ACETYL TRIBUTYL CITRATE

(CAS #77-90-7)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: December 20, 2023

Expiration Date: December 20, 2028

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GreenScreen® Executive Summary for Acetyl tributyl citrate (CAS #77-90-7)

Acetyl tributyl citrate is an ester of citric acid that is most widely used as a plasticizer alternative to phthalates. It is also used as a flavor ingredient in non-alcoholic beverages, and an indirect food additive in adhesives. It is produced from citric acid via the tributyl ester followed by acetylation.

Acetyl tributyl citrate was assigned a **GreenScreen Benchmark™ Score of 3** ("Use but Search for Safer Substitutes"). This score is based on the following hazard score combinations:

- Benchmark 3a
  - Moderate Persistence-P
  - Moderate Ecotoxicity (chronic aquatic-CA)
- Benchmark 3b
  - High Ecotoxicity (acute aquatic-AA)

A data gap (DG) exists for endocrine activity (E). As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), acetyl tributyl citrate meets requirements for a GreenScreen Benchmark™ Score of 3 despite the hazard data gap. In a worst-case scenario, if acetyl tributyl citrate were assigned a High score for the data gap E, it would be categorized as a Benchmark 3 Chemical. The GreenScreen® Benchmark Score for acetyl tributyl citrate has not changed over time. The original GreenScreen® assessment was performed in 2015 under version 1.2 criteria and ToxServices assigned a Benchmark 3 (BM-3) score. The BM-3 score was maintained with version 1.3 and 1.4 updates in 2016, 2018, and 2023.

New Approach Methodologies (NAMs) used in this GreenScreen® include *in vitro* data for genotoxicity and *in silico* modeling for bioaccumulation. 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 Acetyl tributyl citrate's NAMs dataset include a lack of experimental data for endocrine activity, respiratory sensitization, and bioaccumulation, and lack of validated test methods for respiratory sensitization. Acetyl tributyl citrate's Type II (extrapolation output) uncertainties include limitations of *in vitro* genotoxicity data in mimicking *in vivo* metabolism and their focusing on a few events in the genotoxicity process, and the lack of consideration of non-immunological mechanisms for respiratory sensitization.

GreenScreen® Hazard Summary Table for Acetyl Tributyl Citrate

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**	**L**	**L**		**L**	**L**	*L*	**L**	*L*	**H**	**M**	*M*	*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 Acetyl tributyl citrate (CAS #77-90-7)

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

GreenScreen® Assessment (v.1.2) Prepared By:
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Date: August 22, 2018

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Date: August 22, 2018

GreenScreen® Assessment (v.1.4) Updated By:
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Organization: ToxServices LLC
Date: October 10, 2023

Quality Control Performed By:
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Organization: ToxServices LLC
Date: December 20, 2023

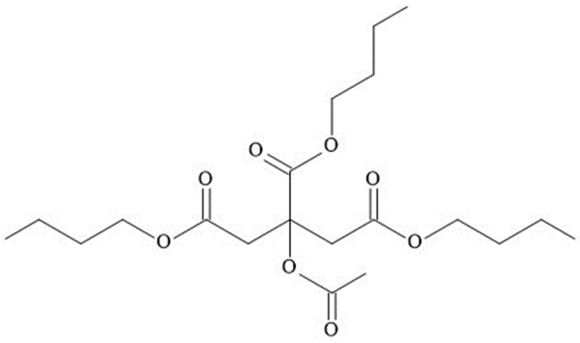
Expiration Date: December 20, 2028[2]

Chemical Name: Acetyl tributyl citrate

CAS Number: 77-90-7

[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).

Chemical Structure(s):



Also called:
Tributyl 2-acetoxypropane-1,2,3-tricarboxylate; Tributyl acetylcitrate; Tributyl O-acetylcitrate; Citroflex A; Blo-trol; Citroflex A 4; 2-Acetyltributylcitrate; Acetyl tributyl citrate; 1,2,3-Propanetricarboxylic acid, 2-(acetyloxy)-, tributyl ester; Tributyl 2-acetylcitrate; Acetylcitric acid, tributyl ester; Citric acid, tributyl ester, acetate; o-Acetylcitric acid tributyl ester; Uniplex 84; Tributyl 2-(acetyloxy)-1,2,3-propanetricarboxylate; Acetyl tri-n-butyl citrate; 1,2,3-Propanetricarboxylic acid, 2-(acetyloxy)-, 1,2,3-tributyl ester; Estaflex; Estaflex ATC; Pfizer citroflex A-4 (PubChem 2023).

Suitable surrogates or moieties of chemicals used in this assessment (CAS #'s):
Acetyl tributyl citrate has a sufficiently complete toxicological dataset to assign a GreenScreen Benchmark™ Score; therefore, no surrogates were used as part of this assessment.

Identify Applications/Functional Uses:
1. Flavoring ingredient
2. Plasticizer used in packaging films for food
(PubChem 2023)

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

GreenScreen® Summary Rating for Acetyl Tributyl Citrate[4,5 6,7]: Acetyl tributyl citrate was assigned a **GreenScreen Benchmark™ Score of 3** ("Use but Search for Safer Substitutes") (CPA 2018b). This score is based on the following hazard score combinations:

- Benchmark 3a
  - Moderate Persistence-P
  - Moderate Ecotoxicity (chronic aquatic-CA)
- Benchmark 3b
  - High Ecotoxicity (acute aquatic-AA)

[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.

A data gap (DG) exists for endocrine activity (E). As outlined in GreenScreen® Guidance (CPA 2018b) Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), acetyl tributyl citrate meets requirements for a GreenScreen Benchmark™ Score of 3 despite the hazard data gap. In a worst-case scenario, if acetyl tributyl citrate were assigned a High score for the data gap E, it would be categorized as a Benchmark 3 Chemical.

Figure 1: GreenScreen® Hazard Summary Table for Acetyl Tributyl Citrate

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**	**L**	**L**		**L**	**L**	*L*	**L**	*L*	**H**	**M**	*M*	*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

No relevant and feasible transformation products were identified. Acetyl tributyl citrate is expected to biodegrade slowly, but no biodegradation products have been experimentally identified. Both the EAWAG-BBD Pathway Prediction System (EAWAG 2014) and OECD Toolbox (OECD 2023) predict a large number of potential degradation products (53 microbial metabolites and 13 hydrolysis products per OECD Toolbox). Considering the large number of potential products and lack of information regarding the relative quantity or likelihood of formation of each, and in the absence of experimental data, it is not possible to determine which of the many products meet criteria to be considered feasible and relevant (i.e. "could potentially result in increased risk from the use of the parent chemical across its life cycle"). Therefore, the Benchmark score of acetyl tributyl citrate is not modified.

Introduction

Acetyl tributyl citrate is an ester of citric acid that is most widely used as a plasticizer alternative to phthalates. It is also used as a flavor ingredient in non-alcoholic beverages, and an indirect food additive in adhesives (HSDB 2015). The United States Food and Drug Administration (U.S. FDA) recognizes acetyl tributyl citrate as an acceptable direct food additive under 21 CFR §172.515; an acceptable indirect food additive under 21 CFR §175.105, §175.300, §175.320, and §178.3910; and as a prior-sanctioned food ingredient under 21 CFR §181.27 (U.S. FDA 2023). It is produced from citric acid via the tributyl ester followed by acetylation (HSDB 2015).

ToxServices assessed acetyl tributyl citrate 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 2023a). It can be accessed at: http://www2.epa.gov/saferchoice/safer-ingredients. Chemicals on the SCIL have been assessed for compliance with the Safer Choice Standard and Criteria for Safer Chemical Ingredients (U.S. EPA 2015).

Acetyl tributyl citrate is not listed on the SCIL.

GreenScreen® List Translator Screening Results

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

- Acetyl tributyl citrate is an LT-P1 chemical when screened using Pharos, and therefore a full GreenScreen® is required.
- Acetyl tributyl citrate is not listed on the U.S. DOT list.
- Acetyl tributyl citrate is on the following list for multiple endpoints.
    - German FEA – Substance Hazardous to Water: Class 2 – Hazard to Waters
- Specified lists for single endpoints are reported in individual hazard endpoints in the hazard assessment section below.

Hazard Statement and Occupational Control

No Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard statements were identified for acetyl tributyl citrate, as indicated in Table 1. General personal protective equipment (PPE) recommendations are presented in Table 2, below. No occupational exposure limits (OELs) were identified.

Table 1: GHS H Statements for Acetyl Tributyl Citrate (CAS #77-90-7) (ECHA 2023)

H Statement	H Statement Details
No harmonized GHS H statements are reported by the European Chemicals Agency (ECHA). According to the notifications provided by companies to ECHA in REACH registrations, no hazards have been classified.	

Table 2: Occupational Exposure Limits and Recommended Personal Protective Equipment for Acetyl Tributyl Citrate (CAS #77-90-7)

Personal Protective Equipment (PPE)	Reference	Occupational Exposure Limits (OEL)	Reference
PVC gloves, safety goggles and protective clothing	Vertellus 2014	None identified	

Physicochemical Properties of Acetyl Tributyl Citrate

Acetyl tributyl citrate is a colorless viscous liquid at room temperature. Its measured vapor pressure of 1.7×10^{-4} Pa indicates that it has the potential to volatilize. It is slightly soluble in water (4.49 mg/L), and its measured log K_{ow} of 4.86 indicates that it is lipophilic and may have the potential to bioaccumulate.

[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 Acetyl Tributyl Citrate (CAS #77-90-7)		
Property	**Value**	**Reference**
Molecular formula	$C_{20}H_{34}O_8$	PubChem 2023
SMILES Notation	CCCCOC(=O)CC(CC(=O)OCCCC)(C(=O)OCCCC)OC(=O)C	PubChem 2023
Molecular weight	402.5 g/mol	PubChem 2023
Physical state	Liquid	PubChem 2023
Appearance	Colorless, slightly viscous	PubChem 2023
Melting point	-80°C	ECHA 2023
Boiling point	331°C	ECHA 2023
Vapor pressure	1.7×10^{-4} Pa at 25°C (experimental)	ECHA 2023
Water solubility	4.49 mg/L at 20°C (experimental)	ECHA 2023
Dissociation constant	pKa = -6.53 at 20°C (estimated)	ECHA 2023
Density/specific gravity	1.0528 g/cm^3 at 20°C (experimental)	ECHA 2023
Partition coefficient	Log K_{ow} = 4.86 at 40°C (experimental)	ECHA 2023

Toxicokinetics

- Absorption
  - CPSC 2010, ECHA 2023
    - Acetyl tributyl citrate is rapidly and extensively absorbed. In an *in vivo* absorption and elimination study, male Sprague-Dawley rats (4-5/dose) were administered 70 mg [^{14}C]Acetyl tributyl citrate/kg once via gavage. Urine, feces, cage wash, expired organics and [^{14}C]CO_2, blood, tissues (including GI tract and contents) and carcass were analyzed for the presence of radiolabeled material [^{14}C] and/or unchanged Acetyl tributyl citrate. The absorption of dosed [^{14}C] was rapid with a half-time of 1 hour determined for its absorption from the gastrointestinal tract. The absorption was also extensive with at least 67% of the administered dose being absorbed after an oral exposure.
- Distribution
  - CPSC 2010, ECHA 2023
    - In the previously described rat absorption study, an elimination half-life of 3.4 hours was calculated for [^{14}C] in blood. Less than 1 % of the radioactivity remained in tissues and the carcass 48 hours post-dosing.
- Metabolism
  - CPSC 2010, ECHA 2023
    - Acetyl tributyl citrate is quickly and almost completely metabolized, primarily by hydrolysis to polar metabolites including acetyl citrate, monobutyl citrate, acetyl monobutyl citrate, dibutyl citrate and acetyl dibutyl citrate (two 1,4 isomers), along with several other unidentified metabolites. In the previously described rat absorption study, at least 9 radiolabeled metabolites were found in urine and 3 in feces. Urinary metabolites positively identified were acetyl citrate, mono-butyl citrate (tentatively the major metabolite), acetyl mono-butyl citrate, dibutyl citrate, and acetyl dibutyl citrate. *In vitro* studies found that acetyl tributyl citrate is metabolized by human serum and rat liver homogenates to citric, acetic, and butyric acids with the hydrolysis rate being much faster in rat liver (half-life < 30 minutes compared to 7 hours in human serum).
- Excretion
  - ECHA 2023

- Acetyl tributyl citrate is virtually completely excreted by the rat. In the previously described rat absorption study, more than 87% of the administered radioactivity was excreted during the initial 24 hours after dosing. The principal route of [^{14}C] excretion was the urine (59- 70% of the [^{14}C] dose), while 25-36% were excreted via feces and 2% as [^{14}C]CO_2. Accordingly, it was suggested by the study authors that the low toxicity of acetyl tributyl citrate could be attributed to its rapid clearance in the rat.

Hazard Classification Summary

Group I Human Health Effects (Group I Human)

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

Acetyl tributyl citrate was assigned a score of Low for carcinogenicity based on no carcinogenic effects seen in an oral carcinogenicity study in rats. 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 high as it is based on reliable experimental data from a well-characterized study.

- 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 2023
  - *Oral*: In a GLP-compliant chronic oral toxicity study conducted according to 875/318/EEC; 83/571/EEC; 91/507/EEC in male and female Wistar rats, animals (50/sex/dose) were administered Acetyl tributyl citrate (purity not reported) in the diet to yield doses of 0, 100, 300, or 1,000 mg/kg/day for 2 years. Histopathological examination to evaluate neoplastic effects were performed on all animals in the control and high dose groups, on animals that died or were sacrificed in extremis, and on all gross lesions. No neoplastic changes were observed (Klimisch 1, reliable with restrictions).
- CPSC 2010, U.S. EPA 2014
  - *Oral*: In a chronic oral study in Sherman rats (sex not specified), animals (20/dose, 40/control) were administered 0, 200, 2,000, or 20,000 ppm (equivalent to 10, 100, and 1,000 mg/kg/day respectively, as calculated by the study authors) acetyl tributyl citrate (99.4% purity) in the diet for 2 years. Body weights were transiently reduced in all dose groups from weeks 5-15, but this effect could not be reproduced in a separate one-year study of 10 rats/dose administered 200 or 2,000 ppm in the diet. In the main study, 20/60 rats died (compared to 8/40 in the control group), and pulmonary lesions indicated possible infection. Lymphomas were observed in both treated and control rats, and the incidence in the control group (6) exceeded those in the treatment groups (1, 0, and 2 for the low, mid, and high doses, respectively). There were no treatment-related lesions, but the Consumer Product Safety Commission (CPSC) notes that the study is limited by small sample sizes, deaths due to infection, and because doses did not reach the maximum tolerated dose (MTD). No additional details were provided (Klimisch score not reported).
  - *Oral*: No effects on hematology or gross or microscopic abnormalities were observed in 2 mongrel dogs that were fed gelatin capsules containing 140 mg (approximately 7-10 mg/kg-day) acetyl tributyl citrate for 2 years, but the CPSC notes that the study is limited by small sample sizes and lack of a control group. No additional details were provided (Klimisch score not reported).

- Based on the weight of evidence, a score of Low was assigned as there was no evidence of carcinogenicity in a well-conducted oral toxicity study in rats. Two additional studies in rats and dogs are limited by small samples but also showed no evidence of a carcinogenic effect.

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

Acetyl tributyl citrate was assigned a score of Low for mutagenicity/genotoxicity based on negative results in *in vitro* and *in vivo* mutagenicity and clastogenicity assays. GreenScreen® criteria classify chemicals as a Low hazard for mutagenicity/genotoxicity when negative data are available for both gene mutations and chromosome aberrations, and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data.

- 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 2023
  - *In vitro:* Acetyl tributyl citrate was negative for mutagenicity in a GLP-compliant bacterial reverse mutation assay according to OECD Guideline 471/472 in *Salmonella typhimurium* test strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strain WP_2. Bacteria were exposed to the test substance (98.8% purity; dimethyl sulfoxide (DMSO) vehicle) at concentrations of 0-5,000 µg/plate with and without metabolic activation (phenobarbital and 5,6-benzoflavone induced rat liver S9). The positive controls were 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, sodium azide, 9-aminoacridine, and 2-aminoanthracene. Positive, negative, and vehicle controls were reported as valid. There were no increases in the frequency of revertants observed in any strain at any concentration with or without metabolic activation (Klimisch 1, reliable without restrictions).
  - *In vitro*: Acetyl tributyl citrate was negative for mutagenicity in a GLP-compliant cell mutation assay according to OECD Guideline 476. Mouse lymphoma L5178Y cells ($TK^{+/-}$) were exposed to acetyl tributyl citrate (purity not reported) in DMSO at concentrations of 200-550 µg/mL with metabolic activation and 10-310 µg/mL without metabolic activation (S9 mix obtained from the liver of Aroclor-induced rats). Positive controls of 3-methylchloranthrene and ethyl methanesulfonate, as well as vehicle controls, were used. While there was a dose-dependent increase in cytotoxicity, there was no dose-dependent increase in mutation frequency with or without metabolic activation. The vehicle and positive controls were reported as valid (Klimisch 2, reliable with restrictions).
  - *In vitro*: Negative results for clastogenicity were obtained in a GLP-compliant chromosomal aberration test conducted according to OECD Guideline 473. Chinese hamster lung (CHL/IU) cells were exposed to acetyl tributyl citrate (98.8% purity) in DMSO at concentrations of 0-1 µg/mL with and without metabolic activation (phenobarbital and 5,6-benzoflavone induced rat liver S9). Positive controls of benzo(a)pyrene and mitomycin C and vehicle controls were used. No cytotoxicity was observed and positive and vehicle controls were reported as valid. No increase in the frequency of chromosome aberrations was identified following treatment in the presence or absence of metabolic activation (Klimisch 1, reliable without restrictions).
  - *In vivo*: Negative results for clastogenicity were obtained in a GLP-compliant chromosomal aberration assay conducted according to OECD Guideline 475. Male and female Wistar rats (10/sex/dose) were administered single doses of 2,000 mg/kg/dose via gavage in polyethylene glycol via gavage. A positive control (cyclophosphamide) was used and was reported as valid. There were no increases in the frequency of chromosomal aberrations seen (Klimisch 1, reliable without restrictions).

- ECHA 2023, U.S. EPA 2003
    - *In vitro:* Acetyl tributyl citrate was negative for mutagenicity in a GLP-compliant bacterial reverse mutation assay according to OECD Guideline 471 in *S. typhimurium* test strains TA98, TA100, TA1535, TA1537, and TA1538. Bacteria were exposed to the test substance (purity not reported) in DMSO at concentrations of 333-10,000 µg/plate with and without metabolic activation (S9 from Aroclor 1254-induced Sprague-Dawley rats and Syrian Golden Hamsters). The positive controls were 9-aminoacridine, 2-nitrofluorene, sodium azide, and 2-aminoanthracene. Positive, negative, and vehicle controls were reported as valid. There were no increases in the frequency of revertants observed in any strain at any concentration with or without metabolic activation (Klimisch 2, reliable with restrictions).
    - *In vitro:* Acetyl tributyl citrate was negative for mutagenicity in a GLP-compliant bacterial reverse mutation assay according to OECD Guideline 471 in *S. typhimurium* test strains TA98, TA100, TA1535, TA1537, and TA1538. Bacteria were exposed to the test substance (purity not disclosed) in DMSO at concentrations of 9-495 µg/plate with and without metabolic activation (metabolic system not disclosed). The positive controls were nitrofluorene. The validity of the controls were not reported. There were no increases in the frequency of revertants observed in any strain at any concentration with or without metabolic activation (Klimisch 2, reliable with restrictions).
    - *In vitro*: Acetyl tributyl citrate was negative for mutagenicity in a GLP-compliant cell mutation assay (conducted against an undisclosed guideline). Mouse lymphoma L5178Y ($TK^{+/-}$) cells were exposed to acetyl tributyl citrate (purity not reported) in DMSO at concentrations of 200-480 µg/mL with metabolic activation and 10-230 µg/mL without metabolic activation (S9 mix). Positive controls of ethylmethanesulfonate and 3-methylcholanthrene, as well as vehicle controls, were used. No increase in the mutation frequency was detected with treatment in the presence or absence of metabolic activation (Klimisch 2, reliable with restrictions).
    - *In vitro*: Acetyl tributyl citrate was negative for mutagenicity in a GLP-compliant cell mutation assay according to OECD Guideline 476. Chinese hamster ovary (CHO) cells were exposed to acetyl tributyl citrate (purity not reported) in an undisclosed solvent at concentrations of 25-400 µg/mL with metabolic activation and 10-310 µg/mL without metabolic activation (rat liver S9). Positive controls (identity not provided), as well as vehicle controls, were used. No increase in the mutation frequency was detected with treatment in the presence or absence of metabolic activation (Klimisch 2, reliable with restrictions).
    - *In vitro*: Negative results for clastogenicity were obtained in a GLP-compliant chromosomal aberration test conducted according to OECD Guideline 473. Rat lymphocytes were exposed to acetyl tributyl citrate (99.02% purity) in DMSO at concentrations of 4-400 µg/mL with and without metabolic activation (S9 liver homogenate prepared from Aroclor 1254-treated Sprague-Dawley rats). Positive controls (identity not provided), as well as vehicle controls, were used. No cytotoxicity was observed and positive and vehicle controls were reported as valid. No increase in the frequency of chromosome aberrations was identified following treatment in the presence or absence of metabolic activation (Klimisch 2, reliable with restrictions).

- U.S. EPA 2003
    - *In vitro*: Acetyl tributyl citrate (98.55% purity) was negative in a GLP-compliant bacterial mutagenicity assay that was conducted according to OECD Guideline 471 in *S. typhimurium* test strains TA98, TA100, TA1535, and TA1537 when tested at concentrations of 50- 5,000 µg/plate with and without metabolic activation. No cytotoxicity was observed and there was no evidence of mutagenicity (Klimisch 1, reliable without restrictions).

Reproductive Toxicity (R) Score (H, M, or L): *L*
Acetyl tributyl citrate was assigned a score of Low for reproductive toxicity based on a lack of effects on mating and fertility parameters in two 2-generation oral toxicity 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 low as marginally reduced implantation rates and litter size were observed at a very high dose (> 1,000 mg/kg) in one rat study, which were not reproducible in another study at the same dose.
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 2003, 2014, ECHA 2023
    - *Oral*: In a GLP-compliant 2-generation reproductive toxicity study conducted according to OECD Guideline 416 in Sprague-Dawley rats, animals (30/sex/dose) were administered acetyl tributyl citrate (99.4% purity) in the diet to yield nominal doses of 0, 100, 300, or 1,000 mg/kg/day (actual doses of 103, 306 and 1,013 mg/kg/day, respectively, for males and 102, 306, and 1,024 mg/kg/day, respectively, for females). The F0 males were treated for 11 weeks prior to mating through sacrifice and the F0 females were treated for 3 weeks prior to mating, through mating, gestation, and lactation. The F1 males and females were treated for 10 weeks prior to mating through sacrifice (males) or through mating, gestation, and lactation (females). There were no clinical signs of toxicity. Body weights were consistently reduced in F1 males of the mid and high dose groups, but there were no effects in the F0 generation or in females of either generation. Water consumption was reduced in both sexes and generations at the high dose. There were no effects on any mating, gestation, or fertility parameters. The United States Environmental Protection Agency (U.S. EPA) identified a NOAEL of 100 mg/kg/day for parental toxicity based on effects on male body weight and a NOAEL of 1,000 mg/kg/day for reproductive toxicity based on a lack of effects at the highest dose tested (Klimisch 1, reliable without restrictions).
    - *Oral*: In a GLP-compliant reproductive and developmental toxicity study that was conducted according to US EPA OPPTS 870.3100/OECD Method 408/EC Method B26, male and female Han Wistar rats (25/sex/dose) were administered acetyl tributyl citrate (99.9% purity) through the diet to yield nominal doses of 0, 100, 300, or 1,000 mg/kg/day (actual doses of 0, 103, 306, and 1,013 mg/kg/day, respectively, for males and 0, 102, 306, and 1,024 mg/kg/day, respectively, for females) for 4 weeks prior to mating through mating (males) or through gestation and lactation (females). There were no effects on estrous cycles, mating performance, fertility, gestation length and parturition. Sperm motility, counts, and morphology were also unaffected. Litter size and the number of implantations were marginally reduced at the high dose, but the statistical significance was not reported. U.S. EPA reported a NOAEL of 300 mg/kg/day and LOAEL of 1,000 mg/kg/day for reproductive toxicity based on effects on litter size and implantations (Klimisch 1, reliable without restrictions).
    - *Oral*: In a GLP-compliant 2-generation reproduction toxicity study (no guideline followed)

in male and female Sprague-Dawley rats (30/sex/dose) were administered acetyl tributyl citrate (99.4% purity) through the diet to yield nominal doses of 0, 100, 300, or 1,000 mg/kg/day (actual doses of 0, 108.8, 316.2, and 1,069.1 mg/kg/day, respectively, for males and 0, 108.8, 363, and 1,119.1 mg/kg/day, respectively, for females). The F0 males were treated for 11 weeks prior to mating through sacrifice and the F0 females were treated for 3 weeks prior to mating, through mating, gestation, and lactation. The F1 males and females were treated for 10 weeks prior to mating through sacrifice (males) or through mating, gestation, and lactation (females). Body weights were consistently reduced in F1 males of the mid and high dose groups, but did not affect the F0 parents of both sexes. Water consumption was reduced in both sexes and generations at the high dose. There were no effects on any mating, gestation, or fertility parameters. Based on these effects, ToxServices identified a NOAEL of 100 mg/kg/day for parental toxicity based on effects on male body weight and a NOAEL of 1,000 mg/kg/day for reproductive toxicity based on a lack of effects at the highest dose tested (Klimisch 2, reliable with restrictions).

- U.S. EPA 2003
    - *Oral*: In an oral study (not conducted according to guidelines or GLP) in male and female rats (strain not specified), animals (number not reported) were administered acetyl tributyl citrate (purity not reported) in a milk solution providing 0, 50, or 250 mg/kg/day. Animals were cross mated in the 9th month of the study and male gonads were evaluated (including evaluation of spermatogenesis index). Fertility rates and number of pups per female were also determined. No treatment-related effects were observed (Klimisch score not reported).
    - *Oral*: In an oral study (not conducted according to guidelines or GLP) in male and female mice (strain not specified), animals (number not reported) were administered Acetyl tributyl citrate (purity not reported) in a milk solution providing 0, 50, or 250 mg/kg/day. Animals were cross mated in the 9th month of the study and male gonads were evaluated (including evaluation of spermatogenesis index). Fertility rates and number of pups per female were also determined. No treatment-related effects were observed (Klimisch score not reported).
- Based on the weight of evidence, a score of Low was assigned as there were no effects on any mating or fertility parameters in a well-conducted, GLP-compliant 2-generation oral study in rats. Although a one-generation study reported a reduction in litter size and implantations at a dose of 1,000 mg/kg/day, the statistical significance was not reported and the magnitude was reported to be "marginal." In addition, this effect was not reproduced in either generation of the 2-generation study that tested the same doses. A lack of effects on fertility parameters, including litter size, in less well-reported studies in rats and mice also supports a Low score for reproductive toxicity.

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

Acetyl tributyl citrate was assigned a score of Low for developmental toxicity based on a lack of effects on development in both one- and two-generation studies in rats. 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 high as it is based on reliable experimental data.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- U.S. EPA 2003, 2014, ECHA 2023
    - *Oral*: In the previously described GLP-compliant 2-generation reproductive toxicity study (guideline not reported) in Sprague-Dawley rats, animals (30/sex/dose) were administered acetyl tributyl citrate (99.4% purity) in the diet to yield nominal doses of 0, 100, 300, or

1,000 mg/kg/day (actual doses of 103, 306, and 1,013 mg/kg/day, respectively, for males and 102, 306, and 1,024 mg/kg/day, respectively, for females). The F0 males were treated for 11 weeks prior to mating through sacrifice and the F0 females were treated for 3 weeks prior to mating, through mating, gestation, and lactation. The F1 males and females were treated for 10 weeks prior to mating through sacrifice (males) or through mating, gestation, and lactation (females). In terms of developmental toxicity, there were no treatment-related effects in the number and sex of pups, stillbirths, live births, postnatal mortality, presence of gross anomalies, and physical or behavioral abnormalities. However, there was a "slight" reduction in pup weight and a slight increase in pup mortality at the mid and high doses (values and statistical significance not reported). Authors noted that these effects were likely to be the result of decreased water intake by the dams. The U.S. EPA reported a NOAEL of 1,000 mg/kg/day for reproductive toxicity. ToxServices assigned a NOAEL of 1,000 mg/kg/day for developmental toxicity, which is the highest dose tested (Klimisch 1, reliable without restrictions).

- *Oral*: In the previously described GLP-compliant reproductive and developmental toxicity study that was conducted according to US EPA OPPTS 870.3100/OECD Method 408/EC Method B26, male and female Han Wistar rats (25/sex/dose) were administered acetyl tributyl citrate (99.9% purity) through the diet to yield nominal doses of 0, 100, 300, or 1,000 mg/kg/day (actual doses of 0, 103, 306, and 1,013 mg/kg/day, respectively, for males and 0, 102, 306, and 1,024 mg/kg/day, respectively, for females) for 4 weeks prior to mating through mating (males) or through gestation and lactation (females). In terms of developmental toxicity exanimation, F1 animals were evaluated for sexual maturation (balano-preputial separation, vaginal opening, anogenital distance, retained areolae in males, sperm assessments), estrous cyclicity, physical appearance, ophthalmologic effects, neurobehavioral effects, growth, food consumption, survival, hematology, blood chemistry, urinalysis, peroxisome proliferation, organ weights, gross pathology, and histopathology. There were no treatment related effects in any of these parameters. However, litter size and the number of implantations were marginally reduced at the high dose, but the statistical significance was not reported. There were no effects on anogenital distance, or retained areolae in offspring. U.S. EPA reported a NOAEL of 300 mg/kg/day and LOAEL of 1,000 mg/kg/day based on effects on litter size and implantations (Klimisch 1, reliable without restrictions).

- U.S. EPA 2003
  - *Oral*: In the previously described oral study (not conducted according to guidelines or GLP) in male and female rats (strain not specified), animals (number not reported) were administered acetyl tributyl citrate (purity not reported) in a milk solution providing 0, 50, or 250 mg/kg/day. Animals were cross mated in the 9th month of the study. Evaluations of early and late embryonic deaths, the number of normal, resorptive and deformed tissues, length of newborns, size and weight of the placenta, and physiological development of the progeny (ear openings; eye openings, appearance of body hair and teeth, behavior, and body weight) were made. Weight and length of progeny and placental weight were increased at the high dose. There were no additional treatment related effects. Authors reported a NOAEL of 250 mg/kg/day for developmental toxicity (Klimisch score not reported).
  - *Oral*: In the previously described oral study (not conducted according to guidelines or GLP) in male and female rats (strain not specified), animals (number not reported) were administered acetyl tributyl citrate (purity not reported) in a milk solution providing 0, 50, or 250 mg/kg/day. Animals were cross mated in the 9th month of the study. Evaluations of early and late embryonic deaths, the number of normal, resorptive and deformed tissues,

length of newborns, size and weight of the placenta, and physiological development of the progeny (ear openings; eye openings, appearance of body hair and teeth, behavior, and body weight) were made. No treatment related effects were observed. Authors reported a NOAEL of 250 mg/kg/day (Klimisch score not reported).

- Based on the weight of evidence, a score of Low was assigned. Reductions in pup body weight and an increase in pup mortality were reported in a 2-generation study in rats, but effects were reported to be "slight" and authors noted that they could be the results of reduced water consumption by dams; U.S. EPA identified a NOAEL of 1,000 mg/kg/day, the highest dose tested. Similarly, a reduction in litter size and implantations was reported in a 1-generation study in rats at high doses (1,000 mg/kg/day), but the statistical significance was not reported and the magnitude was reported to be "marginal". In both studies, no adverse developmental effects were seen and the weight of evidence indicates a Low hazard for developmental toxicity. .

Endocrine Activity (E) Score (H, M, or L): DG
Acetyl tributyl citrate was assigned a score of Data Gap for endocrine activity based on insufficient data identified for this endpoint. The negative prediction for estrogen activity is insufficient to determine its *in vivo* activity, and there are no data on its activity on the androgen and thyroid pathways.

- 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 2023b
  - Acetyl tributyl citrate is predicted to be inactive for estrogen agonism, antagonism, and binding (Appendix D).
  - Acetyl tributyl citrate was active in 0/18 estrogen receptor (ER) assays, 1/14 androgen receptor (AR) assays, 0/2 steroidogenesis assays, and 2/15 thyroid receptor assays performed as part of the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) in the 21st Century (Appendix E).
- Although the available data indicate acetyl tributyl citrate is not likely to interact with endocrine receptors, insufficient data are available to determine if it affects circulating estrogen, androgen, or thyroid hormone levels. Therefore, a data gap is assigned.

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
Acetyl tributyl citrate was assigned a score of Low for acute toxicity based on oral LD_{50} > 31,500 mg/kg in rats and cats. GreenScreen® criteria classify chemicals as a Low hazard for acute toxicity when oral LD_{50} values are > 2,000 mg/kg (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023
  - *Oral:* LD_{50} > 31,500 mg/kg in rats (sex and strain not specified) (non-GLP, standard acute method) (Klimisch 2, reliable with restrictions)

- *Oral:* LD_{50} > 30 mL/kg[9] in Wistar rats (sex not specified) (non-GLP, OECD Guideline 401) (Klimisch 2, reliable with restrictions)
- *Oral:* LD_{50} > 50,000 mg/kg in cats (sex and strain not specified) (non-GLP, standard acute method) (Klimisch 2, reliable with restrictions)
- *Dermal:* LD_{50} > 1,000 mg/kg in male albino rabbits (GLP compliance not specified, no guideline followed) (Klimisch 2, reliable with restrictions)

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

Acetyl tributyl citrate was assigned a score of Low for systemic toxicity (single dose) based on the lack of systemic toxicity in rats after a single oral exposure > 5,000 mg/kg. GreenScreen® criteria classify chemicals as a Low hazard for systemic toxicity (single dose) when adequate data are available and negative, and they are not classified under GHS (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023
  - *Oral*: In an older acute oral study in Wistar rats (sex not specified), 5 animals were administered a single dose of 31,500 mg/kg acetyl tributyl citrate (purity not reported) via gavage and were observed for 21 days. Shortly after administration, the test substance leaked from the rectum and transient sluggishness was reported. There were no additional clinical signs. No deaths occurred at any dose level throughout the study and an oral LD_{50} of > 31,500 mg/kg was established. No additional details regarding body weight or gross pathology were provided (Klimisch 2, reliable with restrictions).
  - *Oral*: In a non-GLP compliant acute oral study conducted according to OECD Guideline 401, rats (sex and strain not reported, 5/dose) were administered doses of 10-30 mL/kg via gavage. Shortly after administration, the test substance leaked from the rectum and transient sluggishness was reported. There were no additional clinical signs. No deaths occurred at any dose level throughout the study and an oral LD_{50} of > 30 mL/kg was established. No additional details regarding body weight or gross pathology were provided (Klimisch 2, reliable with restrictions).
  - *Oral*: In an acute oral study in cats (sex not specified), 8 animals were administered single doses ranging from 30,000-50,000 mg/kg acetyl tributyl citrate (purity not reported) via gavage and were observed for up to 2 months. No deaths occurred. The only clinical signs seen were nausea and diarrhea for 24 hours. There were no other clinical signs of toxicity and there were no effects on blood counts, clinical chemistry, or urinalysis. Study authors established an oral LD_{50} of > 50,000 mg/kg (Klimisch 2, reliable with restrictions).
  - *Dermal:* In a skin irritation study, which likewise could be used as acute dermal toxicity study, three male albino rabbits were dermally administered acetyl tributyl citrate (purity not reported) on the intact skin of the abdomen at a dose of 1 mL/kg (equivalent to 1,000 mg/kg) daily for 4 days. There were no deaths during the 36-hour observation period and no clinical signs of systemic toxicity. Authors identified a dermal LD_{50} of > 1,000 mg/kg (Klimisch 2, reliable with restrictions).

[9] Equivalent to > 31,584 mg/kg based on a density of 1.0528 g/cm^3: 30 mg/kg * 1.0526 g/mL * 1,000 mg/1 g = 31,584 mg/kg.

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

Acetyl tributyl citrate was assigned a score of Low for systemic toxicity (repeated dose) based on the most conservative NOAEL of 100 mg/kg/day for body weight reductions at the LOAEL of 300 mg/kg/day in a 1-year oral toxicity study in rats. GreenScreen® criteria classify chemicals as a Low hazard for systemic toxicity (repeated dose) when there is no evidence of systemic toxicity below 100 mg/kg/day in a 90-day oral study or not classified as per GHS (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data from several studies.

- 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 2023
  - *Oral*: In a GLP-compliant chronic oral toxicity study conducted according to 875/318/EEC; 83/571/EEC; 91/507/EEC in male and female Wistar rats, animals were administered acetyl tributyl citrate (purity not reported) in the diet to yield nominal doses of 0, 100, 300, or 1,000 mg/kg/day (actual doses of 100.2, 302.5, and 1,004 mg/kg/day, respectively, for males and 100.9, 304.1, and 1,021 mg/kg/day, respectively, for females) for 52 weeks (20/sex/dose) or 104 weeks (50/sex/dose, previously described under Carcinogenicity). Endpoints evaluated and results for the two portions of the study are described separately below (Klimisch 1, reliable without restrictions):
    - In the 104-week portion of the study, animals were evaluated for mortality, clinical signs, body weight, food consumption, organ weights, and gross pathology. Histopathological evaluations were performed on all animals in the control and high dose groups, on animals that died or were sacrificed in extremis, and on all gross lesions. Hematological evaluations were also performed. Mean body weights were significantly decreased in males in the mid and high dose groups (11% and 16%) and females in the high dose group (13%) at termination. Food consumption was also reduced in males at these doses. In the high dose group, relative liver weights were increased in males by 18% and females by 16%. Minimal to moderate centrilobular hypertrophy was observed in 5 males and a minimal to moderate single cell necrosis of hepatocytes was observed in 7 males and 1 female of the high dose group. Authors reported a NOAEL of 300 mg/kg/day based on effects on body weight, liver weight, and liver histopathology at the LOAEL of 1,000 mg/kg/day.
    - For the 52-week systemic toxicity portion of the study, histopathological evaluations were performed on all animals. Animals were evaluated for mortality, clinical signs, body weight, and food consumption. Ophthalmoscopic, hematological, clinical chemistry, and urinalysis evaluations were conducted during weeks 26 and 52. Terminal body weights were reduced in both sexes in the mid (7% in males and 11% in females) and high (15% in males and 8% in females) dose groups. Food consumption was slightly reduced in all treatment groups, but was not considered by authors to be biologically relevant. Changes in clinical chemistry parameters related to metabolic activation were observed in the mid and high dose groups. Urine volumes were increased in males of all dose groups at termination, and urinary pH was reduced at the high dose and protein content was reduced at the mid and high doses. An accentuated lobular pattern was observed in the livers of were enlarged in 5 males and 5 females in the high dose group. Minimal centrilobular hepatocellular hypertrophy was observed in 2 males and 1 female in the high dose group. Authors reported a NOEL of 100 mg/kg/day based on effects on body weight.

- *Oral*: In a range-finding study that was similar to OECD Guideline 408 but included only limited histopathological evaluations and no neurotoxicity examination, male and female Wistar rats (10/sex/dose) were administered acetyl tributyl citrate (purity not reported) in the diet to yield nominal doses of 0, 100, 300, or 1,000 mg/kg/day (actual doses of 0, 96.02, 287.50, and 961.16 mg/kg/day, respectively) for 90 days. There were no effects on mortality, clinical sign, body weight and weight gain, or ophthalmoscopic examination. At the high dose, bilirubin levels were decreased in both sexes. Aspartate aminotransferase and lactate dehydrogenase activity, chloride and calcium levels, and globulin levels were decreased in males at the mid and high doses. Sodium levels were increased in males at these doses. Liver weights were significantly increased in both sexes at the high dose, and livers were enlarged and showed minimal hepatocellular hypertrophy in some animals. Authors reported a NOAEL of 1,000 mg/kg/day, stating that treatment-related changes were the result of hepatic metabolic adaptation (Klimisch 1, reliable without restrictions).
- *Oral*: In an 8-week study in male and female rats (strain not specified) that was not conducted according to recognized guidelines, animals (4/sex/dose) were administered acetyl tributyl citrate (purity not reported) in the diet at concentrations of 5 or 10% for 8 weeks. There were no effects on differential blood counts, gross pathology, or microscopic pathology of the heart, lungs, gastrointestinal tract, liver, pancreas, spleen, or kidneys. No additional endpoints were evaluated. Authors identified a NOAEL of 10% in the diet (equivalent to 9,600 mg/kg/day[10]) (Klimisch 2, reliable with restrictions).
- *Oral*: In a 6-week study in male and female rats (strain not specified) that was not conducted according to recognized guidelines, animals (4/sex/dose) were administered acetyl tributyl citrate (purity not reported) in the diet at concentrations of 5 or 10% for 6 weeks. Body weights were reduced at the high dose (statistical analyses not performed due to small sample size). Authors noted that this effect could have been the result of diarrhea in the animals. Authors identified a NOEL of 5% and LOEL of 10% (4,800 and 9,600 mg/kg/day) based on effects on body weight (Klimisch 2, reliable with restrictions).
- *Oral*: In an 8-week study in male and female cats that was not conducted according to recognized guidelines, animals (2/sex/dose) were administered acetyl tributyl citrate (purity not reported) via gavage at a dose of 5 mL/kg daily for 8 weeks. There were no effects on urine, red blood cell counts, white blood cell counts, hemoglobin, blood sugar, blood non protein nitrogen, and blood creatinine. Body weights were reduced in treated animals (statistical analyses not performed due to small sample size). Authors reported a LOEL of 5 mL/kg (equivalent to 5,264 mg/kg[11]) (Klimisch 2, reliable with restrictions).

- U.S. EPA 2003, 2014
  - *Oral*: In a previously described GLP-compliant 2-generation reproductive toxicity study (guideline not reported) in Sprague-Dawley rats, animals (30/sex/dose) were administered acetyl tributyl citrate (99.4% purity) in the diet to yield nominal doses of 0, 100, 300, or 1,000 mg/kg/day (actual doses of 103, 306, and 1,013 mg/kg/day for males and 102, 306, and 1,024 mg/kg/day for females). The F0 males were treated for 11 weeks prior to mating through sacrifice and the F0 females were treated for 3 weeks prior to mating, through mating, gestation, and lactation. The F1 males and females were treated for 10 weeks prior to mating through sacrifice (males) or through mating, gestation, and lactation (females). There were no clinical signs of toxicity. Body weights were consistently reduced in F1 males of the mid and high dose groups, but there were no effects in the F0 generation or in

[10] 10% = 100,000 mg/kg food x 0.096 kg food/kg BW/day = 9,600 mg/kg/day (rat average food factor value from http://www.tera.org/Tools/ratmousevalues.pdf)
[11] 1,052.8 kg/m^3 x 1 m^3/10^6 mL = 0.00105 kg/mL x 106 mg/kg = 1,052.8 mg/mL x 5 mL/kg = 5,264 mg/kg

females of either generation. The U.S. EPA identified a NOAEL of 100 mg/kg/day for parental toxicity based on effects on male body weight at the LOAEL of 300 mg/kg/day (Klimisch 1, reliable without restrictions).

- *Oral*: In the subchronic (with *in utero* exposure) portion of the previously described GLP-compliant repeated dose toxicity and reproductive and developmental toxicity study that was conducted according to US EPA OPPTS 870.3100/OECD Method 408/EC Method B26, male and female Han Wistar rats (25/sex/dose) were administered acetyl tributyl citrate (99.9% purity) through the diet to yield nominal doses of 0, 100, 300, or 1,000 mg/kg/day (actual doses of 0, 103, 306, and 1,013 mg/kg/day, respectively, for males and 0, 102, 306, and 1,024 mg/kg/day, respectively, for females) for 4 weeks prior to mating through mating (males) or through gestation and lactation (females). Offspring (20/sex/dose, 10/sex/dose for recovery group) were then selected for a subsequent 13-week study and were fed diets providing the parental doses beginning at weaning on postnatal day 21. There were no clinical signs of toxicity. Body weight gain was slightly reduced (magnitude and statistical significance not specified) at the high dose. Liver weights were increased at this dose, and histopathology revealed hepatic hypertrophy that authors considered to be indicative of enzyme induction. Weak peroxisome proliferation was observed in males at the mid dose and both sexes at the high dose. There were slight variations in urinary composition and plasma electrolyte concentration at the mid and high doses; authors speculated that effects on kidney function were the result of adaptation to excretion of high levels of the test material and metabolites. U.S. EPA noted that results for the kidney were within the range of historical controls, resolved within the recovery period, and were not accompanied by histopathological changes. U.S. EPA reported a NOAEL of 300 mg/kg/day (Klimisch 1, reliable without restrictions).
- *Oral*: In a GLP-compliant subchronic oral toxicity study that was conducted according to OECD Guideline 408 in male and female Sprague-Dawley rats, animals (20/sex/dose) were administered acetyl tributyl citrate (> 98% purity) in the diet to yield nominal doses of 0, 100, 300, or 1,000 mg/kg/day (actual doses of 101, 302, and 996 mg/kg/day, respectively, for males and 100, 296, and 999 mg/kg/day, respectively, for females) for 90 days (doses selected based on results of a prior range finding study demonstrating cytoplasmic eosinophilia in in periportal hepatocytes at a dose of 5,000 mg/kg/day). There were no deaths or clinical signs of toxicity. Body weights were slightly reduced in females at the mid dose and both sexes at the high dose (statistical significance not reported). Urinary pH was decreased in males at the high dose, and crystals were observed in the urine of males at the mid and high doses and females at the high dose. Serum alkaline phosphatase activity was increased in males at the high dose, fasting glucose was decreased in females at the mid and high doses, and alanine aminotransferase activity and bilirubin concentrations were decreased in females at the high dose. Relative kidney weights were increased in males at the high dose. Relative liver weights were increased in males at the mid and high doses and in females at the high dose. There were no histopathological changes observed. The U.S. EPA identified a NOAEL of 300 mg/kg/day and LOAEL of 1,000 mg/kg/day based on changes in body and organ weights (Klimisch 1, reliable without restrictions).

- CPSC 2010, U.S. EPA 2014
  - *Oral*: In a chronic oral study in Sherman rats (sex not specified), animals (20/dose, 40/control) were administered 0, 200, 2,000, or 20,000 ppm (contributing doses of 10, 100, and 1,000 mg/kg/day, respectively) acetyl tributyl citrate (purity not reported) in the diet for 2 years. Body weights were transiently reduced in all dose groups during weeks 5-15, but this effect could not be reproduced in a separate 1 year study of 10 rats/dose administered

200 or 2,000 ppm in the diet. In the main study, 20/60 rats died (compared to 8/40 in the control group), and pulmonary lesions indicated possible infection. There were no clinical signs of toxicity or effects on pathology. The U.S. EPA notes that a NOAEL and LOAEL cannot be identified due to the lack of sufficient study details (Klimisch 2, reliable with restrictions).

- Based on the weight of evidence, a score of Low was assigned. Several subchronic and chronic oral toxicity studies were identified, and reported effects on body weight and clinical chemistry and pathology changes generally consistent with metabolic enzyme induction. These effects were observed at high doses (1,000 mg/kg/day) that greatly exceed guidance values for classification. One 2-year study in rats reported evidence of liver necrosis, which is more consistent with liver damage than with enzyme induction, with a NOAEL of 300 and LOAEL of 100 mg/kg/day. The companion one-year study did not show evidence of necrosis, but reported a decrease in body weight with a NOAEL of 100 mg/kg/day and LOAEL of 300 mg/kg/day. As all effects were observed at doses greater than the GHS guidance value of 100 mg/kg/day for subchronic oral studies (UN 2023), a score of Low was assigned.

Neurotoxicity (single dose, N-single) (Group II) Score (vH, H, M, or L): DG
Acetyl tributyl citrate was assigned a score of Data Gap for neurotoxicity (single dose) based on the lack of adequate data 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.
- ECHA 2023
  - *Oral*: In the previously described acute oral study in Wistar rats (sex not specified), 5 animals were administered a single dose of 31,500 mg/kg acetyl tributyl citrate (purity not reported) via gavage and were observed for 21 days. Shortly after administration, the test substance leaked from the rectum and transient sluggishness was reported. There were no additional clinical signs. No additional details were provided (Klimisch 2, reliable with restrictions).
- Based on the weight of evidence, a Data Gap was assigned. Transient sluggishness was observed in an acute oral study in rats, but as this effect may result from gavage administration of a large volume of the test substance, ToxServices did not consider it to be sufficient evidence of neurological effects. In the absence of additional data, a Data Gap was assigned.

Neurotoxicity (repeated dose, N-repeated) (Group II*) Score (H, M, or L): L
Acetyl tributyl citrate was assigned a score of Low for neurotoxicity (repeated dose) based on a lack of effects on appearance, behavior, motor activity, sensory activity, and autonomic activity at up to 1,000 mg/kg/day in a 90-day oral toxicity study in rats. GreenScreen® criteria classify chemicals as a Low hazard for neurotoxicity (repeated dose) when there is no evidence of neurotoxicity at doses below 100 mg/kg/day in a 90-day study (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- U.S. EPA 2003, 2014
  - *Oral*: In the previously described GLP-compliant subchronic oral toxicity study that was conducted according to OECD Guideline 408 in male and female Sprague-Dawley rats, animals (20/sex/dose) were administered acetyl tributyl citrate (> 98% purity) in the diet to yield nominal doses of 0, 100, 300, or 1,000 mg/kg/day (actual doses of 101, 302, and 996

mg/kg/day, respectively, for males and 100, 296, and 999 mg/kg/day, respectively, for females) for 90 days (doses selected based on results of a prior range finding study demonstrating cytoplasmic eosinophilia in in periportal hepatocytes at a dose of 5,000 mg/kg/day). There were no effects on functional observations of appearance, behavior, motor activity, sensory activity, or autonomic activity. No additional details were provided. ToxServices assigned a NOAEL of 1,000 mg/kg/day based on a lack of effects on neurological endpoints at the highest dose tested (Klimisch 1, reliable without restrictions).

- Based on the weight of evidence, a score of Low was assigned. Only one of the repeated dose toxicity studies reported on neurological effects; the study is a well-conducted guideline study and showed no effects on appearance, behavior, motor activity, sensory activity, and autonomic activity at up to 1,000 mg/kg/day.

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

Acetyl tributyl citrate was assigned a score of Low for skin sensitization based on the lack of dermal sensitization seen in guinea pigs. 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 experimental data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023
  - A non-GLP compliant guinea pig maximization test was conducted following Magnusson and Kligman in male guinea pigs (strain not specified, 10/dose) administered doses of acetyl tributyl citrate (100% purity) at 2.5% intradermally and 100% epicutaneously, with a challenge dose of 50% applied epicutaneously under occlusive conditions. There were no signs of skin sensitization seen. The study authors concluded that acetyl tributyl citrate is not dermally sensitizing under the test conditions (Klimisch 1, reliable without restrictions).

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

Acetyl tributyl citrate was assigned a score of Low for respiratory sensitization based on extrapolation from the lack of dermal sensitization potential according to ECHA guidance (ECHA 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
  - Acetyl tributyl citrate does not contain any structural alerts for respiratory sensitization (Appendix F)
- 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 acetyl tributyl citrate 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 acetyl tributyl citrate, and as acetyl tributyl citrate does not contain any structural alerts for respiratory sensitization (OECD 2023), acetyl tributyl citrate is not expected to be a respiratory sensitizer.

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

Acetyl tributyl citrate was assigned a score of Low for skin irritation/corrosivity based on no irritation effects seen in dermal irritation studies. GreenScreen® criteria classify chemicals as a Low hazard for skin irritation/corrosivity when adequate negative data are available and are not classified per GHS (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023
  - No indication of skin irritation was reported in male albino rabbits (2-3/group) that were administered 1 mL/kg acetyl tributyl citrate to intact abdominal skin daily (occlusion not specified) for four days or 6 days/week for a total of 18 applications, or to abraded skin 6 days/week for a total of 18 applications. No additional details were provided (Klimisch 2, reliable with restrictions).
- ECHA 2011
  - Acetyl tributyl citrate was not irritating to the skin of rabbits in a test conducted according to OECD Guideline 404. No additional details were provided (Klimisch score not reported).
  - One study in guinea pigs showed no evidence of skin irritation, while and older study showed slight edema. No additional details were provided (Klimisch score not reported).

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

Acetyl tributyl citrate was assigned a score of Low for eye irritation/corrosivity based on a very slight, reversible effect seen in rabbits. GreenScreen® criteria classify chemicals as a Low hazard for eye irritation/corrosivity when adequate and negative data and no GHS classification are available (CPA 2018b). The confidence in the score is low as it is based on poorly described ocular irritation studies.

- 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 2023
  - Acetyl tributyl citrate was slightly irritating when 0.1 mL was instilled into the eyes of three male albino rabbits. Eyes were observed after 20 minutes and for 3-72 hours after instillation. Moderate erythema (location not specified) was observed in 2/3 rabbits within 20 minutes and persisted through the 3-hour observation. It subsided in one of two rabbits 5 hours post instillation. At 24 hours, moderate erythema was slightly increased in this rabbit. All effects resolved within 72 hours (Klimisch 2, reliable without restrictions).
- ECHA 2011
  - Acetyl tributyl citrate was not irritating to the eyes of rabbits in a test conducted according to OECD Guideline 405. Older irritation studies show slight to moderate irritation. No additional details were provided (Klimisch score not reported).
- Based on the weight of evidence, a score of Low was assigned. ECHA reports that old studies showed slight to moderate irritation, but no additional details were provided. One study reported slight irritation in rabbits, but few details were reported and effects were described simply as "erythema". Because effects were seen in only two animals, and resolved within 5 hours in one

animal and 72 hours in the second animal, acetyl tributyl citrate would not meet the criteria for irritant effects in 2/3 animals at 24/48/72 hours as specified for GHS classification. In addition, ECHA reports that it was negative in a guideline study. Therefore a score of Low was assigned based on results from the most well-reported study, but confidence is reduced due to the lack of details provided and due to conflicting results in older, poorly reported studies.

Ecotoxicity (Ecotox)

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

Acetyl tributyl citrate was assigned a score of High for acute aquatic toxicity based on a measured EC_{50} value of 7.85 mg/L in daphnia. GreenScreen® criteria classify chemicals as a High hazard for acute aquatic toxicity when the most conservative L/EC_{50} values are between 1 and 10 mg/L (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* GHS – New Zealand – Hazardous to the aquatic environment – Acute category 1
- ECHA 2023
  - 96-hour LC_{50} = 38-60 mg/L (*Lepomis macrochirus*, bluegill sunfish, nominal) (non-GLP compliant, equivalent to OECD Guideline 203) (Klimisch 2, reliable with restrictions)
  - 96-hour LC_{50} = 59 mg/L (*Fundalus heteroclitus*, mummichog, nominal) (GLP-compliant, no guideline followed) (Klimisch 2, reliable with restrictions)
  - 24-hour mobility EC_{50} > 1 mg/L (*Daphnia magna*, water flea, nominal) (GLP compliance not specified, OECD Guideline 202) (Klimisch 2, reliable with restrictions)
  - 48-hour mobility EC_{50} = 7.82 mg/L (*Ceriodaphnia dubia,* measured) (GLP-compliant , EPA OPPTS 850.1010) (Klimisch 1, reliable without restrictions)
  - 72-hour EC_{50} = 74.4 mg/L (growth), 11.5 mg/L (biomass) (mean measured concentration of aqueous phase) (*Desmodesmus subspicatus*, green algae) (GLP-compliant, OECD Guideline 201) (Klimisch 1, reliable without restrictions)

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

Acetyl tributyl citrate was assigned a score of Moderate for chronic aquatic toxicity based on NOEC values of 1.28 mg/L in fish and 4.65 mg/L in algae. GreenScreen® criteria classify chemicals as a Moderate hazard for chronic aquatic toxicity when the most conservative chronic NOEC values are between 1 and 10 mg/L (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* GHS – New Zealand – Hazardous to the aquatic environment – Chronic category 1
- ECHA 2023
  - 7-day NOEC = 1.28 mg/L (*Pimephales promelas,* fathead minnow, larval survival) (GLP-compliant U.S. EPA Method 1000.0) (Klimisch 1, reliable without restrictions)
  - 21-day mobility NOEC > 1.11 mg/L (*Daphnia magna*, water flea, nominal) (non-GLP compliant, EU Method C.20/OECD Guideline 211) (Klimisch 1, reliable without restrictions)
  - 72-hour NOEC = 4.65 mg/L (*Desmodesmus subspicatus*, green algae, growth and biomass) (mean measured concentration of aqueous phase) (GLP-compliant OECD Guideline 201/EU Method C.3) (Klimisch 1, reliable without restrictions)

Environmental Fate (Fate)

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

Acetyl tributyl citrate was assigned a score of Moderate for persistence based on 83% biodegradation occurring within 52 days. GreenScreen® criteria classify chemicals as a Moderate hazard for persistence when the half-life in soil is between 16 and 60 days (CPA 2018b). The confidence in the score is low based on the variability in biodegradation rates between different types of studies.

- 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 2023
  - Acetyl tributyl citrate (purity not reported, 30 mg/L starting concentration) was inherently biodegradable in a test similar to OECD Guideline 302 C (Inherent Biodegradability: Modified MITI Test (II)) using activated sludge inoculum (100 mg/L, adaptation not specified). The substance reached 82% biodegradation in 28 days (Klimisch 2, reliable with restrictions).
  - Acetyl tributyl citrate (purity not reported, 1.642 mg/L starting concentration) was not readily biodegradable in a GLP-compliant test that was conducted according to OECD Guideline 301 D (Ready Biodegradability: Closed Bottle Test), reaching 16% biodegradation in 28 days. Authors noted that the lack of biodegradability may be the result of low water solubility (Klimisch 2, reliable with restrictions).
  - In a biodegradation in soil test that was conducted according to EPA OPPTS 835.3300 (Soil Biodegradation)/ASTM D 5988 acetyl tributyl citrate (≥ 99.0% purity) had half-lives of 8.4, 10.9, 19.9, 25.8, and 57.9 days when tested at concentrations of 0.8, 1.6, 3.2, 6, or 12 mg C/g soil, respectively. Authors noted that decay rates decreased as a result of substrate concentration and that acetyl tributyl citrate is considered to be readily biodegradable at concentrations < 3.2 mg C/g soil (Klimisch 1, reliable without restrictions).
  - In a biodegradation in soil test that was conducted according to ASTM D 5338; ASTM D 5988, acetyl tributyl citrate (≥ 99.0% purity) had a half-life of 3.5 days when tested at a starting concentration of 1.9 mg C/g soil or a half-life of 14.6 days when tested at a starting concentration of 10.8 mg C/g soil (Klimisch 1, reliable without restrictions).
  - In a biodegradation in soil test that was conducted according to ASTM D 5338/ASTM D 5988, acetyl tributyl citrate (≥ 99.0% purity) reached 37% biodegradation in 35 days when tested at a starting concentration of 17.2 mg c/g soil (Klimisch 1, reliable without restrictions).
  - Acetyl tributyl citrate (purity not reported) reached 128, 125, 90, and 83% biodegradation in 52 days when tested at starting concentrations of 40, 80, 160, and 300 mg C/g soil. Authors concluded that the substance is rapidly degradable (Klimisch 2, reliable with restrictions).
- U.S. EPA 2014
  - U.S. EPA reported biodegradation rates of 26% in 21 days, > 90% in 5 hours, 72.9% in 42 days, 37% in 45 days (compost soil), and 82% in 28 days (OECD 301 C). No additional details were provided.
  - Acetyl tributyl citrate is expected to have low persistence.

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

Acetyl tributyl citrate was assigned a score of Very Low for bioaccumulation based on a predicted BCF of 12.52. GreenScreen® criteria classify chemicals as a Very Low hazard for bioaccumulation when the

BCF/BAF values are below 100 (CPA 2018b). The confidence in the score is low as it is based on modeled data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA 2023
  - Acetyl tributyl citrate has a measured log K_{ow} of 4.86 at 40°C obtained from a HPLC method conducted according to EU Method A.8 or OECD Guideline 117 (Klimisch 1, reliable without restrictions).
- U.S. EPA 2017
  - BCFBAF predicts a BCF of 1.5 using the regression based model based on a measured log K_{ow} of 4.92, and a BCF of 12.52 using the Arnot-Gobas model for the upper trophic level, taking metabolism into consideration (Appendix G).
- Based on the weight of evidence, a score of Very Low was assigned. Although the log K_{ow} of 4.92 corresponds to a High score, ToxServices considered the modeled data, which take into consideration both the log K_{ow} and *in vivo* metabolism, to be more relevant to assessing the bioaccumulation potential. The modeled BCF of 12.52 corresponds to a Very Low.

Physical Hazards (Physical)

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

Acetyl tributyl citrate was assigned a score of Low for reactivity based on the lack of structural alerts for oxidizing and explosive properties. GreenScreen® criteria classify chemicals as a Low hazard for reactivity when it does not warrant GHS classification for any of the reactivity sub-endpoints and the chemical is not present on authoritative or screening lists (CPA 2018b). The confidence in the score was low based on the lack of measured data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- No measured data were identified. Therefore, screening procedures for explosivity were used here to estimate the reactivity property of acetyl tributyl citrate. These procedures are listed in the GHS (UN 2023).
  - Based on the structure of its components or moieties, acetyl tributyl citrate is not considered explosive or self-reactive due to lack of functional groups associated with explosive or self-reactive properties (See Appendix H).
  - Based on the structure of its components or moieties, acetyl tributyl citrate is not considered to have oxidizing properties as it does not contain any structural groups known to be correlated with a tendency to react exothermally with combustible materials. Specifically, organic substances which contain oxygen, fluorine, or chlorine where these elements are chemically bonded only to carbon or hydrogen, classification as an oxidizing liquid need not be applied. Therefore, as the molecular structure of acetyl tributyl citrate has 8 oxygens, which are all bonded only to carbon and hydrogen, classification is not warranted.

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

Acetyl tributyl citrate was assigned a score of Low for flammability based on not being classified as a flammable liquid. GreenScreen® criteria classify chemicals as a Low hazard for flammability when there are adequate data available and not classified per GHS as a flammable liquid (CPA 2018b). The confidence in the score was high as it is based on a measured flash point.

- 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 2023
    - Acetyl tributyl citrate has a measured flash point of 217.9°C obtained in a non-GLP compliant closed-cup method conducted according to EU Method A.9 (Klimisch 1, reliable without restriction).
        - According to GHS criteria (UN 2023), the flash point of 217°C is above the GHS Guidance value for Category 4 flammable liquid (93°C). Therefore, acetyl tributyl citrate is not classified as per GHS.

Use of New Approach Methodologies (NAMs)[12] in the Assessment, Including Uncertainty Analyses of Input and Output

New Approach Methodologies (NAMs) used in this GreenScreen® include *in vitro* data for genotoxicity and *in silico* modeling for bioaccumulation. 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 acetyl tributyl citrate's NAMs dataset include a lack of experimental data for endocrine activity, respiratory sensitization, and bioaccumulation, and lack of validated test methods for respiratory sensitization. Acetyl tributyl citrate's Type II (extrapolation output) uncertainties include limitations of *in vitro* genotoxicity data in mimicking *in vivo* metabolism and their focusing on a few events in the genotoxicity process, uncertain *in vivo* relevance of *in silico* receptor binding activity predictions and *in vitro* receptor binding activity assays, and the lack of consideration of non-immunological mechanisms for respiratory sensitization. Some of acetyl tributyl citrate'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	**Endocrine activity:** No experimental data are available. **Respiratory sensitization**: No experimental data are available and there are no validated test methods. **Bioaccumulation:** No experimental BCF data are available.
Type II Uncertainty: Extrapolation Output	**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[13]. The mammalian cell gene mutation assay (as defined in OECD Guideline 476) only detects gene mutations, and the exogenous metabolic activation system does not entirely mirror *in vivo* metabolism (i.e., the liver S9 mix contains enzymes present in the endoplasmic reticulum but not the cytosol of liver cells).[14] The *in*

[12] 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).
[13] https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1614097593&id=id&accname=guest&checksum=89925F80B9F4BD2FFC6E90F94A0EE427
[14] https://www.oecd-ilibrary.org/docserver/9789264264809-en.pdf?expires=1614097800&id=id&accname=guest&checksum=C0DE371FB9C5A878E66C9AB7F84E6BBE

	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[15]. **Endocrine activity**: The ToxCast models do not define applicability domains. The *in vivo* relevance of EDSP Tox 21 screening assays and *in silico* modeling of receptor binding is unknown due to lack of consideration of metabolism and other toxicokinetic factors. EDSP Tox 21 assays do not cover all critical endocrine pathways. **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	N	
Mutagenicity	Y	*In vitro* data: Bacterial reverse mutation assay/*in vitro* gene mutation assay/*in vitro* chromosome aberration assay
Reproductive toxicity	N	
Developmental toxicity	N	
Endocrine activity	Y	*In vitro* high throughput data: EDSP Tox 21 screening assays
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/Danish QSAR
Skin irritation	N	
Eye irritation	N	
Acute aquatic toxicity	N	
Chronic aquatic toxicity	N	
Persistence	N	
Bioaccumulation	Y	*In silico* modeling: EPI Suite™

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

References

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

Consumer Product Safety Commission (CPSC). 2010. Review of exposure and toxicity data for phthalate substitutes. Available: https://www.cpsc.gov/s3fs-public/phthalsub.pdf

European Chemicals Agency (ECHA). 2011. Annex XV Restriction Report. Proposal for a Restriction. Substance Name: Bis(2-Ehtylhexyl)Phthlate (DEHP), Benzyl Butylphthalate (BBP), Dibutyl Phthalate (DBP), Diisobutyl Phthalate (DIBP). 12 August 2011. Available: http://echa.europa.eu/documents/10162/c6781e1e-1128-45c2-bf48-8890876fa719

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). 2023. Tributyl O-acetylcitrate (CAS #77-90-7). Available: https://echa.europa.eu/registration-dossier/-/registered-dossier/13143

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/

Hazardous Substances Data Bank (HSDB). 2015. Acetyl tributyl citrate. United States National Library of Medicine. Available: https://pubchem.ncbi.nlm.nih.gov/source/hsdb/656

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. 2023. Pharos chemical and material library entry for acetyltributyl citrate (CAS #77-90-7). Available: http://www.pharosproject.net/material/

PubChem. 2023. Acetyl tributyl citrate (CAS #77-90-7). United States National Library of Medicine. Available: https://pubchem.ncbi.nlm.nih.gov/

The Swiss Federal Institute of Aquatic Science and Technology (EAWAG). 2014. EAWAG-BBD Pathway Prediction System. Available: http://eawag-bbd.ethz.ch/predict/

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

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

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

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

United States Environmental Protection Agency (U.S. EPA). 2003. Robust summaries of reliable studies and QSAR model data. Acetyl tributyl citrate (Acetyl tributyl citrate). Available: http://toxplanet.com

United States Environmental Protection Agency (U.S. EPA). 2014. Screening-Level hazard characterization. Acetyl tributyl citrate (CASRN 77-90-7). Available: https://nepis.epa.gov/Exe/ZyPDF.cgi/P100ZPVM.PDF?Dockey=P100ZPVM.PDF

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

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

United States Environmental Protection Agency (U.S. EPA). 2023b. CompTox Dashboard. Available: https://comptox.epa.gov/dashboard

United States Food and Drug Administration (U.S. FDA). 2023. Tributyl acetylcitrate (CAS # 77-90-7). Substances Added to Food (formerly EAFUS). Available:

https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=FoodSubstances&id=TRIBUTYLACETYLCITRATE

Vertellus. 2009. Safety data sheet for Citroflex® A-4. Revision Date: December 3, 2009. Available: https://www.b2bcomposites.com/msds/ted/78549.pdf

APPENDIX A: Hazard Classification Acronyms
(in alphabetical order)

(AA) Acute Aquatic Toxicity

(AT) Acute Mammalian Toxicity

(B) Bioaccumulation

(C) Carcinogenicity

(CA) Chronic Aquatic Toxicity

(D) Developmental Toxicity

(E) Endocrine Activity

(F) Flammability

(IrE) Eye Irritation/Corrosivity

(IrS) Skin Irritation/Corrosivity

(M) Mutagenicity and Genotoxicity

(N) Neurotoxicity

(P) Persistence

(R) Reproductive Toxicity

(Rx) Reactivity

(SnS) Sensitization- Skin

(SnR) Sensitization- Respiratory

(ST) Systemic/Organ Toxicity

APPENDIX B: Results of Automated GreenScreen® Score Calculation for Acetyl Tributyl Citrate (CAS #77-90-7)

			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	Acetyl tributyl citrate	77-90-7	L	L	*L*	L	DG	L	L	L	DG	L	L	*L*	L	*L*	H	M	*M*	*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	Yes	Yes	No	No			
4	STOP						

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
Acetyl tributyl citrate	3

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

Table 6

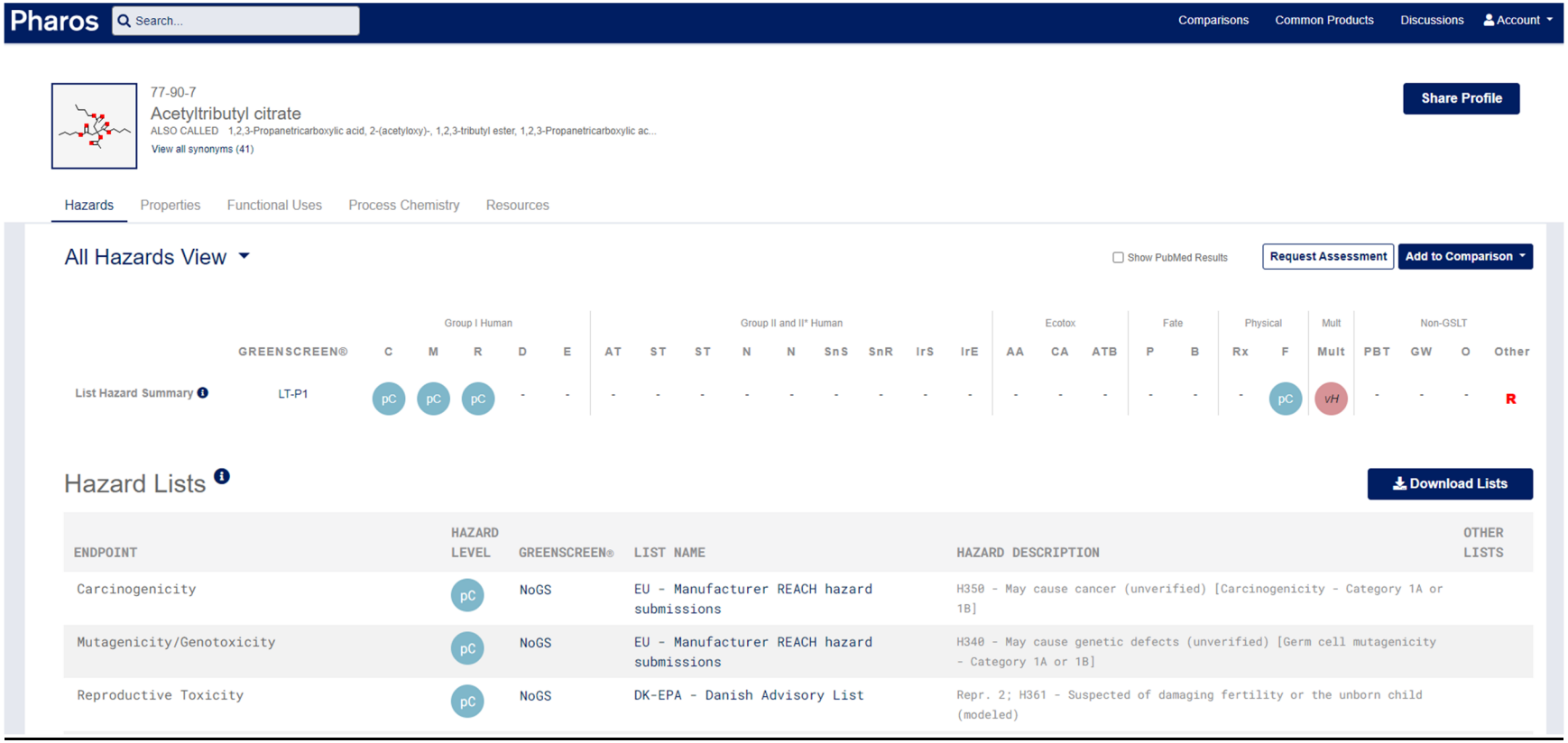
Chemical Name	Final GreenScreen® Benchmark Score
Acetyl tributyl citrate	3

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 Acetyl Tributyl Citrate (CAS #77-90-7)



Flammability	pC	NoGS	EU - Manufacturer REACH hazard submissions	H220 - Extremely flammable gas (unverified) [Flammable gases - Category 1]	
T & P and/or B [(Chronic Aquatic Toxicity and Persistence) or (Acute Aquatic Toxicity and Persistence and/or Bioaccumulation)]	vH	LT-UNK	GHS - New Zealand	Hazardous to the aquatic environment - acute category 1	+2
	vH	LT-P1	GHS - New Zealand	Hazardous to the aquatic environment - chronic category 1	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H412 - Harmful to aquatic life with long lasting effects (unverified) [Hazardous to the aquatic environment (chronic) - Category 3]	
Human and/or Aquatic toxicity and/or Persistence and/or Bioaccumulation	U	LT-P1	German FEA - Substances Hazardous to Waters	Class 2 - Hazard to Waters	

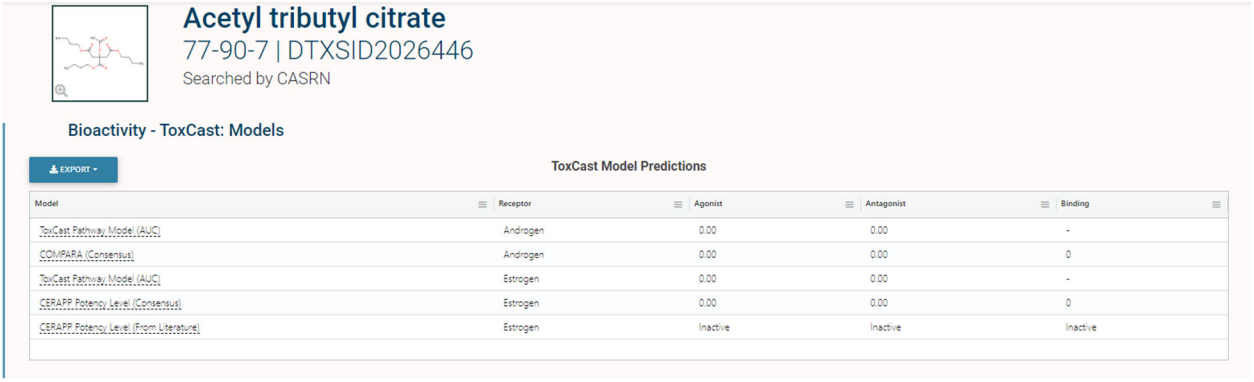
Restricted Substance Lists (4)

- EU - PACT-RMOA Substances: Substances selected for RMOA or hazard assessment
- Food Contact Chemicals Database (FCCdb): Food Contact Chemicals Database Version 5.0
- GSPI - Six Classes Precautionary List: Some Solvents
- TSCA Chemical Substance Inventory (Active-Inactive): TSCA Chemical Substance Inventory - Active

Positive Lists (4)

- Cosmetic Ingredient Review (CIR): Safe as Used
- GB 9685 National Food Safety Standard (2016): GB 9685 National Food Safety Standard (2016)
- Inventory of Existing Cosmetic Ingredients in China (IECIC 2021): Cosmetic Ingredients
- TCO Certified potential candidate list (tentative - awaiting assessment): TCO Certified potential candidate list (tentative - awaiting assessment)

APPENDIX D: ToxCast Endocrine Modeling Results for Acetyl Tributyl Citrate (CAS #77-90-7)



Acetyl tributyl citrate
77-90-7 | DTXSID2026446
Searched by CASRN

Bioactivity - ToxCast: Models

EXPORT

ToxCast Model Predictions

Model	Receptor	Agonist	Antagonist	Binding
ToxCast Pathway Model (AUC)	Androgen	0.00	0.00	-
COMPARA (Consensus)	Androgen	0.00	0.00	0
ToxCast Pathway Model (AUC)	Estrogen	0.00	0.00	-
CERAPP Potency Level (Consensus)	Estrogen	0.00	0.00	0
CERAPP Potency Level (From Literature)	Estrogen	Inactive	Inactive	Inactive

APPENDIX E: U.S. EPA Endocrine Disruptor Screening Program in the 21st Century (EDSP21) Results for Acetyl Tributyl Citrate (CAS #77-90-7)

Name	Description	Endpoint Name	Active	Details	Rep. Plot	All Plots	Gene	Intended Target	Cell Line	Cell Format
(1) EDSP ER										
EDSP ER	Estrogen receptor assays...	OT_ER_ERbERb_0480	Inactive				ESR2	steroidal	kidney	cell line
EDSP ER	Estrogen receptor assays...	OT_ER_ERbERb_1440	Inactive				ESR2	steroidal	kidney	cell line
EDSP ER	Estrogen receptor assays...	OT_ER_ERaERa_1440	Inactive				ESR1	steroidal	kidney	cell line
EDSP ER	Estrogen receptor assays...	ACEA_ER_AUC_viability	Inactive				-	cytotoxicity	breast	cell line
EDSP ER	Estrogen receptor assays...	TOX21_ERa_LUC_VM7_Antagonist_0.5...	Inactive				-	cytotoxicity	ovary	cell line
EDSP ER	Estrogen receptor assays...	TOX21_ERa_LUC_VM7_Antagonist_0.5...	Inactive				ESR1	steroidal	ovary	cell line
EDSP ER	Estrogen receptor assays...	ATG_ERa_TRANS_up	Inactive				ESR1	steroidal	liver	cell line
EDSP ER	Estrogen receptor assays...	OT_ER_ERaERb_0480	Inactive				ESR2	steroidal	kidney	cell line
EDSP ER	Estrogen receptor assays...	TOX21_ERa_BLA_Antagonist_viability	Inactive				-	cytotoxicity	kidney	cell line
EDSP ER	Estrogen receptor assays...	OT_ER_ERaERb_1440	Inactive				ESR2	steroidal	kidney	cell line
EDSP ER	Estrogen receptor assays...	ATG_ERE_CIS_up	Inactive				ESR1	steroidal	liver	cell line
EDSP ER	Estrogen receptor assays...	OT_ERa_EREGFP_0480	Inactive				ESR1	steroidal	cervix	cell line
EDSP ER	Estrogen receptor assays...	OT_ER_ERaERa_0480	Inactive				ESR1	steroidal	kidney	cell line
EDSP ER	Estrogen receptor assays...	OT_ERa_EREGFP_0120	Inactive				ESR1	steroidal	cervix	cell line

Rows: 18 of 920 Total Rows: 920 Filtered: 18

Name	Description	Endpoint Name	Active	Details	Rep. Plot	All Plots	Gene	Intended Target	Cell Line	Cell Format
(1) EDSP AR										
EDSP AR	Androgen receptor assa...	UPITT_HCI_U2OS_AR_TIF2_Nucleoli_A...	Inactive				AR	steroidal	bone	cell line
EDSP AR	Androgen receptor assa...	TOX21_AR_LUC_MDAKB2_Antagonist_...	Active				AR	steroidal	breast	cell line
EDSP AR	Androgen receptor assa...	OT_AR_ARSRC1_0960	Inactive				AR	steroidal	kidney	cell line
EDSP AR	Androgen receptor assa...	TOX21_AR_BLA_Antagonist_viability	Inactive				-	cytotoxicity	kidney	cell line
EDSP AR	Androgen receptor assa...	ATG_AR_TRANS_up	Inactive				AR	steroidal	liver	cell line
EDSP AR	Androgen receptor assa...	TOX21_AR_BLA_Agonist_ratio	Inactive				AR	steroidal	kidney	cell line
EDSP AR	Androgen receptor assa...	TOX21_AR_LUC_MDAKB2_Agonist	Inactive				AR	steroidal	breast	cell line
EDSP AR	Androgen receptor assa...	TOX21_AR_LUC_MDAKB2_Antagonist_...	Inactive				-	cytotoxicity	breast	cell line
EDSP AR	Androgen receptor assa...	TOX21_AR_BLA_Antagonist_ratio	Inactive				AR	steroidal	kidney	cell line
EDSP AR	Androgen receptor assa...	OT_AR_ARSRC1_0480	Inactive				AR	steroidal	kidney	cell line
EDSP AR	Androgen receptor assa...	UPITT_HCI_U2OS_AR_TIF2_Nucleoli_A...	Inactive				AR	steroidal	bone	cell line
EDSP AR	Androgen receptor assa...	TOX21_AR_LUC_MDAKB2_Antagonist_...	Inactive				-	cytotoxicity	breast	cell line
EDSP AR	Androgen receptor assa...	TOX21_AR_LUC_MDAKB2_Antagonist_...	Inactive				AR	steroidal	breast	cell line
EDSP AR	Androgen receptor assa...	OT_AR_ARELUC_AG_1440	Inactive				AR	steroidal	ovary	cell line

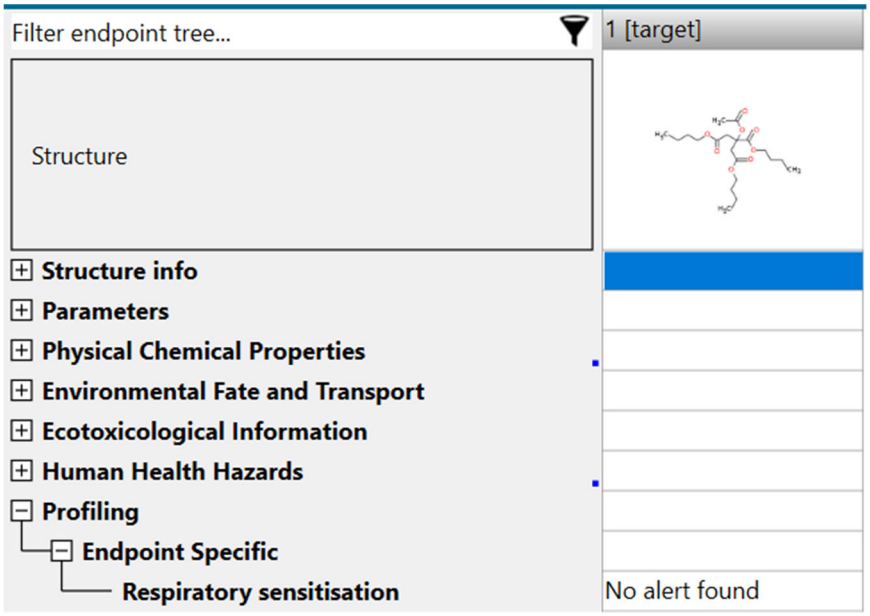
Rows: 14 of 920 Total Rows: 920 Filtered: 14

Name	Description	Endpoint Name	Active	Details	Rep. Plot	All Plots	Gene	Intended Target	Cell Line	Cell Format
(1) EDSP steroidogenesis										
EDSP steroidogenesis	Steroidogenesis pathway...	TOX21_Aromatase_Inhibition_viability	Inactive				-	cytotoxicity	breast	cell line
EDSP steroidogenesis	Steroidogenesis pathway...	TOX21_Aromatase_Inhibition	Inactive				CYP19A1	steroidogenesis-rela	breast	cell line

Name	Description	Endpoint Name	Active	Details	Rep. Plot	All Plots	Gene	Intended Target	Cell Line	Cell Format
(1) EDSP thyroid										
EDSP thyroid	Thyroid pathway assays ...	NVS_GPCR_rTRH	Inactive				Trhr	rhodopsin-like recep	brain	tissue-base...
EDSP thyroid	Thyroid pathway assays ...	CPHEA_Stoker_NIS_Inhibition_RAIU	Inactive				SLC5A5	sodium-iodide symp	kidney	cell line
EDSP thyroid	Thyroid pathway assays ...	LTEA_HepaRG_THRSP_up	Inactive				THRSP	NR mediated metab	liver	cell line
EDSP thyroid	Thyroid pathway assays ...	TOX21_TR_LUC_GH3_Antagonist	Active				THRB	non-steroidal	pituitary gl...	cell line
EDSP thyroid	Thyroid pathway assays ...	TOX21_TRHR_HEK293_Antagonist	Inactive				TRHR	thyrotropin-releasing	kidney	cell line
EDSP thyroid	Thyroid pathway assays ...	LTEA_HepaRG_THRSP_dn	Inactive				THRSP	NR mediated metab	liver	cell line
EDSP thyroid	Thyroid pathway assays ...	TOX21_TSHR_HTRF_Agonist_ratio	Inactive				TSHR	thyrotropin-releasing	kidney	cell line
EDSP thyroid	Thyroid pathway assays ...	TOX21_TSHR_HTRF_wt_ratio	Inactive				TSHR	thyrotropin-releasing	kidney	cell line
EDSP thyroid	Thyroid pathway assays ...	ATG_THRa1_TRANS_up	Inactive				THRA	non-steroidal	liver	cell line
EDSP thyroid	Thyroid pathway assays ...	TOX21_TR_LUC_GH3_Agonist	Inactive				THRB	non-steroidal	pituitary gl...	cell line
EDSP thyroid	Thyroid pathway assays ...	TOX21_TR_LUC_GH3_Antagonist_viabil...	Inactive				-	cytotoxicity	pituitary gl...	cell line
EDSP thyroid	Thyroid pathway assays ...	TOX21_TSHR_HTRF_Antagonist_ratio	Active				TSHR	thyrotropin-releasing	kidney	cell line
EDSP thyroid	Thyroid pathway assays ...	ATG_THRa1_TRANS_dn	Inactive				THRA	non-steroidal	liver	cell line
EDSP thyroid	Thyroid pathway assays ...	CPHEA_Stoker_NIS_Cytotoxicity	Inactive				-	cytotoxicity	kidney	cell line

Rows: 15 of 920 Total Rows: 920 Filtered: 15

APPENDIX F: OECD Toolbox Respiratory Sensitization Results for Acetyl Tributyl Citrate (CAS #77-90-7)



APPENDIX G: EPI Suite™ Modeling Results for Acetyl Tributyl Citrate (CAS #77-90-7)

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

CAS Number: 000077-90-7
SMILES : O=C(OC(C(=O)OCCCC)(CC(=O)OCCCC)CC(=O)OCCCC)C
CHEM : ACETYL TRIBUTYL CITRATE
MOL FOR: C20 H34 O8
MOL WT : 402.49
---------- EPI SUMMARY (v4.11) ----------
Physical Property Inputs:
Log Kow (octanol-water): 4.92
Boiling Point (deg C) : 331.00
Melting Point (deg C) : -80.00
Vapor Pressure (mm Hg) : 1.52E-005
Water Solubility (mg/L): 5
Henry LC (atm-m3/mole) : ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.69 estimate) = 4.29
Log Kow (Exper. database match) = 4.92
Exper. Ref: EPA HPV Robust Summary

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPVP v1.43):
Boiling Pt (deg C): 410.75 (Adapted Stein & Brown method)
Melting Pt (deg C): 94.35 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 0.000371 (Modified Grain method)
VP (Pa, 25 deg C) : 0.0494 (Modified Grain method)
MP (exp database): -80 deg C
BP (exp database): 172 @ 1 mm Hg deg C
VP (exp database): 1.52E-05 mm Hg (2.03E-003 Pa) at 25 deg C

Water Solubility Estimate from Log Kow (WSKOW v1.42):
Water Solubility at 25 deg C (mg/L): 2.045
log Kow used: 4.92 (user entered)
melt pt used: -80.00 deg C
Water Sol (Exper. database match) = 5 mg/L (deg C)
Exper. Ref: CHEMICALS INSPECTION AND TESTING INSTITU (1992)

Water Sol Estimate from Fragments:
Wat Sol (v1.01 est) = 2.4396 mg/L

ECOSAR Class Program (ECOSAR v1.11):
Class(es) found:
Esters

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:
Bond Method : 3.78E-010 atm-m3/mole (3.83E-005 Pa-m3/mole)
Group Method: Incomplete

Exper Database: 1.27E-06 atm-m3/mole (1.29E-001 Pa-m3/mole)
For Henry LC Comparison Purposes:
User-Entered Henry LC: not entered
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:
HLC: 1.610E-006 atm-m3/mole (1.631E-001 Pa-m3/mole)
VP: 1.52E-005 mm Hg (source: User-Entered)
WS: 5 mg/L (source: User-Entered)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:
Log Kow used: 4.92 (user entered)
Log Kaw used: -4.285 (exp database)
Log Koa (KOAWIN v1.10 estimate): 9.205
Log Koa (experimental database): None

Probability of Rapid Biodegradation (BIOWIN v4.10):
Biowin1 (Linear Model) : 1.3940
Biowin2 (Non-Linear Model) : 1.0000
Expert Survey Biodegradation Results:
Biowin3 (Ultimate Survey Model): 3.5535 (days-weeks)
Biowin4 (Primary Survey Model) : 4.8367 (hours)
MITI Biodegradation Probability:
Biowin5 (MITI Linear Model) : 1.1677
Biowin6 (MITI Non-Linear Model): 0.9591
Anaerobic Biodegradation Probability:
Biowin7 (Anaerobic Linear Model): 0.2037
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): 0.00203 Pa (1.52E-005 mm Hg)
Log Koa (Koawin est): 9.205
Kp (particle/gas partition coef. (m3/ug)):
Mackay model : 0.00148
Octanol/air (Koa) model: 0.000394
Fraction sorbed to airborne particulates (phi):
Junge-Pankow model : 0.0508
Mackay model : 0.106
Octanol/air (Koa) model: 0.0305

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 14.4562 E-12 cm3/molecule-sec
Half-Life = 0.740 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 8.879 Hrs
Ozone Reaction:
No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi):

0.0783 (Junge-Pankow, Mackay avg)
0.0305 (Koa method)
Note: the sorbed fraction may be resistant to atmospheric oxidation

Soil Adsorption Coefficient (KOCWIN v2.00):
Koc : 8.765E+004 L/kg (MCI method)
Log Koc: 4.943 (MCI method)
Koc : 3276 L/kg (Kow method)
Log Koc: 3.515 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:
Total Kb for pH > 8 at 25 deg C : 5.755E-002 L/mol-sec
Kb Half-Life at pH 8: 139.394 days
Kb Half-Life at pH 7: 3.816 years
(Total Kb applies only to esters, carbmates, alkyl halides)

Bioaccumulation Estimates (BCFBAF v3.01):
Log BCF from regression-based method = 1.539 (BCF = 34.59 L/kg wet-wt)
Log Biotransformation Half-life (HL) = -3.0488 days (HL = 0.0008938 days)
Log BCF Arnot-Gobas method (upper trophic) = 1.098 (BCF = 12.52)
Log BAF Arnot-Gobas method (upper trophic) = 1.098 (BAF = 12.52)
log Kow used: 4.92 (user entered)

Volatilization from Water:
Henry LC: 1.27E-006 atm-m3/mole (Henry experimental database)
Half-Life from Model River: 926.9 hours (38.62 days)
Half-Life from Model Lake : 1.028E+004 hours (428.3 days)

Removal In Wastewater Treatment:
Total removal: 75.01 percent
Total biodegradation: 0.66 percent
Total sludge adsorption: 74.34 percent
Total to Air: 0.01 percent
(using 10000 hr Bio P,A,S)

Level III Fugacity Model: (MCI Method)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	1.63	17.8	1000
Water	17.9	208	1000
Soil	55.1	416	1000
Sediment	25.4	1.87e+003	0

Persistence Time: 386 hr

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

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	1.63	17.8	1000
Water	17.9	208	1000

water (15.8)
biota (0.0656)
suspended sediment (2.07)
Soil 55.1 416 1000
Sediment 25.4 1.87e+003 0
Persistence Time: 386 hr

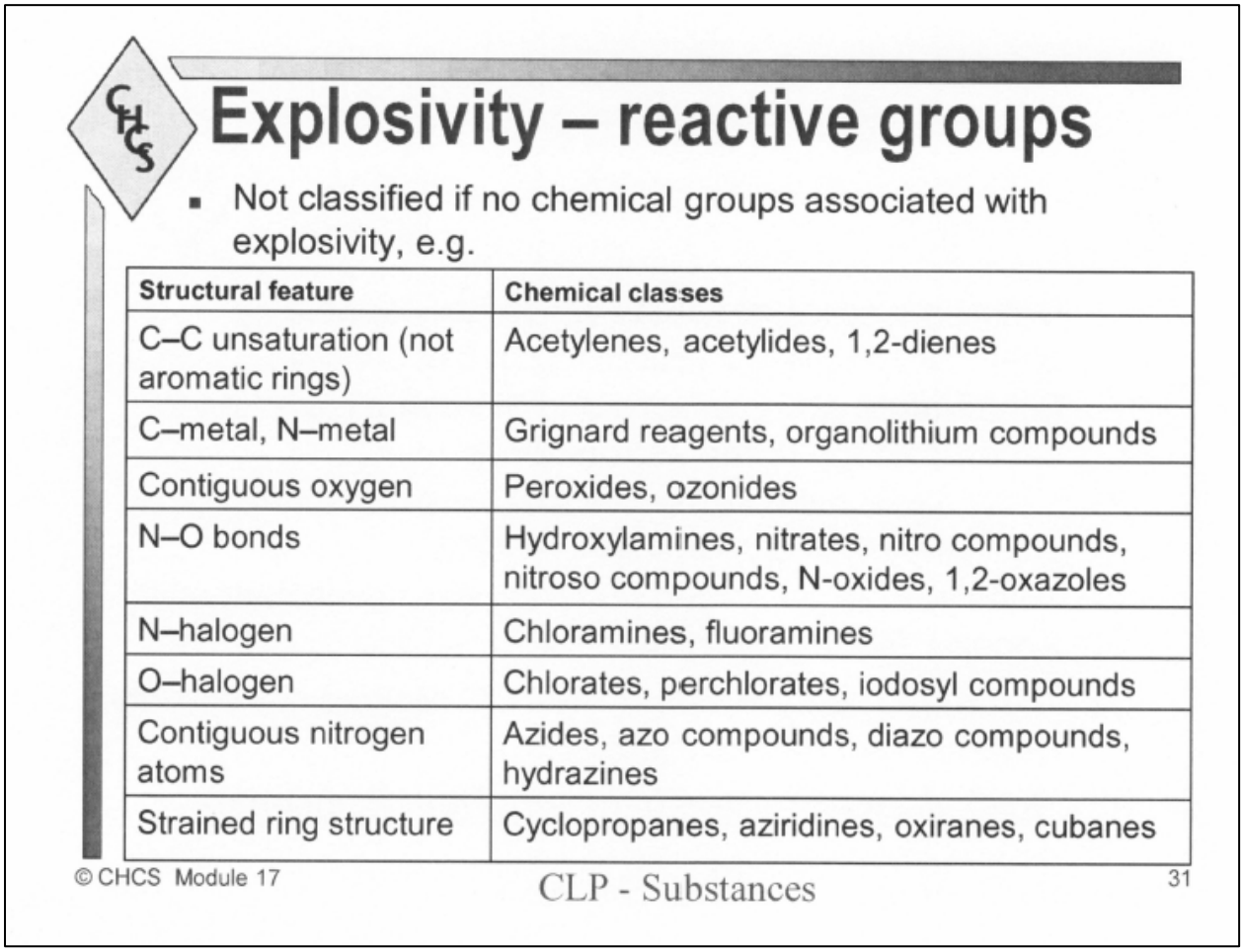
Level III Fugacity Model: (EQC Default)

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	1.86	17.8	1000
Water	21.9	208	1000
water	(20.8)		
biota	(0.0865)		
suspended sediment	(1.06)		
Soil	63.1	416	1000
Sediment	13.1	1.87e+003	0

Persistence Time: 337 hr

APPENDIX H: Known Structural Alerts for Reactivity

Explosivity – Abbreviated List



Explosivity – reactive groups

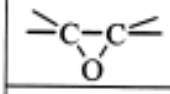
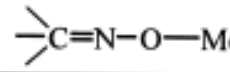
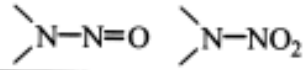
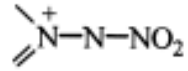
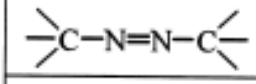
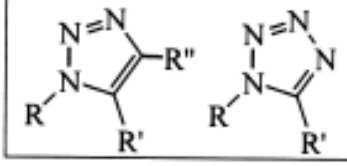
- Not classified if no chemical groups associated with explosivity, e.g.

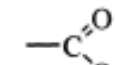
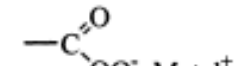
Structural feature	Chemical classes
C–C unsaturation (not aromatic rings)	Acetylenes, acetylides, 1,2-dienes
C–metal, N–metal	Grignard reagents, organolithium compounds
Contiguous oxygen	Peroxides, ozonides
N–O bonds	Hydroxylamines, nitrates, nitro compounds, nitroso compounds, N-oxides, 1,2-oxazoles
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

© CHCS Module 17 CLP - Substances 31

Explosivity – Full List

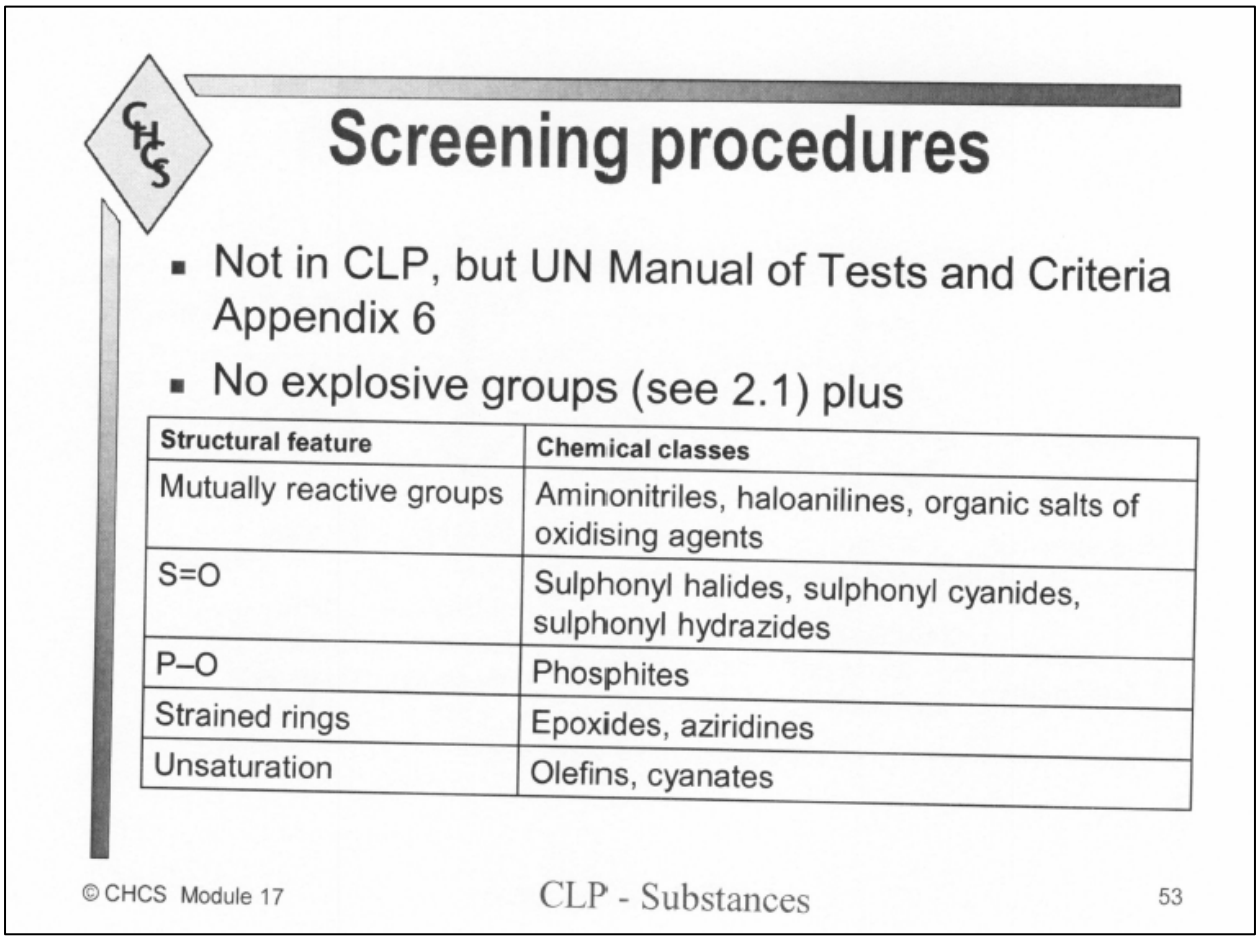
Table R.7.1-28 Chemical groups associated with explosive properties

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 I: Change in Benchmark Score

Table 5 provides a summary of changes to the GreenScreen® Benchmark™ for acetyl tributyl citrate. The GreenScreen® Benchmark Score for acetyl tributyl citrate has not changed over time. The original GreenScreen® assessment was performed in 2015 under version 1.2 criteria and ToxServices assigned a Benchmark 3 (BM-3) score. The BM-3 score was maintained with version 1.3 and 1.4 updates in 2016, 2018, and 2023.

Table 5: Change in GreenScreen® Benchmark™ for Acetyl Tributyl Citrate

Date	GreenScreen® Benchmark™	GreenScreen® Version	Comment
November 17, 2015	BM-3	v.1.2	New assessment.
November 15, 2016	BM-3	v.1.3	No change in BM score. The GreenScreen® assessment was updated with a v.1.3 template.
August 22, 2018	BM-3	v.1.4	No change in BM score. The GreenScreen® assessment was updated with a v.1.4 template.
November 20, 2023	BM-3	v.1.4	No change in BM score. The GreenScreen® assessment was updated with a current v.1.4 template.

Licensed GreenScreen® Profilers

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