CITRIC ACID

(CAS #77-92-9)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: March 18, 2024

Expiration Date: March 18, 2029

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GreenScreen® Executive Summary for Citric Acid (CAS #77-92-9)

Citric acid is a white, crystalline solid under standard temperature and pressure that is not reactive or flammable. It is not a volatile organic chemical (VOC) since it decomposes prior to boiling. Citric acid functions as an antimicrobial agent, anticoagulant, antioxidant, buffering agent, flavor enhancer, flavoring agent or adjuvant, leavening agent, pH control agent, chelant/sequestrant, solvent or vehicle, surface-active agent, and fragrance agent. The United States Food and Drug Administration (U.S. FDA) recognizes citric acid as a direct food additive, as an indirect food additive, and as generally recognized as safe (GRAS).

Citric acid was assigned a **GreenScreen Benchmark™ Score of 3** ("Use but Still Opportunity for Improvement"). This score is based on the following hazard score combinations:

- Benchmark 3c
    - Moderate Group II Human Health hazard (single dose systemic toxicity-STs)
    - High Group II Human Health hazard (eye irritation-IrE)

Data gaps (DG) exist for endocrine activity-E and repeated dose neurotoxicity-Nr*. As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), citric acid meets requirements for a GreenScreen Benchmark™ Score of 3 despite the hazard data gaps. In a worst-case scenario, if citric acid were assigned a High score for the data gap E, it would be categorized as a Benchmark 1 Chemical

The GreenScreen® Benchmark Score for citric acid has changed over time. The original GreenScreen® assessment was performed in 2015 under version 1.2 criteria and ToxServices assigned a Benchmark 2 (BM-2) score. Most recently, ToxServices changed the GreenScreen® benchmark score to a BM-3 due to reclassification of the eye irritation endpoint from **Very High** (high confidence) to **High** (high confidence) following a weight of evidence evaluation of this chemical's eye irritation dataset, including the harmonized EU classification for this endpoint.

New Approach Methodologies (NAMs) used in this GreenScreen® include *in silico* modeling for carcinogenicity, respiratory sensitization, chronic aquatic toxicity, persistence and biodegradation, and bioaccumulation and *in vitro* assays for genotoxicity. The quality, utility, and accuracy of NAM predictions are greatly influenced by two primary types of uncertainties:

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

Type I (input data) uncertainties in citric acid's NAMs dataset include citric acid's NAMs dataset include insufficient experimental data for carcinogenicity, endocrine activity, and respiratory sensitization, and lack of established test methods for respiratory sensitization. Citric acid's Type II (extrapolation output) uncertainties include limitation of *in vitro* genotoxicity assays in mimicking *in vivo* metabolism and their focusing on one or only a few types of genotoxicity events, the limitation of Toxtree and OECD Toolbox in identifying structural alerts without defining the applicability domains, the inability of Oncologic models to evaluate citric acid's carcinogenic potential, and the limitations in the examination of structural alerts for respiratory sensitization evaluation that does not account for non-immunologic mechanisms of respiratory sensitization. Some of citric acid's type II uncertainties were alleviated by the use of *in vitro* test batteries and/or in combination of *in vivo* data.

GreenScreen® Hazard Summary Table for Citric Acid

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

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

GreenScreen® Chemical Assessment for Citric Acid (CAS #77-92-9)

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

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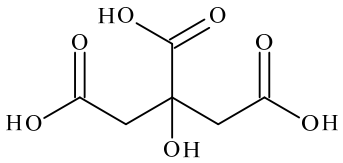
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: March 18, 2023

Expiration Date: March 18, 2029[2]

Chemical Name: Citric Acid

CAS Number: 77-92-9

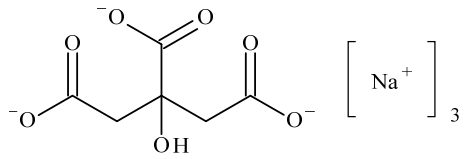
Chemical Structure(s):



Also called:
1,2,3-Propanetricarboxylic acid, 2-hydroxy-; 2-Hydroxypropane-1,2,3-tricarboxylic acid; 2-Hydroxy-1,2,3-propanetricarboxylic acid; 2-Hydroxypropan-1,2,3-tricarboxylic acid; 3-Carboxy-3-hydroxypentane-1,5-dioic acid; 2-Hydroxy-1,2,3-propanetricarboxylate; Propane-1,2,3-tricarboxylic acid, 2-hydroxy-; Zitronensaure; Uro-trainer; CITRONENSAEURE; Hydrocerol A; CITRIC-ACID, ANHYDRIDE CRISTO; CITRIC ACID ANHYDROUS; Chemfill; Citretten; Aciletten; Celenex 3P6; Acide citrique; acido citrico (U.S. EPA 2024a). EC 201-069-1, EC 680-681-4 (ECHA 2024).

Suitable surrogates or moieties of chemicals used in this assessment (CAS #'s):
Due to its irritating properties, limited citric acid data are available for the skin sensitization endpoint. Therefore, ToxServices used data for the sodium salt of citric acid, trisodium citrate (CAS #68-04-2), in a weight of evidence approach to assign the skin sensitization hazard score.

[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).



Surrogate: Trisodium citrate (CAS #68-04-2)

Identify Applications/Functional Uses (HSDB 2014, EC 2024, U.S. FDA 2024)**:**
1. Antimicrobial agent
2. Anticoagulant
3, Antioxidant
4. Buffering agent
5. Flavor enhancer
6. Flavoring agent or adjuvant
7. Leavening agent
8. pH control agent
9. Chelant/sequestrant
10. Solvent or vehicle
11. Surface-active agent
12. Fragrance agent

Known Impurities[3]:
Citric acid contains < 1% w/w water, < 0.15% w/w sulfate, < 0.035% w/w oxalates, < 0.02% w/w calcium, < 0.005% w/w iron, and < 0.005% w/w chloride (UNEP 2004). The screen is performed on the theoretical pure substance.

GreenScreen® Summary Rating for Citric Acid[4,5 6,7]: Citric acid was assigned a **GreenScreen Benchmark™ Score of 3** ("Use but Still Opportunity for Improvement") (CPA 2018b). This score is based on the following hazard score combinations:

- Benchmark 3c
    - Moderate Group II Human Health hazard (single dose systemic toxicity-STs)
    - High Group II Human Health hazard (eye irritation-IrE)

Data gaps (DG) exist for endocrine activity-E and repeated dose neurotoxicity-Nr*. As outlined in GreenScreen® Guidance (CPA 2018b) Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), citric acid meets requirements for a GreenScreen Benchmark™ Score of 3 despite the hazard data gaps. In a worst-case scenario, if citric acid were assigned a High score for the data gap E, it would be categorized as a Benchmark 1 Chemical.

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

Figure 1: GreenScreen® Hazard Summary Table for Citric Acid

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

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

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 citric acid is readily biodegradable, it is not expected to have relevant transformation products.

Introduction

Citric acid functions as an antimicrobial agent, anticoagulant, antioxidant, buffering agent, flavor enhancer, flavoring agent or adjuvant, leavening agent, pH control agent, chelant/sequestrant, solvent or vehicle, surface-active agent, and fragrance agent (HSDB 2014, EC 2024, U.S. FDA 2024). It is produced via fermentation of carbohydrates or extracted from citrus juices (HSDB 2014). The United States Food and Drug Administration (U.S. FDA) recognizes citric acid as a direct food additive under 21 CFR §172.755, §172.861, §173.160, §173.165, and §173.280; as an indirect food additive under 21 CFR §178.1010; and as generally recognized as safe (GRAS) under 21 CFR §184.1033 (U.S. FDA 2024). The Cosmetic Ingredient Review (CIR) Expert Panel concluded citric acid is safe in the present practices of use and concentration (≤ 4% for leave-on products, ≤ 10% for rinse-off products, and ≤ 39% in diluted for bath use products) (CIR 2014).

ToxServices assessed citric acid 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 (SCIL)

The SCIL is a list of chemicals that meet the Safer Choice standard (U.S. EPA 2024b). 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).

Citric acid is listed on the SCIL as an antimicrobial active, chelating agent, and processing aid and additive with a full green circle (FGC).

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 2024) 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 citric acid can be found in Appendix C.

- Citric acid is an LT-UNK chemical when screened using Pharos, and therefore a full GreenScreen® is required.
- Citric acid is not listed on the U.S. DOT list.
- Citric acid is on the following list for multiple endpoints:
    - German FEA - Substances Hazardous to Waters - Class 1 - Low Hazard to Waters.
- Specified lists for single endpoints are reported in individual hazard endpoints in the hazard assessment section below.

Hazard Statement and Occupational Control

The European Chemicals Agency (ECHA) established harmonized Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard statements of H319 and H335 for citric acid, as indicated in Table 1. General personal protective equipment (PPE) recommendations are presented in Table 2, below. No occupational exposure limits (OELs) were identified for citric acid.

Table 1: GHS H Statements for Citric Acid (CAS #77-92-9) (ECHA, CAS #77-92-9, 2024)

H Statement	H Statement Details
H319	Causes serious eye irritation
H335	May cause respiratory irritation

Table 2: Occupational Exposure Limits and Recommended Personal Protective Equipment for Citric Acid (CAS #77-92-9)

Personal Protective Equipment (PPE)	Reference	Occupational Exposure Limits (OEL)	Reference
Gloves, goggles or face shield, dust mask	HSDB 2014	None identified	N/A

Physicochemical Properties of Citric Acid

Citric acid is a white, crystalline solid under standard temperature and pressure. It has a low vapor pressure (1.65E-8 mm Hg) indicating it exists mostly in the solid phase. It is highly water soluble (592,000 mg/L) and is more soluble in water than in octanol (log K_{ow} = -1.8 to -0.2).

Table 3: Physical and Chemical Properties of Citric Acid (CAS #77-92-9)

Property	Value	Reference
Molecular formula	$C_6H_8O_7$ $CH_2COOH\text{-}C(OH)COOH\text{-}CH_2COOH$	PubChem 2024
SMILES Notation	C(C(=O)O)C(CC(=O)O)(C(=O)O)O	PubChem 2024
Molecular weight	192.12 g/mol	PubChem 2024
Physical state	Solid	ECHA, CAS #77-92-9, 2024
Appearance	White, crystalline	ECHA, CAS #77-92-9, 2024
Melting point	153°C	ECHA, CAS #77-92-9, 2024

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

Table 3: Physical and Chemical Properties of Citric Acid (CAS #77-92-9)		
Property	**Value**	**Reference**
Boiling point	Decomposes prior to boiling	ECHA, CAS #77-92-9, 2024
Vapor pressure	2.2E-6 Pa (1.65E-8 mm Hg) at 25°C	ECHA, CAS #77-92-9, 2024
Water solubility	592 g/L (592,000 mg/L) at 20°C	ECHA, CAS #77-92-9, 2024
Dissociation constant	pKa 1 = 3.13 pKa 2 = 4.76 pKa 3 = 6.4	ECHA, CAS #77-92-9, 2024
Density/specific gravity	Relative density = 1.665 at 20°C	ECHA, CAS #77-92-9, 2024
Partition coefficient	Log K_{ow} = -1.8 to -0.2	ECHA, CAS #77-92-9, 2024

Toxicokinetics

Absorption
Citric acid is readily absorbed following oral administration (CIR 2014).

Distribution
Citric acid is found in all body tissues, with the greatest percentage in the hard tissue of bones (ECHA 2018).

Metabolism
Citric acid originating endogenously or exogenously is metabolized in the cellular energy processes and serves as an intermediate in the Krebs or citric acid cycle (CIR 2014).

Excretion/Elimination
Approximately 65-90% of circulating citric is reabsorbed in the glomerulus of the kidneys, and the remaining 10-35% is excreted in the urine (CIR 2014). Metabolized citric acid is eliminated as exhaled carbon dioxide (ECHA 2018).

Hazard Classification Summary

Group I Human Health Effects (Group I Human)

Carcinogenicity (C) Score (H, M, or L): *L*
Citric acid was assigned a score of Low for carcinogenicity based on negative carcinogenicity results in limited studies supported by negative modeling predictions. GreenScreen® criteria classify chemicals as a Low hazard for carcinogenicity when adequate negative data are available and they are not GHS classified (CPA 2018b). The confidence in the score is low as it is based on limited experimental evidence and modeling.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- UNEP 2004
  - In a two-year dietary study in 20 male rats that received 3% or 5% citric acid in feed (contributing doses of 1,200 or 2,000 mg/kg/day, respectively, according to the SIDS dossier authors), no evidence of carcinogenicity was reported. The SIDS dossier reports this study with a reliability score of 4 (not assignable) (Horn et al. 1957).

  - Insufficient or negative evidence of a tumor-promoting effect was noted in several non-standard studies in which rats were co-treated with citric acid or citrate salt and a known carcinogen.
  - Based on the limited evidence available, UNEP concluded that citric acid is not a potential carcinogen.
- Toxtree 2018
  - Citric acid does not contain structural alerts for genotoxic carcinogenicity but does contain a structural alert for non-genotoxic carcinogenicity (n-alkylcarboxylic acids) (Appendix D).
- VEGA 2023
  - ToxServices predicted the carcinogenicity potential of citric acid using the following five VEGA v1.2.3 models: CAESAR v2.1.10, ISS v.1.0.3, IRFMN/ISSCAN-CGX v1.0.2, IRFMN/Antares v1.0.2, IRFMN oral classification v1.0.1, and IRFMN inhalation classification v1.0.1 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 index range from 0 (worst case) to 1 (best case). Generally, AD index values of > 0.70 indicate that the prediction has moderate or better predictivity (Gad 2016). The CAESAR, ISS, IRFMN-ISSCAN-CGX, and IRFMN-Antares models indicate citric acid is not a carcinogen based on experimental data. The results for these models are not discussed further herein (Appendix E).
  - Citric acid is within the AD of the IRFMN oral classification model (global AD index = 0.893) and the model predicts that it is a non-carcinogen. The similarity index of 0.797 and the accuracy and concordance indices of 1 support the use of this model. Therefore, ToxServices concluded the IRFMN oral classification model's prediction of citric acid as a non-carcinogen is reliable (Appendix E).
  - Citric acid is within the AD of the IRFMN inhalation classification model (global AD index = 0.758) and the model predicts that it is a non-carcinogen. The similarity index of 0.808 and the accuracy index of 1 support the use of this model, while the concordance index of 0.506 does not support the use of this model due to disagreement between the measured and predicted values for similar chemicals. Therefore, ToxServices concluded the IRFMN inhalation classification model's prediction of citric acid as a non-carcinogen is not reliable (Appendix E).
- U.S. EPA 2019, 2021
  - ToxServices attempted to evaluate the carcinogenic potential of citric acid using OncoLogic™ v9.0 (U.S. EPA 2021). However, this chemical belongs to a class of compounds not supported by the software at the time of writing (Appendix F). Additionally, citric acid does not belong to the organic chemical classes included in the OncoLogic™ v8.0 (U.S. EPA 2019). Therefore, ToxServices could not use OncoLogic™ to determine the carcinogenic potential of citric acid.
- DTU 2024
  - Citric acid is inside of the applicability domains of all seven E Ultra FDA RCA carcinogenicity models included in the Danish (Q)SAR Models, and is predicted to be negative in all seven (male rats, female rats, rats, male mice, female mice, mice, and rodents). Additionally, it is inside the applicability domains for four of the seven Leadscope FDA RCA carcinogenicity models included in the Danish (Q)SAR Models, and is predicted to be negative in all four (female rats, rats, mice, and rodents). Finally, citric acid is outside the applicability domain for the battery of liver-specific cancer models in mice or rats, with a negative, in domain prediction from the SciQSAR model (Appendix G).

- In summary, citric acid was not carcinogenic in a limited two-year carcinogenicity study in male rats (the current OECD Guideline 451 recommends testing be performed with 50 animals per sex, link) and did not exhibit tumor-promoting activity in non-standard tests performed with known carcinogens. Although it contains a structural alert for non-genotoxic carcinogenicity (n-alkylcarboxylic acids) as identified with Toxtree (2018), in domain modeling predictions from VEGA (2023) and Danish (Q)SAR Models (DTU 2024) indicate citric acid is not likely to be carcinogenic. Therefore, ToxServices concludes citric acid is not likely to possess carcinogenic potential.

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

Citric acid was assigned a score of Low for mutagenicity/genotoxicity based on negative *in vitro* mutagenicity results, negative *in vivo* clastogenicity results, and ToxServices not classifying it as genotoxic under GHS criteria. GreenScreen® criteria classify chemicals as a Low hazard for mutagenicity/genotoxicity when negative data are available for both gene mutations and chromosome aberrations, and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on reliable measured data for the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- UNEP 2004
  - Citric acid was not mutagenic in *in vitro* tests with *Salmonella typhimurium*, *Escherichia coli*, or *Saccharomyces cerevisiae*, in both the presence and absence of metabolic activation. It was negative for chromosomal damage in human and hamster cell cultures and in a dominant lethal assay in rats.
- ECHA, CAS #77-92-9, 2024
  - *In vitro*: Negative results for mutagenicity were obtained in a bacterial reverse mutation assay (GLP status not specified) performed in a manner similar to OECD Guideline 471 (no specific positive controls included). *S. typhimurium* tester strains TA92, TA94, TA98, TA100, TA1535, and TA1537 were exposed to citric acid (99.9% purity) in phosphate buffer at up to 5,000 µg/plate with and without exogenous metabolic activation (S9 mix from livers of polychlorinated biphenyl-induced rats). Treatment did not increase the mutation frequency in the presence or absence of metabolic activation. Results for the vehicle and untreated negative controls were not specified; positive results were obtained with other test substances evaluated at the same time as citric acid. The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) (Ishidate et al. 1984).
  - *In vitro*: Positive results for clastogenicity were obtained in a mammalian cell micronucleus test (GLP status not specified) conducted in a manner similar to OECD Guideline 487. Human peripheral lymphocytes were exposed to citric acid (purity not specified) in water at 50-3,000 µg/mL without metabolic activation. Treatment induced cytotoxicity at 3,000 µg/mL and statistically significantly increased the percentage of binucleated cells with micronuclei at 50 (1.65%), 100 (2.35%), and 200 µg/mL (2.60%) relative to the negative control (0.30%) in a concentration-dependent manner. The vehicle and positive (cyclophosphamide) controls were reported as valid. The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) (Yilmaz et al. 2008).
  - *In vitro*: Positive results for genotoxicity were obtained in a comet assay (GLP status not specified). Human lymphocytes were exposed to citric acid (≥ 99% purity) in distilled water at 50-3,000 µg/mL without exogenous metabolic activation. Treatment induced cytotoxicity

at 3,000 µg/mL and statistically significantly increased the mean tail intensity and mean tail length at 200 µg/mL. The vehicle control was reported as valid, but the positive control (hydrogen peroxide) was not considered valid due to the lack of historical data for this chemical and other genotoxicity tests not using it as a positive control. The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) (Yilmaz et al. 2014).

- *In vitro*: Negative results for mutagenicity were obtained in a bacterial reverse mutation assay (GLP status not specified) conducted in a manner similar to OECD Guideline 471 (only 4 strains and 3 concentrations used). *S. typhimurium* tester strains TA97, TA98, TA100, and TA104 were exposed to citric acid (purity and vehicle not specified) at 0, 500, or 1,000 µg/plate with and without exogenous metabolic activation (S9 mix from livers of phenobarbital-induced rats). Treatment did not increase the mutation frequency in the presence or absence of metabolic activation. The vehicle and one positive (2-aminoanthracene) controls were reported as valid. The REACH dossier reports this study with a reliability score of 4 (not assignable) (Al-ani and Al-Lami 1988).
- *In vitro*: Positive results for clastogenicity were obtained in a mammalian cell chromosome aberration test (GLP-compliance not specified) conducted in a manner similar to OECD Guideline 473 (no activation, sister chromatid unions scored as aberrations). Human peripheral lymphocytes were exposed to citric acid (purity not specified) in water at 50-3,000 µg/mL without exogenous metabolic activation. After the 24- or 48-hour exposures, treatment at 3,000 µg/mL induced cytotoxicity and treatment with 50, 100, or 200 µg/mL statistically significantly increased the percentage of abnormal cells and the number of chromosome aberrations per cell. Concentration-dependent increases were identified for both endpoints at the 24-hour exposure and for the number of chromosome aberrations/cell at the 48-hour exposure. The vehicle and positive controls were reported as valid. The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) (Yilmaz et al. 2008).
- *In vitro*: Negative results for clastogenicity were obtained in a mammalian cell chromosome aberration test (GLP status not specified) conducted in a manner similar to OECD Guideline 473 (no activation). Chinese hamster lung cells were exposed to citric acid (99.9% purity) in phosphate buffer at up to 5.0 mg/mL without exogenous metabolic activation. Treatment did not increase the frequency of chromosome aberrations in the absence of metabolic activation. The vehicle and untreated negative controls were not specified, but other substances tested at the same time provided positive results. The REACH dossier reports this study with a reliability score of 4 (not assignable) (Ishidate et al. 1984).
- *In vivo*: Negative results for clastogenicity were obtained in a non-GLP-compliant mammalian bone marrow chromosome aberration test conducted in a manner similar to OECD Guideline 475 (only 50 cells evaluated per animal). Male Sprague-Dawley rats (5/dose group) were administered gavage doses of citric acid (purity not specified) in physiological saline as a single dose (acute study) or daily for 5 days (subacute study). For the acute study, the animals were dosed with 300, 500, 3,000, or 3,500 mg/kg and were sacrificed 6, 24, or 48 hours after dosing. For the subacute study, the animals were dosed with 1.2, 12, or 120 mg/kg/day on 5 sequential days and were sacrificed 6 hours after administration of the final dose. Treatment did not increase the frequency of chromosome aberrations in the acute or subacute studies. The vehicle and positive (triethylenemelamine) controls were reported as valid. The REACH dossier reports this study with a reliability score of 2 (reliable with restriction) (Unnamed study 1975).
- *In vivo*: Negative results for genotoxicity were obtained in a non-GLP-compliant dominant lethal assay performed in a manner similar to EU Method B.22 (no information regarding mating). Male Sprague-Dawley rats (10/group) were administered gavage doses of citric

acid (purity not specified) in physiological saline either as a single dose or daily for 5 consecutive days. The single doses were 0, 300, 500, or 3,500 mg/kg and the subacute doses were 1.2, 12.0 or 120 mg/kg/day. Treated males were then mated with two virgin females each week for 7 or 8 weeks. The females were sacrificed two weeks after mating and the fertility indices, pre-implantation loss, and lethal effects were evaluated. Treatment did not adversely affect these parameters in the acute study. Treatment with 1.2 and 12.0 mg/kg/day in the subacute study increased the preimplantation losses per female during week 4 but not during week 1 or week 7 and no adverse effects were noted on this endpoint at the high dose during week 4. The vehicle and positive (triethylenemelamine) controls were reported as valid. The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) (Unnamed study 1975).

- In summary, citric acid was not mutagenic *in vitro* but produced positive results in some *in vitro* clastogenicity assays. However, it was not clastogenic in an *in vivo* bone marrow chromosome aberration test and was not genotoxic in a dominant lethal assay, indicating citric acid is not likely to be genotoxic in intact organisms. Therefore, ToxServices did not classify citric acid as genotoxic under GHS criteria (UN 2023).

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

Citric acid was assigned a score of Low for reproductive toxicity based on the lack of adverse effects on reproductive parameters in one- and two-generation studies in rats. GreenScreen® criteria classify chemicals as a Low hazard for reproductive toxicity when adequate negative data are available and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on measured data for the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- U.S. EPA 2024c
  - In a two-generation study, rats were provided feed containing 0 or 1.2% citric acid (contributing doses of 0 or 600 mg/kg/day). Exposure began 29 weeks prior to mating and continued for a few months after mating. Treatment did not adversely affect reproduction; therefore, the authors assigned the reproductive toxicity NOAEL as 600 mg/kg/day, the only dose tested.
  - In a one-generation study, female rats (strain and number not specified) and female mice (strain and number not specified) were provided feed containing 5% citric acid (contributing a dose of 2,500 mg/kg/day) prior to, during, and after mating. Treatment reduced the mouse body weight gain and survival time (statistical significance not provided). Treatment did not adversely affect pregnancy rate, litter size, or pup survival during the postnatal period. No effects identified in rats were specifically stated. The authors identified a LOAEL of 2,500 mg/kg/day for mice based on the reduced body weight gain and survival. ToxServices identified a reproductive toxicity NOAEL of 2,500 mg/kg/day based on the lack of adverse effects on reproductive parameters at the only dose tested.

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

Citric acid was assigned a score of Low for developmental toxicity based on the lack of developmental toxicity in experimental animals up to the highest dose tested in prenatal developmental toxicity studies. GreenScreen® criteria classify chemicals as a Low hazard for developmental toxicity when adequate negative data are available and they are not GHS classified (CPA 2018b). The confidence in the score is low because although the REACH dossier assigned Klimisch 2 scores to the developmental toxicity

studies on citric acid, OECD assigned lower reliability scores, the studies were not GLP-compliant or according to guidelines, and the secondary sources provided limited and conflicting details.

- Authoritative and Screening Lists
  - *Authoritative:*
    - MAK Pregnancy Risk Group C ("Damage to the embryo or foetus is unlikely when the MAK value or the BAT value is observed").
  - *Screening:* Not present on any screening lists for this endpoint.
- UNEP 2004; ECHA, CAS #77-92-9, 2024
  - No developmental toxicity was detected in a non-GLP-compliant study in which pregnant female hamsters (30/dose group) were administered gavage doses of citric acid (purity not specified) at ≤ 272 mg/kg/day on GD 6-10. The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) while the SIDS dossier reports this study with a reliability score of 4 (not assignable) (Food & Drug Research Laboratories, Inc. 1973 or Unnamed summary report 1973). *ToxServices notes that, in the REACH dossier, the experimental animals are identified as albino CD-1 mice in the test animals section and as pregnant hamsters in the 'Applicant's summary and conclusion' section.*
  - No developmental toxicity was detected in a non-GLP-compliant study in which pregnant female Wistar rats (25/dose group) were administered gavage doses of citric acid (purity not specified) at ≤ 295 mg/kg/day on GD 6-15. The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) while the SIDS dossier reports this study with a reliability score of 4 (not assignable) (Food & Drug Research Laboratories, Inc. 1973 or Unnamed summary report 1973).
  - No developmental toxicity was detected in a non-GLP-compliant study in which pregnant female Dutch belted rabbits (25/dose group) were administered gavage doses of citric acid (purity not specified) at ≤ 425 mg/kg/day on GD 6-15. The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) while the SIDS dossier reports this study with a reliability score of 4 (not assignable) (Food & Drug Research Laboratories, Inc. 1973 or Unnamed summary report 1973).
  - No developmental toxicity was detected in a non-GLP-compliant study in which pregnant female albino CD-1 mice (30/dose group) were administered gavage doses of citric acid (purity not specified) at ≤ 241 mg/kg/day on GD 6-15. The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) while the SIDS dossier reports this study with a reliability score of 4 (not assignable) (Food & Drug Research Laboratories, Inc. 1973 or Unnamed summary report 1973). *ToxServices notes that the 'Applicant's summary and conclusion' section of the REACH dossier study entry identifies the animals as pregnant rats.*

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

Citric acid was assigned a score of Data Gap for endocrine activity based on the lack of data for endocrine receptor binding or circulating endocrine hormone levels.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- UNEP 2004
  - Citric acid is a natural component of eukaryotic cellular energy metabolism as part of the citric acid or Krebs cycle. It is unlikely to exhibit endocrine activity.

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

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

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

Citric acid was assigned a score of Low for acute toxicity based on based on oral LD_{50} values ≥ 5,400 mg/kg in rats and mice and a dermal LD_{50} > 2,000 mg/kg in rats. GreenScreen® criteria classify chemicals as a Low hazard for acute toxicity when oral and dermal LD_{50} values are > 2,000 mg/kg (CPA 2018b). The confidence in the score is high as it is based on reliable measured data for the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA, CAS #77-92-9, 2024
  - *Oral*: LD_{50} (mouse, Füllinsdorf Albino (SPF), male/female) = 5,400 mg/kg. The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) (Unnamed study 1981).
  - *Oral*: LD_{50} (rat, ICR-JCL, male) = 11,700 mg/kg. The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) (Yokotani et al. 1971).
  - *Oral*: LD_{50} (mice, SD-JCL, male) = 5,790 mg/kg. The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) (Yokotani et al. 1971).
  - *Dermal*: LD_{50} (rats, Sprague-Dawley, male/female) > 2 000 mg/kg (GLP-compliant, OECD Guideline 402). The REACH dossier reports this study with a reliability score of 1 (reliable without restriction (Unnamed study 2006).

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

Citric acid was assigned a score of Moderate for systemic toxicity (single dose) based on the harmonized EU classification of citric acid as a GHS Category 3 specific target organ toxicant following single exposures for respiratory irritation. GreenScreen® criteria classify chemicals as a Moderate hazard for systemic toxicity (single dose) when they are classified as GHS Category 3 specific target organ toxicants following single exposures for respiratory irritation (CPA 2018b). The confidence in the score is high as it is based on the harmonized EU classification.

- Authoritative and Screening Lists
  - *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].
    - GHS - New Zealand - Specific target organ toxicity - single exposure category 3.
      - Based on respiratory irritation (CCID 2024).
- UNEP 2004:
  - *Oral*: A young woman ingested a single dose of 25 g (417 mg/kg) of citric acid, causing her to vomit and nearly die (Nazario 1952).

  - *Inhalation*: Exposure to an unspecified concentration of citric acid caused bronchoconstriction in dogs, "which have non-specific airway hyperactivity." No additional details were provided (Lindemann et al. 1989).
  - *Inhalation*: In human asthmatics, unspecific concentrations of citric acid produced bronchoconstriction. No additional details were provided (Lindemann et al. 1989).
- ECHA, CAS #77-92-9, 2024
  - *Oral*: In the acute oral toxicity study that identified an oral LD_{50} value of 5,400 mg/kg in Füllinsdorf Albino (SPF) mice (5/sex/group), the animals were dosed via gavage with 3,000, 4,200, 6,000, 8,500, or 12,000 mg/kg. Treatment with 6,000 mg/kg produced "slight relaxation" two hours after dosing. No clinical signs of toxicity were identified at ≤ 4,200 mg/kg and all animals dosed with ≥ 8,500 mg/kg died. No data for body weights were presented and gross pathological observations were not conducted. The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) (Unnamed study 1981).
  - *Oral*: In the acute oral toxicity studies that identified oral LD_{50} values of 11,700 mg/kg and 5,790 mg/kg in male ICR-JCL rats (6/group) and SD-JCL mice (6/group), respectively. The rats were dosed (presumably via gavage) with 1,800 or 12,500 mg/kg and the mice were dosed with 5,790 or 7,000 mg/kg. The spontaneous movement of the animals in the cages increased several minutes following dosing. Motor ataxia, mydriasis (dilation of the pupil), and decreased rate of respiration were observed approximately 50 minutes after dosing. Deaths observed were caused by respiratory failure. Animals that survived to the scheduled sacrificed showed full recovery within several hours of dosing and exhibited no adverse clinical signs of toxicity 24 hours after dosing. Hemorrhage of the gastric mucosa was the only gross pathological change observed at necropsy. The lowest doses tested were 5,790 mg/kg for mice and 1,800 mg/kg for rats. The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) (Yokotani et al. 1971).
  - *Dermal*: In the GLP-compliant, OECD Guideline 402 acute dermal toxicity test that identified a dermal LD_{50} value greater than 2,000 mg/kg in Sprague-Dawley rats (5/sex/group), the only dose tested was 2,000 mg/kg. Treatment did not induce clinical signs of toxicity, changes to body weights, or gross pathological abnormalities. The REACH dossier reports this study with a reliability score of 1 (reliable without restriction (Unnamed study 2006).
- UNEP 2004; ECHA, CAS #77-92-9, 2024
  - *Inhalation*: In a study in guinea pigs, citric acid aerosol at the concentration of 0.93 M or 75 mg/mL induced 90 +/-1.9 coughs during a three-minute exposure. Bronchoconstriction occurred after 3-4 minutes. No additional details were provided. The REACH dossier reports this study with a reliability score of 4 (not assignable) (Forsberg and Karlsson 1986).
- ECHA 2019
  - *Inhalation*: In several human volunteer studies where subjects were exposed to citric acid aerosol, the main treatment-related effect was cough response.
- In summary, the only gross pathological effect noted with oral exposure to citric acid was hemorrhaging of the gastric mucosa which is likely a local effect following ingestion of an irritating substance. Single oral and dermal dosing did not produce evidence of systemic toxicity based on limited evaluations. However, inhalation exposures to citric acid produced evidence of respiratory irritation in guinea pigs. Additionally, citric acid produces a cough response in exposed humans. Since it is classified as a GHS Category 3 specific target organ toxicant following single exposure for respiratory irritation in the EU harmonized classification (ECHA, CAS #77-92-9, 2024), ToxServices classified it as a Category 3 specific target organ toxicant following single exposure for

respiratory irritation under GHS criteria (UN 2023).

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

Citric acid was assigned a score of Low for systemic toxicity (repeated dose) based on ToxServices not classifying it as a specific target organ toxicant following repeated doses under GHS criteria. GreenScreen® criteria classify chemicals as a Low hazard for systemic toxicity (repeated dose) when adequate and negative data and no GHS classification are available (CPA 2018b). The confidence in the score is high as it is based on measured data for the target chemical. Although all of the studies were reported with limited details, consistently negative results across multiple studies and species, in conjunction with citric acid's endogenous functions, support high confidence.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- UNEP 2004
  - *Oral*: No treatment-related effects were reported in a repeated dose toxicity study in which rabbits (15/dose group, strain and sex not specified) were provided feed containing 7.7% sodium citrate (approximately 1,500 mg citric acid/kg/day) for 150 days. No further details were provided. *ToxServices identified a NOAEL of 1,500 mg/kg/day based on the available information for this study*.
  - *Oral*: No treatment-related effects were reported in a repeated dose toxicity study in which dogs (3/dose group, strain and sex not specified) were provided feed containing citric acid at 1,380 mg/kg/day for 120 days. No further details were provided. *ToxServices identified a NOAEL of 1,380 mg/kg/day based on the available information for this study*.
  - *Oral*: In a two-year repeated dose toxicity study, male rats (20/dose group, strain not specified) were provided feed containing 3% or 5% citric acid (equivalent to 1,200 and 2,000 mg/kg/day, respectively). Slightly decreased growth was measured in both dose groups and food consumption decreased in the high dose group. No gross pathological abnormalities were observed at necropsy. The study authors identified a NOAEL of 1,200 mg/kg/day.
- U.S EPA 2024c
  - *Oral*: Rats given 600 mg/kg/day orally for 90 days had no weight, blood, histopathological or reproductive effects.
  - *Oral*: In a 1-year three-generation rat oncogenic/chronic toxicity feeding study, no adverse effects were noted on growth, reproduction, mortality, hematology, or metabolism at the highest dose level (800 mg/kg/day citric acid).
- GHS criteria (UN 2023) identifies oral guidance values of 10 and 100 mg/kg/day for subchronic repeated oral dose toxicity studies. Since the available subchronic and chronic repeated oral dose toxicity data identified NOAELs > 100 mg/kg/day, ToxServices did not classify citric acid as a specific target organ toxicant following repeated doses under GHS criteria (UN 2023).

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

Citric acid was assigned a score of Low for neurotoxicity (single dose) based on ToxServices not classifying it as a specific target organ toxicant following single exposures for neurotoxicity under GHS criteria. GreenScreen® criteria classify chemicals as a Low hazard for neurotoxicity (single dose) when adequate and negative data and no GHS classification are available (CPA 2018b). The confidence in the score is low as the acute toxicity studies did not include detailed functional analyses.

- Authoritative and Screening Lists

  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA, CAS #77-92-9, 2024
  - *Oral*: In the acute oral toxicity study that identified an oral LD_{50} value of 5,400 mg/kg in Füllinsdorf Albino (SPF) mice (5/sex/group), the animals were dosed with 3,000, 4,200, 6,000, 8,500, or 12,000 mg/kg. Treatment with 6,000 mg/kg produced "slight relaxation" two hours after dosing. No clinical signs of toxicity were identified at ≤ 4,200 mg/kg and all animals dosed with ≥ 8,500 mg/kg died. No gross pathological observations were conducted. The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) (Unnamed study 1981).
  - *Oral*: In the acute oral toxicity studies that identified oral LD_{50} values of 11,700 mg/kg and 5,790 mg/kg in male ICR-JCL rats (6/group) and SD-JCL mice (6/group), respectively. The rats were dosed with 1,800 or 12,500 mg/kg and the mice were dosed with 5,790 or 7,000 mg/kg. The spontaneous movement of the animals in the cages increased several minutes following dosing. Motor ataxia, mydriasis (dilation of the pupil), and decreased rate of respiration were observed approximately 50 minutes after dosing. Deaths observed were caused by respiratory failure. Animals that survived to the scheduled sacrificed showed full recovery within several hours of dosing and exhibited no adverse clinical signs of toxicity 24 hours after dosing. Hemorrhage of the gastric mucosa was the only gross pathological change observed at necropsy. The lowest doses tested were 5,790 mg/kg for mice and 1,800 mg/kg for rats. The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) (Yokotani et al. 1971).
  - *Dermal*: In the GLP-compliant, OECD Guideline 402 acute dermal toxicity test that identified a dermal LD_{50} value greater than 2,000 mg/kg in Sprague-Dawley rats (5/sex/group), the only dose tested was 2,000 mg/kg. Treatment did not induce clinical signs of toxicity or gross pathological abnormalities. The REACH dossier reports this study with a reliability score of 1 (reliable without restriction (Unnamed study 2006).
- GHS criteria (UN 2023) define chemicals as specific target organ toxicant following single doses when they produce non-lethal neurotoxicity following single oral or dermal doses ≤ 2,000 mg/kg or inhalation exposures to aerosols ≤ 5.0 mg/L (Categories 1 or 2) or reversible narcotic effects (defined as ataxia, narcosis, lethargy, and lack of coordination righting reflex) at any dose/concentration (Category 3). Based on the lack of clear neurobehavioral or neuropathological changes following single exposures to citric acid, ToxServices did not classify citric acid as a specific target organ toxicant following single exposures for neurotoxicity under GHS criteria (UN 2023). Although one study reported potential neurological signs (e.g., ataxia, pupil dilation, and altered respiratory rate), these effects occurred at very high and lethal doses that also resulted in hemorrhage of the gastric mucosa; therefore, these effects are likely due to severe discomfort.

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

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

Skin Sensitization (SnS) (Group II*) Score (H, M, or L): L
Citric acid was assigned a score of Low for skin sensitization based on the lack of skin sensitization

identified in clinical tests with the target chemical and a lack of skin sensitization reactions identified in a guinea pig maximization test performed with the surrogate sodium citrate. GreenScreen® criteria classify chemicals as a Low hazard for skin sensitization when adequate and negative data and no GHS classification are available (CPA 2018b). The confidence in the score is high as it is based on reliable measured data for the surrogate supported by clinical data for the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- UNEP 2004:
  - Patch testing of 60 eczema patients with 2.5% citric acid in petrolatum did not produce any irritant or allergic reactions (Niinimäki 1987).
- CIR 2014
  - A human repeat insult patch test (HRIPT) was performed with 56 patients administered topical application of a cuticle cream containing 4% citric acid under semi-occlusive dressing three times per week for three weeks. A challenge dose was applied two weeks after the last induction dose. Citric acid was not sensitizing to the skin under the tested conditions (Clinical Research Laboratories Inc. 2007).
  - A skin prick test was performed with 91 patients with chronic angioedema or urticaria exposed to a 2.5% aqueous solution of citric acid. Three patients (3%) exhibited positive dermal reactions, with one of these patients also reacting to propionic and benzoic acids (Malanin and Kalimo 1989).
- ECHA, CAS #68-04-2, 2024
  - *Surrogate: Sodium citrate (CAS #68-04-2):* A GLP-compliant, OECD Guideline 406 guinea pig maximization test was performed with male Himalayan Spotted (Ibm:GOHI) guinea pigs (10 vehicles, 20 test animals) administered dermal doses of sodium citrate (purity not specified). The induction doses were administered as intradermal injections of 5% sodium citrate and topical applications of 75% sodium citrate in water under occlusive dressing for 48 hours. The challenge dose was applied on day 22 as topical applications of 25%, 50%, or 75% citric acid in water under occlusive dressing for 24 hours. At readings 24 and 48 hours after the challenge dose, challenge treatment did not include any positive dermal reactions. Therefore, the authors concluded sodium citrate was not sensitizing to the skin under the tested conditions. The REACH dossier reports this study with a reliability score of 1 (reliable with restrictions) (Unnamed study 1995).

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

Citric acid was assigned a score of Low for respiratory sensitization based on the lack of dermal sensitization potential according to the ECHA guidance (2017). GreenScreen® criteria classify chemicals as a Low hazard for respiratory sensitization when they are not GHS classified (CPA 2018b). The confidence in the score is low as this evaluation does not include non-immunologic mechanisms of respiratory sensitization, and no specific data are available for respiratory sensitization.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- OECD 2023
  - Citric acid does not contain any structural alerts for respiratory sensitization (Appendix H).
- 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 citric acid 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 citric acid, and as citric acid does not contain any structural alerts for respiratory sensitization (OECD 2023), citric acid is not expected to be a respiratory sensitizer.

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

Citric acid was assigned a score of Low for skin irritation/corrosivity based on ToxServices' conclusion that it does not warrant classification as a skin irritant under GHS criteria (UN 2023) based on results of several irritation studies in rabbits. GreenScreen® criteria classify chemicals as a Low hazard for skin irritation/corrosivity when adequate and negative data and no GHS classification are available (CPA 2018b). The confidence in the score is high as it is based on reliable measured data on the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - GHS – Australia - H315 - Causes skin irritation [Skin corrosion/irritation - Category 2].
- ECHA, CAS #77-92-9, 2024
  - A GLP-compliant, OECD Guideline 404 dermal irritation test was performed with New Zealand White rabbits (6 total, sex not specified) administered topical applications of 0.5 g (500 mg) citric acid (purity not specified) to shaved skin under semi-occlusive dressing moistened with water for 4 hours. The dermal reactions were evaluated at 1, 24, 48, and 72 hours. The mean primary dermal irritation index (PDII) at 1, 24, 48, and 72 hours was 0.3/8 (mild skin irritation) and the mean overall irritation score at 24, 48, and 72 hours was 0.3/8. Well-defined erythema (mean score of 1.67/4 at 24, 48, and 72 hours) was observed in one animal at 1-48 hours and mild edema (mean score of 0.33/4 at 24, 48, and 72 hours) was observed in the same animal at 72 hours. The remaining animals did not exhibit erythema or edema at 24, 48, or 72 hours. The study authors concluded that citric acid was not irritating to the skin under the tested conditions. The REACH dossier reports this study with a reliability score of 1 (reliable without restriction) (Unnamed study 1990).
  - A GLP-compliant dermal irritation test conducted in a manner similar to OECD Guideline 404 was performed with New Zealand rabbits (6 males) administered topical applications of an unspecified amount of citric acid (purity not specified) to shaved skin under semi-occlusive dressing for 4 hours. The dermal reactions were evaluated at 0.5, 1, 24, 48, and 72 hours after the exposure period. The PDII at 72 hours was 0.33/2. One animal exhibited well-defined erythema at 0.5, 1, 24, and 48 hours after exposure. By 72 hours, this animal exhibited slight erythema. The REACH dossier reports this study with a reliability score of 1 (reliable without restriction) (Unnamed study 1990).
  - A non-GLP-compliant dermal irritation test conducted in a manner similar to OECD Guideline 404 was performed with New Zealand White rabbits (3 total, sex not specified) administered topical applications of an unspecified amount of citric acid (purity not specified) in water to clipped and abraded or non-abraded skin under occlusive dressing for 4 hours. The dermal reactions were evaluated at 0, 20, and 44 hours after the exposure period. The mean PDII for intact and abraded skin was 0.8/8 and the mean overall irritation

score for non-abraded skin was 0/8. The study authors concluded that citric acid was slightly irritating to abraded skin and not irritating to intact skin. The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) (Unnamed study 1984).
    - A non-GLP-compliant dermal irritation test conducted in a manner similar to OECD Guideline 404 was performed with New Zealand White rabbits (6 total, sex not specified) administered topical applications of an unspecified amount of citric acid (purity not specified) in water to shaved skin under occlusive dressing for 4 hours. An observation period of 44 hours followed the exposure period. The mean overall irritation score was 0. The study authors concluded that citric acid was not irritating to the skin in this study. The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) (Unnamed study 1979).
- UNEP 2004
    - In a Draize test, citric acid was not irritating to the skin when applied as a 30% aqueous solution to the skin of three New Zealand White rabbits following a 4-hour exposure under occlusive dressing. The overall primary irritation index was 0.84. The SIDS dossier reports this study with a reliability score of 1 (reliable without restriction) (Hoffmann 1984).
    - Application of a 50% citric acid solution to the tongue of dogs for 5 minutes caused severe ulceration and tissue damage (Lilly and Cutcher 1972).
    - Citric acid has been reported to cause an irritant skin dermatitis to waiters and bakers. 2% stated in one case to case pain or "sting", patch testing of 60 eczema patients with 2.5% citric acid in petrolatum did not produce any irritant or allergic reactions; thus, the reaction could be mainly due to the acid effect, which in unbuffered 2% to 2.5% aqueous solution results in a pH of approximately 2. The SIDS dossier reports this study with a reliability score of 4 (not assignable) (Niinimäki 1987).
    - The authors of the SIDS document concluded pure citric acid and citric acid aqueous solutions should not be judged as dermal irritants.
- GHS criteria define skin irritants as chemicals that produce mean scores ≥ 1.5 for erythema and/or edema in at least 2 of 3 animals following readings at 24, 48, and 72 hours (UN 2023). Although it is classified as a GHS Category 2 skin irritant by Australia, citric acid did not produce sufficient dermal irritation to warrant classification under GHS criteria based on the results of available skin irritation studies. Additionally, the ECHA Committee for Risk Assessment (RAC) did not classify citric acid as a skin irritant based on the available data.

Eye Irritation/Corrosivity (IrE) (Group II) Score (vH, H, M, or L): H
Citric acid was assigned a score of High for eye irritation/corrosivity based on the harmonized EU classification of citric acid as a GHS Category 2A eye irritant. GreenScreen® criteria classify chemicals as a High hazard for eye irritation/corrosivity when they are classified as GHS Category 2A eye irritants (CPA 2018b). The confidence in the score is high as it is based on an EU harmonized GHS classification.

- Authoritative and Screening Lists
    - *Authoritative:*
        - EU - GHS (H-Statements) Annex 6 Table 3-1 - H319 - Causes serious eye irritation [Serious eye damage/eye irritation - Category 2A].
    - *Screening:*
        - GHS - New Zealand - Serious eye damage category 1.
            - Based on it being highly irritating to the eyes of rabbits (CCID 2024).
        - GHS – Australia - H319 - Causes serious eye irritation [Serious eye damage/eye

irritation - Category 2A].

- ECHA, CAS #77-92-9, 2024
    - A GLP-compliant, OECD Guideline 405 ocular irritation test was performed with New Zealand White rabbits (3 total, sex not specified) administered ocular instillations of 0.1 mL 10% or 30% citric acid (purity not specified) in water. An observation period of 14 days followed the exposure period. The mean overall irritation scores at 1, 24, 48, and 72 hours were 9.3/110 and 16/110 for the 10% and 30% solutions, respectively. The effects were fully reversible within 7 days for the 10% solution, but were not fully reversible within 14 days for the 30% solution in one animal. At 24, 48, and 72 hours, the 10% solution produced a mean corneal score of 0/4, a mean iris score of 0/2, a mean conjunctival score of 1/3 (individual animal scores of 1, 1, and 1), and a mean chemosis score of 0/4. At 24, 48, and 72 hours, the 30% solution produced a mean corneal score of 0/4, a mean iris score of 0/2, a mean conjunctival score of 3/3 (individual animal scores of 3, 3, and 3), and a mean chemosis score of 2.43/4 (individual animal scores of 2.3, 2.7, and 2.3). The REACH dossier authors assumed the ocular irritation effects would have resolved after 21 days for the third animal and classified citric acid as a GHS Category 2 eye irritant based on these results. The REACH dossier reports this study with a reliability score of 1 (reliable without restriction) (Unnamed study 1984). *Based on the effects observed with 30% solution not being reversible at the end of the observation period of 14 days, ToxServices classified citric acid to GHS Category 1 for eye irritation.*
    - An ocular irritation test was performed with albino rabbits (5 total, sex not specified) administered ocular instillations of 0.5% (right eye) or 2% (left eye) citric acid (purity not specified) daily over 7 days. An observation period of 1 month followed the exposure period. A cloudy cornea and edematous lids were observed 30 minutes after instillation of the 2% solution. Similar effects were observed after instillation of the 0.5% solution but to a lesser magnitude. The opacity was localized within the internal layers of the cornea. After 24 hours, the 2% solution produced solid opacity and 100% necrosis while the 0.5% solution caused the eye to be cloudy with 80% necrosis. After two weeks the left eye was opaque and had a thickened cornea. Dense necrosis surrounded by opacity was observed over the pupillary area, with some vascularization from the dorsal limbus. Based on the production of necrosis, the study authors concluded that citric acid was irritating to the eye. The REACH dossier reports this study with a reliability score of 4 (not assignable) (Carpenter et al. 1946).
- UNEP 2004; ECHA, CAS #77-92-9, 2024
    - A 5% citric acid aqueous solution (50g/L) has a pH of 1.8 at 25°C. The REACH dossier reports this study with a reliability score of 4 (not assignable) while the SIDS dossier reports the reliability score as 2 (reliable with restrictions) (Jungbunzlauer 1993). *Per GHS criteria (UN 2023), chemicals with pH <2 are classified to GHS Category 1 for eye irritation in the absence of other data. ECHA (2019) concluded that, based on the available in vivo data, "a pH < 2 cannot be used as a predictor of skin irritation/corrosion" for citric acid.*
- UNEP 2004
    - Instillation of 750 µg (0.75 mg) citric acid to the eyes of rabbits for 24 hours results in severe irritation effects. No further details were provided. The SIDS dossier reported this study with a reliability score of 4 (not assignable) (Marhold 1986).
    - The authors of the SIDS document concluded pure citric acid and citric acid aqueous solutions should be judged as ocular irritants.

Ecotoxicity (Ecotox)

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

Citric acid was assigned a score of Low for acute aquatic toxicity based on acute aquatic toxicity values > 100 mg/L. GreenScreen® criteria classify chemicals as a Low hazard for acute aquatic toxicity when acute aquatic toxicity values > 100 mg/L (CPA 2018b). The confidence in the score is high as it is based on reliable measured data for the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- UNEP 2004; ECHA, CAS #77-92-9, 2024
  - 96-hour LC_{50} (*Lepomis macrochirus,* bluegill) = 1,516 mg/L (US EPA (1973) EPA -600/2-74-003) (nominal or measured not specified). The REACH dossier reported this study with a reliability score of 4 (not assignable) while the SIDS dossier reported it with a reliability score of 2 (reliable with restrictions) (Schwarz 1973).
  - Nominal 48-hour or 96-hour LC_{50} (*Leuciscus idus melanotus*, ide*)* = 440-760 mg/L (non-GLP-compliant, OECD 203). The REACH and SIDS dossiers reported this study with a reliability score of 2 (reliable with restrictions) (Deutsche Einheitsverfahren zur Wasser- 1976 and Juhnke and Ludemann 1978). *ToxServices notes that the SIDS dossier reports the exposure duration as 96 hours while the REACH dossier reports the exposure duration as 48 hours.*
  - Nominal 24-hour EC_{50} (*Daphnia magna*) = 1,535 mg/L (non-GLP-compliant, neutralized conditions). The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) while the SIDS dossier reports this study with a reliability score of 4 (not assignable) (Bringmann and Kühn 1982).
  - Nominal 48-hour LC_{50} (*Carcinus maenas*, European green crab) = 160 mg/L (non-GLP-compliant). The REACH dossier reports this study with a reliability score of 4 (not assignable) while the SIDS dossier reports this study with a reliability score of 2 (reliable with restrictions) (Portmann and Wilson 1971).
  - Nominal 7-day or 8-day cell density toxicity threshold (EC_0) (*Scenedesmus quadricauda*, algae) = 640 mg/L (non-GLP-compliant). The REACH and SIDS dossiers report this study with a reliability score of 2 (reliable with restrictions) (Bringmann and Kühn 1978 and 1980). *ToxServices notes the SIDS dossier reports the exposure duration as 7 days while the REACH dossier reports the exposure duration as 8 days*.
- UNEP 2004
  - 24-hour EC_{50} (*D. magna*) = 85 mg/L (not neutralized). The SIDS dossier reports this study with a reliability score of 4 (not assignable) (Bringmann and Kühn 1982).
- ECHA, CAS #77-92-9, 2024
  - 48-hour LC_{50} (*L. idus*, ide) = 2,600 mg/L (nominal or measured not specified). The REACH dossier reports this study with a reliability score of 4 (not assignable) (Schöberl et al. 1988).
  - Nominal 96-hour LC_{50} (*Pimephales promelas*, fathead minnow) > 100 mg/L (non-GLP-compliant). The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) (Terhaar et al. 1972).
  - Nominal 48-hour attachment to substrate EC_{50} (*Dreissena polymorpha*, zebra mussel) > 50 mg/L (non-GLP-compliant, ASTM (1993) PCN 03-547093-16). The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) (Cope et al. 1997).
  - Estimated 72-hour mobility EC_{50} (*D. magna*) = 120 mg/L (non-GLP-compliant). The REACH dossier reports this study with a reliability score of 4 (not assignable) (Ellis 1937).

  - Nominal 24-hour LC_{50} (*Artemia franciscana*, brine shrimp) = 190-270 mg/L (non-GLP-compliant). The REACH dossier reports this study with a reliability score of 4 (not assignable) (Nelson and Kursar 1999).
- With one exception, the acute aquatic toxicity values for citric acid are > 100 mg/L. One 24-hour EC_{50} of 85 mg/L was identified for daphnia under non-neutralized conditions, suggesting that the acute toxic effects were due to decreased pH rather than a direct chemical effect. As natural surface waters have buffering capacity,[9] citric acid is not likely to cause acute toxicity towards aquatic organisms.

Chronic Aquatic Toxicity (CA) Score (vH, H, M, or L): *L*
Citric acid was assigned a score of Low for chronic aquatic toxicity based on measured or estimated chronic aquatic toxicity values > 10 mg/L. GreenScreen® criteria classify chemicals as a Low hazard for chronic aquatic toxicity when chronic aquatic toxicity values are > 10 mg/L (CPA 2018b). The confidence in the score is low as measured data are available for only one trophic level.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA, CAS #77-92-9, 2024
  - Nominal estimated 8-day cell density NOAEC (*S. quadricauda*, green algae) = 425 mg/L (non-GLP-compliant). The REACH dossier reports this study with a reliability score of 2 (reliable with restrictions) (Bringmann and Kühn 1978 and 1980).
  - Nominal 8-day growth rate toxicity threshold (*Microcystis aeruginosa*, cyanobacteria) = 80 mg/L (non-GLP-compliant). The REACH dossier reports this study with a reliability score of 4 (not assignable) (Bringmann and Kühn 1978).
- U.S. EPA 2022
  - Citric acid belongs to the neutral organics ECOSAR chemical class. The most conservative predicted chronic values (ChVs) are 44,600 (4.46E04) mg/L in fish, 48,200 (4.82E04) mg/L in daphnia, and 28,700 (2.87E04) mg/L in green algae (Appendix I).

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): *vL*
Citric acid was assigned a score of Low for persistence based modeling that predicts that it is readily biodegradable, with support from numerous studies demonstrating that it meets the pass level in ready biodegradability tests. GreenScreen® criteria classify chemicals as a Very Low hazard for persistence when they meet the 10-day window (CPA 2018b). The confidence in the score is low as it is based in part on modeling, as experimental studies did not report on the 10-day window.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA, CAS #77-92-9, 2024
  - An OECD Guideline 301 D ready biodegradability (closed bottle) test was performed with citric acid (purity not specified) at 1 mg/L for 30 days under aerobic conditions. The test substance degraded 90% at the end of the exposure period. No additional details were

[9] https://www.usgs.gov/special-topics/water-science-school/science/alkalinity-and-water; https://www.epa.gov/caddis-vol2/ph

provided. The REACH dossier reports this study with a reliability score of 2 reliable with restrictions) (Gerike and Fischer 1979).
    - An EPA OTS 796.3180 ready biodegradability (modified AFNOR) test was performed with citric acid (purity not specified) at 40 mg/L for 42 days under aerobic conditions. The test substance degraded 100% based on DOC removal at the end of the exposure period. No additional details were provided. The REACH dossier reports this study with a reliability score of 2 reliable with restrictions) (Gerike and Fischer 1979).
    - An OECD Guideline 301 E ready biodegradability (modified OECD screening) test was performed with 0.05% effluent (no further details provided) exposed to citric acid (purity not specified) at 3-20 mg/L for 19 days. At the end of the exposure period, the level of degradation was 100% based on DOC removal. The study authors concluded citric acid was readily biodegradable under the tested conditions. No information regarding the 10-day window was provided for this test. The REACH dossier reports this study with a reliability score of 2 reliable with restrictions) (Gerike and Fischer 1979).
    - An OECD Guideline 302 B inherent biodegradability (Zahn-Wellens/EMPA) test was performed with sludge (1g/L) exposed to citric acid (purity not specified) at 400 mg/L for 14 days. At the end of the exposure period, the level of degradation was 85% based on DOC removal. The study authors concluded that citric acid was inherently biodegradable under the tested conditions. The REACH dossier reports this study with a reliability score of 2 reliable with restrictions) (Gerike and Fischer 1979).
    - An OECD Guideline 301 B ready biodegradability (CO_2 Evolution Test) test was performed with effluents after acclimation (no further details provided) exposed to citric acid (purity not specified) at 10 mg/L for 28 days. The level of degradation after the exposure period was 97%. The study authors concluded that citric acid was readily biodegradable in this test. No information regarding the 10-day window was provided for this test. The REACH dossier reports this study with a reliability score of 2 reliable with restrictions) (Gerike and Fischer 1979).
- U.S. EPA 2017
    - The BIOWIN modeling Ready Biodegradable Predictor indicates that citric acid is expected to be readily biodegradable. Fugacity modeling (MCI method) predicts 71.8% will partition to soil with a half-life of 416 hours (17 days), 28.1% will partition to water with a half-life of 208 hours (8.7 days), and 0.0592% will partition to sediment with a half-life of 1,870 hours (77.9 days) (Appendix J).
- The available data indicates that citric acid reaches the 60%/70% degradation threshold for GHS rapid degradation but no data regarding the 10-day window were provided for the reviewed studies. However, modeling predicts that it is readily biodegradable, indicating that it is expected to meet the 10-day window. Therefore, ToxServices assigned a Very Low score for this endpoint.

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

Citric acid was assigned a score of Very Low for bioaccumulation based on measured log K_{ow} values ≤ -0.2 and estimated BCF values ≤ 3.162. GreenScreen® criteria classify chemicals as a Very Low hazard for bioaccumulation when log K_{ow} values are ≤ 4 and BCF values are ≤ 100 (CPA 2018b). The confidence in the score is high as it is based in part on measured log K_{ow} data for the target chemical.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- ECHA, CAS #77-92-9, 2024
    - Measured log K_{ow} values for citric acid ranged from -1.8 to -0.2 from multiple sources.

- U.S. EPA 2017
    - BCFBAF predicts a BCF of 3.162 using the regression based model based on a measured log K_{ow} of -1.64, and a BCF of 0.8942 using the Arnot-Gobas model for the upper trophic level, taking metabolism into consideration (Appendix J).

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M, or L): L
Citric acid was assigned a score of Low for reactivity based on ToxServices not classifying it as a reactive chemical under GHS criteria due to its lack of explosive and oxidizing properties. GreenScreen® criteria classify chemicals as a Low hazard for reactivity when no GHS classification is warranted (CPA 2018b). The confidence in the score is low as it is based on data for the target chemical.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- UNEP 2004
    - Citric acid is not explosive. Minimum ignition energy of citric acid (particle size range 3 to 150 µm) was between 1,300 mJ (no ignition) and 4,000 mJ (ignition).
    - Citric acid has no oxidizing properties.
- ECHA, CAS #77-92-9, 2024
    - Citric acid does not contain functional groups associated with explosive or oxidizing properties.
- Based on the information presented above, ToxServices did not classify citric acid as a reactive chemical under GHS criteria (UN 2023).

Flammability (F) Score (vH, H, M, or L): L
Citric acid was assigned a score of Low for flammability based on its lack of ignition in a burning test. GreenScreen® criteria classify chemicals as a Low hazard for flammability when no GHS classification is warranted (CPA 2018b). The confidence in the score is high as it is based on measured data for the target chemical.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- ECHA, CAS #77-92-9, 2024
    - Citric acid did not ignite in a burning test performed with deposited citric acid dust. The test substance melted and evaporated.
- Based on the lack of ignition during a burning test, ToxServices did not classify citric acid as a flammable solid under GHS criteria (UN 2023).

Use of New Approach Methodologies (NAMs)[10] in the Assessment, Including Uncertainty Analyses of Input and Output

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

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

As shown in Table 4, Type I (input data) uncertainties in citric acid's NAMs dataset include insufficient experimental data for carcinogenicity, endocrine activity, and respiratory sensitization, and lack of established test methods for respiratory sensitization. Citric acid's Type II (extrapolation output) uncertainties include limitation of *in vitro* genotoxicity assays in mimicking *in vivo* metabolism and their focusing on one or only a few types of genotoxicity events, the limitation of Toxtree and OECD Toolbox in identifying structural alerts without defining the applicability domains, the inability of Oncologic models to evaluate citric acid's carcinogenic potential, and the limitations in the examination of structural alerts for respiratory sensitization evaluation that does not account for non-immunologic mechanisms of respiratory sensitization. Some of citric acid'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**: Only limited experimental data are available. **Endocrine activity**: No experimental data are available. **Respiratory sensitization**: No experimental data are available and there are no validated test methods.
Type II Uncertainty: Extrapolation Output	**Carcinogenicity**: Toxtree only identifies structural alerts (SAs), and no applicability domain can be defined (Toxtree 2018). OncoLogic™ could not evaluate the carcinogenic potential of this chemical. **Genotoxicity:** The bacterial reverse mutation assay (as defined in OECD Guideline 471) only tests point-mutation inducing activity in non-mammalian cells, and the exogenous metabolic activation system does not entirely mimic *in vivo* conditions[11].

[10] 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).

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

	The *in vitro* chromosome aberration assay (OECD Guideline 473) does not measure aneuploidy and it only measures structural chromosomal aberrations. The exogenous metabolic activation system does not entirely mirror *in vivo* metabolism[12]. The *in vitro* mammalian cell micronucleus test (as defined in OECD Guideline 487) detects chromosomal damage only in cells that have undergone cell division during or after exposure to the test chemical, and may overestimate chromosomal damage potential because aberrations measured in metaphase cells may not necessarily be transmitted to daughter cells. Additionally, the exogenous metabolic activation system does not entirely mirror *in vivo* conditions[13]. **Respiratory sensitization**: The OECD Toolbox only identifies structural alerts, and does not define applicability domains. Additionally, the ECHA guidance (2017), on which the use of OECD Toolbox structural alerts is based, does not evaluate non-immunologic mechanisms for respiratory sensitization.	
Endpoint	**NAMs Data Available and Evaluated? (Y/N)**	**Types of NAMs Data (*in silico* modeling/*in vitro* biological profiling/frameworks)**
Carcinogenicity	Y	*In silico* modeling: VEGA/Toxtree/OncoLogic™/Danish QSAR
Mutagenicity	Y	*In vitro* data: Bacterial reverse mutation assay/*in vitro* gene mutation assay/*in vitro* chromosome aberration assay/*in vitro* micronucleus assay/comet assay
Reproductive toxicity	N	
Developmental toxicity	N	
Endocrine activity	N	
Acute mammalian toxicity	N	
Single exposure systemic toxicity	N	
Repeated exposure systemic toxicity	N	
Single exposure neurotoxicity	N	
Repeated exposure neurotoxicity	N	
Skin sensitization	N	
Respiratory sensitization	Y	*In silico* modeling: OECD Toolbox structural alerts
Skin irritation	N	

[12] https://www.oecd-ilibrary.org/docserver/9789264264649-en.pdf?expires=1614098015&id=id&accname=guest&checksum=6A4F9CE52EA974F5A74793DD54D54352
[13] https://www.oecd.org/chemicalsafety/test-no-487-in-vitro-mammalian-cell-micronucleus-test-9789264264861-en.htm

Eye irritation	N	
Acute aquatic toxicity	N	
Chronic aquatic toxicity	Y	*In silico* modeling: ECOSAR
Persistence	Y	*In silico* modeling: EPI Suite™ Non-animal testing: OECD 301 B, D, E Biodegradation tests
Bioaccumulation	Y	*In silico* modeling: EPI Suite™

References

Chemical Classification and Information Database (CCID). 2024. Citric acid (CAS #77-92-9). Environmental Protection Authority, Government of New Zealand. Available: https://www.epa.govt.nz/database-search/chemical-classification-and-information-database-ccid/view/6BFD11CF-B9B9-4647-8669-8FCAFB1C599D

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

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

Cosmetic Ingredient Review (CIR). 2014. Safety assessment of citric acid, inorganic citrate salts, and alkyl citrate esters as used in cosmetics. *Int. J. Toxicol*. 33(2 suppl): 16S-46S. Available: https://journals.sagepub.com/doi/10.1177/1091581814526891

Danish Technical University (DTU). 2024. National Food Institute. Danish QSAR Database. Available: http://qsar.food.dtu.dk/.

European Chemicals Agency (ECHA). 2017. Guidance on information requirements and Chemical Safety Assessment. Chapter R.7a: Endpoint specific guidance. Version 6.0. Dated: July 2017. Available: https://echa.europa.eu/documents/10162/17224/information_requirements_r7a_en.pdf/e4a2a18f-a2bd-4a04-ac6d-0ea425b2567f?t=1500286622893

European Chemicals Agency (ECHA). 2018. CLH report for citric acid (CAS #77-92-9). Proposal for Harmonised Classification and Labelling based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2. Version 3, dated October 30, 2018. Available: https://echa.europa.eu/documents/10162/7b5164b3-6025-874c-7b14-bbb62ee465fc

European Chemicals Agency (ECHA). 2019. Opinion proposing harmonised classification and labelling at EU level of citric acid (CAS #77-92-9). Committee for Risk Assessment (RAC). CLH-O-0000001412-86-299/F. Adopted September 20, 2019. Available: https://echa.europa.eu/documents/10162/039eb0b3-d5a4-c474-915e-0cf4ed07ccba

European Chemicals Agency (ECHA). 2024. Information on chemicals. Available: https://echa.europa.eu/information-on-chemicals

European Commission (EC). 2024. Citric acid (CAS #77-92-9). CosIng database. Available: https://ec.europa.eu/growth/tools-databases/cosing/details/32858

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

Gad, S. 2016. QSAR Tools for Drug Safety. Chapter 10. In, Drug Safety Evaluation. Third Edition. New York: Wiley. 209-224.

Hazardous Substances Data Bank (HSDB). 2014. Citric acid. United States National Library of Medicine. Available: https://pubchem.ncbi.nlm.nih.gov/source/hsdb/911

Madden, J.C., S.J. Enoch, A. Paini, and M.T.D. Cronin. 2020. A review of *in silico* tools as alternatives to animal testing: principles, resources, and applications. *Alt. Lab. Animals.* 1-27. Available: https://journals.sagepub.com/doi/pdf/10.1177/0261192920965977

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

Organisation for Economic Co-operation and Development (OECD). 2023. OECD QSAR Toolbox for Grouping Chemicals into Categories Version 4.6. Available: https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm#Download_qsar_application_toolbox

Pharos. 2024. Pharos chemical and material library entry for citric acid (CAS #77-92-9). Available: http://www.pharosproject.net/material/.

PubChem. 2024. Citric acid (CAS #77-92-9). United States National Library of Medicine. Available: https://pubchem.ncbi.nlm.nih.gov/compound/311

Sahigara, F. 2007. Defining the Applicability Domain of QSAR models: An overview. Available: http://www.moleculardescriptors.eu/tutorials/T7_moleculardescriptors_ad.pdf

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

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

United Nations (UN). 2023. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Tenth revised edition.

United Nations Environmental Programme (UNEP). 2004. Screening Information Data Set (SIDS) dossier for citric acid (CAS #77-92-9). Organization for Economic Cooperation and Development (OECD) SIDS. Published July 2004. Available: https://hpvchemicals.oecd.org/UI/SIDS_Details.aspx?id=EE84E16E-D3E9-456F-907C-E1CCE3CFB138

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

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

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

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

United States Environmental Protection Agency (U.S. EPA). 2019. OncoLogic™. Version 8.0.16 Washington, DC, USA.

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

United States Environmental Protection Agency (U.S. EPA). 2021. OncoLogic™. Version 9.0 Washington, DC, USA.

United States Environmental Protection Agency (U.S. EPA). 2022. ECOSAR 2.2. Washington, DC, USA. Available: http://www.epa.gov/oppt/newchems/tools/21ecosar.htm/.

United States Environmental Protection Agency (U.S. EPA). 2024a. CompTox Chemicals Dashboard v2.3.0 entry for citric acid (CAS #77-92-9). Available: https://comptox.epa.gov/dashboard/chemical/details/DTXSID3020332

United States Environmental Protection Agency (U.S. EPA). 2024b. Safer Chemical Ingredients List (SCIL). Available: https://www.epa.gov/saferchoice/safer-ingredients

United States Environmental Protection Agency (U.S. EPA). 2024c. High Production Volume Information System (HPVIS) entry for citric acid, anhydrous (CAS #77-92-9). Available: https://chemview.epa.gov/chemview#

United States Food and Drug Administration (U.S. FDA). 2024. Citric acid (CAS #77-92-9). Substances added to food (formerly EAFUS). Available: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=FoodSubstances&id=CITRICACID

Virtual Models for Evaluating the Properties of Chemicals within a Global Architecture (VEGA). 2023. Predictive Model Platform version 1.2.3. Available: https://www.vegahub.eu/download/vega-qsar-download/

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 Citric Acid (CAS #77-92-9)

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	Citric Acid	77-92-9	*L*	L	L	*L*	DG	L	M	L	*L*	DG	L	*L*	L	H	L	*L*	*vL*	vL	L	L

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	No	No
3	No	No	Yes	No			
4	STOP						

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
Citric Acid	3

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

Table 6

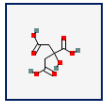
Chemical Name	Final GreenScreen® Benchmark Score
Citric Acid	3

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												
3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		3
4												

APPENDIX C: Pharos Output for Citric Acid (CAS #77-92-9)



77-92-9
Citric acid
ALSO CALLED .beta.-Hydroxytricarballylic acid, 1,2,3-Propanetricarboxylic acid, 2-hydroxy-, 1,2,3-Propanetricarb...
View all synonyms (115)

Share Profile

Hazards | Properties | Functional Uses | Process Chemistry | Resources

All Hazards View

Show PubMed Results | Request Assessment | Add to Comparison

		Group I Human					Group II and II* Human								Ecotox			Fate		Physical		Mult	Non-GSLT			
	GREENSCREEN®	C	M	R	D	E	AT	ST	ST	N	N	SnS	SnR	IrS	IrE	AA	CA	ATB	P	B	Rx	F	Mult	PBT	GW	O	Other
List Hazard Summary	LT-UNK	-	-	-	M-L	-	pC	M	-	-	-	-	-	H	H	-	-	-	-	-	-	-	M	-	-	-	R

Hazard Lists

Download Lists

ENDPOINT	HAZARD LEVEL	GREENSCREEN®	LIST NAME	HAZARD DESCRIPTION	OTHER LISTS
Developmental Toxicity incl. developmental neurotoxicity	M-L	LT-UNK	MAK	Pregnancy Risk Group C	
Acute Mammalian Toxicity	pC	NoGS	US EPA - OPP - Registered Pesticides	FIFRA Registered Pesticide	
Systemic Toxicity/Organ Effects-Single Exposure	M	LT-UNK	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]	+2
	M	LT-UNK	GHS - Australia	H335 - May cause respiratory irritation [Specific target organ toxicity - single exposure; Respiratory tract irritation - Category 3]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H335 - May cause respiratory irritation (unverified) [Specific target organ toxicity - single exposure; Respiratory tract irritation - Category 3]	
Skin Irritation/Corrosivity	H	LT-UNK	GHS - Australia	H315 - Causes skin irritation [Skin corrosion/irritation - Category 2]	+1
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H315 - Causes skin irritation (unverified) [Skin corrosion/irritation - Category 2]	

Eye Irritation/Corrosivity	H	LT-UNK	EU - GHS (H-Statements) Annex 6 Table 3-1	H319 - Causes serious eye irritation [Serious eye damage/eye irritation - Category 2A]	+4
	vH	LT-UNK	GHS - New Zealand	Serious eye damage category 1	
	H	LT-UNK	GHS - Australia	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]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H319 - Causes serious eye irritation (unverified) [Serious eye damage/eye irritation - Category 2A]	
Systemic Toxicity/Organ Effects [Single Exposure] and/or Neurotoxicity [Single Exposure]	M	LT-UNK	GHS - New Zealand	Specific target organ toxicity - single exposure category 3 narcotic effects	
Reactivity and/or Eye Irritation/Corrosivity and/or Skin Irritation/Corrosivity	U	LT-UNK	Québec CSST - WHMIS 1988	Class E - Corrosive materials	
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	

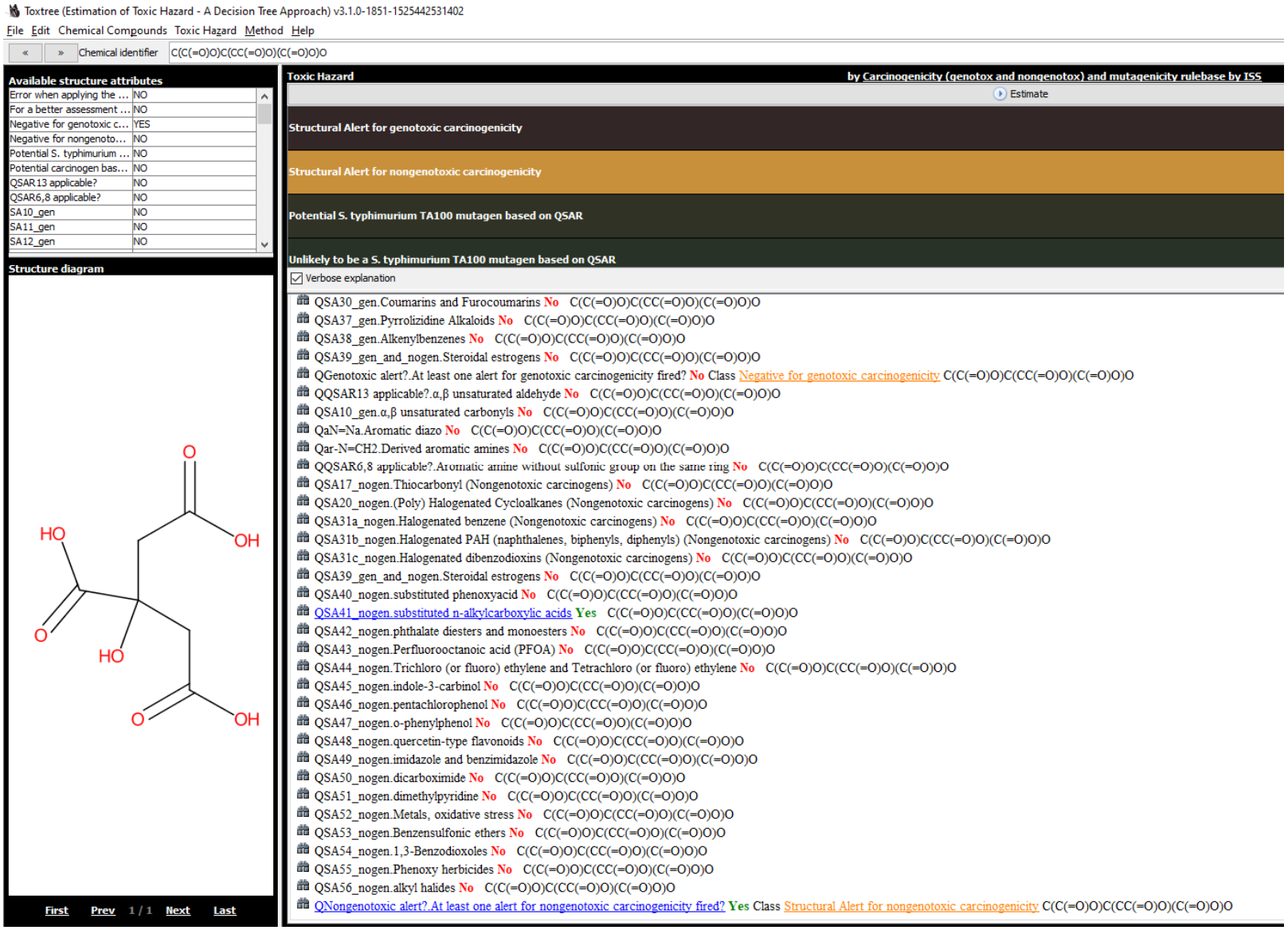
Restricted Substance Lists (6)

- Apple Regulated Substances Specification: Reportable Substances and Future Restrictions in Products ✱
- Campaign for Safe Cosmetics' Red List of Chemicals of Concern: Tier 3 Asthmagens, Allergens, & Irritants
- EU - PACT-RMOA Substances: Substances selected for RMOA or hazard assessment
- Food Contact Chemicals Database (FCCdb): Food Contact Chemicals Database Version 5.0
- Health Canada - Cosmetic Ingredient Hotlist: Ingredients that are Restricted for Use in Cosmetic Products
- TSCA Chemical Substance Inventory (Active-Inactive): TSCA Chemical Substance Inventory - Active

Positive Lists (5)

- Cosmetic Ingredient Review (CIR): Safe as Used
- Inventory of Existing Cosmetic Ingredients in China (IECIC 2021): Cosmetic Ingredients
- US EPA - DfE Safer Chemicals Ingredients list (SCIL): Antimicrobial Actives - Green Circle (Verified Low Concern)
- US EPA - DfE Safer Chemicals Ingredients list (SCIL): Chelating Agents - Green Circle (Verified Low Concern)
- US EPA - DfE Safer Chemicals Ingredients list (SCIL): Processing Aids-Additives - Green Circle (Verified Low Concern)

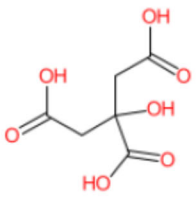
APPENDIX D: Toxtree Carcinogenicity Results for Citric Acid (CAS #77-92-9)



APPENDIX E: VEGA Carcinogenicity Results for Citric Acid (CAS #77-92-9)

1. Prediction Summary

Prediction for compound Molecule 0 -

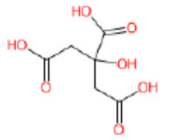


EXPERIMENTAL DATA

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

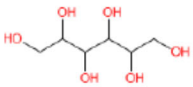
Compound: Molecule 0
Compound SMILES: O=C(O)CC(O)(C(=O)O)CC(=O)O
Experimental value: NON-Carcinogen
Predicted Carcinogen activity: Carcinogen
P(Carcinogen): 0.549
P(NON-Carcinogen): 0.451
Reliability: The predicted compound is outside the Applicability Domain of the model
Remarks:
none

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



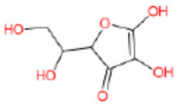
Compound #1

CAS: 77-92-9
Dataset id:173 (Training Set)
SMILES: O=C(O)CC(O)(C(=O)O)CC(=O)O
Similarity: 1
Experimental value : NON-Carcinogen
Predicted value : Carcinogen



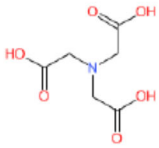
Compound #2

CAS: 69-65-8
Dataset id:421 (Training Set)
SMILES: OCC(O)C(O)C(O)C(O)CO
Similarity: 0.817
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen



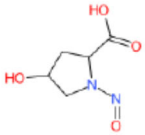
Compound #3

CAS: 50-81-7
Dataset id:58 (Training Set)
SMILES: O=C1C(O)=C(O)OC1C(O)CO
Similarity: 0.802
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen



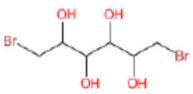
Compound #4

CAS: 139-13-9
Dataset id:498 (Training Set)
SMILES: O=C(O)CN(CC(=O)O)CC(=O)O
Similarity: 0.8
Experimental value : Carcinogen
Predicted value : Carcinogen



Compound #5

CAS: 30310-80-6
Dataset id:593 (Training Set)
SMILES: O=NN1CC(O)CC1(C(=O)O)
Similarity: 0.758
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen



Compound #6

CAS: 10318-26-0
Dataset id:213 (Training Set)
SMILES: OC(CBr)C(O)C(O)C(O)CBr
Similarity: 0.755
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 = 1 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..
✔	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 = 0.098 Explanation: model class assignment is uncertain..
⚠	Neural map neurons concordance Neurons concordance = 0.75 Explanation: predicted value disagrees with experimental values of training set compounds laying in the same neuron..

Symbols explanation:

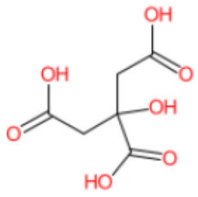
✔ The feature has a good assessment, model is reliable regarding this aspect.

⚠ 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 -

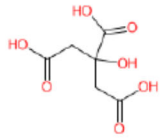


EXPERIMENTAL DATA

E xperimental value is NON-Carcinogen. Model prediction is Carcinogen (LOW reliability).
The following alerts have been found: SA41 Substituted n-alkylcarboxylic acids

Compound: Molecule 0
Compound SMILES: O=C(O)CC(O)(C(=O)O)CC(=O)O
Experimental value: NON-Carcinogen
Predicted Carcinogen activity: Carcinogen
Structural Alerts: SA41 Substituted n-alkylcarboxylic acids
Reliability: The predicted compound is outside the Applicability Domain of the model
Remarks:
none

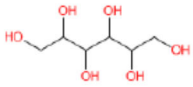
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

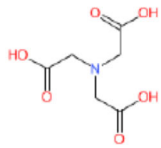
CAS: 77-92-9
Dataset id:829 (Training Set)
SMILES: O=C(O)CC(O)(C(=O)O)CC(=O)O
Similarity: 1
Experimental value : NON-Carcinogen
Predicted value : Carcinogen

Alerts (found also in the target): SA41 Substituted n-alkylcarboxylic acids



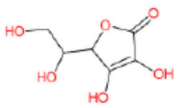
Compound #2

CAS: 69-65-8
Dataset id:86 (Training Set)
SMILES: OCC(O)C(O)C(O)C(O)CO
Similarity: 0.817
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen



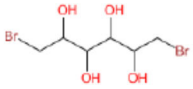
Compound #3

CAS: 139-13-9
Dataset id:206 (Training Set)
SMILES: O=C(O)CN(CC(=O)O)CC(=O)O
Similarity: 0.8
Experimental value : Carcinogen
Predicted value : NON-Carcinogen



Compound #4

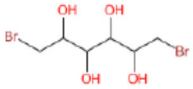
CAS: 50-81-7
Dataset id:31 (Training Set)
SMILES: O=C1OC(C(O)=C1(O))C(O)CO
Similarity: 0.777
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen



Compound #5

CAS: 10318-26-0
Dataset id:445 (Training Set)
SMILES: OC(CBr)C(O)C(O)C(O)CBr
Similarity: 0.755
Experimental value : Carcinogen
Predicted value : Carcinogen

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



Compound #6

CAS: 488-41-5
Dataset id:484 (Training Set)
SMILES: OC(CBr)C(O)C(O)C(O)CBr
Similarity: 0.755
Experimental value : Carcinogen
Predicted value : Carcinogen

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

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 = 1 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.

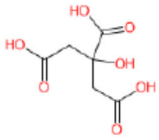
4.1 Reasoning:
Relevant Chemical Fragments and Moieties

(Molecule 0) Reasoning on fragments/structural alerts :.

Fragment found: SA41 Substituted n-alkylcarboxylic acids

Substituted n-alkylcarboxylic acids

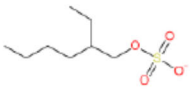
Following, the most similar compounds from the model's dataset having the same fragment.



CAS: 77-92-9
Dataset id:829 (Training Set)
SMILES: O=C(O)CC(O)(C(=O)O)CC(=O)O
Similarity: 1

Experimental value : NON-Carcinogen
Predicted value : Carcinogen

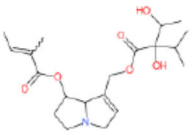
Alerts (found also in the target): SA41 Substituted n-alkylcarboxylic acids



CAS: 126-92-1
Dataset id:77 (Training Set)
SMILES: O=S(=O)([O-])OCC(CC)CCCC
Similarity: 0.675

Experimental value : Carcinogen
Predicted value : Carcinogen

Alerts (found also in the target): SA41 Substituted n-alkylcarboxylic acids



CAS: 22571-95-5
Dataset id:486 (Training Set)
SMILES: O=C(OC2CCN1CC=C(COC(=O)C(O)(C(O)C)C(C)C)C12)C(=CC)C
Similarity: 0.616

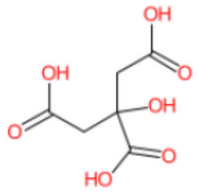
Experimental value : Carcinogen
Predicted value : Carcinogen

Alerts (found also in the target): SA41 Substituted n-alkylcarboxylic acids

Alerts (not found also in the target): SA37 Pyrrolizidine Alkaloids

1. Prediction Summary

Prediction for compound Molecule 0 -

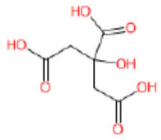


EXPERIMENTAL DATA

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

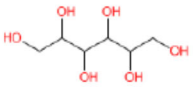
Compound: Molecule 0
Compound SMILES: O=C(O)CC(O)(C(=O)O)CC(=O)O
Experimental value: NON-Carcinogen
Predicted Carcinogenic activity: Possible NON-Carcinogen
No. alerts for carcinogenicity: 0
Structural Alerts: -
Reliability: The predicted compound is into the Applicability Domain of the model
Remarks:
none

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



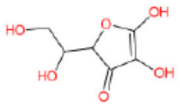
Compound #1

CAS: 77-92-9
Dataset id:745 (Training Set)
SMILES: O=C(O)CC(O)(C(=O)O)CC(=O)O
Similarity: 1
Experimental value : NON-Carcinogen
Predicted value : Possible NON-Carcinogen



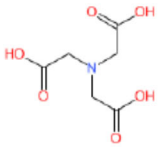
Compound #2

CAS: 69-65-8
Dataset id:69 (Training Set)
SMILES: OCC(O)C(O)C(O)C(O)CO
Similarity: 0.817
Experimental value : NON-Carcinogen
Predicted value : Possible NON-Carcinogen



Compound #3

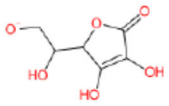
CAS: 50-81-7
Dataset id:25 (Training Set)
SMILES: O=C1C(O)=C(O)OC1C(O)CO
Similarity: 0.802
Experimental value : NON-Carcinogen
Predicted value : Possible NON-Carcinogen



Compound #4

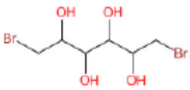
CAS: 139-13-9
Dataset id:167 (Training Set)
SMILES: O=C(O)CN(CC(=O)O)CC(=O)O
Similarity: 0.8
Experimental value : Carcinogen
Predicted value : Carcinogen

Alerts (not found also in the target): Carcinogenity alert no. 34



Compound #5

CAS: 6381-77-7
Dataset id:943 (Training Set)
SMILES: O=C1OC(C(O)=C1(O))C(O)C[O-]
Similarity: 0.767
Experimental value : NON-Carcinogen
Predicted value : Possible NON-Carcinogen



Compound #6

CAS: 10318-26-0
Dataset id:369 (Training Set)
SMILES: OC(CBr)C(O)C(O)C(O)CBr
Similarity: 0.755
Experimental value : Carcinogen
Predicted value : Possible 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..
✓	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:

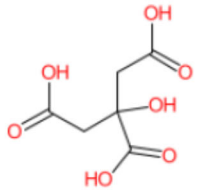
✓ The feature has a good assessment, model is reliable regarding this aspect.

⚠ 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 -

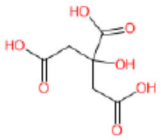


EXPERIMENTAL DATA

E xperimental value is NON-Carcinogen. Model prediction is Carcinogen (LOW reliability).
The following relevant fragments have been found: Carcinogenity alert no. 21

Compound: Molecule 0
Compound SMILES: O=C(O)CC(O)(C(=O)O)CC(=O)O
Experimental value: NON-Carcinogen
Predicted Carcinogenic activity: Carcinogen
No. alerts for carcinogenicity: 1
Structural Alerts: Carcinogenity alert no. 21
Reliability: The predicted compound is outside the Applicability Domain of the model
Remarks:
none

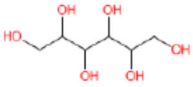
3.1 Applicability Domain:
Similar Compounds, with Predicted and Experimental Values



Compound #1

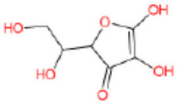
CAS: 77-92-9
Dataset id:173 (Training Set)
SMILES: O=C(O)CC(O)(C(=O)O)CC(=O)O
Similarity: 1
Experimental value : NON-Carcinogen
Predicted value : Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 21



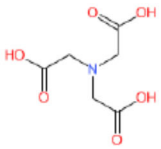
Compound #2

CAS: 69-65-8
Dataset id:421 (Training Set)
SMILES: OCC(O)C(O)C(O)C(O)CO
Similarity: 0.817
Experimental value : NON-Carcinogen
Predicted value : Possible NON-Carcinogen



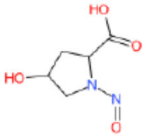
Compound #3

CAS: 50-81-7
Dataset id:58 (Training Set)
SMILES: O=C1C(O)=C(O)OC1C(O)CO
Similarity: 0.802
Experimental value : NON-Carcinogen
Predicted value : Possible NON-Carcinogen



Compound #4

CAS: 139-13-9
Dataset id:498 (Training Set)
SMILES: O=C(O)CN(CC(=O)O)CC(=O)O
Similarity: 0.8
Experimental value : Carcinogen
Predicted value : Possible NON-Carcinogen

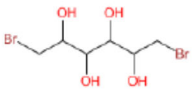


Compound #5

CAS: 30310-80-6
Dataset id:593 (Training Set)
SMILES: O=NN1CC(O)CC1(C(=O)O)
Similarity: 0.758
Experimental value : NON-Carcinogen
Predicted value : Carcinogen

Alerts (not found also in the target): Carcinogenity alert no. 8; Carcinogenity alert no. 11; Carcinogenity alert no. 15; Carcinogenity alert no. 46; Carcinogenity alert no. 47; Carcinogenity alert no. 50; Carcinogenity alert no. 51; Carcinogenity alert no. 54; Carcinogenity alert no. 55; Carcinogenity alert no. 63

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



Compound #6

CAS: 10318-26-0
Dataset id:213 (Training Set)
SMILES: OC(CBr)C(O)C(O)C(O)CBr
Similarity: 0.755
Experimental value : Carcinogen
Predicted value : Carcinogen

Alerts (not found also in the target): Carcinogenity alert no. 58; Carcinogenity alert no. 59

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 = 1
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.

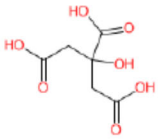
4.1 Reasoning:
Relevant Chemical Fragments and Moieties

(Molecule 0) Reasoning on fragments/structural alerts :.

Fragment found: Carcinogenity alert no. 21

Structural alert for carcinogenity defined by the SMARTS: CC(C)(O)C(O)=O

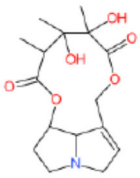
Following, the most similar compounds from the model's dataset having the same fragment.



CAS: 77-92-9
Dataset id:173 (Training Set)
SMILES: O=C(O)CC(O)(C(=O)O)CC(=O)O
Similarity: 1

Experimental value : NON-Carcinogen
Predicted value : Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 21

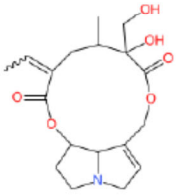


CAS: 315-22-0
Dataset id:488 (Test Set)
SMILES: O=C1OC3CCN2CC=C(COC(=O)C(O)(C)C(O)(C)C1C)C23
Similarity: 0.653

Experimental value : Carcinogen
Predicted value : Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 21

Alerts (not found also in the target): Carcinogenity alert no. 20; Carcinogenity alert no. 41; Carcinogenity alert no. 77; Carcinogenity alert no. 115



CAS: 480-54-6
Dataset id:694 (Training Set)
SMILES: O=C1OC3CCN2CC=C(COC(=O)C(O)(CO)C(C)CC1(=CC))C23
Similarity: 0.629

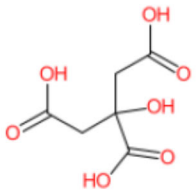
Experimental value : Carcinogen
Predicted value : Carcinogen

Alerts (found also in the target): Carcinogenity alert no. 21

Alerts (not found also in the target): Carcinogenity alert no. 20; Carcinogenity alert no. 41; Carcinogenity alert no. 76; Carcinogenity alert no. 77; Carcinogenity alert no. 106; Carcinogenity alert no. 115

1. Prediction Summary

Prediction for compound Molecule 0 -

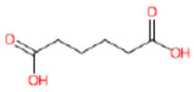


Prediction: Reliability:

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

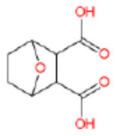
Compound: Molecule 0
Compound SMILES: O=C(O)CC(O)(C(=O)O)CC(=O)O
Experimental value: -
Predicted Oral Carcinogenic class: NON-Carcinogen
Reliability: The predicted compound is into the Applicability Domain of the model
Remarks:
none

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



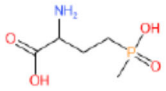
Compound #1

CAS: 124-04-9
Dataset id:541 (Training Set)
SMILES: O=C(O)CCCCC(=O)O
Similarity: 0.816
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen



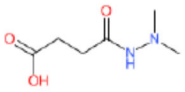
Compound #2

CAS: 145-73-3
Dataset id:494 (Training Set)
SMILES: O=C(O)C2C1OC(CC1)C2(C(=O)O)
Similarity: 0.78
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen



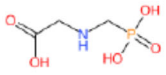
Compound #3

CAS: 77182-82-2
Dataset id:528 (Training Set)
SMILES: O=C(O)C(N)CCP(=O)(O)C
Similarity: 0.765
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen



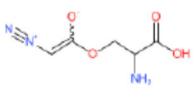
Compound #4

CAS: 1596-84-5
Dataset id:88 (Training Set)
SMILES: O=C(O)CCC(=O)NN(C)C
Similarity: 0.753
Experimental value : Carcinogen
Predicted value : Carcinogen



Compound #5

CAS: 1071-83-6
Dataset id:531 (Training Set)
SMILES: O=C(O)CNCP(=O)(O)O
Similarity: 0.744
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen



Compound #6

CAS: 115-02-6
Dataset id:27 (Training Set)
SMILES: N#[N+]C=C([O-])OCC(N)C(=O)O
Similarity: 0.74
Experimental value : Carcinogen
Predicted value : NON-Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

✓	Global AD Index AD index = 0.893 Explanation: The predicted compound is into the Applicability Domain of the model.
⚠	Similar molecules with known experimental value Similarity index = 0.797 Explanation: Only moderately similar compounds with known experimental value in the training set have been found..
✓	Accuracy of prediction for similar molecules Accuracy index = 1 Explanation: Accuracy of prediction for similar molecules found in the training set is good..
✓	Concordance for similar molecules Concordance index = 1 Explanation: Similar molecules found in the training set have experimental values that agree with the predicted value..
✓	Model's descriptors range check Descriptors range check = True Explanation: descriptors for this compound have values inside the descriptor range of the compounds of the training set..
✓	Atom Centered Fragments similarity check ACF index = 1 Explanation: all atom centered fragment of the compound have been found in the compounds of the training set..

Symbols explanation:

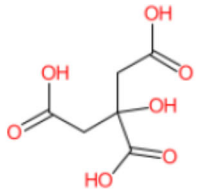
✓ The feature has a good assessment, model is reliable regarding this aspect.

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

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

1. Prediction Summary

Prediction for compound Molecule 0 -

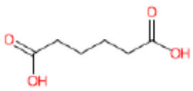


Prediction: Reliability:

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

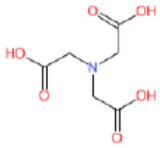
Compound: Molecule 0
Compound SMILES: O=C(O)CC(O)(C(=O)O)CC(=O)O
Experimental value: -
Predicted Inhalation Carcinogenic class: NON-Carcinogen
Reliability: The predicted compound could be out of the Applicability Domain of the model
Remarks:
none

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



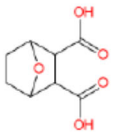
Compound #1

CAS: 124-04-9
Dataset id:517 (Training Set)
SMILES: O=C(O)CCCCC(=O)O
Similarity: 0.816
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen



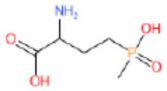
Compound #2

CAS: 139-13-9
Dataset id:178 (Training Set)
SMILES: O=C(O)CN(CC(=O)O)CC(=O)O
Similarity: 0.8
Experimental value : Carcinogen
Predicted value : Carcinogen



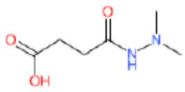
Compound #3

CAS: 145-73-3
Dataset id:466 (Training Set)
SMILES: O=C(O)C2C1OC(CC1)C2(C(=O)O)
Similarity: 0.78
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen



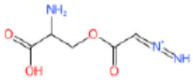
Compound #4

CAS: 77182-82-2
Dataset id:503 (Training Set)
SMILES: O=C(O)C(N)CCP(=O)(O)C
Similarity: 0.765
Experimental value : NON-Carcinogen
Predicted value : NON-Carcinogen



Compound #5

CAS: 1596-84-5
Dataset id:74 (Training Set)
SMILES: O=C(O)CCC(=O)NN(C)C
Similarity: 0.753
Experimental value : Carcinogen
Predicted value : Carcinogen



Compound #6

CAS: 115-02-6
Dataset id:24 (Training Set)
SMILES: O=C(O)C(N)COC(=O)C=[N+]=N
Similarity: 0.749
Experimental value : Carcinogen
Predicted value : Carcinogen

3.2 Applicability Domain:
Measured Applicability Domain Scores

⚠	Global AD Index AD index = 0.758 Explanation: The predicted compound could be out of the Applicability Domain of the model.
✓	Similar molecules with known experimental value Similarity index = 0.808 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 = 0.506 Explanation: similar molecules found in the training set have experimental values that disagree with the predicted value..
✓	Model's descriptors range check Descriptors range check = True Explanation: descriptors for this compound have values inside the descriptor range of the compounds of the training set..
✓	Atom Centered Fragments similarity check ACF index = 1 Explanation: all atom centered fragment of the compound have been found in the compounds of the training set..

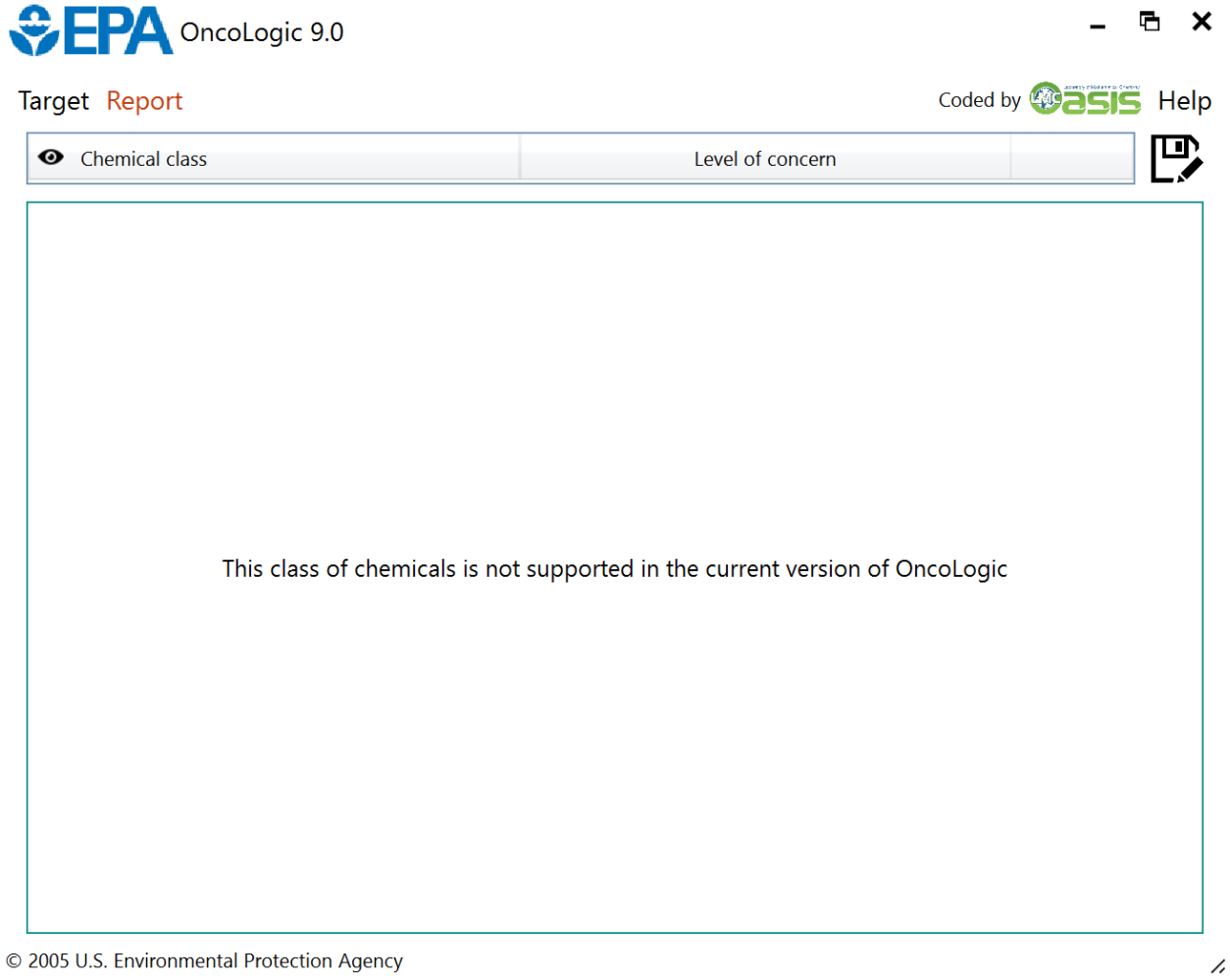
Symbols explanation:

✓ 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 Results for Citric Acid (CAS #77-92-9)



APPENDIX G: Danish QSAR Carcinogenicity Results for Citric Acid (CAS #77-92-9)

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

Commercial models from CASE Ultra and Leadscope

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

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

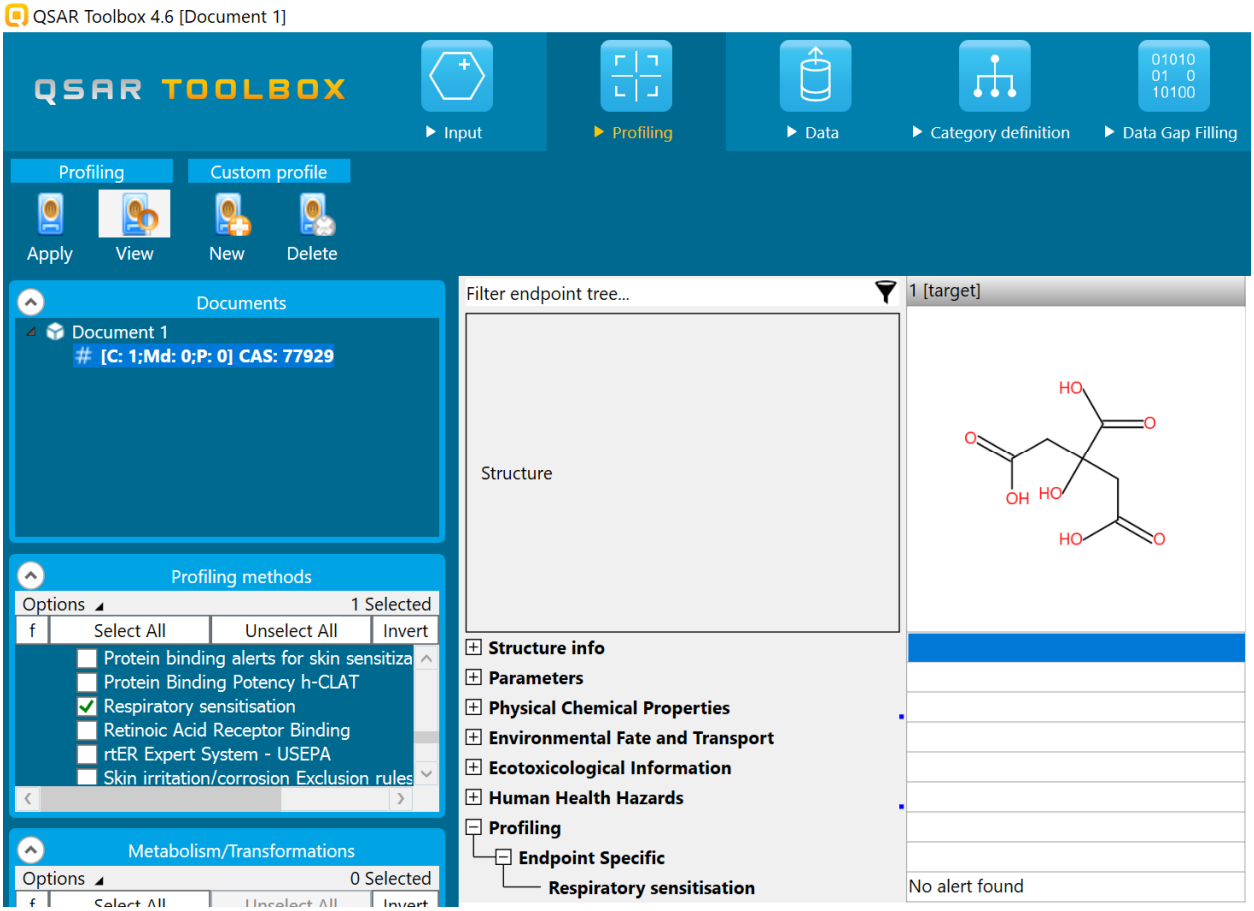
OECD QSAR Toolbox v.4.2 profilers

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	POS_OUT	NEG_OUT	NEG_IN

DTU-developed models

APPENDIX H: OECD Toolbox Respiratory Sensitization Results for Citric Acid (CAS #77-92-9)



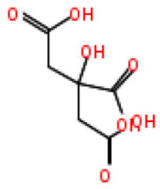
APPENDIX I: ECOSAR Modeling Results for Citric Acid (CAS #77-92-9)

Organic Module Report

Results of Organic Module Evaluation

CAS	Name	SMILES
77929	1,2,3-Propanetricarboxylic acid, 2-hydroxy-	O=C(O)C(O)(CC(=O)O)CC(=O)O

Structure



Details	
Mol Wt	192.13
Selected LogKow	
Selected Water Solubility (mg/L)	
Selected Melting Point (°C)	
Estimated LogKow	-1.67
Estimated Water Solubility (mg/L)	999999.94
Measured LogKow	-1.64
Measured Water Solubility (mg/L)	1000000
Measured Melting Point (°C)	153

Class Results:	

Neutral Organics

Organism	Duration	End Point	Concentration (mg/L)	Max Log Kow	Flags

1/ 2

Class Results:	

Organism	Duration	End Point	Concentration (mg/L)	Max Log Kow	Flags
Fish	96h	LC50	3.14E06	5	• Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported
Daphnid	48h	LC50	1.27E06	5	• Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported
Green Algae	96h	EC50	2.33E05	6.4	
Fish		ChV	2.06E05	8	
Daphnid		ChV	4.82E04	8	
Green Algae		ChV	2.87E04	8	
Fish (SW)	96h	LC50	3.87E06	5	• Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported
Mysid	96h	LC50	3.46E07	5	• Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported
Fish (SW)		ChV	4.46E04	8	
Mysid (SW)		ChV	8.89E06	8	• Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported
Earthworm	14d	LC50	8.03E03	6	

APPENDIX J: EPI Suite™ Modeling Results for Citric Acid (CAS #77-92-9)

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

CAS Number: 77-92-9
SMILES : O=C(O)C(O)(CC(=O)O)CC(=O)O
CHEM : 1,2,3-Propanetricarboxylic acid, 2-hydroxy-
MOL FOR: C6 H8 O7
MOL WT : 192.13
---------- EPI SUMMARY (v4.11) ----------
Physical Property Inputs:
Log Kow (octanol-water): ------
Boiling Point (deg C) : ------
Melting Point (deg C) : 153.00
Vapor Pressure (mm Hg) : 1.65E-008
Water Solubility (mg/L): 5.92E+005
Henry LC (atm-m3/mole) : ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = -1.67
Log Kow (Exper. database match) = -1.64
Exper. Ref: AVDEEF,A (1997)

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 407.16 (Adapted Stein & Brown method)
Melting Pt (deg C): 169.23 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 5.64E-009 (Modified Grain method)
VP (Pa, 25 deg C) : 7.52E-007 (Modified Grain method)
MP (exp database): 153 deg C
VP (exp database): 1.66E-08 mm Hg (2.21E-006 Pa) at 25 deg C
Subcooled liquid VP: 3.04E-007 mm Hg (25 deg C, user-entered VP)
: 4.06E-005 Pa (25 deg C, user-entered VP)

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 1e+006
log Kow used: -1.64 (expkow database)
melt pt used: 153.00 deg C
Water Sol (Exper. database match) = 3.83e+005 mg/L (25 deg C)
Exper. Ref: YALKOWSKY,SH ET AL. (2010)
Water Sol (Exper. database match) = 1e+006 mg/L (25 deg C)
Exper. Ref: YALKOWSKY,SH & DANNENFELSER,RM (1992)

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 1e+006 mg/L

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

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 8.33E-018 atm-m3/mole (8.44E-013 Pa-m3/mole)
Group Method: Incomplete
Exper Database: 1.10E-14 atm-m3/mole (1.11E-009 Pa-m3/mole)
For Henry LC Comparison Purposes:
User-Entered Henry LC: not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 7.046E-015 atm-m3/mole (7.139E-010 Pa-m3/mole)
VP: 1.65E-008 mm Hg (source: User-Entered)
WS: 5.92E+005 mg/L (source: User-Entered)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: -1.64 (exp database)
Log Kaw used: -12.347 (exp database)
Log Koa (KOAWIN v1.10 estimate): 10.707
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 0.6902
Biowin2 (Non-Linear Model) : 0.6193
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 3.6563 (days-weeks)
Biowin4 (Primary Survey Model) : 4.5738 (hours-days)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 1.1307
Biowin6 (MITI Non-Linear Model): 0.9754
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): 1.1142
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): 4.05E-005 Pa (3.04E-007 mm Hg)
Log Koa (Koawin est): 10.707
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 0.074
Octanol/air (Koa) model: 0.0125
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 0.728
Mackay model : 0.856
Octanol/air (Koa) model: 0.5

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 7.0238 E-12 cm3/molecule-sec
Half-Life = 1.523 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 18.274 Hrs

Ozone Reaction:
No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi):
0.792 (Junge-Pankow, Mackay avg)
0.5 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 10 L/kg (MCI method)
Log Koc: 1.000 (MCI method)
Koc : 0.06873 L/kg (Kow method)
Log Koc: -1.163 (Kow method)

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

Bioaccumulation Estimates (BCFBAF v3.01):
Log BCF from regression-based method = 0.500 (BCF = 3.162 L/kg wet-wt)
Log Biotransformation Half-life (HL) = -1.6429 days (HL = 0.02275 days)
Log BCF Arnot-Gobas method (upper trophic) = -0.049 (BCF = 0.8942)
Log BAF Arnot-Gobas method (upper trophic) = -0.049 (BAF = 0.8942)
log Kow used: -1.64 (expkow database)

Volatilization from Water:
Henry LC: 1.1E-014 atm-m3/mole (Henry experimental database)
Half-Life from Model River: 7.378E+010 hours (3.074E+009 days)
Half-Life from Model Lake : 8.048E+011 hours (3.353E+010 days)

Removal In Wastewater Treatment:
Total removal: 1.85 percent
Total biodegradation: 0.09 percent
Total sludge adsorption: 1.75 percent
Total to Air: 0.00 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model: (MCI Method)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	**1.43e-006**	**36.6**	**1000**
Water	**28.1**	**208**	**1000**
Soil	**71.8**	**416**	**1000**
Sediment	**0.0592**	**1.87e+003**	**0**

Persistence Time: 414 hr

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

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	1.43e-006	36.6	1000
Water	28.1	208	1000

water (28.1)
biota (3.22e-008)
suspended sediment (0.000422)
Soil 71.8 416 1000
Sediment 0.0592 1.87e+003 0
Persistence Time: 414 hr

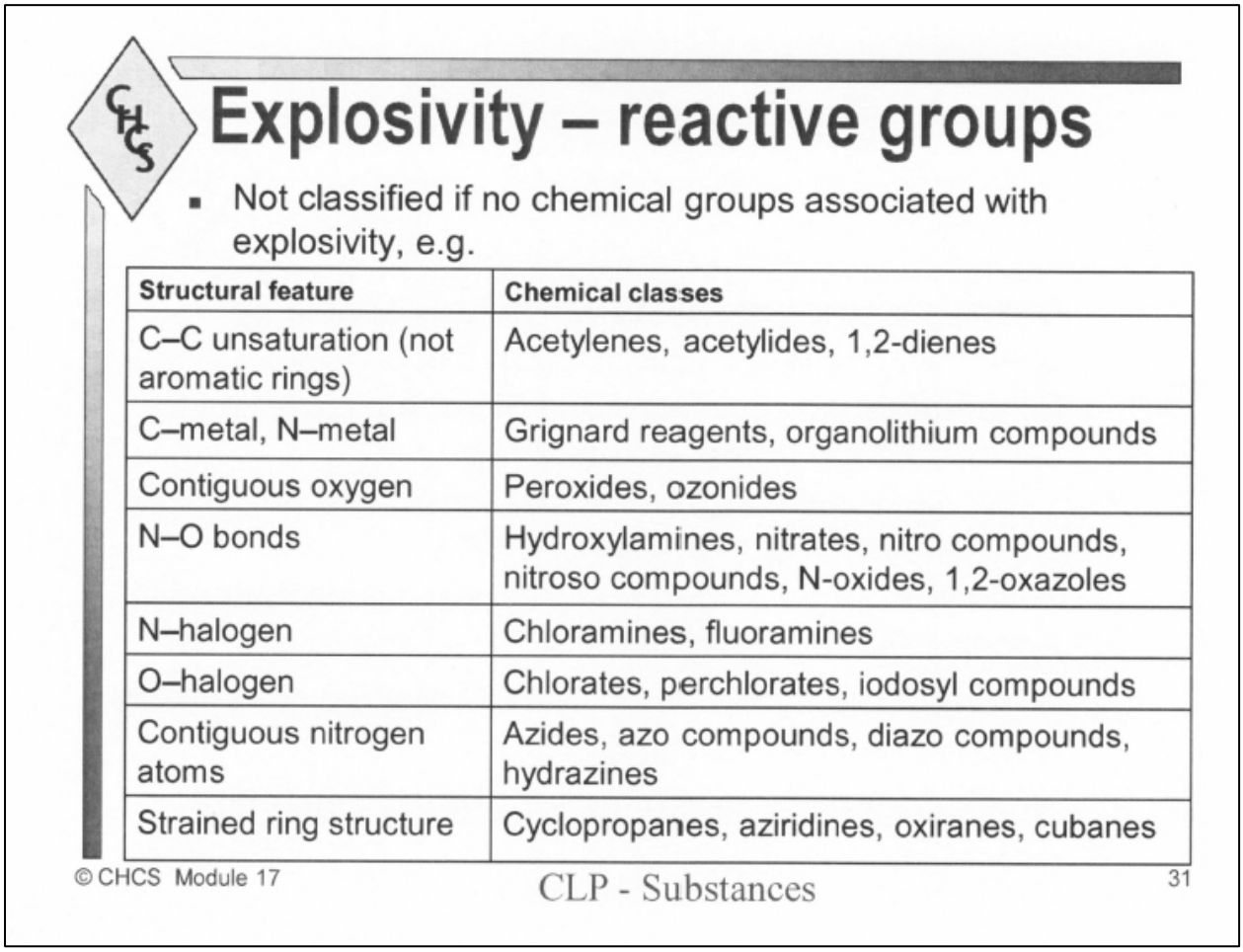
Level III Fugacity Model: (EQC Default)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	1.53e-006	36.6	1000
Water	34.5	208	1000
water	(34.5)		
biota	(3.95e-008)		
suspended sediment	(4.85e-007)		
Soil	65.5	416	1000
Sediment	0.0596	1.87e+003	0

Persistence Time: 387 hr

APPENDIX K: Known Structural Alerts for Reactivity

Explosivity – Abbreviated List



Explosivity – reactive groups

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

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

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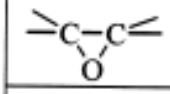
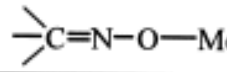
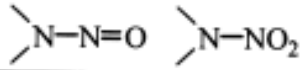
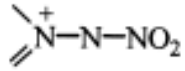
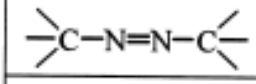
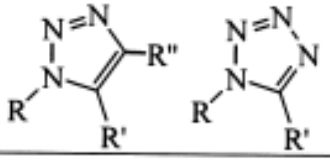
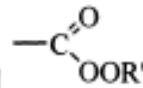
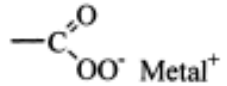
Explosivity – Full List

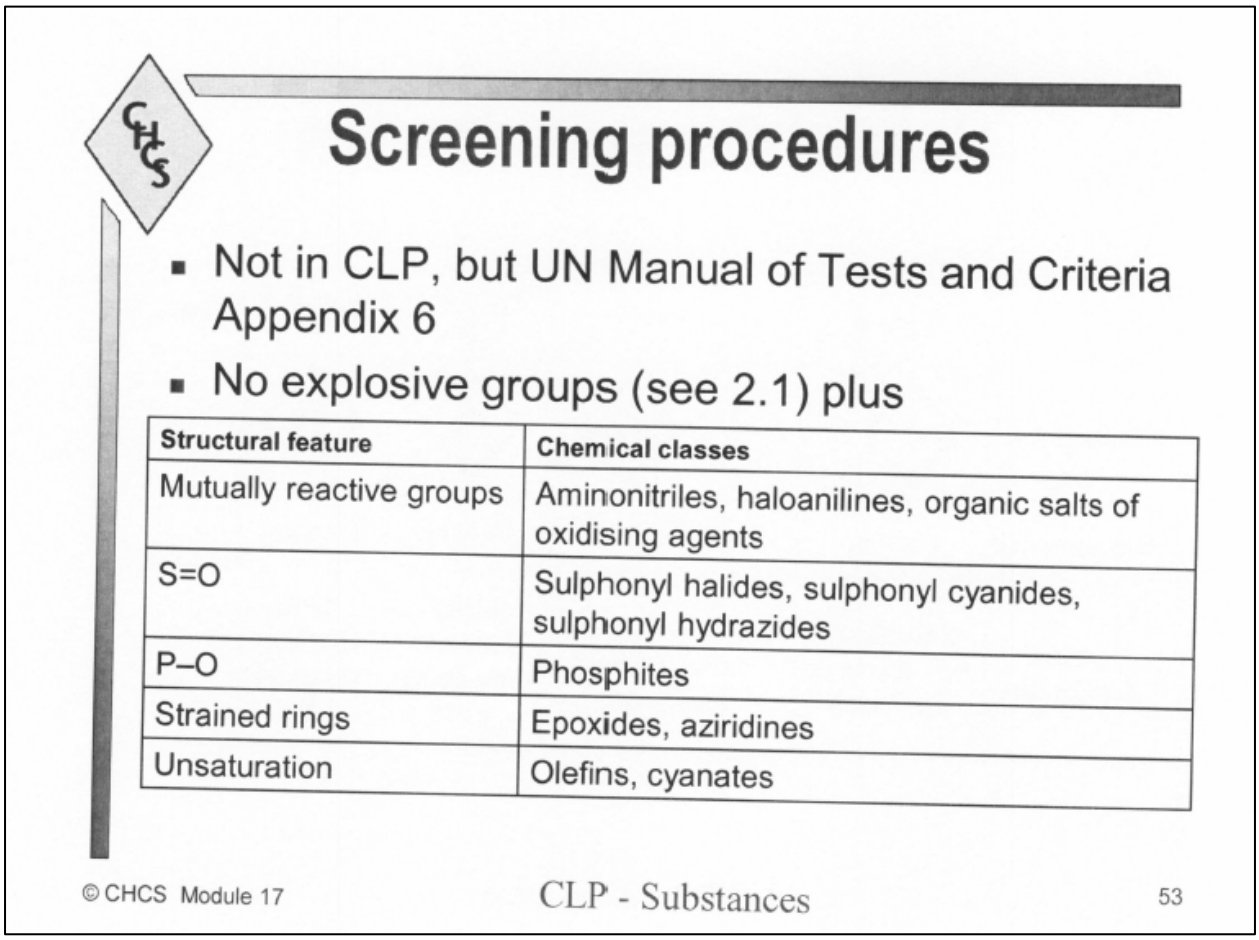
Table R.7.1-28 Chemical groups associated with explosive properties

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

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

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

Self-Reactive Substances



APPENDIX L: Change in Benchmark Score

Table 5 provides a summary of changes to the GreenScreen® Benchmark™ for citric acid. The GreenScreen® Benchmark Score for citric acid has changed over time. The original GreenScreen® assessment was performed in 2015 under version 1.2 criteria and ToxServices assigned a Benchmark 2 (BM-2) score. Most recently, ToxServices changed the GreenScreen® benchmark score to a BM-3 due to reclassification of the eye irritation endpoint from **Very High** (high confidence) to **High** (high confidence) following a weight of evidence evaluation of this chemical's eye irritation dataset, including the harmonized EU classification for this endpoint.

Table 5: Change in GreenScreen® Benchmark™ for Citric Acid

Date	GreenScreen® Benchmark™	GreenScreen® Version	Comment
June 17, 2015	BM-2	v.1.2	New assessment.
March 18, 2024	BM-3	v. 1.4	BM score changed to a BM-3 due to reclassification of eye irritation endpoint from **Very High** (high confidence) to **High** (high confidence) based on the harmonized EU GHS classification. Additionally, the skin irritation score was reclassified from *Moderate* (low confidence) to **Low** (high confidence) and the single exposure systemic toxicity was reclassified from **Low** (high confidence) to **Moderate** (high confidence); these changes had no impacts on the BM score.

Licensed GreenScreen® Profilers

Citric Acid GreenScreen® v. 1.2 Evaluation Prepared by:

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