CETRIMONIUM CHLORIDE

(CAS #112-02-7)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: February 24, 2025[1]

Expiration Date: February 24, 2030

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[1] The report was last updated on February 24, 2025. However, the last complete literature search was conducted on November 18, 2024.

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GreenScreen® Executive Summary for Cetrimonium Chloride (CAS #112-02-7)

Cetrimonium chloride has several uses in industry, including as a preservative, softener, processing aid, and a surfactant. In personal care products, it functions as an antimicrobial, antistatic, surfactant – emulsifying, and preservative. Cetrimonium chloride is a solid under standard temperature and pressure. It has a low measured vapor pressure, indicating that it is not expected to exist in the vapor phase. It is moderately soluble in water.

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

- Benchmark 2f
    - Very High Group II Human Toxicity (skin irritation-IrS and eye irritation-IrE)
    - Very High Ecotoxicity (acute aquatic-AA and chronic aquatic-CA)

Data gaps (DG) exist for reproductive toxicity-R, and endocrine activity-E. The lack of data for single dose neurotoxicity is not counted as a data gap as sufficient data are available for repeated dose neurotoxicity. As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), cetrimonium chloride meets requirements for a GreenScreen Benchmark™ Score of 2 despite the hazard data gaps. In a worst-case scenario, if cetrimonium chloride were assigned a High score for the data gaps R or E, it would be categorized as a Benchmark 1 Chemical.

The GreenScreen® Benchmark Score for cetrimonium chloride has not changed over time. The original GreenScreen® assessment was performed in 2019 under version 1.4 criteria and ToxServices assigned a Benchmark 2 (BM-2) score. The BM-2 score was maintained with a version 1.4 update in 2024, with minor updates in 2025.

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

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

Type I (input data) uncertainties in cetrimonium chloride's NAMs dataset include insufficient data for carcinogenicity and respiratory sensitization, and the lack of validated test methods for respiratory sensitization. Cetrimonium chloride's Type II (extrapolation output) uncertainties include lack of defined applicability domains by Toxtree and OECD Toolbox structural alerts, the inability of VEGA models to evaluate ionic substances such as the target chemical, the inability of OncoLogic to evaluate the structural features of the target chemical, the limitations of *in vitro* genotoxicity assays to truly mimic *in vivo* metabolism and their focus on only individual aspects of the genotoxicity process, and the lack of consideration of non-immunological mechanisms of respiratory sensitization by the examination of structural alerts only. Some of cetrimonium chloride's type II uncertainties were alleviated by the use of *in vitro* test batteries and/or in combination of *in vivo* data.

GreenScreen® Hazard Summary for Cetrimonium Chloride

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**	DG	**L**	DG	**M**	*M*	**L**		*L*	**L**	*L*	**vH**	**vH**	**vH**	**vH**	**vL**	**vL**	*L*	**L**

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

GreenScreen® Chemical Assessment for Cetrimonium Chloride (CAS #112-02-7)

Method Version: GreenScreen® Version 1.4
Assessment Type[2]: Certified
Assessor Type: Licensed GreenScreen® Profiler

GreenScreen® Assessment (v.1.4) Prepared By:
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Date: June 14, 2019

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Date: June 17, 2019

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Name: Megan B. Boylan, M.S.
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Date: November 13, 2024; February 19, 2025

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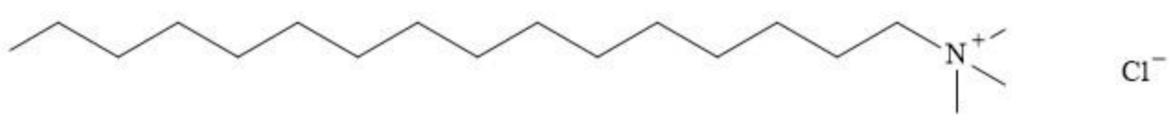
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: November 18, 2024; February 24, 2025

Expiration Date: February 24, 2030[3]

Chemical Name: Cetrimonium chloride

CAS Number: 112-02-7

Chemical Structure(s):



Also called: 1-Hexadecyltrimethylammonium chloride; Cetavlon; Cetrimide; Cetriminium; Cetrimonium; Cetyltrimethylammonium chloride (PubChem 2024).

Suitable surrogates or moieties of chemicals used in this assessment (CAS #'s):
Limited data were identified for centrimonium chloride, a C16 quaternary ammonium compound. ToxServices utilized data for similar quaternary ammonium compounds, alkyltrimethyl chlorides, namely quaternary ammonium compounds, C12-18 (even numbered) alkyltrimethyl chlorides (CAS #68391-03-7), quaternary ammonium compounds and (C16-18 and C18-unsatd. alkyl)trimethyl, chlorides (CAS #68002-61-9), and tetramethylstearylammonium chloride (CAS #112-03-8), which were presented as analogs in the REACH dossier for cetrimonium chloride. These chemicals share a high degree of structural similarity and ToxServices expects that they will have similar toxicological properties.

[2] GreenScreen® reports are either "UNACCREDITED" (by unaccredited person), "AUTHORIZED" (by Authorized GreenScreen® Practitioner), or "CERTIFIED" (by Licensed GreenScreen® Profiler or equivalent).
[3] 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).

Identify Applications/Functional Uses (PubChem 2024, EC 2024)**:**
1. Preservative
2. Softener
3. Processing aid
4. Surfactant
5. Antimicrobial
6. Antistatic

Known Impurities[4]:
No information is available. The screen is performed on the theoretical pure substance.

GreenScreen® Summary Rating for Cetrimonium Chloride[5,6 7,8]: Cetrimonium chloride was assigned a **GreenScreen Benchmark™ Score of 2** ("Use but Search for Safer Substitutes") (CPA 2018b). This score is based on the following hazard score combinations:

- Benchmark 2f
    - Very High Group II Human Toxicity (skin irritation-IrS and eye irritation-IrE)
    - Very High Ecotoxicity (acute aquatic-AA and chronic aquatic-CA)

Data gaps (DG) exist for reproductive toxicity-R, and endocrine activity-E. The lack of data for single dose neurotoxicity is not counted as a data gap as sufficient data are available for repeated dose neurotoxicity. As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), cetrimonium chloride meets requirements for a GreenScreen Benchmark™ Score of 2 despite the hazard data gaps. In a worst-case scenario, if cetrimonium chloride were assigned a High score for the data gaps R or E, it would be categorized as a Benchmark 1 Chemical.

Figure 1: GreenScreen® Hazard Summary for Cetrimonium Chloride

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**	DG	**L**	DG	**M**	*M*	**L**		*L*	**L**	*L*	**vH**	**vH**	**vH**	**vH**	**vL**	**vL**	*L*	**L**

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

[4] Impurities of the chemical will be assessed at the product level instead of in this GreenScreen®.
[5] 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.
[6] See Appendix A for a glossary of hazard endpoint acronyms.
[7] For inorganic chemicals only, see GreenScreen® Guidance v1.4 Section 12 (Inorganic Chemical Assessment Procedure).
[8] 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.

Environmental Transformation Products

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

Introduction

Cetrimonium chloride has several uses in industry, including as a preservative, softener, processing aid, and a surfactant (PubChem 2024). In personal care products, it functions as an antimicrobial, antistatic, surfactant – emulsifying, and preservative (EC 2024). It is manufactured by the reaction of n-hexadecylamine with methyl chloride in the presence of sodium hydroxide or via the reaction of n-hexadecylchloride with trimethylamine (PubChem 2024).

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

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

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

Cetrimonium chloride is not listed on the U.S. EPA SCIL.

GreenScreen® List Translator Screening Results

The GreenScreen® List Translator identifies specific authoritative or screening lists that should be searched to identify GreenScreen Benchmark™ 1 chemicals (CPA 2018b). Pharos (Pharos 2024) is an online list-searching tool that is used to screen chemicals against all of the lists in the List Translator electronically. ToxServices also checks the U.S. Department of Transportation (U.S. DOT) lists (U.S. DOT 2008a,b),[9] which are not considered GreenScreen® Specified Lists but are additional information sources, in conjunction with the Pharos query. The output indicates benchmark or possible benchmark scores for each human health and environmental endpoint. The output for cetrimonium chloride can be found in Appendix C.

- Cetrimonium chloride is an LT-P1 chemical when screened using Pharos, and therefore a full GreenScreen® is required.
- Cetrimonium chloride is not listed on the U.S. DOT list.
- Cetrimonium chloride is on the following lists for multiple endpoints.
    - EC – CEPA DSL: Inherently Toxic in the Environment
    - German FEA – Substances Hazardous to Waters: Class 2 – Hazard to Waters
- Specified lists for single endpoints are reported in individual hazard endpoints in the hazard assessment section below.

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

Hazard Statement and Occupational Control

No harmonized Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard statements were identified for cetrimonium chloride. The self-classifications by its ECHA CHEM dossier authors are presented 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 Cetrimonium Chloride (CAS #112-02-7) (ECHA CHEM, CAS #112-02-7, 2024)	
H Statement	**H Statement Details**
H302	Harmful if swallowed
H314	Causes severe skin burns and eye damage
H410	Very toxic to aquatic life with long lasting effects

Table 2: Occupational Exposure Limits and Recommended Personal Protective Equipment for Cetrimonium Chloride (CAS #112-02-7)			
Personal Protective Equipment (PPE)	**Reference**	**Occupational Exposure Limits (OEL)**	**Reference**
Goggles/face shield, gloves Face shield/safety glasses, gloves, protective clothing, full-face particle respirator (where appropriate)	HSDB 2003	N/A	N/A

Physicochemical Properties of Cetrimonium Chloride

Cetrimonium chloride is a solid under standard temperature and pressure. It has a low measured vapor pressure, indicating that it is not expected to exist in the vapor phase. It is moderately soluble in water, and its measured log K_{ow} of 3.08 indicates that it is not likely to bioaccumulate in aquatic biota.

Table 3: Physical and Chemical Properties of Cetrimonium Chloride (CAS #112-02-7)		
Property	**Value**	**Reference**
Molecular formula	$C_{19}H_{42}ClN$	PubChem 2024
SMILES Notation	CCCCCCCCCCCCCCCC[N+](C)(C)C.[Cl-]	PubChem 2024
Molecular weight	320.0 g/mol	PubChem 2024
Physical state	Solid	ECHA, CAS #112-02-7, 2024
Appearance	White powder	ECHA, CAS #112-02-7, 2024
Melting point	99°C (OECD Guideline 102)	ECHA, CAS #112-02-7, 2024
Boiling point	248°C (OECD Guideline 103)	ECHA, CAS #112-02-7, 2024
Vapor pressure	<0.002 Pa at 20°C (OECD Guideline 104)	ECHA, CAS #112-02-7, 2024
Water solubility	240 mg/L at 25°C (DIN 53914)	ECHA, CAS #112-02-7, 2024

Table 3: Physical and Chemical Properties of Cetrimonium Chloride (CAS #112-02-7)		
Property	**Value**	**Reference**
Dissociation constant	Not applicable	ECHA, CAS #112-02-7, 2024
Density/specific gravity	0.96 g/cm^3 at 20°C (NF T20-053)	ECHA, CAS #112-02-7, 2024
Partition coefficient	Log K_{ow} = 3.08 at 25°C (DIN 53914)	ECHA, CAS #112-02-7, 2024

<u>Toxicokinetics</u>

- ECHA CHEM, CAS #112-02-87, 20
  - In a GLP-compliant *in vitro* dermal absorption test conducted according to OECD Guideline 428, a hair-care formulation that contains 14% of a 25% commercial preparation of cetrimonium chloride, excised dermatomed pig skin was exposed to the test substance for 30 minutes before rinse-off. Study authors determined an absorption rate of 10.6 µg/cm^2, and 1.2% of the applied dose was absorbed (Klimisch 2, reliable with restrictions).

Hazard Classification Summary

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

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

Cetrimonium chloride was assigned a score of Low for carcinogenicity based on negative predictions from rule-based and statistical based models. GreenScreen® criteria classify chemicals as a Low hazard for carcinogenicity when adequate negative data are available and they are not GHS classified (CPA 2018b). The confidence in the score is low due to lack of 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, CAS #112-02-7, 2024
  - The ECHA dossier authors did not classify cetrimonium chloride for carcinogenicity under GHS, based on negative genotoxicity data, lack of hyperplastic or pre-neoplastic lesions in any organs in repeated dose toxicity studies along with the overall low general toxicity, and the absence of alerts for carcinogenicity as per the ISS rule of the OECD QSAR Toolbox v.2.3.
- U.S. EPA 2019, 2021
  - Cetrimonium chloride could not be modeled with OncoLogic v9.0 or v8.0 as the molecular structure is outside the scope of both versions.
- Toxtree 2018
  - Based on its default structure, cetrimonium chloride does not have any structural alerts for genotoxic or nongenotoxic carcinogenicity (Appendix D).
- VEGA 2024
  - VEGA does not support unconnected or ionic structures.
- DTU 2024 (Appendix E)
  - For the E Ultra model, cetrimonium chloride was predicted to be negative for carcinogenicity and was within the applicability domain for all datasets (i.e., Male Rat, Female Rat, Rat, Male Mouse, Female Mouse, Mouse, and Rodent).

- For the Leadscope model, the compound was predicted to be negative for carcinogenicity and was within the applicability domain for 5 of 7 datasets (i.e., Female Rat, Male Mouse, Female Mouse, Mouse, and Rodent). The remaining datasets were negative but outside the applicability domain, and the result is not included in the weight of evidence.
- For the liver-specific cancer in rats or mouse model, the CASE Ultra, Leadscope, and SciQSAR models as well as the overall model battery predictions are outside of their respective applicability domains, and therefore the result is not included in the weight of evidence.

- Based on the weight of evidence, a score of Low was assigned. Toxtree (a rule-based program) identified no structural alerts for genotoxic or nongenotoxic carcinogenicity for the target compound. Additionally, cetrimonium chloride was predicted to be a non-carcinogen by all models of the statistical-based E Ultra and Leadscope FDA RCA Cancer models that were within their respective applicability domains.

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

Cetrimonium chloride was assigned a score of Low for mutagenicity/genotoxicity based on negative results for mutagenicity and clastogenicity in bacteria and/or mammalian cells *in vitro*. 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, CAS #112-02-7, 2024
  - *In vitro*: Negative results for mutagenicity were obtained in a GLP-compliant bacterial reverse mutation assay conducted according to OECD Guideline 471. *Salmonella typhimurium* test strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP_2 were exposed to cetrimonium chloride (25% purity, 75% water) in deionized water at 0.01-333 µg/plate with and without metabolic activation. Positive and negative controls were reported as valid; controls used were sodium azide, 4-nitro-o-phenylene-diamine, methylmethanesulfonate, and 2-aminoanthracene. No increase in the mutation frequency was detected following treatment in the presence or absence of metabolic activation. Vehicle and positive controls were reported as valid (Klimisch 1, reliable without restrictions).
  - *In vitro*: Negative results for mutagenicity were obtained in a GLP-compliant mammalian cell gene mutation assay conducted according to OECD Guideline 476. Chinese hamster lung fibroblasts (V79) were exposed to cetrimonium chloride (25% purity, 75% water) in deionized water at 0.2-4.7 µg/mL without metabolic activation and 1.6-18.8 µg/mL with metabolic activation. Positive and negative controls were used; controls used were ethylmethanesulfonae and 7,12-dimethylbenzanthracene. No increase in the mutation frequency was detected following treatment in the presence or absence of metabolic activation. Positive and negative controls were valid (Klimisch 2, reliable with restrictions).
  - *In vitro*: Negative results for clastogenicity were obtained in a GLP-compliant mammalian chromosome aberration test conducted according to OECD Guideline 473. Chinese hamster lung fibroblasts (V79) were exposed to cetrimonium chloride (24-26% aqueous solution) in water at 1.0-3.0 µg/mL without metabolic activation and 1.0-10.0 µg/mL with metabolic activation. Positive and negative controls were used; controls used were ethylmethanesulfonate and cyclophosphamide. No increase in the frequency of chromosome

aberrations was identified following treatment in the presence or absence of metabolic activation. Positive and vehicle controls were valid (Klimisch 1, reliable without restrictions).

- U.S. EPA 2008
    - *In vitro*: Negative results for mutagenicity were obtained in a bacterial reverse mutation assay. *S. typhimurium* test strains TA98 and TA100 were exposed to cetrimonium chloride (purity not specified) at 0.05-10 µg/plate with and without metabolic activation. No increase in the mutation frequency was detected following treatment in the presence or absence of metabolic activation.

Reproductive Toxicity (R) Score (H, M, or L): DG
Cetrimonium chloride was assigned a score of Data Gap for reproductive toxicity based on the lack of data identified for this endpoint.

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

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): L
Cetrimonium chloride was assigned a score of Low for developmental toxicity based on a lack of developmental effects seen in two prenatal developmental toxicity studies in rabbits (dermal) and rats (oral). 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 on the target chemical.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* Not present on any screening lists for this endpoint.
- ECHA, CAS #112-02-7, 2024
    - *Dermal:* A GLP-compliant prenatal developmental toxicity test conducted in a manner similar to OECD Guideline 414 (exposure on gestation days 7-18 only) was performed with pregnant female New Zealand White rabbits (20/group) administered dermal applications of cetrimonium chloride (as 1-hexadecanaminium, N,N,N-trimethyl-, chloride, 25% aqueous solution) in water at 0, 10, 20, or 40 mg active ingredient (a.i.)/kg/day on gestation days (GD) 7-18. Maternal examinations included clinical signs of toxicity, assessment of dermal irritation, body weights, food consumption, and ovarian and uterine content. Fetal evaluations included examinations of body weight, sex, and external, visceral, and skeletal malformations. No treatment-related effects were detected on maternal health or developmental endpoints. Therefore, the study authors identified maternal toxicity and developmental toxicity NOAELs of 40 mg/kg/day (Klimisch 1, reliable without restrictions).
- ECHA CHEM, CAS #112-02-7, 2024
    - *Oral:* In a GLP-compliant developmental toxicity test, conducted in accordance with OECD Guideline 414, pregnant female Sprague-Dawley rats (25/group) received the test substance at doses of 0, 18.75, 37.5, or 75 mg/kg/day via gavage. Statistically significant reductions in body weight, gravid uterus weight, and absolute weight gain at the high dose were slight in magnitude and hence not considered of toxicological significance. A 4% and 5% decrease in fetal body weight was measured in female and male offspring, respectively. The weights were within historical control ranges and not considered toxicologically relevant. No other

developmental toxicity was reported and the authors assigned a NOAEL of 75 mg/kg/day for both maternal and developmental toxicities (Klimisch 1, reliable without restriction).

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

Cetrimonium chloride was assigned a score of Data Gap for endocrine activity based on a lack of sufficient data for this endpoint. No effects on thyroid hormones in dams or effects on anogenital distance (AGD) in either sex of the offspring were found in a prenatal developmental toxicity study in rats with surrogate cetrimonium chloride, but the exposure period was very limited for the offspring in this study to thoroughly evaluate effects on the estrogen and androgen pathways, and no data were available on steroidogenesis.

- 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 2024b
    - Cetrimonium chloride was not evaluated as part of the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) in the 21st Century.
- ECHA CHEM, CAS #112-02-7, 2024
    - *Oral:* In the previously described GLP-compliant developmental toxicity test, conducted in accordance with OECD Guideline 414, pregnant female Sprague-Dawley rats (25/group) received the test substance at doses of 0, 18.75, 37.5, or 75 mg/kg/day via gavage on gestational days 3-19. Thyroid hormones (T3, T4 and thyroid stimulating hormone (TSH)) were measured on gestational day 20 in the blood; thyroid from the dams was weighed and histologically examined. In additional to standard fetal examinations, the anogenital distance (AGD) was measured in all fetuses. No toxicologically significant effects were detected on these parameters (Klimisch 1, reliable without restriction).

<u>Group II and II* Human Health Effects (Group II and II* Human)</u>

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

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

Cetrimonium chloride was assigned a score of High for acute toxicity based on oral LD_{50} values as low as 450 mg/kg in rats. GreenScreen® criteria classify chemicals as a Moderate hazard for acute toxicity when oral LD_{50} values are > 300 mg/kg to 2,000 mg/kg (CPA 2018b). Korea classified cetrimonium chloride to a higher GHS Category 3, but no basis was provided. Therefore, this classification was not considered in the weight of evidence. The confidence in the score is high as it is based on reliable experimental data for the target chemical.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:*
        - GHS Korea: H301 – Toxic if swallowed [Acute toxicity (oral) – Category 3]
            - Basis unspecified[10]
        - GHS Australia: H302 – Harmful if swallowed [Acute toxicity (oral) – Category 4]
        - GHS Australia: H312 – Harmful in contact with skin [Acute toxicity (dermal) – Category 4]
        - GHS Japan: H302 – Harmful if swallowed [Acute toxicity (oral) – Category 4]

[10] https://kreach.me.go.kr/repwrt/mttr/en/mttrDetail.do

    - GHS Korea: H302 – Harmful if swallowed [Acute toxicity (oral) – Category 4]
    - GHS New Zealand – Acute oral toxicity category 4
- ECHA, CAS #112-02-7, 2024
  - *Oral:* LD_{50} = 450 mg/kg in female Wistar rats, 861 mg/kg in male Wistar rats (GLP-compliant, OECD Guideline 401/EU Method B.1) (Klimisch 1, reliable without restrictions)
  - *Oral:* LD_{50} > 250 mg/kg in male and female Sprague-Dawley rats (GLP compliance not specified, 16 CFR §1500.3) (Klimisch 2, reliable with restrictions)
- SCCS 2009
  - *Oral:* LD_{50} = 2,000 mg/kg (equivalent to 480-520 mg a.i./kg, Sprague-Dawley rats[11]).

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

Cetrimonium chloride was assigned a score of Moderate for systemic toxicity (single dose) based on respiratory tract irritation in humans that correlates to a classification of GHS Category 3, as well as a GHS (Japan) classification of GHS Category 3. GreenScreen® criteria classify chemicals as a Moderate hazard for systemic toxicity (single dose) when classified as GHS Category 3 following single exposures (CPA 2018b). The confidence in the score is low due to limited experimental data available and its presence on a screening list.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* GHS Japan: H335 – May cause respiratory irritation [Specific target organ toxicity – Single exposure – Category 3]
- ECHA, CAS #112-02-7, 2024
  - *Oral*: In the acute oral toxicity test that identified an oral LD_{50} of 450-861 mg/kg in Wistar rats, animals (5/sex/dose) were administered via gavage doses of 0, 630, 1,000, 1,600, 2,500, 3,150, or 4,000 mg/kg test substance (containing 29% a.i. in water) and observed for 14 days. Deaths occurred during day 1 and day 13 (mortality at each dose level not reported). Clinical signs of toxicity included hunched posture, retracted flanks, rough fur, quiet behavior, noisy un-regular breathing, slimy feces, and myosis (excessive constriction of the pupil of the eye) during the first days of the observation period. Reduced body weights were identified "in individual animals at most dosage groups, in particular after 7 days." However, the doses at which these effects were observed were not reported. Animals that survived until the scheduled sacrifice did not exhibit any significant gross pathological change. Animals that died during the observation period exhibited bleeding of the lungs, very full bladders, darkened adrenal glands, bleeding in the stomach mucous membrane, swollen stomach, stomach filled with liquid, slime, or feed, glassy appearance of the small intestine, small intestine filled with yellowish-clear mass, and reddened small intestine mucous membrane (Klimisch 1, reliable without restrictions).
  - *Oral*: In the acute oral toxicity test that identified an oral LD_{50} > 250 mg/kg in Sprague-Dawley rats, clinical signs of toxicity detected during the first three days after dosing included transient diarrhea, soft feces, and anogenital staining. One animal that died on day 2 exhibited anogenital staining, lethargy, and hunched posture prior to death. All other animals appeared normal on day four of the observation period. All of the surviving males gained weight during the observation period, while one female lost 2 g of body weight. The one decedent animal exhibited discoloration of the liver, kidney, lungs, and spleen and

[11] The test substance contains 24-26% a.i., Therefore, the LD_{50} of 2,000 mg/kg is equivalent to 480 mg/kg (2,000 mg/kg * 24%) to 520 mg/kg a.i.

accumulation of fluid and distention of the gastrointestinal tract. No gross pathological data were provided for the surviving animals (Klimisch 2, reliable with restrictions).

- SCCS 2009
  - *Oral:* In a GLP-compliant, OECD Guideline 420 acute toxicity study, Sprague-Dawley rats (5/sex/dose) were administered via gavage doses of 0, 500 or 2,000 mg/kg test substance (containing 24-26% a.i. in water) and observed for 14 days. None of the animals died during the observation period at 500 mg/kg, while 5/10 animals died at 2,000 mg/kg. Clinical signs observed included decreased motor activity, ataxia, hunched back, half-closed eyes, piloerection, salivation, and diarrhea at 2,000 mg/kg. Body weight decreased at the top dose for the first 3 days of observation. No gross pathological changes were noted in surviving animals.
- HSDB 2003
  - *Inhalation:* The vapor of centrimonium chloride is irritating to the nose and throat.
- Based on the lack of gross pathological changes detected in animals that survived until the scheduled sacrifice following single exposures, and insufficient reported signs of toxicity at specific doses to identify LOAELs , ToxServices did not classify cetrimonium chloride as a specific target organ toxicant Categories 1 or 2 following single exposures under GHS criteria (UN 2023). However, limited human information indicates that centrimonium chloride is irritating to the respiratory tract, probably due to its irritating potential. This warrants classification to GHS Category 3.

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

Cetrimonium chloride was assigned a score of Low for systemic toxicity (repeated dose) based on an oral NOAEL of 113 mg/kg/day from a subchronic oral toxicity study for a structural surrogate. GreenScreen® criteria classify chemicals as a Low hazard for systemic toxicity (repeated dose) when the oral LOAEL is > 100 mg/kg/day in subchronic toxicity studies (CPA 2018b). The confidence in the score is low as it is based on reliable experimental data for weak surrogate, whereas cetrimonium chloride was not tested up to the guidance values in the available 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, CAS #112-02-7, 2024
  - *Oral: <u>Surrogate: Quaternary ammonium compounds, C12-18 (even numbered) alkyltrimethyl chlorides (CAS #68391-03-7)</u>:* A GLP-compliant subchronic repeated dose toxicity study conducted according to OECD Guideline 408/EU Method B.26/EPA OPPTS 870.3100 was performed with Sprague-Dawley rats (10/sex/group) provided diets containing quaternary ammonium compounds, C12-18 (even numbered) alkyltrimethyl chlorides (as coco alkyl trimethyl ammonium chloride or C12-18 TMAC, 35.5% aqueous solution) at 0, 100, 500, or 2,000 ppm (equivalent to 0, 22, 113, and 273 mg/kg/day, respectively, for the active ingredient) for 90 days. The high dose of 2,000 ppm was reduced to 1,000 ppm from day 29 onwards due to deteriorating health of the animals. The animals were evaluated for clinical signs of toxicity, body weight, food and water consumption, ophthalmology, hematology, clinical chemistry, gross pathology, and histopathology. No clinical signs of toxicity were detected in the low or mid dose groups. High dose animals exhibited hunched posture, piloerection, tiptoe gait, diarrhea and red/brown staining of the fur from day 7 onwards. Following reduction of the dose from 2,000 ppm to 1,000 ppm, clinical signs of toxicity included pallor of the extremities and generalized fur loss. During the first five

weeks of the study, high dose animals exhibited reduced body weight gains. Mid dose animals also exhibited reduced body weight gains but only during the first two weeks of the study. Following reduction of the high dose, the animals recovered body weights. However, terminal body weights of mid dose males and high dose males and females were less than controls (statistical significance not specified). Reduced food consumption was identified for mid and high dose animals throughout the study. High dose animals also exhibited reduced food efficiency during the first three weeks of the study. No treatment-related effects were identified for ophthalmology, hematology, or clinical chemistry parameters. Changes to organ weights were identified with treatment in the high dose group but were considered by the study authors to be consequences of reduced body weight development rather than test substance toxicity. No treatment-related effects were identified on gross pathological findings. Lower severities of pigment accumulation were detected in the spleens of high dose males (statistically significant, $p < 0.05$), but not in high dose females. An increased incidence of pigment accumulation was detected in the kidneys of mid and high dose males. In both tissues, the pigment reacted positively to Perl's staining technique and was considered by the study authors to be hemosiderin. The study authors identified a NOAEL of 113 mg/kg/day based on clinical signs of toxicity, reduced body weight gain, and histopathological changes detected in the high dose group (Klimisch 1, reliable without restrictions).

  - *Dermal*: A repeated dose toxicity study conducted in a manner similar to OECD Guideline 410 was performed with New Zealand White rabbits (5/sex/group) administered topical applications of cetrimonium chloride (purity not specified) in water at 0 or 10 mg/kg/day without coverage for 6.5-7 hours 5 days/week for 4 weeks. The animals were evaluated for clinical signs of toxicity, dermal irritation, body weight, hematology, gross pathology, organ weights (liver and kidneys only), and histopathology. At the application sites, mild to marked acanthosis with active mitosis, hyperkeratosis, partial to extensive necrosis of the epidermis and hair follicles, and encrustation were detected. No evidence of systemic toxicity was detected and the study authors identified a NOAEL of 10 mg/kg/day (equivalent to 7.1 mg/kg/day for a 7-day/week exposure frequency) (Klimisch 2, reliable with restrictions).

- SCCS 2009, ECHA, CAS #112-02-7, 2024
  - *Oral:* A GLP-compliant repeated dose toxicity study conducted in a manner similar to OECD Guideline 407 was performed with Sprague-Dawley rats (10/sex/group) administered gavage doses of cetrimonium chloride (24-26% aqueous solution) at 0, 30, 100, or 300 mg/kg/day for 28 days. Additional groups of 5 animals/sex were exposed to 0 or 300 mg/kg/day for 28 days and then maintained for at least 27 days without treatment (recovery groups). The animals were evaluated for clinical signs of toxicity, body weight, food consumption, ophthalmology, hematology, clinical chemistry, organ weights, gross pathology, and histopathology. No treatment-related effects were detected on body weight, food consumption, ophthalmology, or hematology. Clinical signs of toxicity were limited to local irritation in the high dose group during the last week of treatment. High dose animals exhibited a significant increase in alanine aminotransferase (ALT) activity. Due to the lack of other correlated hepatic effects, the study authors considered this finding to be incidental. High dose males exhibited changes to the absolute and relative spleen and adrenals (direction and magnitude not specified). Gross pathological changes in the high dose group included edema of the forestomach mucosa, thickening of the mucosa, and ulceration. No irritation effects of the mucosa were detected after the recovery period. Histopathological findings of the gastrointestinal tract of high dose animals identified inflammatory edema of

the submucosa and sporadic ulceration and acanthosis of the mucosa up to papillomatous hyperplasia. The recovery group animals exhibited a complete and regular regeneration of the forestomach mucosa. The study authors identified a NOAEL of 300 mg/kg/day based on the reversibility of the gross pathological and histopathological effects (Klimisch 2, reliable with restrictions).

- *Note: The ECHA entry for this study indicates that there is uncertainty as to the quantification of the dose. Specifically, it is unclear if the doses mentioned are for the aqueous solution or are for the undiluted active ingredient. The SCCS (2009) authors state that the doses are for the trade name containing 24-26% cetrimonium chloride. Therefore, the actual NOAEL is 300 mg/kg/day * 24% = 72 mg a.i. /kg/day.*

- Based on the weight of evidence, a score of Low was assigned. The subacute dermal and oral studies performed with cetrimonium chloride did not identify systemic effects, but the highest tested doses (40 and 72 mg/kg/day for the dermal and oral study, respectively) were far below the tripled GHS Category 2 guidance values for 28-day studies (i.e., 600 and 300 mg/kg/day for dermal and oral routes, respectively). The NOAEL of 113 mg/kg/day from the 90-day study on the surrogate quaternary ammonium compounds, C12-18 (even numbered) alkyltrimethyl chlorides was above the GHS Category 2 value of 100 mg/kg/day and supports a Low score. However, as the surrogate is a mixture and the impact of carbon chain length on toxicity is unclear. Therefore, the confidence in the score is reduced.

Neurotoxicity (single dose, N-single) (Group II) Score (vH, H, M, or L): DG
Cetrimonium chloride was assigned a score of Data Gap for neurotoxicity (single dose) based on insufficient data identified.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA, CAS #112-02-7, 2024
  - *Oral*: In the acute oral toxicity test that identified an oral LD_{50} of 450-861 mg/kg in Wistar rats, animals (5/sex/dose) were administered via gavage doses of 0, 630, 1,000, 1,600, 2,500, 3,150, or 4,000 mg/kg test substance (containing 29% a.i. in water) and observed for 14 days. Deaths occurred during day 1 and day 13 (mortality at each dose level not reported). Clinical signs of toxicity included hunched posture, retracted flanks, rough fur, quiet behavior, noisy un-regular breathing, slimy feces, and myosis (excessive constriction of the pupil of the eye) during the first days of the observation period. Animals that survived to the scheduled sacrifice did not exhibit any significant gross pathological change (Klimisch 1, reliable without restrictions).
  - *Oral*: In the acute oral toxicity test that identified an oral LD_{50} greater than 250 mg/kg in Sprague-Dawley rats, clinical signs of toxicity detected during the first three days after dosing included transient diarrhea, soft feces, and anogenital staining. One animal that died on day 2 exhibited anogenital staining, lethargy, and hunched posture prior to death. All other animals appeared normal on day four of the observation period. No gross pathological data were provided for the surviving animals (Klimisch 2, reliable with restrictions).
- HSDB 2003
  - Parenteral exposure to centrinomium chloride is associated with central nervous system (CNS) depression, respiratory muscle paralysis and ultipmately coma.
- Based on the weight of evidence, a score of Data Gap was assigned. Some signs indicative of narcotic effects (e.g., quiet behavior, decreased motor activity, and ataxia) were observed in two

acute oral toxicity studies with cetrimonium chloride. However, there are other clinicals signs in the same animals that the overall weight of evidence indicates general toxicity rather than specific neurotoxicity. Cetrimonium when administered by the parenteral route was associated with CNS depression, but this route is not relevant to the GreenScreen® paradigm. Therefore, data are insufficient to score this endpoint.

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

Cetrimonium chloride was assigned a score of Low for neurotoxicity (repeated dose) based on the lack of neurotoxic effects seen at doses up to 273 mg/kg/day in a subchronic oral toxicity study in rats with a close structural surrogate. GreenScreen® criteria classify chemicals as a Low hazard for neurotoxicity (repeated dose) when adequate data are available and GHS not classified (CPA 2018b). The confidence in the score is low as it is based on reliable experimental data for a weak surrogate.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA, CAS #112-02-7, 2024
  - *Oral: <u>Surrogate: Quaternary ammonium compounds, C12-18 (even numbered) alkyltrimethyl chlorides (CAS #68391-03-7)</u>:* A GLP-compliant subchronic repeated dose toxicity study conducted according to OECD Guideline 408/EU Method B.26/EPA OPPTS 870.3100 was performed with Sprague-Dawley rats (10/sex/group) provided diets containing quaternary ammonium compounds, C12-18 (even numbered) alkyltrimethyl chlorides (as coco alkyl trimethyl ammonium chloride or C12-18 TMAC, 35.5% aqueous solution) at 0, 100, 500, or 2,000 ppm (equivalent to 0, 22, 113, and 273 mg/kg/day, respectively, for the active ingredient) for 90 days. The high dose of 2,000 ppm was reduced to 1,000 ppm from day 29 onwards due to deteriorating health of the animals. The animals were evaluated in a behavioral assessment, functional performance test, and sensory reactivity assessment. No treatment-related effects were detected on the functional performance parameters. High dose females exhibited an increase in startle reflex parameters, but no such effect was detected for high dose males or animals in the lower dose groups. Open-field observations confirmed the hunched posture, piloerection, and tiptoe gait detected in high dose animals during the clinical evaluation (see the repeated dose systemic toxicity section above). Based on the lack of a dose-response and gender correlation in the effects and the lack of neurobehavioral effects at doses not associated with significant clinical toxicity, ToxServices identified a neurotoxicity NOAEL of 273 mg/kg/day (Klimisch 1, reliable without restrictions).

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

Cetrimonium chloride was assigned a score of Low for skin sensitization based on a lack of dermal sensitization seen in a Buehler test conducted in guinea pigs. GreenScreen® criteria classify chemicals as a Low hazard for skin sensitization when adequate negative data are available and no GHS classification (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, CAS #112-02-7, 2024
  - A GLP-compliant Buehler test conducted according to OECD Guideline 406/EU Method B.6 was performed with female Pirbright-White guinea pigs (20 treated animals, 10 controls) administered dermal doses of cetrimonium chloride (as Genamin CTAC, 28.7%

purity, 70% water). The induction doses were applied as 4% w/v solutions in water under occlusive dressing, and the challenge dose was applied as 1% w/v solutions in water under occlusive dressing. No positive dermal reactions were identified following the challenge doses. The study authors concluded that cetrimonium chloride was not sensitizing to the skin under the tested conditions (Klimisch 1, reliable without restrictions).

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

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

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- OECD 2024
  - Cetrimonium chloride 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 cetrimonium chloride 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 cetrimonium chloride, and as cetrimonium chloride does not contain any structural alerts for respiratory sensitization (OECD 2024), cetrimonium chloride is not expected to be a respiratory sensitizer.

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

Cetrimonium chloride was assigned a score of Very High for skin irritation/corrosivity based on it being classified as GHS Category 1C based on experimental data for both cetrimonium chloride and a close structural surrogate. GreenScreen® criteria classify chemicals as a Very High hazard for skin irritation/corrosivity when they are corrosive to skin or classified as GHS Category 1 for skin irritation (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data for a strong structural surrogate.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - GHS Australia: H314 – Causes severe skin burns and eye damage (Skin corrosion/irritation – Category 1A or 1B or 1C)
    - GHS Japan: H315 – Causes skin irritation (Skin corrosion/irritation – Category 2)
    - GHS New Zealand: Skin irritation category 2
- ECHA, CAS #112-02-7, 2024
  - A dermal irritation test (test method and GLP compliance not specified) was performed with New Zealand White rabbits (2 males, 4 females) administered topical applications of 0.5 mL undiluted cetrimonium chloride (as C16 TMAC, purity not specified) to clipped skin under

occlusive dressing for 24 hours. A 72-hour observation period followed the exposure period. The mean primary dermal irritation index (PDII) was 5.42 and the mean erythema and edema scores were 2.5 and 2.33, respectively, and 24 hours and 2.83 and 2.67, respectively, at 72 hours. Thickening of the skin, blacking staining, and brown discoloration were also observed at the application sites. Reversibility of the dermal effects was not assessed. The study authors concluded that cetrimonium chloride was irritating to the skin under the conditions of this test (Klimisch 2, reliable with restrictions).

  - *Surrogate: Quaternary ammonium compounds, (C16-18 and C18-unsatd. Alkyl) trimethyl, chlorides (CAS #68002-61-9):* A dermal irritation test conducted in a manner similar to OECD Guideline 404 (GLP compliance not specified) was performed with New Zealand White rabbits (6 males and females) administered topical applications of 0.5 mL undiluted quaternary ammonium compounds, (C16-18 and C18-unsatd. alkyl)trimethyl, chlorides (as C16-18 and C18-unsatd. TMAC, 50% purity, 15% water, 35% isopropyl alcohol) to intact or abraded skin under occlusive dressing for one or four hours. An observation period of up to 10 days followed the exposure period. After the one-hour exposure, the mean overall irritation was 0.85/8 one hour after the exposure and 1.25/8 48 hours after the exposure. After the four-hour exposure, the overall irritation score was 4/8 four hours after the exposure and 5.4/8 48 hours after the exposure. The irritation effects were fully reversible within 10 days after the 1-hour exposure but not fully reversible within 10 days after the 4-hour exposure (slight to distinct incrustation and decreased hair growth detected at the end of the observation period). The study authors concluded that quaternary ammonium compounds, (C16-18 and C18-unsatd. alkyl)trimethyl, chlorides was corrosive to the skin based on the irreversible of the dermal effects following the four-hour exposure and classified as GHS Category 1C (Klimisch 2, reliable with restrictions).
- Based on the weight of evidence, cetrimonium chloride induced dermal irritation to the sin of rabbits following a 24-hour exposure. Due to the lack of a reversibility assessment in the study design, ToxServices relied on the data for the analog quaternary ammonium compounds, (C16-18 and C18-unsatd. alkyl)trimethyl, chlorides to assign a score for this endpoint. As the analog produced irreversible effects to the skin of rabbits, ToxServices concluded that cetrimonium chloride is corrosive to the skin and classified it as a GHS Category 1 skin irritant. This is in agreement with the authors of the REACH dossier who classified cetrimonium chloride as a GHS Category 1C skin irritant (H314).

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

Cetrimonium chloride was assigned a score of Very High for eye irritation/corrosivity based on ToxServices classifying cetrimonium chloride as a GHS Category 1 eye irritant. GreenScreen® criteria classify chemicals as a Very High hazard for eye irritation/corrosivity when classified under GHS Category 1 and associated with the hazard phrase H318 (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data on the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - GHS Japan: H318 – Causes serious eye damage (Serious eye damage/eye irritation – Category 1)
    - GHS New Zealand: Eye irritation Category 2
- ECHA, CAS #112-02-7, 2024
  - A GLP-compliant ocular irritation test conducted according to OECD Guideline 405 was performed with Kleinrusse Chbb: HM/Fa Thomae rabbits (4 males) administered ocular

instillations of 0.1 mL 2% cetrimonium chloride (as C16 TMAC, purity not specified) in water. An observation period of 14 days followed the instillations. At 24, 48, and 72 hours, the mean opacity grade was 0.33 (time-point scores of 0.5, 0.25, and 0.25), the mean opacity surface score was 0.67 (time point scores of 0.5, 1.0, and 0.5), the mean redness score was 2.67 (time point scores of 2.25, 3.0, and 2.75), the mean chemosis score was 1.08 (time point scores of 1.5, 1.0, and 0.75), and the mean exudation score was 2.75 (time point scores of 3.0, 2.75, and 2.50). The study authors concluded that cetrimonium chloride was highly irritating to the eyes under the tested conditions and classified it as GHS Category 2 (Klimisch 2, reliable with restrictions).

  - An ocular irritation test conducted according to 16 CFR §1500.42 (GLP compliance not specified) was performed with New Zealand White rabbits (3/sex) administered ocular instillations of 0.1 mL undiluted cetrimonium chloride (as cetyltrimethyl ammonium Cl, 5% purity). An observation period of 72 hours followed the instillations. The mean maximum meant total score (MMTS) was 53/110 at 24 hours, 59.7/110 at 48 hours, and 58.3/110 at 72 hours. All treated eyes exhibited corneal opacity, iritis, and conjunctival irritation at the 24-72-hour observations. The study authors concluded that cetrimonium chloride was highly irritating to the eyes under the tested conditions, and classified it as GHS Category 1 (Klimisch 2, reliable with restrictions).
- Based on the weight of evidence, instillation of cetrimonium chloride at 2% or 5% solutions to the eyes of rabbits induced significant ocular irritation. Based on these findings and the classification of this compound as a GHS Category 1 skin irritant, ToxServices classified cetrimonium chloride as a GHS Category 1 eye irritant.

Ecotoxicity (Ecotox)

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

Cetrimonium chloride was assigned a score of Very High for acute aquatic toxicity based on a measured LC_{50} value of 0.59 mg/L in fish, along with being listed with the hazard statement H400 on two screening lists. GreenScreen® criteria classify chemicals as a Very High hazard for acute aquatic toxicity when L/EC_{50} values are <1 mg/L, as well as being associated with the hazard statement H400 (CPA 2018b). The confidence in the score is high as it is based on reliable measured data for both the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:*
    - GHS Japan: H400 – Very toxic to aquatic life [Hazardous to the aquatic environment (acute) – Category 1]
    - GHS Korea: H400 – Very toxic to aquatic life [Hazardous to the aquatic environment (acute) – Category 1]
- ECHA, CAS #112-02-7, 2024
  - 96-hour LC_{50} in fish = 0.59 mg/L (*Danio rerio*, zebrafish, nominal) (GLP-compliant, OECD Guideline 203/EU Method C.1) (Klimisch 1, reliable without restrictions)
  - *Surrogate: Quaternary ammonium compounds, coco alkyltrimethyl, chlorides (CAS #61789-18-2):* 48-hour mortality EC_{50} in daphnid = 0.28 mg/L (*Daphnia magna*, water flea, nominal) (GLP-compliant, OECD Guideline 202) (Klimisch 2, reliable with restrictions)
  - *Surrogate: Quaternary ammonium compounds, (C16-18 and C18-unsatd. Alkyl) trimethyl, chlorides (CAS #68002-61-9):* 72-hour growth rate EC_{50} in algae = 0.113 mg a.i./L (*Raphidocelis subcapitata*, freshwater algae, measured) (GLP-compliant, OECD Guideline

201/ISO 8692) (Klimisch 1, reliable without restrictions)

Chronic Aquatic Toxicity (CA) Score (vH, H, M, or L): vH
Cetrimonium chloride was assigned a score of Very High for chronic aquatic toxicity based on NOEC values as low as 0.058 mg/L with surrogate quaternary ammonium compounds, (C16-18 and C18-unsatd. Alkyl) trimethyl, chlorides. GreenScreen® criteria classify chemicals as a Very High hazard for chronic aquatic toxicity when NOEC values are < 0.1 mg/L (CPA 2018b). The confidence in the score is high as it is based on measured data for a strong structural surrogate.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA, CAS #112-02-7, 2024
  - <u>*Surrogate: Quaternary ammonium compounds, (C16-18 and C18-unsatd. Alkyl) trimethyl, chlorides (CAS #68002-61-9):*</u> 21-day mortality and reproduction NOEC (*D. magna*) = 0.0068 mg/L (well water), 0.0991 mg/L (river water) (both measured) (similar to OECD Guideline 211)
  - <u>*Surrogate: Quaternary ammonium compounds, (C16-18 and C18-unsatd. Alkyl) trimethyl, chlorides (CAS #68002-61-9):*</u> 72-hour growth rate NOEC in algae = 0.058 mg a.i./L (*R. subcapitata*, freshwater algae, measured) (GLP-compliant, OECD Guideline 201/ISO 8692) (Klimisch 1, reliable without restrictions)

<u>Environmental Fate (Fate)</u>

Persistence (P) Score (vH, H, M, L, or vL): vL
Cetrimonium chloride was assigned a score of Very Low for persistence based on meeting the 10-day biodegradation window in an OECD Guideline 301B study. Although environmental partitioning could not be modeled due to surfactants being out of domain for EPI Suite™, the low vapor pressure of cetrimonium chloride suggests that it is not likely to mainly partition to air. GreenScreen® criteria classify chemicals as a Very Low hazard for persistence when soil, water, or sediment is the dominant environmental compartment and it meets the 10-day window for ready biodegradability (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data for the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA CHEM, CAS #112-02-7, 2024
  - In a non-GLP preliminary study for a subsequent OECD Guideline 301D (closed bottle test) study, domestic non-adapted activated sludge and river water was exposed to the test substance (as C16 TMAC 98.5% active) for 42 days. As cetrimonium chloride is known to be toxic to the inoculum, different detoxification conditions were tested, including the addition of sorbents silica gel or humic acid at two different concentrations each. Biodegradation was measured by oxygen consumption. The results demonstrated that silica gel was the best detoxicant and that active sludge performed better than river water, with 57% and 62% degradation at 28 days at 2 and 1 g silica gel/bottle, and 60% and 65% at 42 days, respectively. Therefore, activated sludge and 1 g silica gel/bottle were tested in the subsequent main study (Klimisch 2, reliable with restrictions).
  - A GLP-compliant ready biodegradability test conducted according to OECD Guideline 301D (closed bottle test) was performed with non-adapted activated sludge with 1 g silica gel/bottle as a detoxicant. The test substance (as C16 TMAC 98.5% active) was degraded

by 65% by day 28, but failed the 10-day window (Klimisch 1, reliable without restriction)
  - A GLP-compliant ready biodegradability test conducted according to OECD Guideline 301 B (CO_2 evolution test) was performed with non-adapted, activated sludge exposed to cetrimonium chloride (as C16 TMAC, 28.9% purity, 21.9% total organic carbon content) at 30 mg/L for 28 days. For one replicate, the 10% degradation level was reached after 7 days and the 60% level was reached after 17 days. For the second replicate, the 10% degradation level was reached after 12 days and the 60% level was reached after 19 days. At the end of the exposure period, the level of degradation was 92% and 95% for the two replicates, with an average of 93.5% degradation. The study authors concluded that cetrimonium chloride was readily biodegradable under the conditions of this test, and met the 10-day window (Klimisch 1, reliable without restrictions).

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

Cetrimonium chloride was assigned a score of Very Low for bioaccumulation based on a calculated log K_{ow} of 3.08 and experimental biomagnification factors (BMFs) of 0.02709-0.04603 for surrogate tetramethylstearylammonium chloride. This surrogate is 2 carbons longer than cetrimonium chloride and hence is likely more lipophilic and more bioaccumulative than cetrimonium chloride. GreenScreen® criteria classify chemicals as a Very Low hazard for bioaccumulation when log K_{ow} are no greater than 4 (CPA 2018b). The confidence in the score is high as it is based on an experimental BMF on a conservative surrogate.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA CHEM, CAS #112-02-7, 2024
  - Log K_{ow} = 3.08 at 25°C (DIN 53914), as calculated based on the measured solubility in water (critical micelle concentration, CMC) and octanol (Klimisch 2, reliable with restrictions)
  - *Surrogate: Tetramethylstearylammonium chloride (CAS #112-03-8):* In a bioaccumulation study conducted according to OECD Guideline 305 presumably under GLP, rainbow trout (*Oncorhynchus mykiss*) was exposed to the test substance (as C18 TMAC) at 23.6 mg/kg in feed for 14 days (uptake phase) followed by a 14-day depuration phase. There was no treatment related mortality or abnormal behavior during the study. The steady state biomagnification factors (BMFss), kinetic BMF (BMFk), and growth-corrected BMF (BMFkg) were calculated to be 0.02709, 0.0404 and 0.0463 g/g, respectively, indicating the test substance did not biomagnify. The authors speculated that this was due to the ionization of the chemical and fast depuration (Klimisch 1, reliable without restriction).

Physical Hazards (Physical)

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

Cetrimonium chloride was assigned a score of Low for reactivity based on the lack of structural alerts for oxidizing and explosive properties, and lack of corrosivity to metals. 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 is low based on the lack of measured data on explosivity.

- 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 CHEM, CAS #112-02-7, 2024
  - In a GLP-unspecified stability to metals test conducted according to DIN 50905, Part 4, the test substance (as C16 TMAS, 50% active in isopropanol) caused pitting with hole depth of 30 and 50 µm in 14 days. This is a slower corrosion rate than the GHS cutoff of 6.25 mm/year (equivalent to 240 µm/14 days). Therefore, the test substance was not classified as being corrosive to metals (Klimisch 2, reliable with restrictions).
- No additional measured data were identified. Therefore, screening procedures for explosivity were used here to estimate the reactivity property of cetrimonium chloride. These procedures are listed in the GHS (UN 2023).
  - Based on the structure of its components or moieties, cetrimonium chloride is not considered explosive or self-reactive due to lack of functional groups associated with explosive or self-reactive properties (See Appendix G).
  - Based on the structure of its components or moieties, cetrimonium chloride 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.

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

Cetrimonium chloride was assigned a score of Low for flammability based on the classification as a non-flammable solid, which corresponds to GHS not classified. GreenScreen® criteria classify chemicals as a Low hazard for flammability when adequate data are available and they are GHS not classified (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data on the target chemical.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- ECHA, CAS #112-02-7, 2024
  - Cetrimonium chloride was non-flammable in a flammability (solids) test conducted according to EU Method A.10 (GLP status not reported). The burning times over 100 mm of cetrimonium chloride were never less than 45 seconds in six separate tests (Klimisch 1, reliable without restrictions).

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* genotoxicity tests and *in silico* modeling for carcinogenicity and respiratory sensitization. NAMs are non-animal alternatives that can be used alone or in combination to provide information for safety assessment (Madden et al. 2020). At present, there is not a uniformly accepted framework on how to report and apply individual NAMs (U.S. EPA 2020, OECD 2020). The expanded application of NAMs greatly amplifies the need to communicate uncertainties associated with their use. As defined by 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 cetrimonium chloride's NAMs dataset include insufficient data for carcinogenicity and respiratory sensitization, and the lack of validated test methods for respiratory sensitization. Cetrimonium chloride's Type II (extrapolation output) uncertainties include lack of defined applicability domains by Toxtree and OECD Toolbox structural alerts, the inability of VEGA models to evaluate ionic substances such as the target chemical, the inability of OncoLogic to evaluate the structural features of the target chemical, the limitations of *in vitro* genotoxicity assays to truly mimic *in vivo* metabolism and their focus on only individual aspects of the genotoxicity process, and the lack of consideration of non-immunological mechanisms of respiratory sensitization by the examination of structural alerts only. Some of cetrimonium chloride's type II uncertainties were alleviated by the use of *in vitro* test batteries and/or in combination of *in vivo* data.

Table 4: Summary of NAMs Used in the GreenScreen® Assessment, Including Uncertainty Analyses	
Uncertainty Analyses (OECD 2020)	
Type I Uncertainty: Data/Model Input	**Carcinogenicity**: Only limited experimental data are available. **Respiratory sensitization**: No experimental data are available and there are no validated test methods.
Type II Uncertainty: Extrapolation Output	**Carcinogenicity:** Toxtree only identifies structural alerts (SAs), and no applicability domain can be defined (Toxtree 2018). VEGA tool does not evaluate ionic substances (VEGA 2024). The target chemical is outside the applicability domain of OncoLogic (U.S. EPA 2019, 2021). **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].

[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

	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 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]. **Respiratory sensitization**: The OECD Toolbox only identifies structural alerts, and does not define applicability domains. Additionally, the ECHA guidance (2017), on which the use of OECD Toolbox structural alerts is based, does not evaluate non-immunologic mechanisms for respiratory sensitization.

Endpoint	NAMs Data Available and Evaluated? (Y/N)	Types of NAMs Data (*in silico* modeling/*in vitro* biological profiling/frameworks)
Carcinogenicity	Y	*In silico* modeling: VEGA/Toxtree/OncoLogic™/ /Danish QSAR
Mutagenicity	Y	*In vitro* data: Bacterial reverse mutation assay/*in vitro* gene mutation assay/*in vitro* chromosome aberration assay
Reproductive toxicity	N	
Developmental toxicity	N	
Endocrine activity	N	
Acute mammalian toxicity	N	
Single exposure systemic toxicity	N	
Repeated exposure systemic toxicity	N	
Single exposure neurotoxicity	N	
Repeated exposure neurotoxicity	N	
Skin sensitization	N	
Respiratory sensitization	Y	*In silico* modeling: OECD Toolbox structural alerts
Skin irritation	N	
Eye irritation	N	
Acute aquatic toxicity	N	

[14] https://www.oecd-ilibrary.org/docserver/9789264264809-en.pdf?expires=1614097800&id=id&accname=guest&checksum=C0DE371FB9C5A878E66C9AB7F84E6BBE
[15] https://www.oecd-ilibrary.org/docserver/9789264264649-en.pdf?expires=1614098015&id=id&accname=guest&checksum=6A4F9CE52EA974F5A74793DD54D54352

Chronic aquatic toxicity	N	
Persistence	Y	Non-animal testing: OECD 301 B and D ready biodegradability tests
Bioaccumulation	N	

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APPENDIX A: Hazard Classification Acronyms
(in alphabetical order)

(AA) **Acute Aquatic Toxicity**

(AT) **Acute Mammalian Toxicity**

(B) **Bioaccumulation**

(C) **Carcinogenicity**

(CA) **Chronic Aquatic Toxicity**

(D) **Developmental Toxicity**

(E) **Endocrine Activity**

(F) **Flammability**

(IrE) **Eye Irritation/Corrosivity**

(IrS) **Skin Irritation/Corrosivity**

(M) **Mutagenicity and Genotoxicity**

(N) **Neurotoxicity**

(P) **Persistence**

(R) **Reproductive Toxicity**

(Rx) **Reactivity**

(SnS) **Sensitization- Skin**

(SnR) **Sensitization- Respiratory**

(ST) **Systemic/Organ Toxicity**

APPENDIX B: Results of Automated GreenScreen® Score Calculation for Cetrimonium Chloride (CAS #112-02-7)

TOXSERVICES TOXICOLOGY RISK ASSESSMENT CONSULTING

GREENSCREEN FOR SAFER CHEMICALS

GreenScreen® Score Inspector

Table 1: Hazard Table / Table 2: Chemical Details

			Group I Human					Group II and II* Human									Ecotox		Fate		Physical	
			Carcinogenicity	Mutagenicity/Genotoxicity	Reproductive Toxicity	Developmental Toxicity	Endocrine Activity	Acute Toxicity	Systemic Toxicity		Neurotoxicity		Skin Sensitization*	Respiratory Sensitization*	Skin Irritation	Eye Irritation	Acute Aquatic Toxicity	Chronic Aquatic Toxicity	Persistence	Bioaccumulation	Reactivity	Flammability
									S	R *	S	R *	*	*								
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	Cetrimonium chloride	112-02-7	*L*	L	DG	L	DG	M	*M*	L	DG	*L*	L	*L*	vH	vH	vH	vH	vL	vL	*L*	L

Table 3: Hazard Summary Table

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

Table 4

Chemical Name	Preliminary GreenScreen® Benchmark Score
Cetrimonium chloride	2

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

Table 6

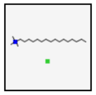
Chemical Name	Final GreenScreen® Benchmark Score
Cetrimonium chloride	2

After Data gap Assessment
Note: No Data gap Assessment Done if Preliminary GS Benchmark Score is 1.

Table 5: Data Gap Assessment Table

Datagap Criteria	a	b	c	d	e	f	g	h	i	j	bm4	End Result
1												
2	Yes	Yes	Yes	Yes	Yes							2
3												
4												

APPENDIX C: Pharos Output for Cetrimonium Chloride (CAS #112-02-7)



112-02-7

Trimethylhexadecylammonium chloride

ALSO CALLED (1-Hexadecyl)trimethylammonium chloride, (C14-C18)Alkyltrimethylammonium chloride, 1-Hexadecanaminiu...

View all synonyms (81)

SHARE PROFILE

HAZARDS PROPERTIES FUNCTIONAL USES RESOURCES

All Hazards View

Show PubMed Results REQUEST ASSESSMENT ADD TO COMPARISON

		Group I Human					Group II and II* Human									Ecotox			Fate		Physical		Mult	Non-GSLT			
	GREENSCREEN®	C	M	R	D	E	AT	ST	ST	N	N	SnS	SnR	IrS	IrE	AA	CA	ATB	P	B	Rx	F	Mult	PBT	GW	O	Other
List Hazard Summary	LT-P1	-	-	-	-	-	H	H	-	-	-	-	-	vH	vH	vH	-	-	-	-	-	-	vH	-	-	-	R

Hazard Lists

DOWNLOAD LISTS

ENDPOINT	HAZARD LEVEL	GREENSCREEN®	LIST NAME	HAZARD DESCRIPTION	OTHER LISTS
Acute Mammalian Toxicity	H	LT-UNK	GHS - Korea	H301 - Toxic if swallowed [Acute toxicity (oral) - Category 3]	+10
	M	LT-UNK	GHS - Australia	H302 - Harmful if swallowed [Acute toxicity (oral) - Category 4]	
	M	LT-UNK	GHS - Australia	H312 - Harmful in contact with skin [Acute toxicity (dermal) - Category 4]	
	M	LT-UNK	GHS - Japan	H302 - Harmful if swallowed [Acute Toxicity (oral) - Category 4]	
	M	LT-UNK	GHS - Korea	H302 - Harmful if swallowed [Acute toxicity (oral) - Category 4]	
	M	LT-UNK	GHS - New Zealand	Acute oral toxicity category 4	
	pC	NoGS	DK-EPA - Danish Advisory List	Acute Tox. 4 - Harmful if swallowed (modeled)	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H301 - Toxic if swallowed (unverified) [Acute toxicity (oral) - Category 3]	

	pC	NoGS	EU - Manufacturer REACH hazard submissions	H302 - Harmful if swallowed (unverified) [Acute toxicity (oral) - Category 4]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H311 - Toxic in contact with skin (unverified) [Acute toxicity (dermal) - Category 3]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H312 - Harmful in contact with skin (unverified) [Acute toxicity (dermal) - Category 4]	
Systemic Toxicity/Organ Effects-Single Exposure	H	LT-UNK	GHS - Japan	H335 - May cause respiratory irritation [Specific target organ toxicity - Single exposure - Category 3]	+1
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H335 - May cause respiratory irritation (unverified) [Specific target organ toxicity - single exposure; Respiratory tract irritation - Category 3]	
Skin Irritation/Corrosivity	vH	LT-UNK	GHS - Australia	H314 - Causes severe skin burns and eye damage [Skin corrosion/irritation - Category 1A or 1B or 1C]	+5
	H	LT-UNK	GHS - Japan	H315 - Causes skin irritation [Skin corrosion / irritation - Category 2]	
	H	LT-UNK	GHS - New Zealand	Skin irritation category 2	
	pC	NoGS	DK-EPA - Danish Advisory List	Skin Irrit. 2 - Causes skin irritation (modeled)	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H314 - Causes severe skin burns and eye damage (unverified) [Skin corrosion/irritation - Category 1A or 1B or 1C]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H315 - Causes skin irritation (unverified) [Skin corrosion/irritation - Category 2]	
Eye Irritation/Corrosivity	vH	LT-UNK	GHS - Japan	H318 - Causes serious eye damage [Serious eye damage / eye irritation - Category 1]	+2
	H	LT-UNK	GHS - New Zealand	Eye irritation category 2	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H318 - Causes serious eye damage (unverified) [Serious eye damage/eye irritation - Category 1]	
Acute Aquatic Toxicity	vH	LT-UNK	GHS - Japan	H400 - Very toxic to aquatic life [Hazardous to the aquatic environment (acute) - Category 1]	+3
	vH	LT-UNK	GHS - Korea	H400 - Very toxic to aquatic life [Hazardous to the aquatic environment (acute) - Category 1]	
	pC	NoGS	DK-EPA - Danish Advisory List	Aquatic Acute1 - Very toxic to aquatic life (modeled)	

	pC	NoGS	EU - Manufacturer REACH hazard submissions	H400 - Very toxic to aquatic life (unverified) [Hazardous to the aquatic environment (acute) - Category 1]	
Systemic Toxicity/Organ Effects [Single Exposure] and/or Neurotoxicity [Single Exposure]	M	LT-UNK	GHS - New Zealand	Specific target organ toxicity - single exposure category 3 narcotic effects	
Acute aquatic toxicity; Chronic aquatic toxicity	U	LT-UNK	EC - CEPA DSL	Inherently Toxic in the Environment (iTE)	
T & P and/or B [(Chronic Aquatic Toxicity and sometimes Persistence) or (Acute Aquatic Toxicity and Persistence and/or Bioaccumulation)]	U	LT-P1	GHS - Japan	H410 - Very toxic to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) - Category 1]	
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
	U	LT-P1	GHS - Korea	H410 - Very toxic to aquatic life with long lasting effects [Hazardous to the aquatic environment (chronic) - Category 1]	
	pC	NoGS	EU - Manufacturer REACH hazard submissions	H410 - Very toxic to aquatic life with long lasting effects (unverified) [Hazardous to the aquatic environment (chronic) - Category 1]	
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 (15)

- Australia - National Industrial Chemicals Notification and Assessments (NICNAS): NICNAS
- C2C Certified v4.0 Product Standard Restricted Substances List (RSL) - Effective July 1, 2022: Cosmetics & Personal Care Products
- CA SCP - Candidate Chemicals: Candidate Chemical List
- Credo Beauty's Restricted Substance List: Restricted Chemicals - see Credo for potential source/use restrictions *
- EC - CEPA DSL: DSL-all
- EU - Cosmetics Regulation: Annex III - Restricted Substances
- EU - PACT-RMOA Substances: Substances selected for RMOA or hazard assessment
- Food Contact Chemicals Database (FCCdb): Food Contact Chemicals Database Version 5.0
- Food Contact Chemicals of Concern (FCCoCL): Food Contact Chemicals of Concern List (FCCoCL) - TIER 3
- FPF Priority Substance List from FCCdb: FPF Priority Substance List from FCCdb
- GreenScreen Certified Standard for Food Service Ware RSL: Food Packaging Forum Priority Substances List
- GSPI - Six Classes Precautionary List: Antimicrobials
- Japan - Chemical Substances Control Law: Japan - CSCL
- TSCA Chemical Substance Inventory (Active-Inactive): TSCA Commercially Active
- TSCA Chemical Substantial Risk Notices: TSCA - Substantial Risk Notices

Positive Lists (3)

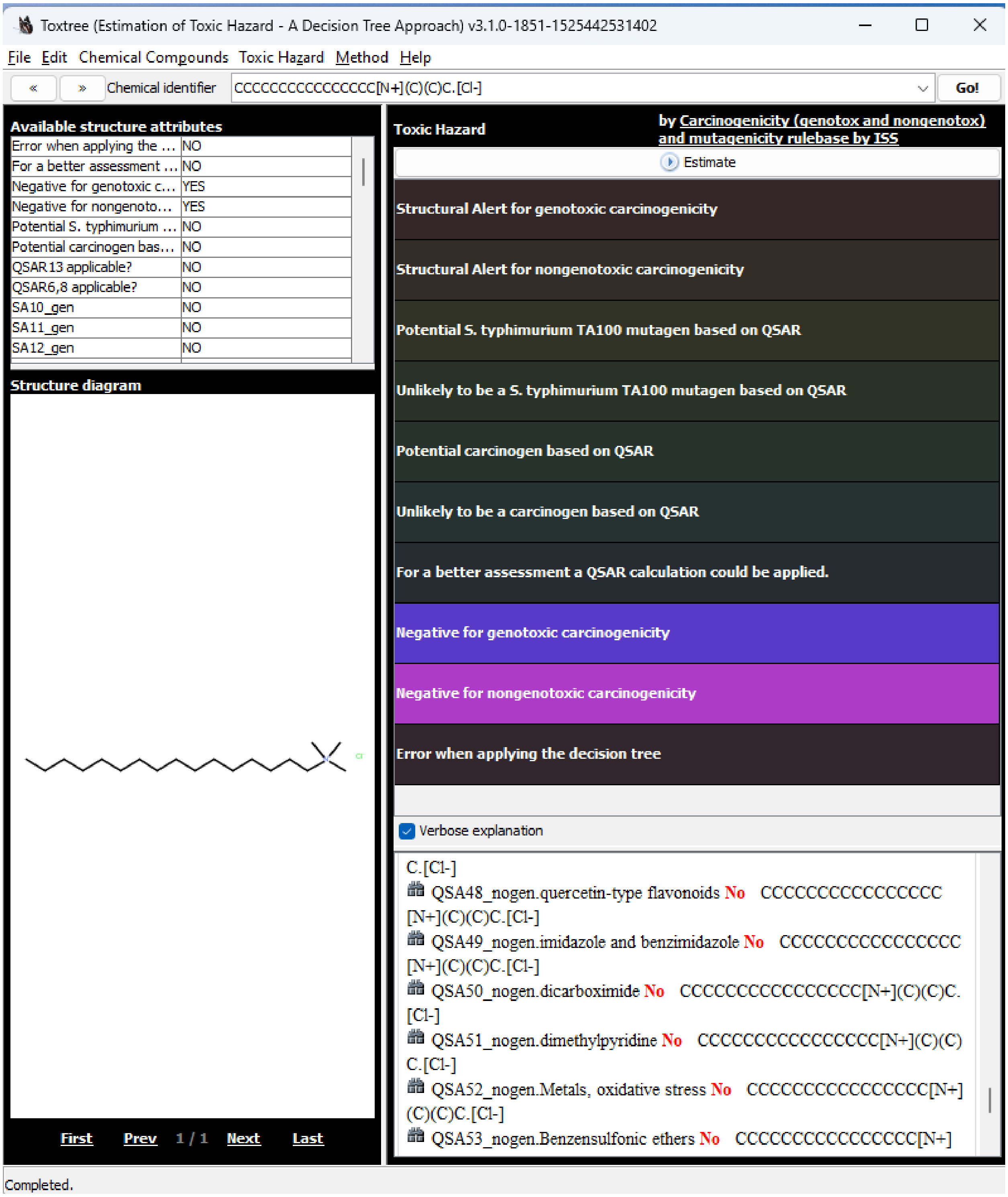
- Cosmetic Ingredient Review (CIR): Safe with Qualifications
- EU - Cosmetics Regulation: Annex V - Preservatives Allowed
- Inventory of Existing Cosmetic Ingredients in China (IECIC 2021): Cosmetic Ingredients

Discussions

No discussions have been posted yet.

Ask a question about this chemical in the forums >

APPENDIX D: Toxtree Carcinogenicity Results for Cetrimonium Chloride (CAS #112-02-7)



APPENDIX E: Danish QSAR Carcinogenicity Results for Cetrimonium Chloride (CAS #112-02-7)

Carcinogenicity

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

Commercial models from CASE Ultra and Leadscope

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

Carcinogenicity (genotox and nongenotox) alerts by ISS, alerts in:	
- parent only	N/A
Oncologic Primary Classification, alerts in:	
- parent only	N/A

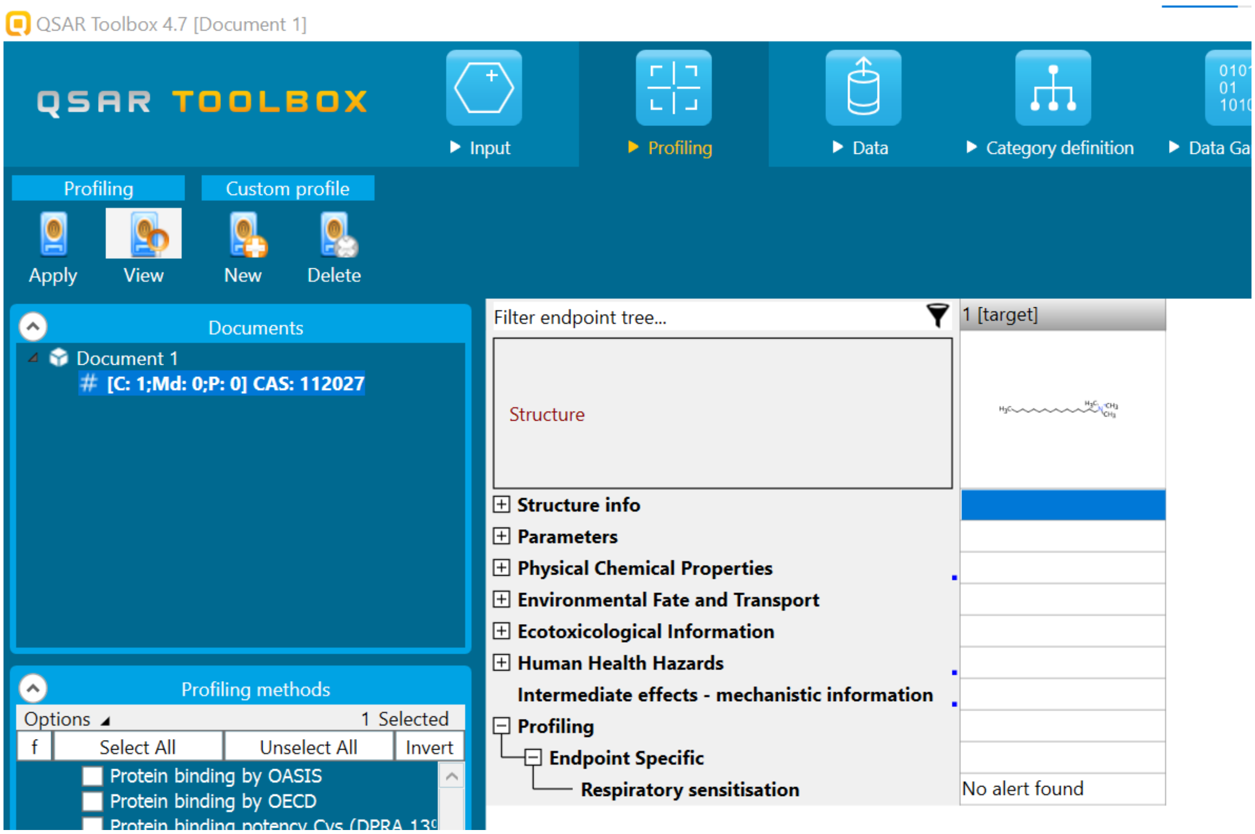
OECD QSAR Toolbox v.4.2 profilers

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

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

DTU-developed models

APPENDIX F: OECD Toolbox Respiratory Sensitization Results for Cetrimonium Chloride (CAS #112-02-7)



APPENDIX G: Known Structural Alerts for Reactivity

Explosivity – Abbreviated List

Explosivity – reactive groups

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

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

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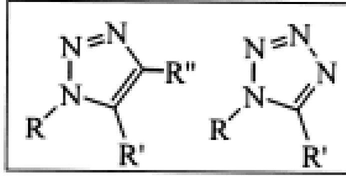
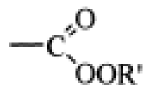
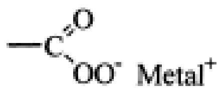
Explosivity – Full List

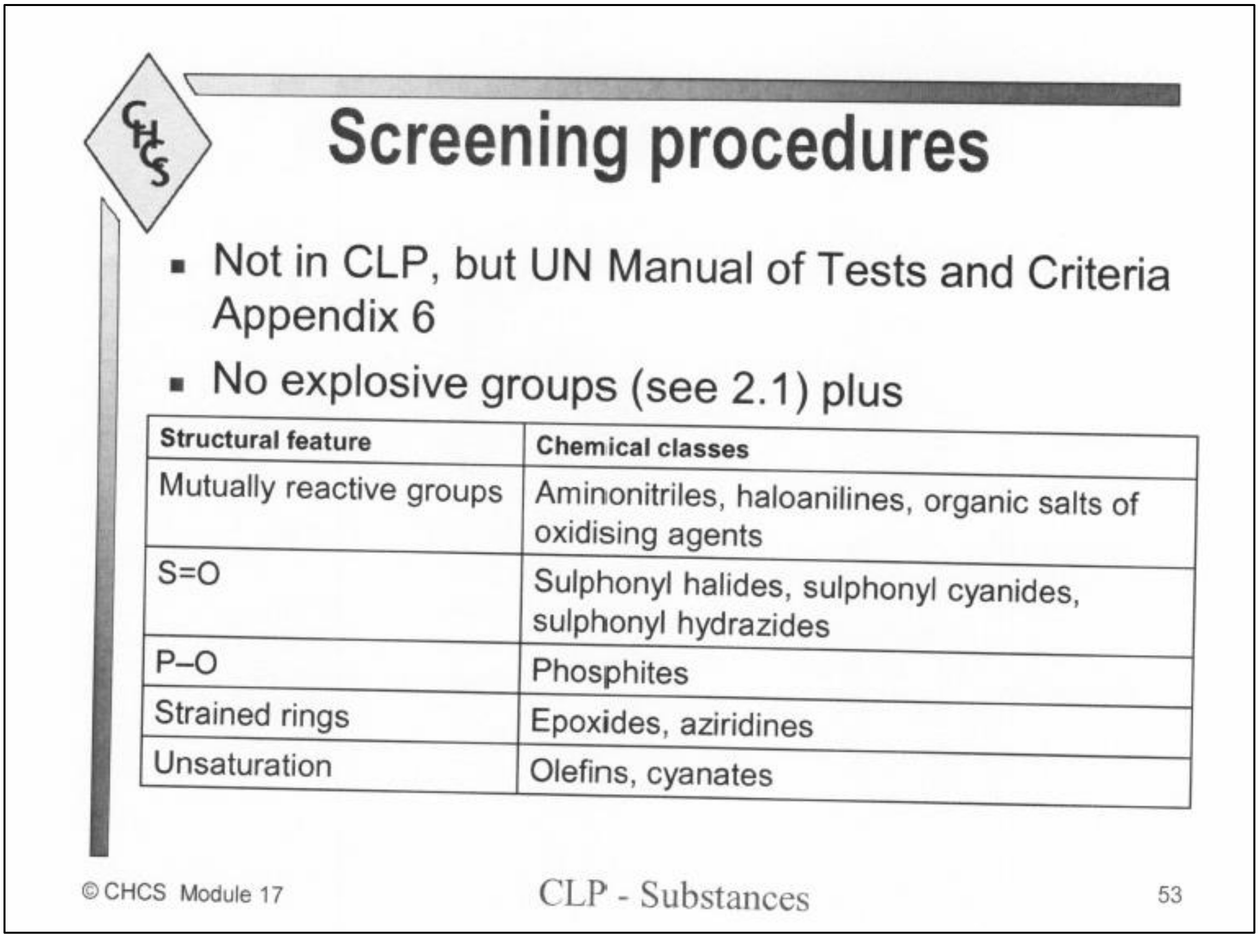
Table R.7.1-28 Chemical groups associated with explosive properties

Chemical group	**Chemical Class**
-C≡C- -C≡C-Metal -C≡C-Halogen	Acetylenic Compounds Metal Acetylides Haloacetylene Derivatives
$>CN_2$	Diazo Compounds
-N=O -NO_2	Nitroso and Nitro Compounds,
R-O-N=O R-O-NO_2	Acyl or Alkyl Nitrites and Nitrates
$>C-C<$ (O bridging)	1,2-Epoxides
$>C=N-O-$Metal	Metal Fulminates or *aci*-Nitro Salts
$>N-$Metal	N-Metal Derivatives (especially heavy metals)
$>N-N=O$ $>N-NO_2$	N-Nitroso and N-Nitro Compounds
$>\overset{+}{N}-N-NO_2$	N-Azolium Nitroimidates
$>C-N=N-C<$	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



Screening procedures

- Not in CLP, but UN Manual of Tests and Criteria Appendix 6
- No explosive groups (see 2.1) plus

Structural feature	Chemical classes
Mutually reactive groups	Aminonitriles, haloanilines, organic salts of oxidising agents
S=O	Sulphonyl halides, sulphonyl cyanides, sulphonyl hydrazides
P–O	Phosphites
Strained rings	Epoxides, aziridines
Unsaturation	Olefins, cyanates

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APPENDIX H: Change in Benchmark Score

Table 5 provides a summary of changes to the GreenScreen® Benchmark™ for cetrimonium chloride. The GreenScreen® Benchmark Score for cetrimonium chloride has not changed over time. The original GreenScreen® assessment was performed in 2019 under version 1.4 criteria and ToxServices assigned a Benchmark 2 (BM-2) score. The BM-2 score was maintained with a version 1.4 update in 2024 which underwent minor updates in 2025.

Table 5: Change in GreenScreen® Benchmark™ for Cetrimonium Chloride

Date	GreenScreen® Benchmark™	GreenScreen® Version	Comment
June 17, 2019	BM-2	v. 1.4	Original GreenScreen® assessment.
November 18, 2024	BM-2	v. 1.4	No change in BM score. The GreenScreen® assessment was updated with a v.1.4 template.
February 24, 2025	BM-2	v. 1.4	No change in BM or hazard score. The GreenScreen® assessment was updated based on Washington Department of Ecology's comments.

Licensed GreenScreen® Profilers

Cetrimonium Chloride GreenScreen® (v.1.4) Evaluation Prepared by:

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