BEHENTRIMONIUM CHLORIDE

(CAS #17301-53-0)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: February 20, 2025[1]

Expiration February 20, 2030

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[1] The report was last updated on February 20, 2025. However, the last complete literature search was conducted on November 15, 2024.

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GreenScreen® Executive Summary for Behentrimonium Chloride (CAS #17301-53-0)

Behentrimonium chloride, also known as docosyltrimethylammonium chloride and N,N,N-trimethyl-1-docosanaminium chloride, is a cationic quaternary ammonium salt with an alkyl chain of 22 carbons. It is a surfactant that functions as an anti-static agent, hair conditioning agent, and a preservative in personal care product formulations.

Behentrimonium 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 (eye irritation-IrE)
  - Very High Ecotoxicity (acute aquatic-AA)

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

New Approach Methodologies (NAMs) used in this GreenScreen® include *in vitro* tests for genotoxicity, 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 behentrimonium chloride's NAMs dataset include the absence of experimental data for carcinogenicity and respiratory sensitization, and lack of established test methods for respiratory sensitization. Behentrimonium chloride's Type II (extrapolation output) uncertainties include limitations of *in vitro* genotoxicity assays to mimic *in vivo* metabolic conditions, VEGA tool not evaluating ionic substances, lack of defined applicability domains for OECD Toolbox and Toxtree, and lack of consideration of non-immunological mechanisms of respiratory sensitization.

GreenScreen® Hazard Summary for Behentrimonium 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**	*L*	*L*	DG	**L**		*M*		*L*	**L**	*L*	**H**	**vH**	**vH**	**H**	**L**	**M**	*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 Behentrimonium Chloride (CAS #17301-53-0)

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

GreenScreen® Assessment (v.1.4) Prepared By:
Name: Mitchell Kelly
Title: Toxicologist
Organization: ToxServices LLC
Date: November 14, 2024; February 18, 2025

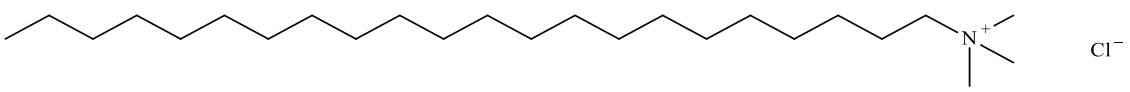
Quality Control Performed By:
Name: Bingxuan Wang, Ph.D., D.A.B.T.
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: November 15, 2024; February 20, 2025

Expiration Date: February 20, 2030[3]

Chemical Name: Behentrimonium Chloride

CAS Number: 17301-53-0

Chemical Structure(s):

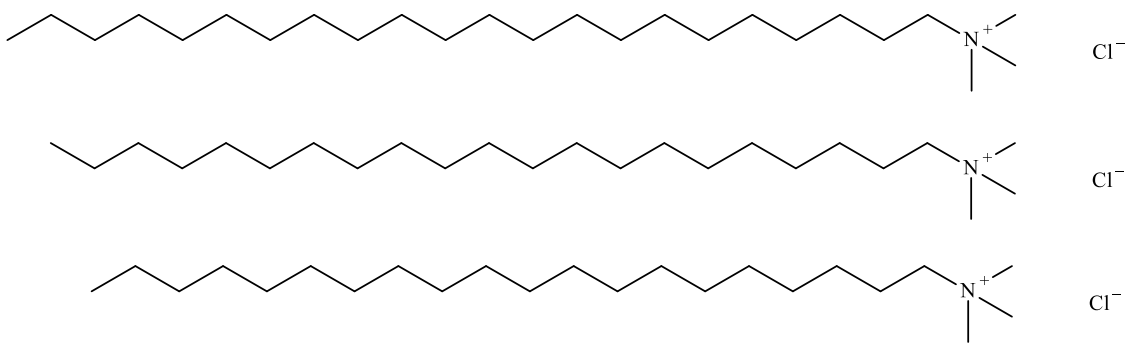


Also called: Docosyltrimethylammonium chloride; N,N,N-Trimethyldocosan-1-aminium chloride; behenyltrimethylammonium chloride (PubChem 2024).

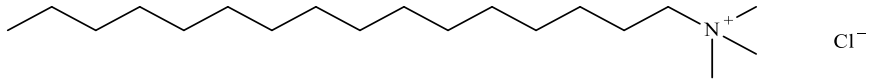
Suitable surrogates or moieties of chemicals used in this assessment (CAS #'s):
Limited data were available for behentrimonium chloride. Therefore, data on quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9) was used to fill data gaps. Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides is a mixture of cationic quaternary ammonium salts with alkyl chains of 20-22 carbons (ECHA CHEM, CAS #68607-24-9, 2024). As behentrimonium chloride is a cationic quaternary ammonium salt with an alkyl chain length of 22 carbons, quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides includes behentrimonium chloride in its mixture and is considered a very strong surrogate. Furthermore, behentrimonium chloride is listed as a synonym for quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides in the ECHA CHEM dossier for the latter.

Additionally, cetrimonium chloride (CAS #112-02-7) is included as a supplemental surrogate when necessary. Cetrimonium chloride is a cationic quaternary ammonium salt with an alkyl chain length of 16 carbons and was evaluated by the Scientific Committee on Consumer Safety (SCCS) in a group with behentrimonium chloride (SCCS 2009).

[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).



Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9) (representative structure)



Cetrimonium chloride (CAS #112-02-7)

Identify Applications/Functional Uses: (EC 2024)
1. Anti-static agent
2. Hair conditioning agent
3. Preservative

Known Impurities[4]:
Free amines and amine hydrochloride (SCCS 2009).

GreenScreen® Summary Rating for Behentrimonium Chloride[5,6 7,8]: Behentrimonium 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 (eye irritation-IrE)
    - Very High Ecotoxicity (acute aquatic-AA)

A data gap (DG) exists for endocrine activity-E. The lack of data for single exposure systemic toxicity and neurotoxicity are not considered data gaps due to sufficient data available for repeated dose systemic toxicity and neurotoxicity, respectively. As outlined in GreenScreen® Guidance (CPA 2018b) Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis) (CPA 2018b), behentrimonium chloride meets requirements for a GreenScreen Benchmark™ Score of 2 despite the hazard data gap. In a worst-case scenario, if behentrimonium chloride were assigned a High score for the data gap E, it would be categorized as a Benchmark 1 Chemical.

[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.

Figure 1: GreenScreen® Hazard Summary for Behentrimonium 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**	*L*	*L*	DG	**L**		*M*		*L*	**L**	*L*	**H**	**vH**	**vH**	**H**	**L**	**M**	*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 transformation products were identified. Based on the structure of surrogate quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9), the authors of the ECHA CHEM dossier suggest that hydrolysis will not occur, and that photochemical degradation of the compound in air or soil will not occur (ECHA CHEM, CAS #68607-24-9, 2024).

Introduction

Behentrimonium chloride, also known as docosyltrimethylammonium chloride and N,N,N-trimethyl-1-docosanaminium chloride, is a cationic quaternary ammonium salt with an alkyl chain of 22 carbons. It is a surfactant that functions as an anti-static agent, hair conditioning agent, and a preservative in personal care product formulations (EC 2024, PubChem 2024).

ToxServices assessed behentrimonium 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).

Behentrimonium chloride 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 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 behentrimonium chloride can be found in Appendix C.

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

- Behentrimonium chloride is an LT-UNK chemical when screened using Pharos, and therefore a full GreenScreen® is required.
- Behentrimonium chloride is not listed on the U.S. DOT list.
- Behentrimonium chloride is on the following list for multiple endpoints.
    - EC-CEPA DSL - Inherently Toxic in the Environment (iTE)

Hazard Statement and Occupational Control

No harmonized Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard statements were identified for behentrimonium chloride, and it does not have an ECHA registration dossier. Self-classifications by the majority of the ECHA notifiers are listed, below, 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 Behentrimonium Chloride (CAS #17301-53-0) (ECHA C&L, CAS #17301-53-0, 2024)

H Statement	H Statement Details
H315	Causes skin irritation (96.3% notifiers)
H319	Causes serious eye irritation (96% notifiers)
H400	Very toxic to aquatic life (86.5% notifiers)
H410	Very toxic to aquatic life with long-lasting effects (86.2% notifiers)

Table 2: Occupational Exposure Limits and Recommended Personal Protective Equipment for Behentrimonium Chloride (CAS #17301-53-0)

Personal Protective Equipment (PPE)	Reference	Occupational Exposure Limits (OEL)	Reference
Safety glasses, protective clothing, protective gloves, respiratory equipment	Interchimie 2021	Not identified	N/A

Physicochemical Properties of Behentrimonium Chloride

Behentrimonium chloride is a solid under normal conditions. It is a surfactant that is slightly water soluble and non-volatile based on surrogate data.

Table 3: Physical and Chemical Properties of Behentrimonium Chloride (CAS #17301-53-0)

Property	Value	Reference
Molecular formula	$C_{25}H_{54}N.Cl$	PubChem 2024
SMILES Notation	CCCCCCCCCCCCCCCCCCCCCCC[N+](C)(C)C.[Cl-]	PubChem 2024
Molecular weight	404.2 g/mol	PubChem 2024
Physical state	Solid	SCCS 2009
Appearance	Not identified	N/A
Melting point	60°C	Interchimie 2021
Boiling point	> 82°C	Interchimie 2021
Vapor pressure	7.0×10^{-5} Pa at 20°C*	ECHA CHEM, CAS #68607-24-9, 2024
Water solubility	Partially soluble 0.01 g/L (exp.)*	SCCS 2009

Table 3: Physical and Chemical Properties of Behentrimonium Chloride (CAS #17301-53-0)		
Property	**Value**	**Reference**
		ECHA CHEM, CAS #68607-24-9, 2024
Dissociation constant	Not identified	N/A
Density/specific gravity	900 kg/cm^3	CIR 2012
Partition coefficient	Accurate measure infeasible Log K_{ow} = 3.29 (calculated from solubility in octanol and water)*	SCCS 2009 ECHA CHEM, CAS #68607-24-9, 2024
Supplier, Tradename(s)	N/A	N/A
Ethoxylated or propoxylated?	No	PubChem 2024
# EO Units	N/A	N/A
# PO Units	N/A	N/A
EO/PO Ratio	N/A	N/A

***:** Data for the surrogate quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides.

Toxicokinetics

No data were available for absorption via the oral or inhalation routes, but dermal absorption data are available that indicate minimal absorption through the skin.

- ECHA CHEM, CAS #68607-24-9, 2024:
  - *In vitro:* In a GLP-compliant skin absorption test conducted according to OECD Guideline 428, donated human skin membranes were treated with a cream containing 3% behentrimonium chloride for 24 hours or 5% behentrimonium chloride for 30 minutes. The total absorption was 0.16% and 0.2%, respectively (Klimisch 1, reliable without restriction).

Hazard Classification Summary

Group I Human Health Effects (Group I Human)

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

Behentrimonium 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.
- U.S. EPA 2019, 2021
  - Behentrimonium 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, behentrimonium 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, it 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, behentrimonium 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

Behentrimonium chloride was assigned a score of Low for mutagenicity/genotoxicity based on negative results for mutagenicity *in vitro* with the target chemical and for clastogenicity *in vitro* with a strong surrogate. 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 measured data with the target compound and a strong 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.
- SCCS 2009
  - *In vitro:* Negative results for mutagenicity were obtained in a GLP-compliant reverse gene mutation assay in bacteria conducted according to OECD Guideline 471. *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 were exposed to the test material in the absence or presence of mammalian metabolic activation at concentrations of 0.8 – 5,000 µg/plate. The test substance did not lead to a relevant increase in the number of revertant colonies either with or without S9 mix.
- ECHA CHEM, CAS #68607-24-9, 2024
  - *Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9): 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 the test substance in water at 1.3-29.6 µg/mL with metabolic activation and 0.6-7.9 µg/mL without metabolic activation. No increase in the mutation frequency was detected following treatment in the presence or absence of metabolic activation (Klimisch 1, reliable without restriction).
  - *Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9): In vitro:* Negative results for clastogenicity were obtained in a GLP-compliant micronucleus test conducted according to a draft version OECD Guideline 487. Chinese hamster lung fibroblasts (V79) were exposed to the test substance in water at 0.8-400.0 µg/mL with and without metabolic activation. No increase in the frequency of

micronuclei was identified following treatment in the presence or absence of metabolic activation (Klimisch 1, reliable without restriction).

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

Behentrimonium chloride was assigned a score of Low for reproductive toxicity based on lack of reproductive effects in a GLP-compliant OECD Guideline 421 reproduction / developmental toxicity screening test in rats exposed to surrogate quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides. 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 it is based on data from a screening test.

- 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 #68607-24-9, 2024
  - *Oral: Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9):* In a GLP-compliant reproduction / developmental toxicity screening test, conducted in accordance with OECD Guideline 421, male and female Wistar rats (10/dose) received the test substance at doses of 0, 10, 30, or 75 mg/kg/day via gavage. Animals were exposed from 2 weeks before mating to postnatal day (PND) 4 for at least 28 days for males and approximately 7 week for females. There was no effect on reproductive performance and the authors assigned a NOAEL of 75 mg/kg/day for reproductive toxicity (Klimisch 1, reliable without restriction).
  - *Oral: Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9):* In a GLP-compliant OECD Guideline 407 study, Wistar rats (5/sex/group) were administered gavage doses of 0, 10, 50, or 150 mg/kg/day test substance in water for 28 days. In terms of organ weights, there were reduced absolute testes and epididymides weights and increased relative weights at the high dose. Upon histopathological examination, there were decreases in myometrial volume of the uterus and uterine glands of the uterus at the mid and high doses, cervix atrophy at the high dose , and reduced sizes of seminal vesicles at the high dose. Study authors established a NOAEL of 10 mg/kg/day and LOAEL of 50 mg/kg/day based on dose-dependent decreases in body weight, hematological parameters, biochemistry parameters, and organ weights (liver, kidney, heart, brain, and thymus), and various macroscopic and microscopic tissue change (lymph nodes, thymus, and lung) (Klimisch 1, reliable without restriction).
  - *Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9):* Dose range finding studies to the studies described above were not included as they were short in duration and less robust than the studies summarized above.
- Based on the weight of evidence, a score of Low was assigned. Although surrogate quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides caused organ weight changes and histopathological changes in male and female reproductive organs in a 28-day oral study, the same surrogate did not cause such effects in an OECD Guideline 421 reproductive/developmental toxicity screening study which also included macroscopic and microscopic examinations of reproductive organs, and additionally examined the reproductive functions in the same strain of rats. In addition, severe toxicity at the high dose was reported in the 28-day oral study. Therefore, ToxServices weighed the OECD Guideline 421 study more heavily than the subacute repeated dose toxicity study.

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): *L*
Behentrimonium chloride was assigned a score of Low for developmental toxicity based on lack of toxicologically significant developmental effects in an OECD Guideline 421 reproductive/developmental toxicity screening study and two GLP-compliant pre-natal developmental toxicity studies with surrogates. GreenScreen® criteria classify chemicals as a Low hazard for developmental toxicity when adequate negative data are available and they are not GHS classified (CPA 2018b). The confidence in the score is low due to slight, but not toxicologically significant reduction in offspring body weight observed at slightly maternally toxic doses in two of the three 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 CHEM, CAS #68607-24-9, 2024
  - *Oral: Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9):* In the previously described GLP-compliant reproduction / developmental toxicity screening test, conducted in accordance with OECD Guideline 421, male and female Wistar rats (10/dose) received the test substance at doses of 0, 10, 30, or 75 mg/kg/day via gavage. Animals were exposed from 2 weeks before mating to postnatal day (PND) 4 for at least 28 days for males and approximately 7 week for females. Mid and high dose males and high dose females of the parental generation had decreased food consumption, and high dose males and females had reduced body weight and body weight gain. Pup weight on PND 4 and pup weight gain were also decreased at the high dose. However, these weight changes were not statistically significant and study authors attributed them to nursing problems due to lower maternal food consumption at this dose. The ECHA CHEM dossier authors assigned a NOAEL of 30 mg/kg/day and LOAEL of 75 mg/kg/day for parental systemic toxicity based on reduced body weight and weight gain, and for developmental toxicity based on reduced viability, body weight, and weight gain of the offspring (Klimisch 1, reliable without restriction).
    - Based on the data table presented, the changes in mean body weight of pups on PND 1 and PND 4, and in pup viability at the highest dose was not statistically significant.
- ECHA CHEM, CAS #112-02-7, 2024
  - *Oral: Surrogate: Cetrimonium chloride (CAS #112-02-7):* 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).
- ECHA CHEM, CAS #112-02-7, 2024, SCCS 2009
  - *Dermal: Surrogate: Cetrimonium chloride (CAS #112-02-7):* 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 the test substance in water at 0, 10, 20, or 40 mg/kg/day on gestation days 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 2, reliable with restrictions).

- Based on the weight of evidence, a score of Low was assigned. Reduced offspring viability and body weight/weight gain that were not statistically significant were found at a maternally toxic dose in an OECD Guideline 421 reproductive/developmental toxicity screening test in rats with surrogate quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides. Non-significant reduction in fetal body weight was also reported at a dose that caused slight but not adverse maternal effects in an oral prenatal developmental toxicity study in rats with the surrogate cetrimonium chloride. Therefore, ToxServices did not consider the reduced offspring viability/weight development to be toxicologically significant.

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

Behentrimonium chloride was assigned a score of Data Gap for endocrine activity based on the lack of sufficient data identified 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 and parameters examined (AGD) were too 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
  - Behentrimonium 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: Surrogate: Cetrimonium chloride (CAS #112-02-7):* 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).

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

Behentrimonium chloride was assigned a score of Low for acute toxicity based on an oral LD_{50} value > 2,000 mg/kg for surrogate quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides . GreenScreen® criteria classify chemicals as a Low hazard for acute toxicity when oral LD_{50} values are greater than 2,000 mg/kg (CPA 2018b). The confidence in the score is high as it is based on measured data for strong surrogates.

- Authoritative and Screening Lists
    - *Authoritative:* Not present on any authoritative lists for this endpoint.
    - *Screening:* GHS-Australia: H302 - Harmful if swallowed.
        - Basis unspecified
    - *Screening:* GHS-Australia: H312 - Harmful in contact with skin.
        - Basis unspecified
- ECHA CHEM, CAS #68607-24-9, 2024
    - *Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9): Oral:* LD_{50} = 3,190 mg/kg (female Wistar rats) (Klimisch 2, reliable with restrictions).
- ECHA CHEM, CAS #112-02-7, 2024
    - *Surrogate: Cetrimonium chloride (CAS #112-02-7): Oral:* LD_{50} = 2,410 mg/kg (Wistar rats) (equivalent to 699 mg/kg active ingredient (a.i.), GLP-compliant, OECD Guideline 401) (Klimisch 1, reliable without restriction).
    - *Surrogate: Cetrimonium chloride (CAS #112-02-7): Oral:* LD_{50} > 5,000 mg/kg (equivalent to 250 mg a.i./kg, Sprague-Dawley rats) (Klimisch 2, reliable with restrictions).
- SCCS 2009
    - *Surrogate: Cetrimonium chloride (CAS #112-02-7): Oral:* LD_{50} = 2,000 mg/kg (equivalent to 480-520 mg a.i./kg, Sprague-Dawley rats[10]).
- Based on the weight of evidence, a score of Low was assigned. An oral study on surrogate quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides reported low acute toxicity (i.e., LD_{50} > 2,000 mg/kg), while three oral studies on the other surrogate cetrimonium chloride reported moderate acute toxicity (LD_{50} between 300 and 2,000 mg/kg a.i.). As quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides is more similar in structure to the target chemical, it is a stronger surrogate compared to cetrimonium chloride, which is smaller than the target chemical and hence likely more toxic/reactive. Therefore, ToxServices relied on the data for quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides to score this endpoint. Although Australia classified it to GHS Category 4 for acute oral and dermal toxicities, the basis of these classifications was unknown. Therefore, ToxServices discounted these classifications.

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

Behentrimonium chloride was assigned a score of Data Gap for systemic toxicity (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 CHEM, CAS #112-02-7, 2024
    - *Surrogate: Cetrimonium chloride (CAS #112-02-7): Oral:* In a GLP-compliant, OECD Guideline 401 acute toxicity study, Wistar rats (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). Study authors calculated an LD_{50} of 2,410 mg/kg, which is equivalent to 699 mg a.i./kg. Clinical signs observed included quiet behavior, hunched posture, closed eyelids, retracted flanks, rough fur, paleness, noisy unregular breathing, myosis and slimy feces. However, the doses at which these effects were observed were not

[10] 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.

reported. No gross pathological changes were noted in surviving animals (Klimisch 1, reliable without restriction).

- SCCS 2009
    - <u>*Surrogate: Cetrimonium chloride (CAS #112-02-7):*</u> *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 weight of evidence, a score of Data Gap was assigned. The only acute toxicity study for the stronger surrogate quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides did not provide details other than mortality to evaluate this endpoint. The other surrogate cetrimonium chloride induced clinical signs of toxicity and/or body weight changes, which either occurred at a lethal dose of 2,000 mg/kg, or at unspecified doses. Therefore, a LOAEL could not be established, but is likely < 2,000 mg/kg, which warrants at least GHS Category 2 classification (LOAEL between 300 and 2,000 mg/kg). Surrogate cetrimonium chloride is irritating to the respiratory tract based on limited human data that might warrant GHS Category 3 classification for respiratory irritation. However, as explained in the acute toxicity section above, cetrimonium chloride is likely more toxic than behentrimonium chloride, and has a higher irritation potential. Therefore, a definitive GHS classification for behentrimonium chloride could not be determined.

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

Behentrimonium chloride was assigned a score of Moderate for systemic toxicity (repeated dose) based on ToxServices classifying a surrogate quaternary ammonium compounds mixture to GHS Category 2. GreenScreen® criteria classify chemicals as a Moderate hazard for systemic toxicity (repeated dose) when classified to GHS Category 2 (CPA 2018b). The confidence in the score is low as the NOAEL of 10 mg/kg/day and the LOAEL of 50 mg/kg/day straddle the adjust GHS Category 1 guidance value of 30 mg/kg/day.

- 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 #68607-24-9, 2024
    - <u>*Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9):*</u> *Oral:* In a GLP-compliant OECD Guideline 407 study, Wistar rats (5/sex/group) were administered gavage doses of 0, 10, 50, or 150 mg/kg/day test substance in water for 28 days. The authors assigned a NOAEL of 10 mg/kg/day and a LOAEL of 50 mg/kg/day based on dose-dependent decreases in body weight, hematological parameters, biochemistry parameters, and organ weights (liver, kidney, heart, brain, and thymus), and various macroscopic and microscopic tissue change (lymph nodes, thymus, and lung) in the mid and high dose treatment groups (Klimisch 1, reliable without restriction).
        - *As the study duration was less than 90 days, ToxServices modified the GHS guidance values of 10 and 100 mg/kg/day for an oral study (UN 2023) by a factor of 3 (28 days is approximately one-third of 90 days) to 30 and 300 mg/kg/day, respectively.*

Since the LOAEL of 50 mg/kg/day is greater than 30 mg/kg/day but less than 300 mg/kg/day, ToxServices classified behentrimonium chloride as a Category 2 oral repeated dose toxicant based on the results of the above study.

Neurotoxicity (single dose, N-single) (Group II) Score (vH, H, M, or L): DG
Behentrimonium 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 CHEM, CAS #112-02-7, 2024
  - *Surrogate: Cetrimonium chloride (CAS #112-02-7): Oral:* In a GLP-compliant, OECD Guideline 401 acute toxicity study, Wistar rats (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). Study authors calculated an LD_{50} of 2,410 mg/kg, which is equivalent to 699 mg a.i./kg. Clinical signs observed included quiet behavior, hunched posture, closed eyelids, retracted flanks, rough fur, paleness, noisy unregular breathing, myosis and slimy feces. However, the doses at which these effects were observed were not reported. No gross pathological changes were noted in surviving animals (Klimisch 1, reliable without restriction).
- SCCS 2009
  - *Surrogate: Cetrimonium chloride (CAS #112-02-7): 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. No gross pathological changes were noted in surviving animals.
- 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 surrogate 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. In addition, as previously described, cetrimonium chloride is a conservative surrogate. Therefore, data are insufficient to score this endpoint.

Neurotoxicity (repeated dose, N-repeated) (Group II*) Score (H, M, or L): *L*
Behentrimonium chloride was assigned a score of Low for neurotoxicity (repeated dose) based on lack of neurobehavior effects in a 28-day oral study in rats exposed to surrogate quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides. GreenScreen® criteria classify chemicals as a Low hazard for neurotoxicity (repeated dose) when negative studies are available and they are not classified under GHS (CPA 2018b). The confidence in the score is low as the NOAEL of 150 mg/kg/day (top dose tested) is below the duration-adjusted GHS Category 2 guidance value of 300 mg/kg/day for 28-day 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 CHEM, CAS #68607-24-9, 2024

- *Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9): Oral:* In a GLP-compliant OECD Guideline 407 study, Wistar rats (5/sex/group) were administered gavage doses of 0, 10, 50, or 150 mg/kg/day test substance in water for 28 days. A battery of functions was tested, including hearing ability, pupillary reflex, static righting reflex, grip strength, and motor activity tests. No effects were observed in these tests (Klimisch 1, reliable without restriction). ToxServices identified a NOAEL of 150 mg/kg/day for neurotoxicity, which was the highest dose tested.

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

Behentrimonium chloride was assigned a score of Low for skin sensitization based on negative results in two Buehler tests. GreenScreen® criteria classify chemicals as a Low hazard for skin sensitization 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 measured data for the target compound.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- SCCS 2009
  - Behentrimonium chloride was not sensitizing in a GLP-compliant OECD Guideline 406 Buehler test with female Pirbright albino guinea pigs (n=20) induced with 20% topical occlusive patches and challenged with a 0.8% topical occlusive patch. No reactions were observed in any animal after challenge.
  - In a second a GLP-compliant OECD Guideline 406 Buehler test, Dunkin Hartley guinea pigs (10/sex) were induced with 10% topical occlusive patches and challenged with a 0.5% topical occlusive patch. Reactions were noted upon challenge but there were no reactions observed upon a rechallenge. The study authors attributed the reactions observed in the first challenge to the irritating effects of behentrimonium chloride.

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

Behentrimonium 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 adequate data are available and 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
  - Behentrimonium 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 behentrimonium 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 behentrimonium chloride, and as behentrimonium chloride does not contain any structural alerts for respiratory sensitization (OECD 2024), behentrimonium chloride is not expected to be a respiratory sensitizer.

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

Behentrimonium chloride was assigned a score of High for skin irritation/corrosivity based on a study in rabbits that warrant GHS Category 2 classification for the surrogate quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides. GreenScreen® criteria classify chemicals as a High hazard for skin irritation/corrosivity when they are classified by GHS Category 2 (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data on a strong 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.
    - Basis not specified
- SCCS 2009
  - In a GLP-compliant OECD Guideline 404 dermal irritation study, male New Zealand White rabbits (n=3) were administered 10% behentrimonium chloride under semi-occlusion for 3 minutes. Individual animal scores were not reported but the mean 24/48/72-hour erythema and edema scores were 0.4 and 0, respectively.
  - In a GLP-compliant OECD Guideline 404 dermal irritation study, male New Zealand White rabbits (n=3) were administered 6.25% behentrimonium chloride under semi-occlusion for 3 minutes. Individual animal scores were not reported but the mean 24/48/72-hour erythema and edema scores were both 0.
- ECHA CHEM, CAS #68607-24-9, 2024
  - *Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9):* In a GLP-compliant acute dermal irritation study conducted according to OECD Guideline 404, New Zealand White rabbits (3 males) received 0.5 g of the test substance moistened with water on the shaved skin under semiocclusion for 4 hours, and were observed for 14 days afterwards. The mean scores across 24, 48 and 72 hours for edema were 1, 1.7, and 0.7 for each of the three animals, and for erythema were 3, 2.7, and 2. All effects were reversible by 14 days. Study authors concluded that the test substance was irritating to the skin (GHS Category 2) (Klimisch 1, reliable without restriction).
    - *According to GHS criteria, the mean erythema or edema scores across 24, 48, and 72 hours of ⩾ 2.3 and < 2.4 in at least 2 of the 3 tested animals warrant classification of Category 2 (UN 2023).*
- Based on the weight of evidence, a score of High was assigned. While data were available for behentrimonium chloride, neither of the two rabbit studies tested up to 100% (they only tested up to 10%), and the exposure duration of 3 minutes is significantly shorter than 4 hours required by OECD Guideline 404. The strong surrogate quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides was tested undiluted in a guideline study in rabbits and the results support a GHS Category 2 (H315) classification. This is consistent with the self-classifications by the majority of the ECHA notifiers for behentrimonium chloride (ECHA C&L, CAS #17301-53-0, 2024). Therefore, ToxServices primarily relied on data for the surrogate to score this endpoint.

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

Behentrimonium chloride was assigned a score of Very High for eye irritation/corrosivity based on irreversible effects in a rabbit study conducted on the surrogate quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides that warrant classification to GHS Category 1. GreenScreen® criteria

classify chemicals as a Very High hazard for eye irritation/corrosivity when a GHS Category 1 classification is warranted (CPA 2018b). The confidence in the score is high as it is based on reliable experimental data for a strong 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.
- SCCS 2009
  - In a GLP-compliant OECD Guideline 405 eye irritation study, male New Zealand White rabbits (n=3) were instilled with 10% behentrimonium chloride for 30 seconds before rinsing. Individual animal scores were not reported but the mean 24/48/72-hour corneal opacity, iris, conjunctiva, and chemosis scores were 0.6, 0.3, 2.3, and 2.2, respectively. Irritation persisted for 22 days.
    - *Although individual animal scores were not reported, behentrimonium chloride can be conservatively classified to GHS Category 2A based on conjunctiva and chemosis scores greater than 2 with effects lasting for more than 7 days.*
  - In a GLP-compliant OECD Guideline 405 eye irritation study, male New Zealand White rabbits (n=3) were instilled with 6.25% behentrimonium chloride for 30 seconds before rinsing. Individual animal scores were not reported but the mean 24/48/72-hour corneal opacity, iris, conjunctiva, and chemosis scores were 0.7, 0.6, 2.6, and 2.3, respectively. Irritation persisted for 15 days.
    - *Although individual animal scores were not reported, behentrimonium chloride can be conservatively classified to GHS Category 2A based on conjunctiva and chemosis scores greater than 2 with effects lasting for more than 7 days.*
  - In a GLP-compliant OECD Guideline 405 eye irritation study, male New Zealand White rabbits (n=3) were instilled with 3.75% behentrimonium chloride for 30 seconds before rinsing. Individual animal scores were not reported but the mean 24/48/72-hour corneal opacity, iris, conjunctiva, and chemosis scores were 0, 0, 0.3, and 0.6, respectively.
- ECHA CHEM, CAS #68607-24-9, 2024
  - *Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9):* In a GLP-compliant acute eye irritation study conducted according to OECD Guideline 405, one male New Zealand White rabbits were exposed to 64.9 mg (~0.1 mL) of the unchanged test substance on the eye for 24 hours without washing. Severe ocular lesions occurred, including discoloration of the eyelid, nictating membrane, and changes of the outside of the eyelids. The animal was humanely sacrificed at 24 hours. Study authors concluded that the test substance was corrosive to the eyes (GHS Category 1) (Klimisch 1, reliable without restriction).
- Based on the weight of evidence, a score of Very High was assigned. The available rabbit studies on behentrimonium chloride warrant classification to GHS Category 2A, but the highest concentration tested was only 10%, and the exposure duration was only 10 seconds with washing, deviating from OECD Guideline 405 which requires 24 hours of contact time without washing. A guideline study with undiluted strong surrogate quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides indicate that a GHS Category 1 classification is more appropriate.

Ecotoxicity (Ecotox)

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

Behentrimonium chloride was assigned a score of Very High for acute aquatic toxicity based on the lowest L/EC_{50} value of 0.5-1 mg/L in fish for the surrogate quaternary ammonium compounds, C20-22-

alkyltrimethyl, chlorides. This is also consistent with the self-classification of GHS Category 1 by the majority of the ECHA notifiers for behentrimonium chloride (ECHA C&L, CAS #17301-53-0, 2024). GreenScreen® criteria classify chemicals as a Very High hazard for acute aquatic toxicity when acute aquatic toxicity values are < 1 mg/L (CPA 2018b). The confidence in the score is high as it is based on measured data for all trophic levels for a strong 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 #68607-24-9, 2024
  - *Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9):* 96-hr LC_{50} (*Danio rerio*, zebrafish) = 3.5 mg/L (measured concentration, GLP-compliant, OECD Guideline 203) (Klimisch 1, reliable without restriction).
  - *Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9):* 96-hr LC_{50} (*D. rerio*, zebrafish) = 0.5-1 mg/L (nominal, OECD Guideline 203) (Klimisch 2, reliable with restrictions).
  - *Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9):* 48-hr mobility EC_{50} (*Daphnia magna*) = 1.39 mg/L (nominal, GLP-compliant, OECD Guideline 202) (Klimisch 1, reliable without restriction).
  - *Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9):* 72-hr growth rate EC_{50} (*Desmodesmus subspicatus*) = 3.48 mg/L (measured, GLP-compliant, OECD Guideline 201) (Klimisch 1, reliable without restriction).

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

Behentrimonium chloride was assigned a score of High for chronic aquatic toxicity based on the lowest chronic value of 0.119 mg/L in daphnia. GreenScreen® criteria classify chemicals as a High hazard for chronic aquatic toxicity when chronic toxicity values are > 0.1 to 1 mg/L (CPA 2018b). The confidence in the score is high based on reliable experimental data for all three trophic levels for a strong 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 #68607-24-9, 2024
  - *Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9):* 9-day mortality NOEC (*D. rerio*, zebrafish) = 0.24 mg/L (measured, GLP-compliant, OECD Guideline 212) (Klimisch 1, reliable without restriction).
  - *Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9):* 21-day reproduction NOEC (*D. magna*) = 0.119 mg/L (measured, GLP-compliant, OECD Guideline 211) (Klimisch 1, reliable without restriction).
  - *Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9):* 72-hr growth rate ErC_{10} (*D. subspicatus*) = 0.927 mg/L (measured, GLP-compliant, OECD Guideline 201) (Klimisch 1, reliable without restriction).

Environmental Fate (Fate)

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

Behentrimonium chloride was assigned a score of Low for persistence based on being readily biodegradable but failing to meet the 10-day window in an OECD Guideline 301 B study. GreenScreen® criteria classify chemicals as a Low hazard for persistence when they have half-lives < 16

days or meet the pass level in ready biodegradation tests without meeting the 10-day window (CPA 2018b). The confidence in the score is high based on measured data for the target compound.

- 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 #68607-24-9, 2024
  - In a GLP-compliant ready biodegradability test conducted according to OECD Guideline 301 B (Ready Biodegradability: CO2 Evolution Test), behentrimonium chloride reached 80% degradation in 28 days with domestic activated sludge (adaption not specified) as the inoculum. It was concluded that the test substance was readily biodegradable but did not meet the 10-dau window in this study (Klimisch 1, reliable without restriction).
  - In a GLP-compliant water: sediment simulation test conducted according to OECD Guideline 308 (aerobic and anaerobic transformation in aquatic sediment systems), fresh natural water/ sediment was exposed to radiolabeled behentrimonium chloride at 99 µg/L (low organic carbon sediment) or 103 µg/L (high organic carbon sediment) for 100 days at 12°C. Degradation was measured by CO_2 evolution, radioactivity monitoring, and HPLC analytical testing. The DT50 (water) was determined to be 4.16 days and 1.19 days, respectively, in the two test systems, and the DT50 (total system) was 29 days and 56.7 days, respectively (Klimisch 1, reliable without restriction).
  - In a GLP-compliant water degradation simulation test conducted according to OECD Guideline 309, natural freshwater was exposed to radiolabeled behentrimonium chloride at 91.65 or 10.68 µg/L for 62 days at 12°C. Degradation was measured by CO_2 evolution, radioactivity monitoring, and HPLC analytical testing. The DT50 was determined to be 101 and 166 days for the high and low application rates, respectively (Klimisch 1, reliable without restriction).
  - In a GLP-compliant soil degradation simulation test conducted according to OECD Guideline 307, three representative field soils (silt loam, clay, and loam) were exposed to radiolabeled behentrimonium chloride at 0.2 mg a.i./kg dry soil for 124 days at 20°C. DT50 was determined to be 23.2, 24, and 41.4 days in the three samples (Klimisch 1, reliable without restriction).
- Based on the weight of evidence, a score of Low was assigned. There were no environmental distribution data, and modeling is not feasible for surfactants with EPI Suite™. However, based on the low vapor pressure, behentrimonium chloride is not expected to mainly partition to the air. In soil, sediment, or water, meeting the pass level in a ready biodegradability test but failing the 10-day window corresponds to a Low score. ToxServices prioritized ready biodegradation data over measured half-lives (DT50) in surface water, soil, and sediment from simulation tests.

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

Behentrimonium chloride was assigned a score of Moderate for bioaccumulation based on a BCF value of 691 for surrogate quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides. GreenScreen® criteria classify chemicals as a Moderate hazard for bioaccumulation when BCF values are between 500 and 1,000 (CPA 2018b). The confidence in the score is high as it is based on measured data for a strong 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 #68607-24-9, 2024

- *Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9):* In a GLP-compliant bioconcentration test conducted according to OECD Guideline 305, *D. rerio* (zebrafish) were exposed to the test substance for 42 days at either 3.97 or 15.9 µg/L. The BCF values were 351 at 15.9 µg/L and 691 at 3.97 µg/L (Klimisch 1, reliable without restriction).

Physical Hazards (Physical)

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

Behentrimonium chloride was assigned a score of Low for reactivity based on structural analysis indicating that it is not explosive and does not have oxidizing potential, and measured data indicating that it is not explosive or self-heating. GreenScreen® criteria classify chemicals as a Low hazard for reactivity when the substance does not require GHS classification for any of the reactivity endpoints (CPA 2018b). The confidence in the score was low as no measured data were 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.
- No measured data were identified. Therefore, screening procedures for explosivity were used here to estimate the reactivity property of behentrimonium chloride. These procedures are listed in the GHS (UN 2023).
  - Based on the structure of its components or moieties, behentrimonium 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, behentrimonium 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. 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 behentrimonium chloride has 0 oxygen, classification is not warranted.

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

Behentrimonium chloride was assigned a score of Low for flammability based on surrogate quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides being non-flammable in a guideline study. GreenScreen® criteria classify chemicals as a Low hazard for flammability when no GHS classification is available (CPA 2018b). The confidence in the score was high based on measured data for a strong 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 #68607-24-9, 2024
  - *Surrogate: Quaternary ammonium compounds, C20-22-alkyltrimethyl, chlorides (CAS #68607-24-9):* In an EU Method A.10 flammability study, ignition of the test substance was not possible and was concluded to be non-flammable (Klimisch 1, reliable without restriction).

Use of New Approach Methodologies (NAMs)[11] in the Assessment, Including Uncertainty Analyses of Input and Output

New Approach Methodologies (NAMs) used in this GreenScreen® include *in vitro* tests for genotoxicity, 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 behentrimonium chloride's NAMs dataset include the absence of experimental data for carcinogenicity and respiratory sensitization, and lack of established test methods for respiratory sensitization. Behentrimonium chloride's Type II (extrapolation output) uncertainties include limitations of *in vitro* genotoxicity assays to mimic *in vivo* metabolic conditions, VEGA tool not evaluating ionic substances, lack of defined applicability domains for OECD Toolbox and Toxtree, and lack of consideration of non-immunological mechanisms of respiratory sensitization.

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

Uncertainty Analyses (OECD 2020)	
Type I Uncertainty: Data/Model Input	**Carcinogenicity**: No experimental data are available. **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 2023). **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[12]. 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*

[11] 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).

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

	metabolism (i.e., the liver S9 mix contains enzymes present in the endoplasmic reticulum but not the cytosol of liver cells).[13] **Respiratory sensitization**: The OECD Toolbox only identifies structural alerts, and does not define applicability domains. Additionally, the ECHA guidance (2017), on which the use of OECD Toolbox structural alerts is based, does not evaluate non-immunologic mechanisms for respiratory sensitization.	
Endpoint	**NAMs Data Available and Evaluated? (Y/N)**	**Types of NAMs Data (*in silico* modeling/*in vitro* biological profiling/frameworks)**
Carcinogenicity	Y	*In silico* modeling: Toxtree/Danish QSAR
Mutagenicity	Y	*In vitro* data: Bacterial reverse mutation assay/*in vitro* gene mutation assay/*in vitro* micronucleus 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	
Chronic aquatic toxicity	N	
Persistence	Y	Non-animal testing: OECD Guideline 301 Biodegradation tests
Bioaccumulation	N	

[13] https://www.oecd-ilibrary.org/docserver/9789264264809-en.pdf?expires=1614097800&id=id&accname=guest&checksum=C0DE371FB9C5A878E66C9AB7F84E6BBE

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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 Behentrimonium Chloride (CAS #17301-53-0)

TOXSERVICES
TOXICOLOGY RISK ASSESSMENT CONSULTING

GreenScreen® Score Inspector

Table 1: Hazard Table

			Group I Human					Group II and II* Human								Ecotox		Fate		Physical	
			Carcinogenicity	Mutagenicity/Genotoxicity	Reproductive Toxicity	Developmental Toxicity	Endocrine Activity	Acute Toxicity	Systemic Toxicity		Neurotoxicity		Skin Sensitization*	Respiratory Sensitization*	Skin Irritation	Eye Irritation	Acute Aquatic Toxicity	Chronic Aquatic Toxicity	Persistence	Bioaccumulation	Reactivity	Flammability
Table 2: Chemical Details									S	R *	S	R *	*	*								
Inorganic Chemical?	Chemical Name	CAS#	C	M	R	D	E	AT	STs	STr	Ns	Nr	SNS*	SNR*	IrS	IrE	AA	CA	P	B	Rx	F
No	Behentrimonium chloride	17301-53-0	*L*	**L**	*L*	*L*	DG	**L**	DG	*M*	DG	*L*	**L**	*L*	**H**	**vH**	**vH**	**H**	**L**	**M**	*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
Behentrimonium chloride	2

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

Table 6

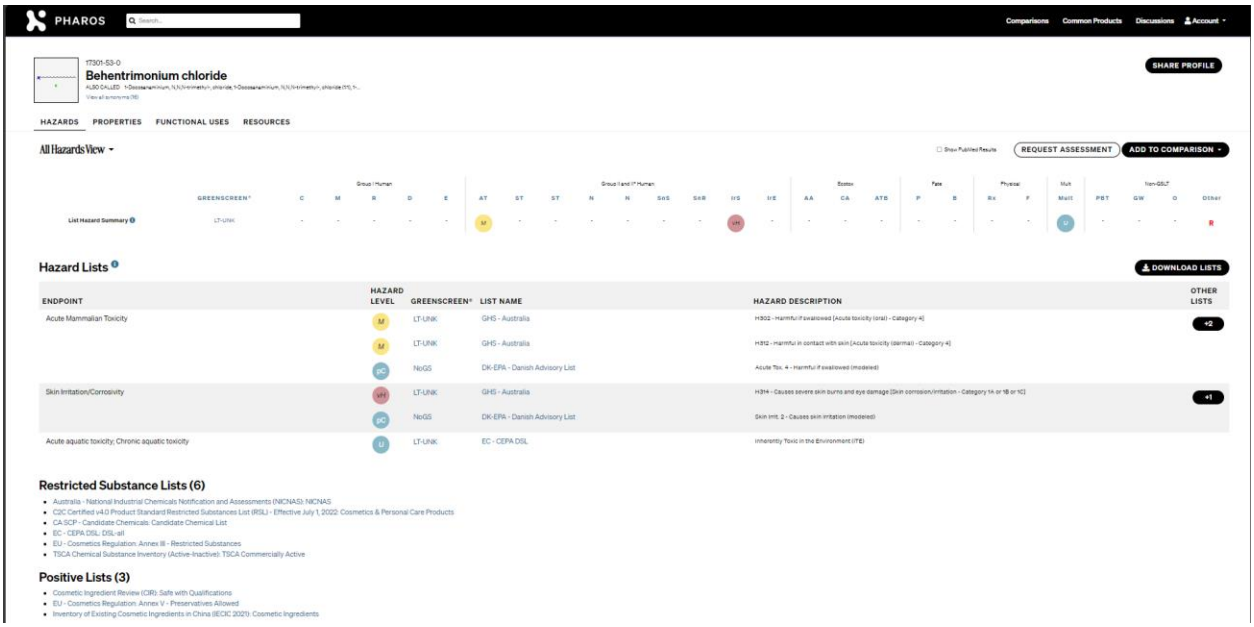
Chemical Name	Final GreenScreen® Benchmark Score
Behentrimonium chloride	2

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

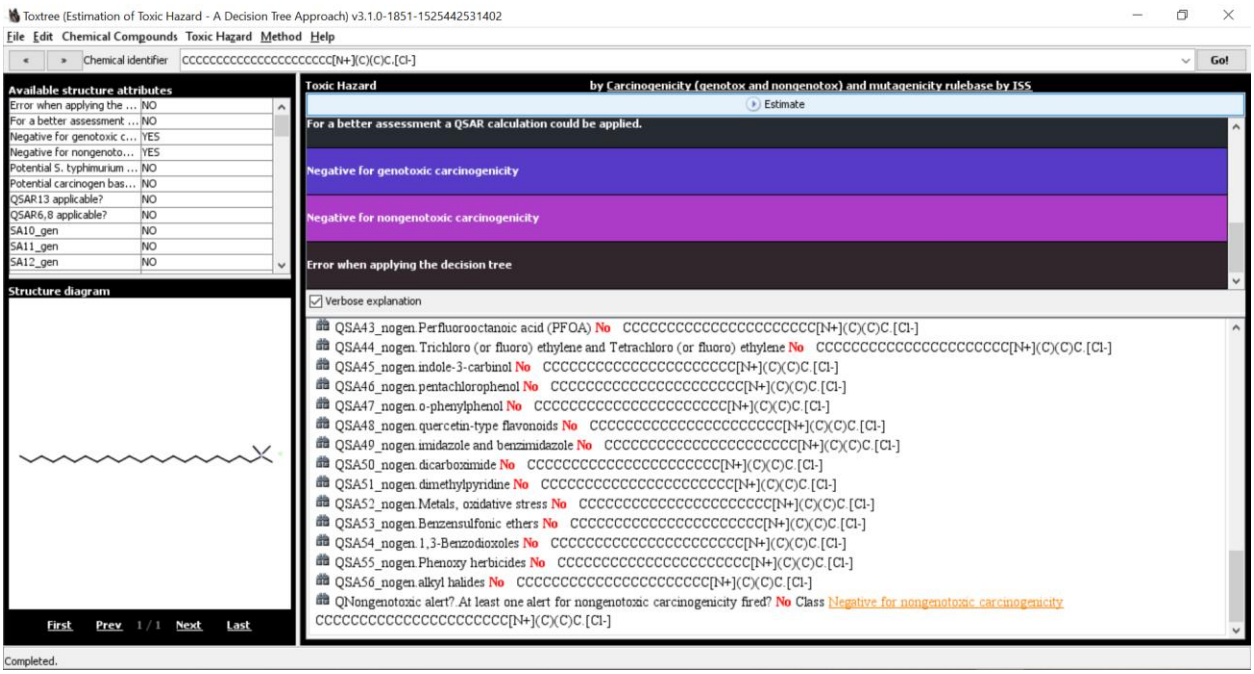
Table 5: Data Gap Assessment Table

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 Behentrimonium Chloride (CAS #17301-53-0)



APPENDIX D: Toxtree Carcinogenicity Results for Behentrimonium Chloride (CAS #17301-53-0)



APPENDIX E: Danish QSAR Carcinogenicity Results for Behentrimonium Chloride (CAS #17301-53-0)

Carcinogenicity

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

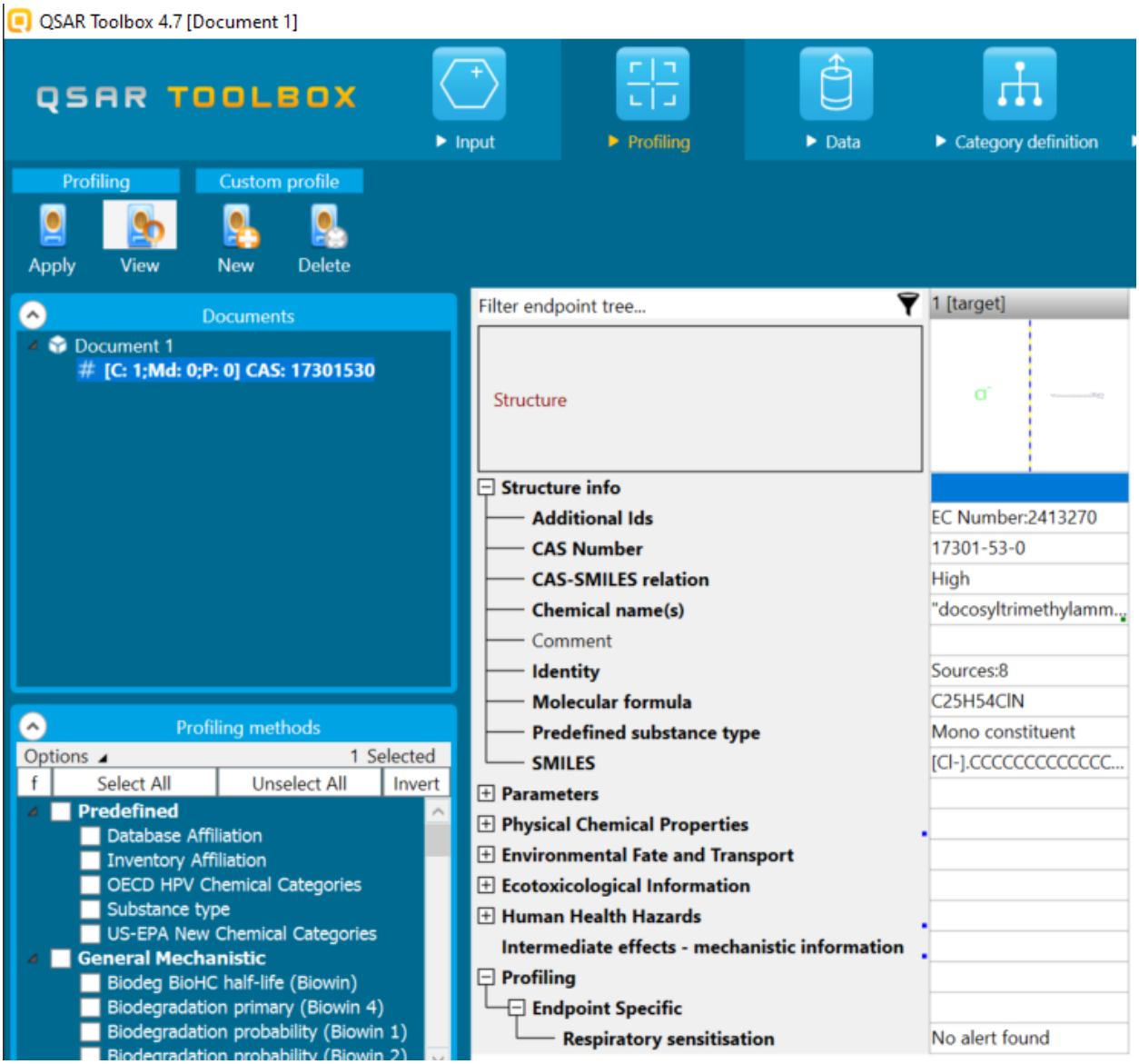
Commercial models from CASE Ultra and Leadscope

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

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

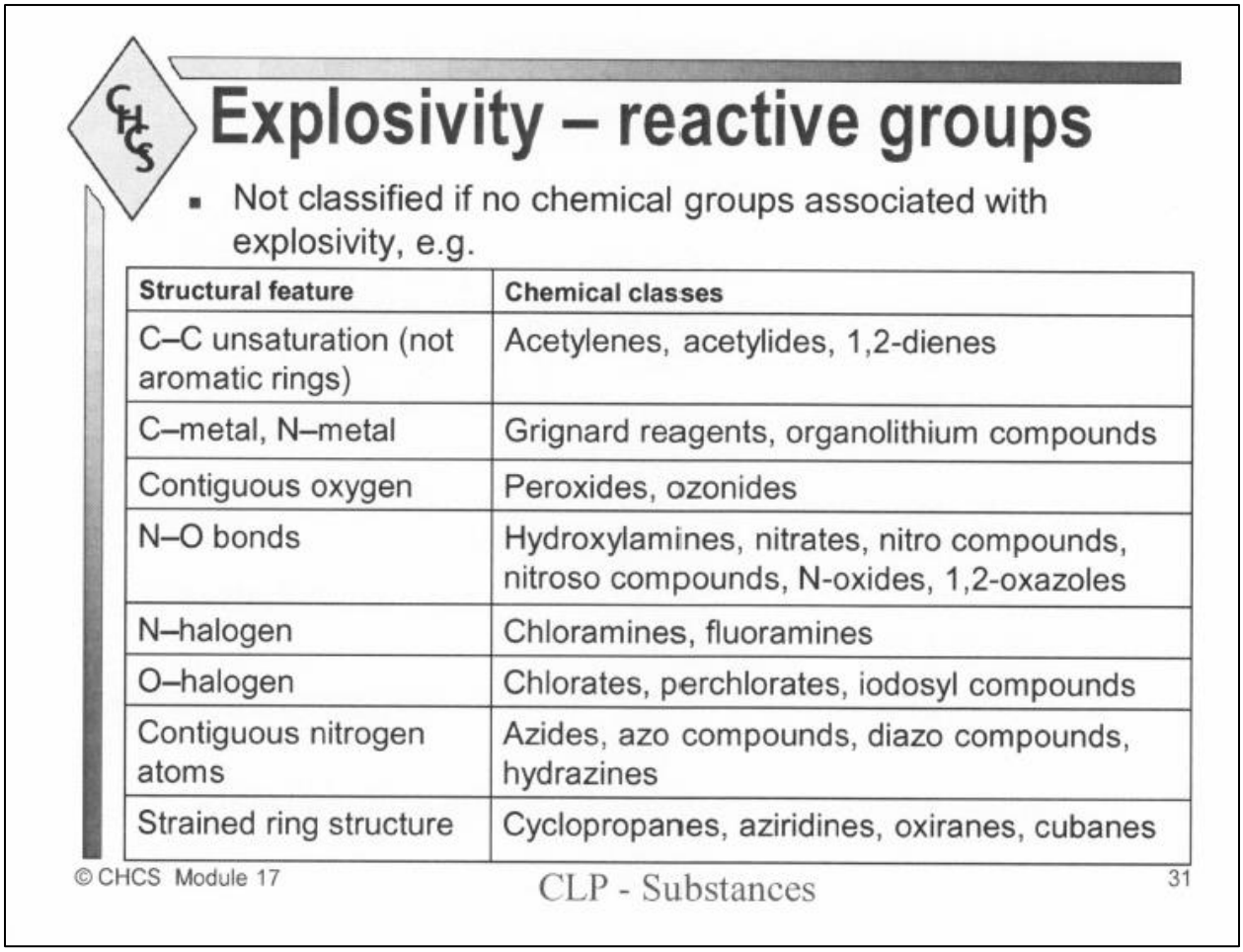
DTU-developed models

APPENDIX F: QSAR Toolbox Respiratory Sensitization Modeling Results for Behentrimonium Chloride (CAS #17301-53-0)



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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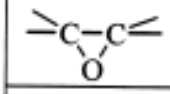
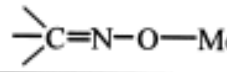
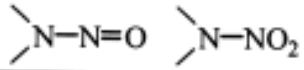
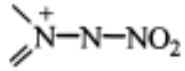
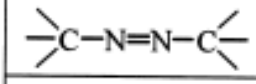
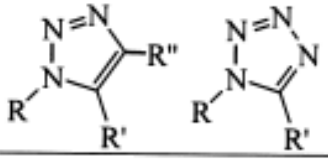
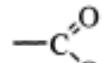
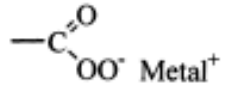
Explosivity – Full List

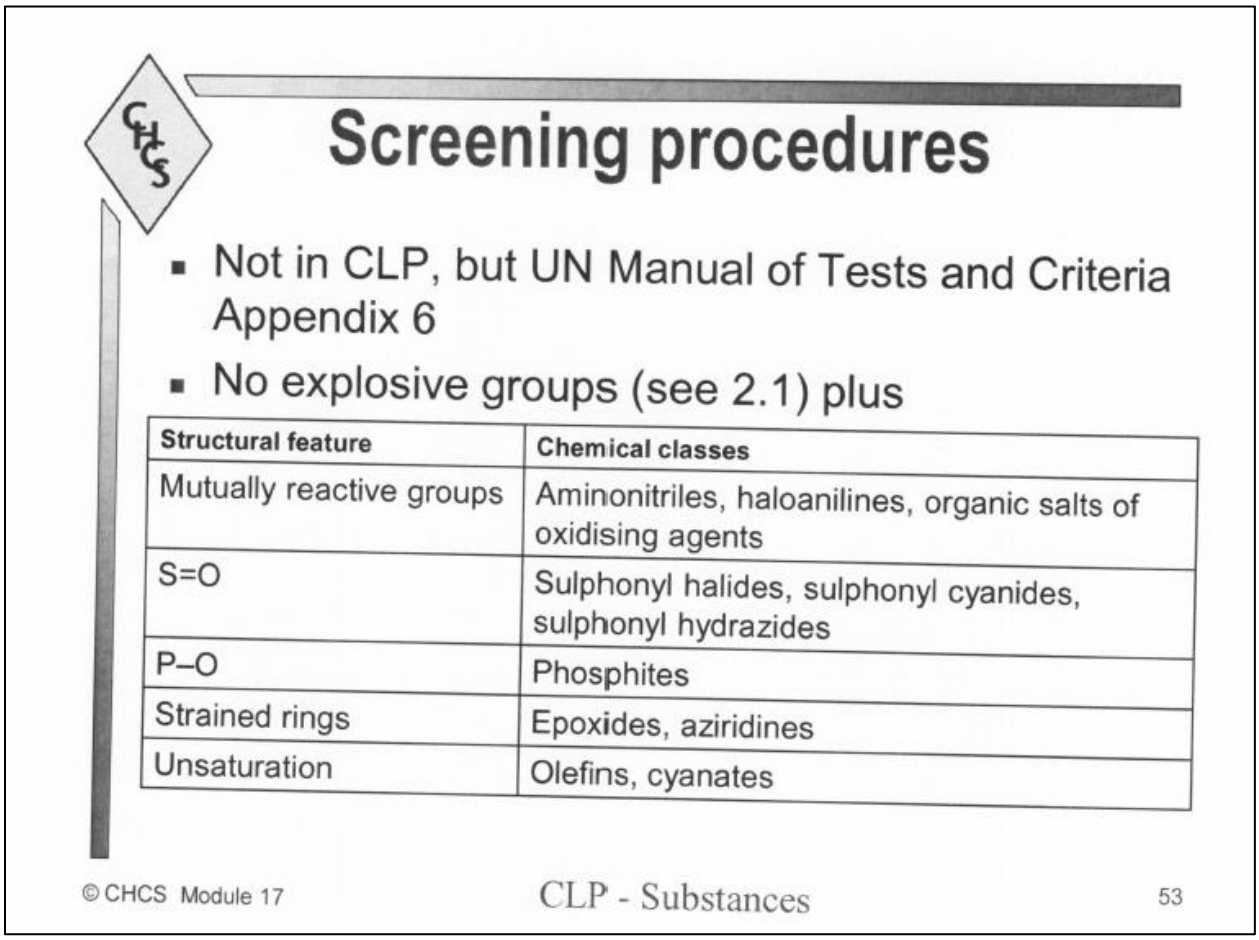
Table R.7.1-28 Chemical groups associated with explosive properties

Chemical group	Chemical Class
-C≡C- -C≡C-Metal -C≡C-Halogen	Acetylenic Compounds Metal Acetylides Haloacetylene Derivatives
	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 H: Change in Benchmark Score

Table 5 provides a summary of changes to the GreenScreen® Benchmark™ for behentrimonium chloride. This is a new GreenScreen® assessment.

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

Date	GreenScreen® Benchmark™	GreenScreen® Version	Comment
November 15, 2024	BM-2	v. 1.4	Original GreenScreen® assessment.
February 20, 2025	B<-2	v. 1.4	Updated to address Washington Department of Ecology comments. No change in hazard scores or benchmark scores.

Licensed GreenScreen® Profilers

Behentrimonium Chloride GreenScreen® Evaluation Prepared by:

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ToxServices LLC

Behentrimonium Chloride GreenScreen® Evaluation QC'd by:

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