The confidence in the score is low due to lack of measured data.

- Authoritative and Screening Lists
  - *Authoritative:* not listed in any authoritative lists for this endpoint.
  - *Screening:* not listed in any screening lists for this endpoint.
- ECHA, CAS #71-36-3, 2023
  - n-Butyl alcohol would not be classified as an oxidizing or explosive chemical as it does not contain structural groups that would cause concern for oxidizing or explosive properties (is not associated with any relevant EU hazard statements).
  - In a GLP-compliant EU Method A.15 (auto-ignition temperature – liquids and gases) test, n-butyl alcohol did not self-ignite at temperatures up to 355°C (Klimisch 2, reliable with restrictions).
- PubChem 2023, OSHA 2021
  - OSHA has reported that n-butyl alcohol has an instability rating of 0 from the National Fire Protection Association (NFPA) ("Normally stable, even under fire exposure conditions, and is not reactive with water")[14].
- Millipore Sigma 2023
  - A material safety data sheet for n-butyl alcohol (as tradename: 1-butanol, ≤ 100% purity) states that it does not have explosive properties; however, it does state that n-butyl alcohol vapor/air-mixtures are explosive with intense heating. Additionally, an upper explosion limit of 11.2% and lower explosion limit of 1.4% were reported for n-butyl alcohol. Furthermore, n-butyl alcohol is stable at room temperature and storage and handling of n-butyl alcohol should avoid exposure to moisture and heating conditions.
- NITE 2006
  - National Institute of Technology (NITE) reports Class 3 Dangerous Goods (DG) packaging group as the rationale for a GHS Not Classified classification for self-heating substances, and corrosivity to metals.

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

n-Butyl alcohol was assigned a score of High for flammability based on association with DOT Class 3, Group II. Classification to GHS Category 3 and association with EU H226 warrant a lower score of Moderate. ToxServices assigned a more conservative score to be protective of human health. GreenScreen® criteria classify chemicals as a High hazard for flammability when associated with DOT Class 3, Group II classification (CPA 2018b). The confidence in the score is high as it is based on an authoritative list.

- Authoritative and Screening Lists

[14] https://www.fm.colostate.edu/files/forms/safety/CH-23.NFPA.ratings.pdf

  - *Authoritative:* EU – GHS (H-Statements) – Flammable liquid and vapor [H226] (Flammable liquids – Category 3).
  - *Authoritative:* Hazard Class 3 chemical, Packing Group II (5L), and III (10L) (U.S. DOT 2008a)
  - *Screening*: GHS – Australia – H226 – Flammable liquid and vapour [Flammable liquids – Category 3].
  - GHS – Japan – H226 – Flammable liquid and vapour [Flammable liquids – Category 3].
  - GHS – Malaysia – H226 – Flammable liquid and vapour [Flammable liquids – Category 3].
  - GHS – New Zealand – Flammable liquids, Category 3.
- ECHA, CAS #71-36-3, 2023
  - n-Butyl alcohol has a flash point of 35°C as determined in a Pensky-Martens closed cup test conducted in a manner similar to ISO 2719 (2002) (Klimisch 2, reliable with restrictions).
  - n-Butyl alcohol has a boiling point of 119°C from a study conducted in a manner similar to OECD Guideline 103 (Klimisch 2, reliable with restrictions).
  - In a GLP-compliant, EU Method A.15 (auto-ignition temperature – liquids and gases) test, n-butyl alcohol did not self-ignite at temperatures up to 355°C (Klimisch 2, reliable with restrictions).
- PubChem 2023, OSHA 2021
  - OSHA has reported that n-butyl alcohol has a NFPA fire rating of 3 ("Liquids and solids that can be ignited under almost all ambient temperature conditions. Materials produce hazardous atmospheres with air under almost all ambient temperatures or, though unaffected by ambient temperatures, are readily ignited under almost all conditions.")
- OECD 2001
  - n-Butyl alcohol has a flammable range of 1.4 – 11.2 volume % in air (14,000 – 112,000 ppm) and a flash point of 98°F (37°C).
- Based on the available data, n-butyl alcohol is classified to GHS Category 3 for flammable liquids (liquids which have a flash point of between 23°C and 60°C) (UN 2021).

Use of New Approach Methodologies (NAMs)[15] in the Assessment, Including Uncertainty Analyses of Input and Output

New Approach Methodologies (NAMs) used in this ChemFORWARD assessment include use of *in silico* modeling for carcinogenicity, endocrine activity, respiratory sensitization, persistence, and bioaccumulation, and *in vitro* testing for genotoxicity, endocrine activity, and skin sensitization. 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 the European Food Safety Authority (EFSA) (2018), uncertainty is "a general term referring to all types of limitations in available knowledge that affect the range and probability of possible answers to an assessment question." The quality, utility, and accuracy of NAM predictions are greatly influenced by two primary types of uncertainties (OECD 2020):

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

As shown in Table 4, Type I (input data) uncertainties in n-butyl alcohol's NAMs dataset include lack or insufficient experimental data to assess carcinogenicity, endocrine activity, and respiratory sensitization, and lack of validated test methods for respiratory sensitization. n-Butyl alcohol's Type II (extrapolation output) uncertainties include reliance on structural alerts or models with undefined applicability domains, reliance on *in vitro* genotoxicity studies that do not fully mimic *in vivo* metabolism and focusing on only a few types of genotoxic events, uncertain *in vivo* relevance of *in silico* receptor binding activity predictions and *in vitro* high throughput screening assays of receptor activities, inability of *in vitro* skin sensitization assays to identify pro- and pre-haptens, and lack of consideration of non-immunological mechanisms of respiratory sensitization. Some of n-butyl alcohol's type II uncertainties were alleviated by the use of *in vitro* test batteries and/or in combination of *in vivo* data.

Table 4: Summary of NAMs Used in the GreenScreen® Assessment, Including Uncertainty Analyses

Uncertainty Analyses (OECD 2020)	
Type I Uncertainty: Data/Model Input	**Carcinogenicity**: No experimental data are available for target chemical or surrogate. **Endocrine activity:** No *in vivo* data for circulating hormones are available. **Respiratory sensitization**: No experimental data are available, and there are no validated tested methods.
Type II Uncertainty: Extrapolation Output	**Carcinogenicity**: OECD Toolbox structural alerts screening does not define applicability domains. **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

[15] 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).

	system does not entirely mimic *in vivo* conditions[16]. The mammalian cell gene mutation assay (as defined in OECD Guideline 476, HPRT locus) only detects gene mutations, and the exogenous metabolic activation system does not entirely mirror *in vivo* metabolism (i.e., the liver S9 mix contains enzymes present in the endoplasmic reticulum but not the cytosol of liver cells).[17] The mammalian cell gene mutation assay (as defined in OECD Guideline 490, TK locus assay) cannot reliably detect aneugens, and the exogenous metabolic activation system does not entirely mirror *in vivo* metabolism (i.e., the liver S9 mix contains enzymes present in the endoplasmic reticulum but not the cytosol of liver cells)[18]. The *in vitro* SCE assay (as defined in OECD Guideline 479, a guideline deleted in 2014) detects reciprocal exchange of DNA without providing the underlying mechanism of action[19]. **Endocrine activity:** The *in vivo* relevance of EDSP Tox 21 screening assays and *in silico* receptor binding modeling is unknown due to lack of consideration of metabolism and other toxicokinetic factors. **Skin sensitization:** The *in chemico* and *in vitro* assays evaluating key events in the skin sensitization adverse outcome pathway (AOP) don't typically include metabolism or abiotic transformation to address chemicals that are pro-haptens or pre-haptens, respectively. Further, each test has their applicable domain such as limitations in test substance solubility or log K_{ow}.[20] **Respiratory sensitization**: The OECD Toolbox only identifies structural alerts and does not define applicability domains. Additionally, ECHA guidance (2017), on which the use of OECD Toolbox structural alerts is based, does not evaluate non-immunologic mechanisms for respiratory sensitization.
Endpoint	**NAMs Data Available and Evaluated? (Y/N)**	**Types of NAMs Data (*in silico* modeling/*in vitro* biological profiling/frameworks)**
Carcinogenicity	Y	*In silico* modeling: VEGA/OncoLogic/OECD Toolbox/Danish QSAR
Mutagenicity	Y	*In vitro* data: Bacterial reverse mutation assay/*in vitro* gene mutation assay/*in vitro* mammalian

[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/9789264264809-en.pdf?expires=1614097800&id=id&accname=guest&checksum=C0DE371FB9C5A878E66C9AB7F84E6BBE
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		cell micronucleus assay/*in vitro* SCE assay
Reproductive toxicity	N	
Developmental toxicity	N	
Endocrine activity	Y	*In vitro* high throughput data: EDSP Tox 21 screening assays, *In silico* modeling: VEGA/Danish QSAR
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 chemico* data: DPRA (OECD Guideline 442 C) test; *In vitro* data: ARE-Nrf2 luciferase test (OECD Guideline 442)
Respiratory sensitization	Y	*In silico* modeling: OECD Toolbox structural alerts/Danish QSAR
Skin irritation	N	
Eye irritation	N	
Acute aquatic toxicity	N	
Chronic aquatic toxicity	N	
Persistence	Y	*In silico* modeling: EPI Suite™ Non-animal testing: Biological oxygen demand (BOD) biodegradability test, OECD 301D and 302B tests
Bioaccumulation	Y	*In silico* modeling: EPI Suite™

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APPENDIX A: Hazard Classification Acronyms
(in alphabetical order)

(AA) Acute Aquatic Toxicity

(AT) Acute Mammalian Toxicity

(B) Bioaccumulation

(C) Carcinogenicity

(CA) Chronic Aquatic Toxicity

(D) Developmental Toxicity

(E) Endocrine Activity

(F) Flammability

(IrE) Eye Irritation/Corrosivity

(IrS) Skin Irritation/Corrosivity

(M) Mutagenicity and Genotoxicity

(N) Neurotoxicity

(P) Persistence

(R) Reproductive Toxicity

(Rx) Reactivity

(SnS) Sensitization- Skin

(SnR) Sensitization- Respiratory

(ST) Systemic/Organ Toxicity

APPENDIX B: Results of Automated GreenScreen® Score Calculation for n-Butyl Alcohol (CAS #71-36-3)

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-Butanol / 71-36-3	*L*	L	L	L	DG	M	M	L	M	L	L	*L*	H	vH	L	M	vL	vL	*L*	H

Table 3: Hazard Summary Table

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

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
n-Butanol	2

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

Table 6

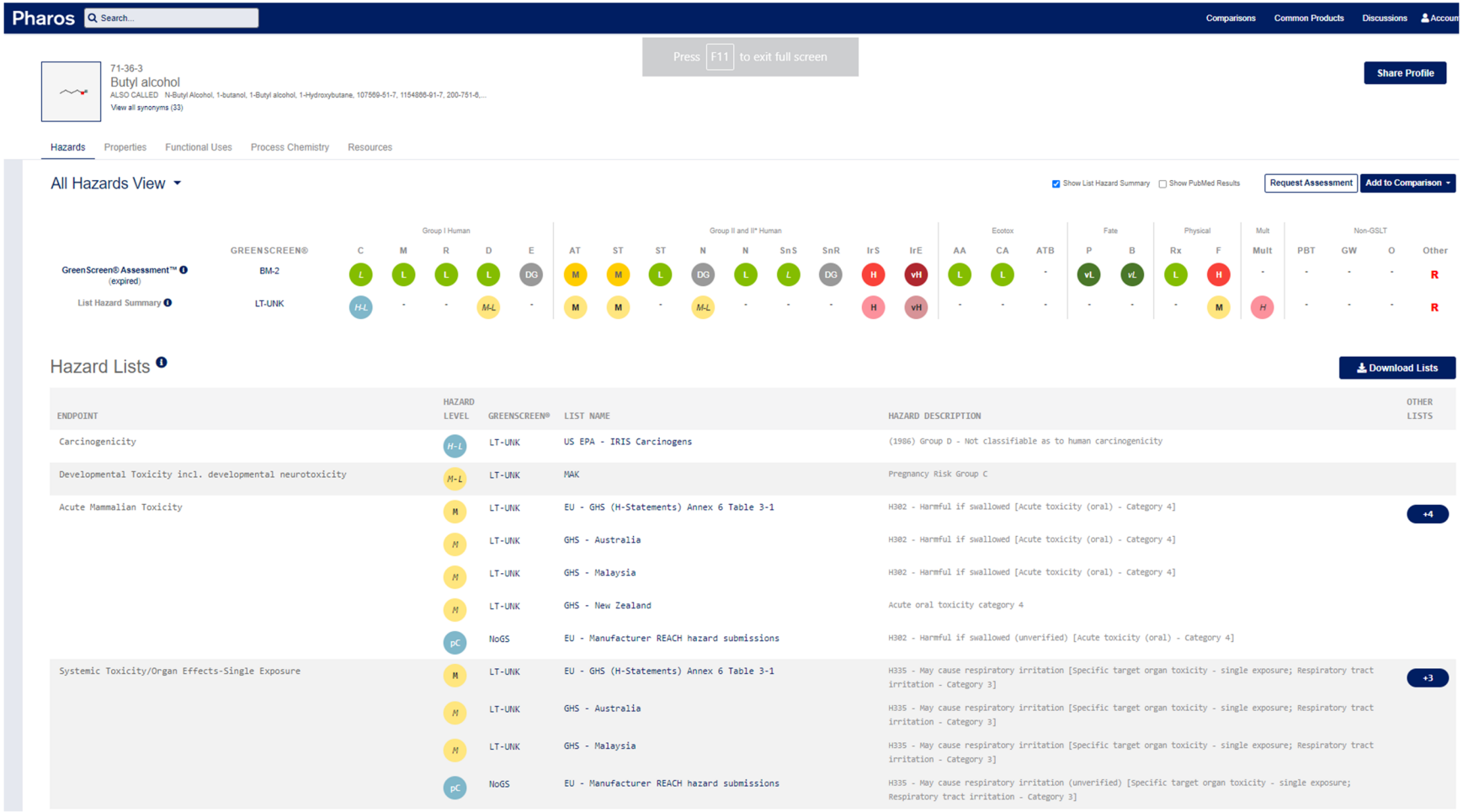
Chemical Name	Final GreenScreen® Benchmark Score
n-Butanol	2

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-Butyl Alcohol (CAS #71-36-3)



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]	+3
	M-L	LT-UNK	GHS - Australia	H336 - May cause drowsiness or dizziness [Specific target organ toxicity - single exposure; Narcotic effects - Category 3]	
	M-L	LT-UNK	GHS - Malaysia	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]	
Skin Irritation/Corrosivity	H	LT-UNK	EU - GHS (H-Statements) Annex 6 Table 3-1	H315 - Causes skin irritation [Skin corrosion/irritation - Category 2]	+5
	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]	
	H	LT-UNK	GHS - Malaysia	H315 - Causes skin irritation [Skin corrosion/irritation - Category 2]	
	H	LT-UNK	GHS - New Zealand	Skin irritation category 2	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H315 - Causes skin irritation (unverified) [Skin corrosion/irritation - Category 2]	
Eye Irritation/Corrosivity	vH	LT-UNK	EU - GHS (H-Statements) Annex 6 Table 3-1	H318 - Causes serious eye damage [Serious eye damage/eye irritation - Category 1]	+5
	vH	LT-UNK	GHS - Australia	H318 - Causes serious eye damage [Serious eye damage/eye irritation - Category 1]	
	vH	LT-UNK	GHS - Malaysia	H318 - Causes serious eye damage [Serious eye damage/eye irritation - Category 1]	
	vH	LT-UNK	GHS - New Zealand	Serious eye damage category 1	
	H	LT-UNK	GHS - Japan	H319 - Causes serious eye irritation [Serious eye damage / eye irritation - Category 2A]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H318 - Causes serious eye damage (unverified) [Serious eye damage/eye irritation - Category 1]	
Flammability	M	LT-UNK	EU - GHS (H-Statements) Annex 6 Table 3-1	H226 - Flammable liquid and vapour [Flammable liquids - Category 3]	+6
	M	LT-UNK	GHS - Australia	H226 - Flammable liquid and vapour [Flammable liquids - Category 3]	
	M	LT-UNK	GHS - Japan	H226 - Flammable liquid and vapour [Flammable liquids - Category 3]	
	M	LT-UNK	GHS - Malaysia	H226 - Flammable liquid and vapour [Flammable liquids - Category 3]	
	M	LT-UNK	GHS - New Zealand	Flammable liquids category 3	
	vH-M	LT-UNK	Québec CSST - WHMIS 1988	Class B2 - Flammable liquids	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H226 - Flammable liquid and vapour (unverified) [Flammable liquids - Category 3]	
Carcinogenicity,Mutagenicity/Genotoxicity Reproductive Toxicity, Developmental Toxicity, Acute Mammalian Toxicity, or System Toxicity/Organ Effects.	U	LT-UNK	Québec CSST - WHMIS 1988	Class D2B - Toxic material causing other toxic effects	+1
	U	LT-UNK	EC - CEPA DSL	Inherently Toxic to Humans (iTH)	
Systemic Toxicity/Organ Effects [Single Exposure] and/or Neurotoxicity [Single Exposure]	M	LT-UNK	GHS - Japan	H335 or H336 [Specific target organs/systemic toxicity following single exposure - Category 3]	+1
	M	LT-UNK	GHS - New Zealand	Specific target organ toxicity - single exposure category 3 narcotic effects	
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]	
Human and/or Aquatic toxicity and/or Persistence and/or Bioaccumulation	U	LT-UNK	German FEA - Substances Hazardous to Waters	Class 1 - Low Hazard to Waters	

APPENDIX D: EPI Suite™ Modeling Results for n-Butyl Alcohol (CAS #71-36-3)

(Estimations Used for Hazard Classification Are Highlighted and Bolded)

CAS Number: 000071-36-3
SMILES : OCCCC
CHEM : 1-BUTANOL
MOL FOR: C4 H10 O1
MOL WT : 74.12
---------- EPI SUMMARY (v4.11) ----------
Physical Property Inputs:
Log Kow (octanol-water): 0.88
Boiling Point (deg C) : 117.70
Melting Point (deg C) : -89.80
Vapor Pressure (mm Hg) : 6.7
Water Solubility (mg/L): 63200
Henry LC (atm-m3/mole) : 8.81E-006

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = 0.84
Log Kow (Exper. database match) = 0.88
Exper. Ref: HANSCH,C ET AL. (1995)

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 113.91 (Adapted Stein & Brown method)
Melting Pt (deg C): -62.33 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 7.91 (Mean VP of Antoine & Grain methods)
VP (Pa, 25 deg C) : 1.05E+003 (Mean VP of Antoine & Grain methods)
MP (exp database): -89.8 deg C
BP (exp database): 118 deg C
VP (exp database): 6.70E+00 mm Hg (8.93E+002 Pa) at 25 deg C

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 8.117e+004
log Kow used: 0.88 (user entered)
melt pt used: -89.80 deg C
Water Sol (Exper. database match) = 6.32e+004 mg/L (25 deg C)
Exper. Ref: TEWARI,YB ET AL. (1982)

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 61389 mg/L

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

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 9.99E-006 atm-m3/mole (1.01E+000 Pa-m3/mole)

Group Method: 9.74E-006 atm-m3/mole (9.87E-001 Pa-m3/mole)
Exper Database: 8.81E-06 atm-m3/mole (8.93E-001 Pa-m3/mole)
For Henry LC Comparison Purposes:
User-Entered Henry LC: 8.810E-006 atm-m3/mole (8.927E-001 Pa-m3/mole)
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 1.034E-005 atm-m3/mole (1.048E+000 Pa-m3/mole)
VP: 6.7 mm Hg (source: User-Entered)
WS: 6.32E+004 mg/L (source: User-Entered)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 0.88 (user entered)
Log Kaw used: -3.443 (user entered)
Log Koa (KOAWIN v1.10 estimate): 4.323
Log Koa (experimental database): 4.190

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.9794
Biowin2 (Non-Linear Model) : 0.9927
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 3.4937 (days-weeks)
Biowin4 (Primary Survey Model) : 4.1393 (days)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 0.6899
Biowin6 (MITI Non-Linear Model): 0.8842
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): 0.6495
Ready Biodegradability Prediction: YES

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

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:
Vapor pressure (liquid/subcooled): 893 Pa (6.7 mm Hg)
Log Koa (Exp database): 4.190
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 3.36E-009
Octanol/air (Koa) model: 3.8E-009
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 1.21E-007
Mackay model : 2.69E-007
Octanol/air (Koa) model: 3.04E-007

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 6.8900 E-12 cm3/molecule-sec
Half-Life = 1.552 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 18.629 Hrs
Ozone Reaction:
No Ozone Reaction Estimation

Fraction sorbed to airborne particulates (phi):
1.95E-007 (Junge-Pankow, Mackay avg)
3.04E-007 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 3.471 L/kg (MCI method)
Log Koc: 0.540 (MCI method)
Koc : 10.01 L/kg (Kow method)
Log Koc: 1.000 (Kow method)
Experimental Log Koc: 0.5 (database)

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

Bioaccumulation Estimates (BCFBAF v3.01):
Log BCF from regression-based method = 0.500 (BCF = 3.162 L/kg wet-wt)
Log Biotransformation Half-life (HL) = -1.1665 days (HL = 0.06816 days)
Log BCF Arnot-Gobas method (upper trophic) = 0.169 (BCF = 1.475)
Log BAF Arnot-Gobas method (upper trophic) = 0.169 (BAF = 1.475)
log Kow used: 0.88 (user entered)

Volatilization from Water:
Henry LC: 8.81E-006 atm-m3/mole (entered by user)
Half-Life from Model River: 58.09 hours (2.421 days)
Half-Life from Model Lake : 705.9 hours (29.41 days)

Removal In Wastewater Treatment:
Total removal: 2.36 percent
Total biodegradation: 0.09 percent
Total sludge adsorption: 1.78 percent
Total to Air: 0.49 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model: (MCI Method)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	**4.58**	**30.2**	**1000**
Water	**40.1**	**208**	**1000**
Soil	**55.2**	**416**	**1000**
Sediment	**0.0747**	**1.87e+003**	**0**

Persistence Time: 240 hr

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

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	4.58	30.2	1000
Water	40.1	208	1000
water	(40.1)		

biota (1.52e-005)
suspended sediment (0.000209)
Soil 55.2 416 1000
Sediment 0.0747 1.87e+003 0
Persistence Time: 240 hr

Level III Fugacity Model: (EQC Default)

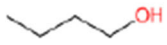
	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	4.6	30.2	1000
Water	40.4	208	1000
water	(40.4)		
biota	(1.53e-005)		
suspended sediment	(0.000188)		
Soil	55	416	1000
Sediment	0.0746	1.87e+003	0

Persistence Time: 239 hr

APPENDIX E: VEGA Carcinogenicity Results for n-Butyl Alcohol (CAS #71-36-3)

1. Prediction Summary

Prediction for compound Molecule 0 -



Prediction: Reliability:

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

Compound: Molecule 0
Compound SMILES: OCCCC
Experimental value: -
Predicted Carcinogen activity: NON-Carcinogen
P(Carcinogen): 0.145
P(NON-Carcinogen): 0.855
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

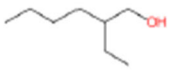
CAS: 67-63-0
Dataset id:406 (Training Set)
SMILES: OC(C)C
Similarity: 0.906
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen

Compound #2

CAS: 75-65-0
Dataset id:108 (Training Set)
SMILES: OC(C)(C)C
Similarity: 0.893
Experimental value : Carcinogen
Predicted value : Carcinogen

Compound #3

CAS: 64-17-5
Dataset id:303 (Test Set)
SMILES: OCC
Similarity: 0.873
Experimental value : Carcinogen
Predicted value : Carcinogen



Compound #4

CAS: 104-76-7
Dataset id:314 (Training Set)
SMILES: OCC(CC)CCCC
Similarity: 0.862
Experimental value : NON-Carcinogen
Predicted value : Carcinogen

Compound #5

CAS: 57-55-6
Dataset id:677 (Training Set)
SMILES: OCC(O)C
Similarity: 0.858
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen

Compound #6

CAS: 106-88-7
Dataset id:295 (Test Set)
SMILES: O1CC1CC
Similarity: 0.852
Experimental value : Carcinogen
Predicted value : Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

⚠	Global AD Index AD index = 0.799 Explanation: The predicted compound could be out of the Applicability Domain of the model.
✓	Similar molecules with known experimental value Similarity index = 0.899 Explanation: Strongly similar compounds with known experimental value in the training set have been ..
✓	Accuracy of prediction for similar molecules Accuracy index = 1 Explanation: Accuracy of prediction for similar molecules found in the training set is good..
⚠	Concordance for similar molecules Concordance index = 0.504 Explanation: some similar molecules found in the training set have experimental values that disagree with the predicted value..
✓	Model's descriptors range check Descriptors range check = True Explanation: descriptors for this compound have values inside the descriptor range of the compounds of the training set..
✓	Atom Centered Fragments similarity check ACF index = 1 Explanation: all atom centered fragment of the compound have been found in the compounds of the training set..
✓	Model class assignment reliability Pos/Non-Pos difference = 0.71 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:

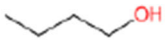
✓ The feature has a good assessment, model is reliable regarding this aspect.

⚠ 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:
- 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: OCCCC
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: 75-65-0
Dataset id:304 (Training Set)
SMILES: OC(C)(C)C
Similarity: 0.893
Experimental value : Carcinogen
Predicted value : NON-Carcinogen

Compound #2

CAS: 64-17-5
Dataset id:467 (Training Set)
SMILES: OCC
Similarity: 0.873
Experimental value : Carcinogen
Predicted value : NON-Carcinogen

Compound #3

CAS: 106-88-7
Dataset id:263 (Training Set)
SMILES: O1CC1CC
Similarity: 0.852
Experimental value : Carcinogen
Predicted value : Carcinogen

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

Compound #4

CAS: 111-76-2
Dataset id:596 (Training Set)
SMILES: OCCOCCCC
Similarity: 0.822
Experimental value : Carcinogen
Predicted value : NON-Carcinogen

Compound #5

CAS: 107-18-6
Dataset id:786 (Training Set)
SMILES: OCC=C
Similarity: 0.82
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen

Compound #6

CAS: 109-99-9
Dataset id:611 (Training Set)
SMILES: O1CCCC1
Similarity: 0.81
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 = 0.883 Explanation: Strongly similar compounds with known experimental value in the training set have been ..
✖	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:

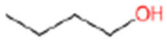
✔ The feature has a good assessment, model is reliable regarding this aspect.

⚠ 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 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: OCCCC
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: 75-65-0
Dataset id:246 (Training Set)
SMILES: OC(C)(C)C
Similarity: 0.893
Experimental value : Carcinogen
Predicted value : Possible NON-Carcinogen

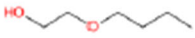
Compound #2

CAS: 64-17-5
Dataset id:390 (Training Set)
SMILES: OCC
Similarity: 0.873
Experimental value : Carcinogen
Predicted value : Possible NON-Carcinogen

Compound #3

CAS: 106-88-7
Dataset id:216 (Training Set)
SMILES: O1CC1CC
Similarity: 0.852
Experimental value : Carcinogen
Predicted value : Carcinogen

Alerts (not found also in the target): Carcinogenity alert no. 23



Compound #4

CAS: 111-76-2
Dataset id:498 (Training Set)
SMILES: OCCOCCCC
Similarity: 0.822
Experimental value : Carcinogen
Predicted value : Possible NON-Carcinogen

Compound #5

CAS: 107-18-6
Dataset id:724 (Training Set)
SMILES: OCC=C
Similarity: 0.82
Experimental value : NON-Carcinogen
Predicted value : Possible NON-Carcinogen

Compound #6

CAS: 109-99-9
Dataset id:508 (Training Set)
SMILES: O1CCCC1
Similarity: 0.81
Experimental value : 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 = 0.871 Explanation: Strongly similar compounds with known experimental value in the training set have been ..
✖	Accuracy of prediction for similar molecules Accuracy index = 0.324 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:

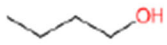
✔ The feature has a good assessment, model is reliable regarding this aspect.

⚠ 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 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: OCCCC
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

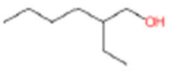
CAS: 67-63-0
Dataset id:406 (Training Set)
SMILES: OC(C)C
Similarity: 0.906
Experimental value : NON-Carcinogen
Predicted value : Possible NON-Carcinogen

Compound #2

CAS: 75-65-0
Dataset id:108 (Training Set)
SMILES: OC(C)(C)C
Similarity: 0.893
Experimental value : Carcinogen
Predicted value : Possible NON-Carcinogen

Compound #3

CAS: 64-17-5
Dataset id:303 (Test Set)
SMILES: OCC
Similarity: 0.873
Experimental value : Carcinogen
Predicted value : Possible NON-Carcinogen



Compound #4

CAS: 104-76-7
Dataset id:314 (Training Set)
SMILES: OCC(CC)CCCC
Similarity: 0.862
Experimental value : NON-Carcinogen
Predicted value : Possible NON-Carcinogen

Compound #5

CAS: 57-55-6
Dataset id:677 (Training Set)
SMILES: OCC(O)C
Similarity: 0.858
Experimental value : NON-Carcinogen
Predicted value : Possible NON-Carcinogen

Compound #6

CAS: 106-88-7
Dataset id:295 (Test Set)
SMILES: O1CC1CC
Similarity: 0.852
Experimental value : Carcinogen
Predicted value : Carcinogen

Alerts (not found also in the target): Carcinogenity alert no. 105

3.2 Applicability Domain:
Measured Applicability Domain Scores

✖	Global AD Index AD index = 0.55 Explanation: The predicted compound is outside the Applicability Domain of the model.
✔	Similar molecules with known experimental value Similarity index = 0.89 Explanation: Strongly similar compounds with known experimental value in the training set have been ..
✖	Accuracy of prediction for similar molecules Accuracy index = 0.34 Explanation: Accuracy of prediction for similar molecules found in the training set is not adequate..
✖	Concordance for similar molecules Concordance index = 0.34 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:

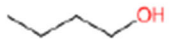
✔ The feature has a good assessment, model is reliable regarding this aspect.

⚠ 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 -



EXPERIMENTAL DATA

E xperimental value is NON-Carcinogen. Model prediction is NON-Carcinogen (GOOD reliability).

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

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

Compound #1

CAS: 71-36-3
Dataset id:362 (Training Set)
SMILES: OCCCC
Similarity: 1
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen

Compound #2

CAS: 78-92-2
Dataset id:363 (Training Set)
SMILES: OC(C)CC
Similarity: 0.968
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen

Compound #3

CAS: 78-83-1
Dataset id:551 (Training Set)
SMILES: OCC(C)C
Similarity: 0.933
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen

Compound #4

CAS: 75-85-4
Dataset id:336 (Training Set)
SMILES: OC(C)(C)CC
Similarity: 0.92
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen

Compound #5

CAS: 67-63-0
Dataset id:553 (Test Set)
SMILES: OC(C)C
Similarity: 0.906
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen

Compound #6

CAS: 57-55-6
Dataset id:661 (Training Set)
SMILES: OCC(O)C
Similarity: 0.858
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

✓	Global AD Index AD index = 1 Explanation: The predicted compound is into the Applicability Domain of the model.
✓	Similar molecules with known experimental value Similarity index = 1 Explanation: Strongly similar compounds with known experimental value in the training set have been ..
✓	Accuracy of prediction for similar molecules Accuracy index = 1 Explanation: Accuracy of prediction for similar molecules found in the training set is good..
✓	Concordance for similar molecules Concordance index = 1 Explanation: Similar molecules found in the training set have experimental values that agree with the predicted value..
✓	Model's descriptors range check Descriptors range check = True Explanation: descriptors for this compound have values inside the descriptor range of the compounds of the training set..
✓	Atom Centered Fragments similarity check ACF index = 1 Explanation: all atom centered fragment of the compound have been found in the compounds of the training set..

Symbols explanation:

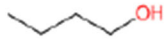
✓ The feature has a good assessment, model is reliable regarding this aspect.

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

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

1. Prediction Summary

Prediction for compound Molecule 0 -



EXPERIMENTAL DATA

E xperimental value is NON-Carcinogen. Model prediction is NON-Carcinogen (GOOD reliability).

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

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

Compound #1

CAS: 71-36-3
Dataset id:314 (Training Set)
SMILES: OCCCC
Similarity: 1
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen

Compound #2

CAS: 78-92-2
Dataset id:316 (Training Set)
SMILES: OC(C)CC
Similarity: 0.968
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen

Compound #3

CAS: 78-83-1
Dataset id:528 (Training Set)
SMILES: OCC(C)C
Similarity: 0.933
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen

Compound #4

CAS: 75-85-4
Dataset id:283 (Training Set)
SMILES: OC(C)(C)CC
Similarity: 0.92
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen

Compound #5

CAS: 67-63-0
Dataset id:531 (Training Set)
SMILES: OC(C)C
Similarity: 0.906
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen

Compound #6

CAS: 57-55-6
Dataset id:650 (Test Set)
SMILES: OCC(O)C
Similarity: 0.858
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

✓	Global AD Index AD index = 1 Explanation: The predicted compound is into the Applicability Domain of the model.
✓	Similar molecules with known experimental value Similarity index = 1 Explanation: Strongly similar compounds with known experimental value in the training set have been ..
✓	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 F: OncoLogic Carcinogenicity Modeling Results for n-Butyl Alcohol (CAS #71-36-3)

OncoLogic Justification Report

SUMMARY :

CODE NUMBER : n-butyl alcohol

SUBSTANCE ID :

JUSTIFICATION:

Aliphatic Alcohols*

Aliphatic alcohols (R-OH) may be loosely divided into (a) high M.W. alcohols (C > 20), (b) medium size alcohols (C = 6 to 20), and (c) low M.W. alcohols (C < 6). In general, high M.W. aliphatic alcohols have low potential to be significant carcinogens. A number of medium size alcohols (e.g., CF3(CF2)6CH2OH; 2-ethylhexanol) that can be oxidized to metabolically persistent aliphatic carboxylic acids (e.g., perfluorinated fatty acid like perfluoroooctanoic; ω – 1 branched fatty acids like 2-ethylhexanoic acid) are potential nongenotoxic carcinogens. Most of these are medium sized with the most potent ones peaking around 7 – 9 carbons. Low M.W. alcohols, especially methanol and ethanol) are of carcinogenic concern because of possible oxidation to reactive aldehydes. The concern for carcinogenic risk is especially higher in individuals who are genetically deficient in aldehyde dehydrogenase which detoxifies aldehydes to carboxylic acids. A number of low M.W. tertiary alcohols (e.g., t-butyl, t-amyl) have been shown to induce kidney tumors in male rats by a mechanism (alpha-2-mu nephropathy) not relevant to humans. In addition, low M.W. alcohols with

(i) terminal double bond or Cl/Br/I,
(ii) α,β-unsaturation,
(iii) monosubstitution with Cl/Br/I at α-carbon are of concern as potential genotoxic carcinogens.

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*This is only a brief summary of the structure activity relationships (SAR) knowledge of this class. A more detailed decision logic will be developed in future version of OncoLogic. If the compound of your interest has been tested in any short-term predictive tests, the results of the tests should be entered into OncoLogic's Functional Arm to give an evaluation of carcinogenic potential based on short-term predictive tests.

APPENDIX G: Danish QSAR Carcinogenicity Results for n-Butyl Alcohol (CAS #71-36-3)

Carcinogenicity

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

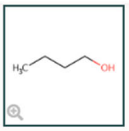
OECD QSAR Toolbox v.4.2 profilers

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_OUT	NEG_IN	NEG_OUT	NEG_OUT

DTU-developed models

APPENDIX H: CompTox EDSP21 Results for n-Butyl Alcohol (CAS #71-36-3)



1-Butanol

71-36-3 | DTXSID1021740

Searched by DTXSID1021740.

Concentration Response Data

Analytical Data on Tox21 Browser

EXPORT

Name	Description	Endpoint Name	Active	Details		All Plots	Gene	Intended Target	Cell Line	Cell Format
(4) EDSP AR,EDSP ER,										
EDSP AR	Androgen receptor as...	ATG_AR_TRANS_up	Inactive				AR	steroidal	liver	cell line
EDSP AR	Androgen receptor as...	TOX21_AR_BLA_Agonist_ratio	Inactive				AR	steroidal	kidney	cell line
EDSP AR	Androgen receptor as...	TOX21_AR_BLA_Antagonist_ratio	Inactive				AR	steroidal	kidney	cell line
EDSP AR	Androgen receptor as...	TOX21_AR_BLA_Antagonist_viability	Inactive				-	cytotoxicity	kidney	cell line
EDSP AR	Androgen receptor as...	TOX21_AR_LUC_MDAKB2_Agonist	Inactive				AR	steroidal	breast	cell line
EDSP AR	Androgen receptor as...	TOX21_AR_LUC_MDAKB2_Antago...	Inactive				AR	steroidal	breast	cell line
EDSP AR	Androgen receptor as...	TOX21_AR_LUC_MDAKB2_Antago...	Inactive				-	cytotoxicity	breast	cell line
EDSP AR	Androgen receptor as...	TOX21_AR_LUC_MDAKB2_Antago...	Inactive				AR	steroidal	breast	cell line
EDSP AR	Androgen receptor as...	TOX21_AR_LUC_MDAKB2_Antago...	Inactive				-	cytotoxicity	breast	cell line
EDSP AR	Androgen receptor as...	UPITT_HCI_U2OS_AR_TIF2_Nucle...	Inactive				AR	steroidal	bone	cell line
EDSP AR	Androgen receptor as...	UPITT_HCI_U2OS_AR_TIF2_Nucle...	Inactive				AR	steroidal	bone	cell line
EDSP ER	Estrogen receptor ass...	ACEA_ER_80hr	Active				ESR1	steroidal	breast	cell line
EDSP ER	Estrogen receptor ass...	ACEA_ER_AUC_viability	Active				-	cytotoxicity	breast	cell line
EDSP ER	Estrogen receptor ass...	ATG_ERa_TRANS_up	Inactive				ESR1	steroidal	liver	cell line
EDSP ER	Estrogen receptor ass...	ATG_ERE_CIS_up	Inactive				ESR1	steroidal	liver	cell line
EDSP ER	Estrogen receptor ass...	TOX21_ERa_BLA_Agonist_ratio	Inactive				ESR1	steroidal	kidney	cell line
EDSP ER	Estrogen receptor ass...	TOX21_ERa_BLA_Antagonist_ratio	Inactive				ESR1	steroidal	kidney	cell line
EDSP ER	Estrogen receptor ass...	TOX21_ERa_BLA_Antagonist_viabil...	Inactive				-	cytotoxicity	kidney	cell line
EDSP ER	Estrogen receptor ass...	TOX21_ERa_LUC_VM7_Agonist	Inactive				ESR1	steroidal	ovary	cell line
EDSP ER	Estrogen receptor ass...	TOX21_ERa_LUC_VM7_Antagonist...	Inactive				ESR1	steroidal	ovary	cell line
EDSP ER	Estrogen receptor ass...	TOX21_ERa_LUC_VM7_Antagonist...	Inactive				-	cytotoxicity	ovary	cell line
EDSP steroidogenesis	Steroidogenesis path...	TOX21_Aromatase_Inhibition	Inactive				CYP19A1	steroidogenesis-rela	breast	cell line
EDSP steroidogenesis	Steroidogenesis path...	TOX21_Aromatase_Inhibition_viabi...	Inactive				-	cytotoxicity	breast	cell line
EDSP thyroid	Thyroid pathway assa...	ATG_THRa1_TRANS_dn	Inactive				THRA	non-steroidal	liver	cell line
EDSP thyroid	Thyroid pathway assa...	ATG_THRa1_TRANS_up	Inactive				THRA	non-steroidal	liver	cell line
EDSP thyroid	Thyroid pathway assa...	TOX21_TRHR_HEK293_Agonist	Inactive				TRHR	thyrotropin-releasin	kidney	cell line
EDSP thyroid	Thyroid pathway assa...	TOX21_TRHR_HEK293_Antagonist	Inactive				TRHR	thyrotropin-releasin	kidney	cell line
EDSP thyroid	Thyroid pathway assa...	TOX21_TR_LUC_GH3_Agonist	Inactive				THRA	non-steroidal	pituitary ...	cell line
EDSP thyroid	Thyroid pathway assa...	TOX21_TR_LUC_GH3_Antagonist	Inactive				THRA	non-steroidal	pituitary ...	cell line
EDSP thyroid	Thyroid pathway assa...	TOX21_TR_LUC_GH3_Antagonist_...	Inactive				-	cytotoxicity	pituitary ...	cell line
EDSP thyroid	Thyroid pathway assa...	TOX21_TSHR_HTRF_Agonist_ratio	Inactive				TSHR	thyrotropin-releasin	kidney	cell line
EDSP thyroid	Thyroid pathway assa...	TOX21_TSHR_HTRF_Antagonist_ra...	Inactive				TSHR	thyrotropin-releasin	kidney	cell line
EDSP thyroid	Thyroid pathway assa...	TOX21_TSHR_HTRF_wt_ratio	Inactive				TSHR	thyrotropin-releasin	kidney	cell line

Rows: 33 of 444 Total Rows: 444 Filtered: 33

APPENDIX I: Danish QSAR Endocrine Results for n-Butyl Alcohol (CAS #71-36-3)

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*)		NEG_OUT	NEG_IN	NEG_OUT	INC_OUT
Estrogen Receptor Activation, CERAPP data (*in vitro*)		N/A	N/A	NEG_IN	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	NEG_IN	N/A
Androgen Receptor Inhibition, CoMPARA data (*in vitro*)		N/A	N/A	NEG_IN	N/A
Androgen Receptor Activation, CoMPARA data (*in vitro*)		N/A	N/A	NEG_IN	N/A
Thyroperoxidase (TPO) inhibition QSAR1 (Rat *in vitro*)		N/A	N/A	NEG_IN	N/A
Thyroperoxidase (TPO) inhibition QSAR2 (Rat *in vitro*)		N/A	N/A	NEG_IN	N/A
Sodium/iodide symporter (NIS), higher sensitivity		N/A	N/A	NEG_IN	N/A
Sodium/iodide symporter (NIS), higher specificity		N/A	N/A	NEG_IN	N/A
Thyroid Receptor α Binding (Human *in vitro*)					
- mg/L		5984.146	11855.92	1084.489	112.3661
- µM		80735.91	159955.8	14631.53	1516.003
- Positive for $IC_{50} \leq 10$ µM					
- Positive for $IC_{50} \leq 100$ µM					
- Domain		IN	IN	OUT	IN
Thyroid Receptor β Binding (Human *in vitro*)					
- mg/L		1321.561	2398.478	75.19095	244.6445
- µM		17830.02	32359.38	1014.449	3300.654
- Positive for $IC_{50} \leq 10$ µM					
- Positive for $IC_{50} \leq 100$ µM					
- Domain		IN	IN	OUT	IN

Arylhydrocarbon (AhR) Activation – Rational final model (Human *in vitro*)		N/A	N/A	NEG_IN	N/A
Arylhydrocarbon (AhR) Activation – Random final model (Human *in vitro*)		N/A	N/A	NEG_IN	N/A
Pregnane X Receptor (PXR) Binding (Human *in vitro*)	N/A	NEG_IN	NEG_IN	NEG_IN	NEG_IN
Pregnane X Receptor (PXR) Binding (Human *in vitro*) NEW		N/A	N/A	NEG_IN	N/A
Pregnane X Receptor (PXR) Activation (Human *in vitro*)	NEG	N/A	N/A	NEG_IN	N/A
Pregnane X Receptor (PXR) Activation (Rat *in vitro*)	NEG	N/A	N/A	NEG_IN	N/A
CYP3A4 Induction (Human *in vitro*)	NEG	N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Activation at max. 20 µM (*in vitro*)		N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Activation at max. 50 µM (*in vitro*)	NEG	N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Inhibition at max. 20 µM (*in vitro*)		N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Inhibition at max. 50 µM (*in vitro*)		N/A	N/A	NEG_IN	N/A

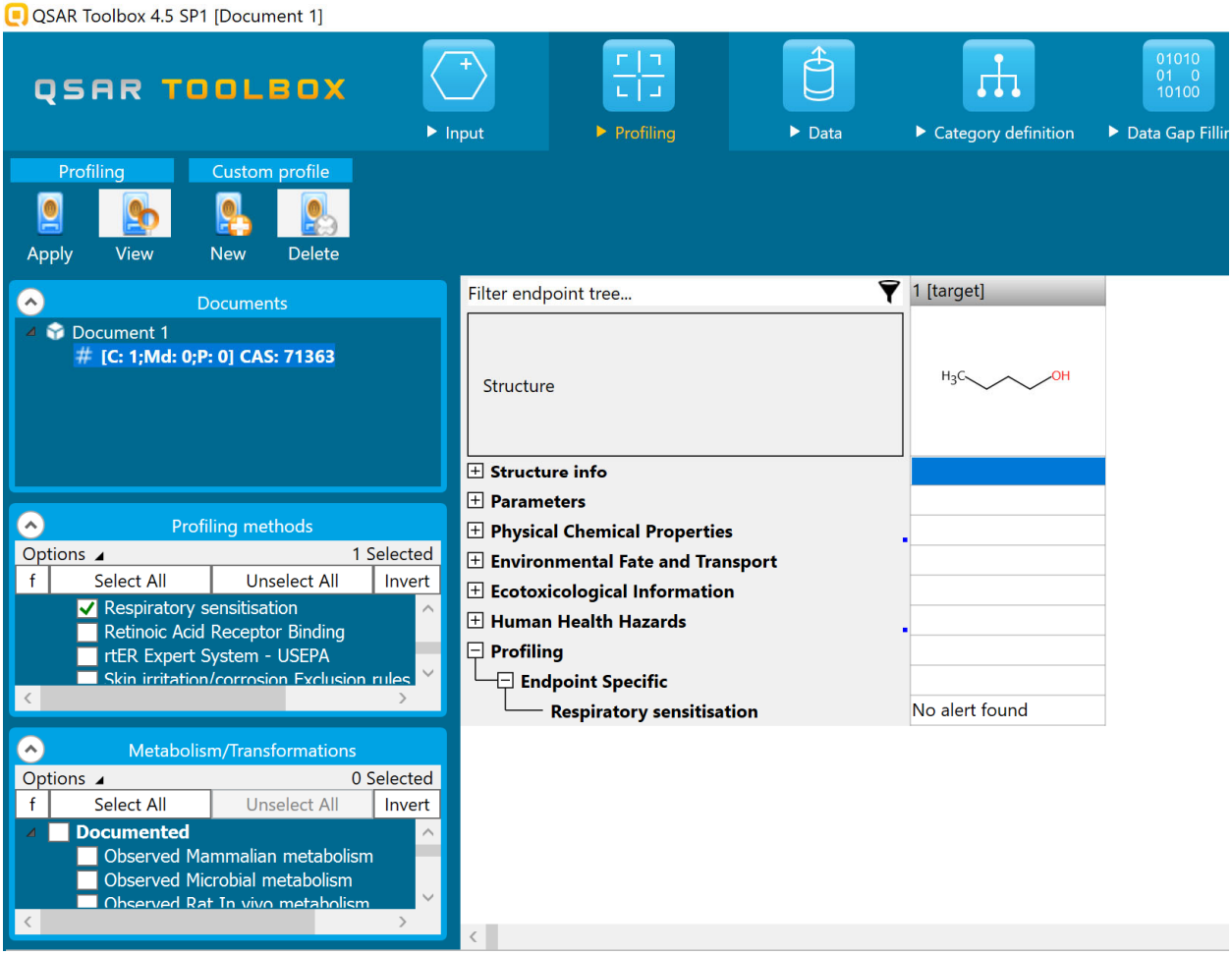
DTU-developed models

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 J: OECD Toolbox Profiling Results for n-Butyl Alcohol (CAS #71-36-3)



APPENDIX K: ECOSAR Modeling Results for n-Butyl Alcohol (CAS #71-36-3)

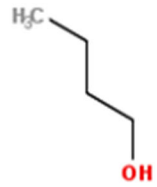
Created on May 10, 2023 2:47:45 PM

Organic Module Report

Results of Organic Module Evaluation

CAS	Name	SMILES
71363	1-Butanol	OCCCC

Structure



Details	
Mol Wt	74.12
Selected LogKow	0.88
Selected Water Solubility (mg/L)	63200
Selected Melting Point (°C)	-89.8
Estimated LogKow	0.84
Estimated Water Solubility (mg/L)	81172.5
Measured LogKow	0.88
Measured Water Solubility (mg/L)	63200
Measured Melting Point (°C)	-89.8

Class Results:	

Neutral Organics

Organism	Duration	End Point	Concentration (mg/L)	Max Log Kow	Flags
Fish	96h	LC50	617.21	5	
Daphnid	48h	LC50	316.02	5	
Green Algae	96h	EC50	153.5	6.4	
Fish		ChV	53.4	8	
Daphnid		ChV	23.11	8	
Green Algae		ChV	31.93	8	
Fish (SW)	96h	LC50	771.82	5	
Mysid	96h	LC50	1224.96	5	

Organism	Duration	End Point	Concentration (mg/L)	Max Log Kow	Flags
Fish (SW)		ChV	42.28	8	
Mysid (SW)		ChV	147.56	8	
Earthworm	14d	LC50	168.38	6	

APPENDIX L: Change in Benchmark Score

Table 5 provides a summary of changes to the GreenScreen® Benchmark™ for n-butyl alcohol. The GreenScreen® Benchmark Score for n-butyl alcohol 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 a version 1.3 update in 2016, and a version 1.4 update in 2022. Most recently, ToxServices maintained the GreenScreen® benchmark score with a version 1.4 update in 2023.

Table 5: Change in GreenScreen® Benchmark™ for n-Butyl Alcohol

Date	GreenScreen® Benchmark™	GreenScreen® Version	Comment
March 17, 2014	BM-2	v. 1.2	Original GreenScreen® assessment.
August 22, 2016	BM-2	v. 1.3	No change in BM score. The GreenScreen® assessment was updated with a v.1.3 template.
December 16, 2022	BM-2	v. 1.4	No change in BM score. The GreenScreen® assessment was updated with a v.1.4 template.
June 2, 2023	BM-2	v. 1.4	No change in BM score. The GreenScreen® assessment was updated with a v.1.4 template for release to the public.

Licensed GreenScreen® Profilers

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