CHITOSAN

(CAS #9012-76-4)

GREENSCREEN® FOR SAFER CHEMICALS (GREENSCREEN®) ASSESSMENT

Prepared by:

ToxServices LLC

Assessment Date: March 18, 2024

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TABLE OF CONTENTS

GreenScreen® Executive Summary for Chitosan (CAS #9012-76-4) i
Chemical Name 1
GreenScreen® Summary Rating for Chitosan 2
Environmental Transformation Products 3
Introduction 3
U.S. EPA Safer Choice Program's Safer Chemical Ingredients List 3
GreenScreen® List Translator Screening Results 4
Hazard Statement and Occupational Control 4
Physicochemical Properties of Chitosan 4
Toxicokinetics 5
Hazard Classification Summary 6
- Group I Human Health Effects (Group I Human) 6
  - Carcinogenicity (C) Score 6
  - Mutagenicity/Genotoxicity (M) Score 6
  - Reproductive Toxicity (R) Score 7
  - Developmental Toxicity incl. Developmental Neurotoxicity (D) Score 9
  - Endocrine Activity (E) Score 10
- Group II and II* Human Health Effects (Group II and II* Human) 10
  - Acute Mammalian Toxicity (AT) (Group II) Score 10
  - Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-single) (Group II) Score 10
  - Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-repeat) (Group II*) Score 11
  - Neurotoxicity (single dose, N-single) (Group II) Score 14
  - Neurotoxicity (repeated dose, N-repeated) (Group II*) Score 14
  - Skin Sensitization (SnS) (Group II*) Score 14
  - Respiratory Sensitization (SnR) (Group II*) Score 15
  - Skin Irritation/Corrosivity (IrS) (Group II) Score 15
  - Eye Irritation/Corrosivity (IrE) (Group II) Score 16
- Ecotoxicity (Ecotox) 16
  - Acute Aquatic Toxicity (AA) Score 16
  - Chronic Aquatic Toxicity (CA) Score 17
- Environmental Fate (Fate) 18
  - Persistence (P) Score 18
  - Bioaccumulation (B) Score 19
- Physical Hazards (Physical) 19
  - Reactivity (Rx) Score 19
  - Flammability (F) Score 20

Use of New Approach Methodologies (NAMs) in the Assessment, Including Uncertainty Analyses of Input and Output 21
References 24
APPENDIX A: Hazard Classification Acronyms 27
APPENDIX B: Results of Automated GreenScreen® Score Calculation for Chitosan (CAS #9012-76-4) 28
APPENDIX C: Pharos Output for Chitosan (CAS #9012-76-4) 29
APPENDIX D: ToxCast Model Results for Chitosan (CAS #9012-76-4) 30
APPENDIX E: OECD Toolbox Respiratory Sensitization Results for Chitosan (CAS #9012-76-4) 31
APPENDIX F: Change in Benchmark Score 32
Licensed GreenScreen® Profilers 33

TABLE OF FIGURES

Figure 1: GreenScreen® Hazard Summary Table for Chitosan 3

TABLE OF TABLES

Table 1: GHS H Statements for Chitosan (CAS #9012-76-4) 4
Table 2: Occupational Exposure Limits and Recommended Personal Protective Equipment for Chitosan (CAS #9012-76-4) 4
Table 3: Physical and Chemical Properties of Chitosan (CAS #9012-76-4) 4
Table 4: Summary of NAMs Used in the GreenScreen® Assessment, Including Uncertainty Analyses 21
Table 5: Change in GreenScreen® Benchmark™ for Chitosan 32

GreenScreen® Executive Summary for Chitosan (CAS #9012-76-4)

Chitosan is a polycationic carbohydrate polymer derived from the deacetylation of chitin, which is a naturally occurring polymer and structural component found in the exoskeleton of arthropods and in the cells walls of fungi and yeast. It is produced by treating seafood shells with a 3-5% sodium hydroxide solution and neutralizing the product with 3-5% hydrochloric acid; deacetylation is done by treatment with a 40-45% sodium hydroxide solution and washing the precipitate with water and dissolving the precipitate in an aqueous 2% acetic acid solution. It can also be produced from the non-viable post-fermentation microbial biomass of fungi and molds such as *Aspergillus niger*. Chitosan is a non-flammable yellow powder at standard temperature and pressure. It varies in molecular weight (~3 – 3,600 kDa) and degree of acetylation (40% - 100%). Technical grade chitosan has a viscosity of 250-600 mPa.s and ~70-80% deacetylation while grades for cosmetic or dietary use have viscosities of 10-100 mPa.s and 85-92% deacetylation.

Chitosan was assigned a **GreenScreen Benchmark™ Score of U** ("Unspecified Due to Insufficient Data"). Prior to the Data Gap Analysis, it was assigned a preliminary score of 2 ("Use but Search for Safer Substitutes"). This preliminary score is based on the following hazard score combinations:

- Benchmark 2f
    - Very High Ecotoxicity (acute aquatic-AA and chronic aquatic-CA)

Data gaps (DG) exist for reproductive toxicity-R, developmental toxicity-D, endocrine activity-E, and neurotoxicity (repeated dose)-Nr*. As outlined in GreenScreen® Guidance Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), chitosan does not meet requirements for a GreenScreen Benchmark™ Score of 2 due to the hazard data gaps. In a worst-case scenario, if chitosan were assigned a High score for the data gaps R, D, or E, it would be categorized as a Benchmark 1 Chemical.

New Approach Methodologies (NAMs) used in this GreenScreen® include *in silico* modeling for endocrine activity and respiratory sensitization, and *in vitro* testing for mutagenicity, skin sensitization, skin irritation, eye irritation, and use of acute to chronic ratios for aquatic toxicity. 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 chitosan's NAMs dataset include lack of experimental data for endocrine activity, and respiratory sensitization, lack of validated test methods for respiratory sensitization, and the availability of *in vitro* data for skin and eye irritation endpoints. Chitosan's Type II (extrapolation output) uncertainties include the lack of a defined applicability domain in ToxCast models, limitation of *in vitro* genotoxicity assays in mimicking *in vivo* metabolism, their focusing on one or only a few types of genotoxicity events, the limitation of OECD Toolbox in identifying structural alerts for respiratory sensitization without accounting for non-immunologic mechanisms of respiratory sensitization, and limitations of *in vitro* skin and eye irritation assays to differentiate between GHS Category classifications. Some of chitosan's type II uncertainties were alleviated by the use of *in vitro* test batteries and/or in combination of *in vivo* data.

GreenScreen® Hazard Summary Table for Chitosan

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	DG	DG	**L**	**L**	**L**	*L*	DG	**L**	*L*	**L**	**L**	*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 Chitosan (CAS #9012-76-4)

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

GreenScreen® Assessment (v.1.4) Prepared By:
Name: Rachel Doerer, M.P.H.
Title: Toxicologist
Organization: ToxServices LLC
Date: January 19, 2024, March 12, 2024

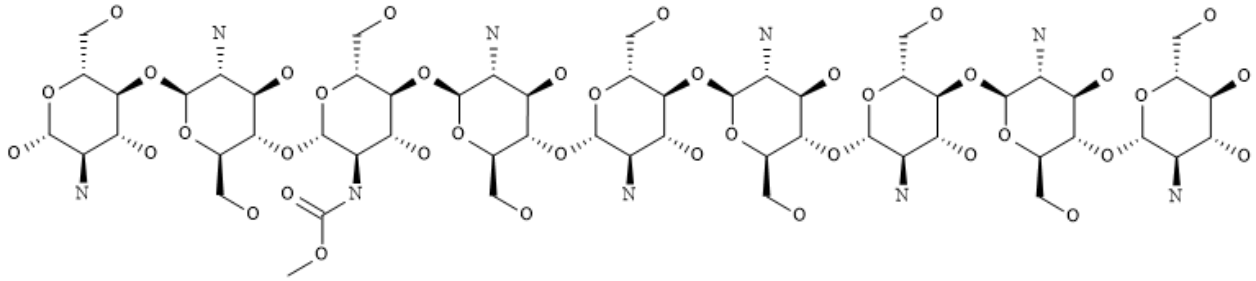
Quality Control Performed By:
Name: Jennifer Rutkiewicz, Ph.D.
Title: Senior Toxicologist
Organization: ToxServices LLC
Date: March 18, 2024

Expiration Date: March 18, 2029[2]

Chemical Name: Chitosan

CAS Number: 9012-76-4

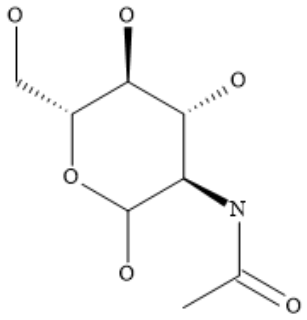
Chemical Structure(s):



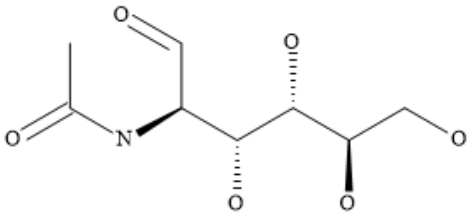
Also called:
Poliglusam (PubChem 2024), 2-Amino-2-deoxy-beta-D-glucosamine; Deacetylated chitin; poly (D-glucosamine) (NTP 2017)

Suitable surrogates or moieties of chemicals used in this assessment (CAS #'s):
Chitosan is a polycationic carbohydrate polymer with the primary units, D-glucosamine and N-acetyl-D-glucosamine, linked by 1-4 glycosidic bonds (NTP 2017). Therefore, data for the monomeric unit N-acetyl glucosamine (CAS #7512-17-6) were used to fill data gaps when available.

[1] GreenScreen® reports are either "UNACCREDITED" (by unaccredited person), "AUTHORIZED" (by Authorized GreenScreen® Practitioner), or "CERTIFIED" (by Licensed GreenScreen® Profiler or equivalent).
[2] Assessments expire five years from the date of completion starting from January 1, 2019. An assessment expires three years from the date of completion if completed before January 1, 2019 (CPA 2018a).



cyclic



open chain

N-Acetyl Glucosamine (CAS #7512-17-6)

Identify Applications/Functional Uses: (NTP 2017)
1. Dietary supplement
2. Flocculating agent
3. Chelating agent
4. Plant growth regulator
5. Antimicrobial agent
6. Wound dressing
7. Skin cleansing agent

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

GreenScreen® Summary Rating for Chitosan[4,5 6,7]: Chitosan was assigned a **GreenScreen Benchmark™ Score of U** ("Unspecified Due to Insufficient Data") (CPA 2018b). This preliminary score is based on the following hazard score combinations:

- Benchmark 2f
    - Very High Ecotoxicity (acute aquatic-AA and chronic aquatic-CA)

Data gaps (DG) exist for reproductive toxicity-R, developmental toxicity-D, endocrine activity-E, and neurotoxicity (repeated dose)-Nr*. As outlined in GreenScreen® Guidance (CPA 2018b) Section 11.6.2.1 and Annex 5 (Conduct a Data Gap Analysis), chitosan does not meet requirements for a GreenScreen Benchmark™ Score of 2 due to the hazard data gaps. In a worst-case scenario, if chitosan were assigned a High score for the data gap s R, D, or E, it would be categorized as a Benchmark 1 Chemical.

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

Figure 1: GreenScreen® Hazard Summary Table for Chitosan

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	DG	DG	**L**	**L**	**L**	*L*	DG	**L**	*L*	**L**	**L**	*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.

Environmental Transformation Products

No transformation products were identified. 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 chitosan is readily biodegradable, it is not expected to have relevant transformation products.

Introduction

Chitosan is a polycationic carbohydrate polymer derived from the deacetylation of chitin, which is a naturally occurring polymer and structural component found in the exoskeleton of arthropods and in the cells walls of fungi and yeast. The primary units of chitosan, D-glucosamine and N-acetyl-D-glucosamine, are linked by 1-4 glycosidic bonds. Chitosan varies in molecular weight (~3 – 3,600 kDa) and degree of acetylation (40% - 100%). Solubility is obtained at approximately 50% deacetylation, but also depends on molecular weight and distribution of the remaining acetyl groups (NTP 2017). Viscosity of the chitosan substance is determined by chain length. Technical grade chitosan has a viscosity of 250-600 mPa.s and ~70-80% deacetylation while grades for cosmetic or dietary use have viscosities of 10-100 mPa.s and 85-92% deacetylation (Ashfords 2024).

Chitosan is produced by treating seafood shells with a 3-5% sodium hydroxide solution and neutralizing the product with 3-5% hydrochloric acid; deacetylation is done by treatment with a 40-45% sodium hydroxide solution and washing the precipitate with water and dissolving the precipitate in an aqueous 2% acetic acid solution (NTP 2017). It can also be produced from the non-viable post-fermentation microbial biomass of fungi and molds such as *Aspergillus niger* (KitoZyme 2011).

ToxServices assessed chitosan 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 2023). 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).

Chitosan is listed on the U.S. EPA SCIL as an antimicrobial active and preservative and antioxidant with a full green circle.

GreenScreen® List Translator Screening Results

The GreenScreen® List Translator identifies specific authoritative or screening lists that should be searched to identify GreenScreen Benchmark™ 1 chemicals (CPA 2018b). Pharos (Pharos 2024) is an online list-searching tool that is used to screen chemicals against all of the lists in the List Translator electronically. ToxServices also checks the U.S. Department of Transportation (U.S. DOT) lists (U.S. DOT 2008a,b),[8] which are not considered GreenScreen® Specified Lists but are additional information sources, in conjunction with the Pharos query. The output indicates benchmark or possible benchmark scores for each human health and environmental endpoint. The output for chitosan can be found in Appendix C.

- Chitosan is an LT-UNK chemical when screened using Pharos, and therefore a full GreenScreen® is required.
- Chitosan is not listed on the U.S. DOT list.
- Chitosan is not on any lists for multiple endpoints. Specified lists for single endpoints are reported in individual hazard endpoints in the hazard assessment section below.

Hazard Statement and Occupational Control

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

Table 1: GHS H Statements for Chitosan (CAS #9012-76-4)

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

Table 2: Occupational Exposure Limits and Recommended Personal Protective Equipment for Chitosan (CAS #9012-76-4)

Personal Protective Equipment (PPE)	Reference	Occupational Exposure Limits (OEL)	Reference
Safety glasses with side shields or goggles; protective gloves and protective clothing	Santa Cruz Biotechnology 2019	None identified	N/A

Physicochemical Properties of Chitosan

Chitosan is a yellow powder at standard temperature and pressure. It is available in several forms with molecular weight ranging from ~3 – 3,600 kDa. The composition of chitosan may vary by manufacturer, depending on the degree of deacetylation. Solubility is obtained at approximately 50% deacetylation, but also depends on molecular weight and distribution of the remaining acetyl groups

Table 3: Physical and Chemical Properties of Chitosan (CAS #9012-76-4)

Property	Value	Reference
Molecular formula	$(C_6H_{11}NO_4)_n$	KitoZyme 2011

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

Table 3: Physical and Chemical Properties of Chitosan (CAS #9012-76-4)		
Property	**Value**	**Reference**
SMILES Notation	COC(=O)N[C@@H]1[C@H]([C@@H]([C@H](O[C@H]1O[C@@H]2[C@H](O[C@H]([C@@H]([C@H]2O)N)O[C@@H]3[C@H](O[C@H]([C@@H]([C@H]3O)N)O)CO)CO)CO)O[C@H]4[C@@H]([C@H]([C@@H]([C@H](O4)CO)O[C@H]5[C@@H]([C@H]([C@@H]([C@H](O5)CO)O[C@H]6[C@@H]([C@H]([C@@H]([C@H](O6)CO)O[C@H]7[C@@H]([C@H]([C@@H]([C@H](O7)CO)O[C@H]8[C@@H]([C@H]([C@@H]([C@H](O8)CO)O[C@H]9[C@@H]([C@H]([C@@H]([C@H](O9)CO)O)O)N)O)N)O)N)O)N)O)N)O)N)O	PubChem 2024
Molecular weight	Variable	
Physical state	Solid	PubChem 2024
Appearance	Yellow powder	PubChem 2024
Melting point	102.5°C	Sigma Aldrich 2023
Boiling point	Not identified	
Vapor pressure	Not identified	
Water solubility	Dependent on deacetylation, molecular weight, and distribution of remaining acetyl groups	NTP 2017
Dissociation constant	Not identified	
Density/specific gravity	1 g/cm^3	Sigma Aldrich 2023
Partition coefficient	Not identified	

Toxicokinetics

Chitosan has poor absorption due to its insoluble chemical properties (KitoZyme 2011). Systemic absorption and distribution of chitosan is influenced by the molecular weight. When Sprague-Dawley rats were administered 20 mg/kg chitosan with molecular weights of 3.8, 7.5, 13, 22, or 230 kDa via gavage, maximum plasma concentrations (C_{max}) peaked at 30 minutes after administration and were 20.23, 9.30, 5.86, 4.32, and <0.5 µg/mL, respectively. This suggests there is low bioavailability of high molecular weight chitosan polymers (NTP 2017, KitoZyme 2011). Female Kunming mice were administered 500 mg/kg fluorescein isothiocyanate-labeled chitosan preparations via gavage as either chitosan oligomer (0.99 kDa), M-chitosan (32.7 kDa), water-soluble chitosan (39.1 kDa), or H-chitosan (760 kDa) and blood samples were taken at 30, 60, 120, and 240 minutes. Absorption was inversely related to the molecular weight with water-soluble chitosan having the highest absorption (KitoZyme 2011). Poor absorption is also supported by *in vitro* studies in which chitosan with molecular weights of 30 kDa or higher were not taken up by intestinal epithelial Caco-2 cells (KitoZyme 2011). However, the *in vitro* and *in vivo* studies have limitations in the study design that include high variability in the labeling of chitosan preparations and the lack of control groups (KitoZyme 2011).

Metabolism of chitosan is suggested to be dependent on the degree of deacetylation, with enzymatic degradation of chitosan depending on the ability to hydrolyze glucosamine-glucosamine, glucosamine-N-acetyl-glucosamine and N-acetyl-glucosamine-N-acetyl-glucosamine linkages (NTP 2017). Chitosan contains beta-glycosidic bonds, which are resistant to hydrolysis by acids in the stomach, but these

bonds may be hydrolyzed by chitosanases to form chitosan oligomers. It is unknown if other digestive enzymes including pepsin, amylase, and lipase are able to hydrolyze chitosan (KitoZyme 2011). Lysozymes and bacterial enzymes in the colon are thought to be the predominant enzymes involved in chitosan degradation (NTP 2017); however, metabolism by endogenous gut microflora is also limited (KitoZyme 2011). There have been eight human chitinases identified, however, their capacity to degrade chitosan has not been investigated (NTP 2017).

Although significant digestion is not expected, putative hydrolysis products are proposed that include chitosan oligomers, glucosamine, N-acetylglucosamine and glucose, all of which are excreted (KitoZyme 2011). Intraperitoneal administration of a dose of 29 mg/kg fluorescein isothiocyanate-labeled chitosan (50% deacetylation) to male ddy mice (n=3) resulted in ~25% of the dose excreted in the urine within 1 hour; nearly the entire dose was accounted for in 14 hours (KitoZyme 2011).

Following intravenous administration of three preparations of [^{125}I]-labeled chitosan (<5 kDa, 5-10 kDa, and >10 kDa) to male Wistar rats, chitosan with molecular weights > 5 kDa had <10% of the administered dose recovered in the plasma and > 50% recovered in the liver at 60 minutes following injection. In contrast, chitosan with molecular weight < 5 kDa had 30% recovered in the plasma and 30% recovered in the liver at 60 minutes after administration (KitoZyme 2011).

Hazard Classification Summary

Group I Human Health Effects (Group I Human)

Carcinogenicity (C) Score (H, M, or L): L
Chitosan was assigned a score of Low for carcinogenicity based on negative results in carcinogenicity studies in rats with the monomeric unit N-acetyl glucosamine. GreenScreen® criteria classify chemicals as a Low hazard for carcinogenicity when adequate negative data are available and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on experimental data for 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.
- NTP 2017
  - *Oral: Surrogate: N-Acetyl Glucosamine (CAS #7512-17-6):* No associated increases in tumor response were observed in F344 rats were fed up to 5% N-acetyl-D-glucosamine in the diet (1,935 mg/kg per day in males and 2,244 mg/kg per day in females) for 104 weeks. No additional details were provided.
  - *Oral: Surrogate: N-Acetyl Glucosamine (CAS #7512-17-6):* No associated increases in tumor response were observed in F344 rats were fed up to 5% N-acetyl-D-glucosamine in the diet (2,323 mg/kg per day in males and 2,545 mg/kg per day in females) for 52 weeks. No additional details were provided.

Mutagenicity/Genotoxicity (M) Score (H, M, or L): L
Chitosan was assigned a score of Low for mutagenicity/genotoxicity based on consistently negative results in *in vitro* and *in vivo* genotoxicity assays. GreenScreen® criteria classify chemicals as a Low hazard for mutagenicity/genotoxicity when negative data are available for both gene mutations and chromosome aberrations, and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on experimental data for the target substance.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- KitoZyme 2011
  - *In vitro:* Chitosan was not mutagenic in an OECD Guideline 471 bacterial reverse mutation assay. *Salmonella Typhimurium* stains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2 pKM101 were exposed to the test substance (highly pure medical grade chitosan derived from *Agaricus bisporus*) at doses of 0, 10, 33, 100, 333, and 1,000 µg/plate with and without metabolic activation. There were no increases in the frequency of revertants in any strain at any concentration.
  - *In vitro:* Chitosan oligomers were not mutagenic in a bacterial reverse mutation assay (guideline not specified). *S. typhimurium* stains TA97, TA98, TA100, and TA102 were exposed to the test substance (derived from shrimp, 1.86 kDa, 85% deacetylation) at doses of 0.5, 5, 50, 500, and 5,000 µg/plate with and without metabolic activation. There were no increases in the frequency of revertants in any strain at any concentration.
  - *In vivo:* Chitosan oligosaccharide were not genotoxic in a micronucleus test in Kunming mice. Animals (5/sex/dose) were administered a single dose of the test substance (derived from shrimp, 1.86 kDa, 85% deacetylation) at doses of 1,200, 2,500, and 5,000 mg/kg via oral gavage. There were no differences in the frequencies of micronuclei in mice.
  - *In vivo:* Chitosan oligosaccharide were not genotoxic in a sperm abnormality test in male Kunming mice. Animals were administered a single dose of the test substance (derived from shrimp, 1.86 kDa, 85% deacetylation) at doses of 1,200, 2,500, and 5,000 mg/kg via oral gavage. There were no differences in frequency of mouse sperm abnormalities.
- KitoZyme 2011, CCRIS 2006
  - *In vivo:* Chitosan oligomers were not genotoxic in male ICR mice (20/group) given 0.01, 0.1, and 1% test substance (<10 kDa, 90% deacetylation) in drinking water for up to 180 days. The authors estimated that 1% w/v was equivalent to 10 mg/kg/day chitosan oligosaccharides. Treated and control mice showed no difference in frequency of micronuclei and chromosomal aberrations. There was also no difference in chromosomal aberrations between treated and control mice in F1, F2, and F3 generations given chitosan oligomers for 180 days.

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

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- KitoZyme 2011
  - *Oral:* Chitosan (water soluble, ~300 kDa, >90% deacetylation) was evaluated at an oral dose of 480 mg/kg/day in B6C3F1 female mice that were induced to ovulate. Animals were dosed daily for 4 days. Chitosan treatment resulted in an increase in the number of ovulated oocytes and normal oocytes and fertilization rates compared to control. The authors suggested that chitosan may improve ovary and oviduct function in mice. A NOAEL cannot be identified since only one dose was evaluated and because the relevance of the effect on ovulation is unclear.
  - *Oral:* Chitosan oligosaccharides were evaluated in four generations of ICR mice given 0.01, 0.1, and 1% in drinking water for up to 180 days. The authors estimated that 1% w/v was

equivalent to 10 mg/kg/day chitosan oligosaccharides. The F1, F2, and F3 generations were given drinking water containing 0, 0.01, 0.1, or 1% chitosan oligomers for 180 days. Although reproductive and developmental endpoints were not specifically examined in this study, the authors did not report any apparent toxicity. Therefore, ToxServices identified a NOAEL of 10 mg/kg/day, the highest dose tested in mice. No additional details were provided for this study.

- *Oral:* In a subchronic study conducted according to Japanese Ministry of Health Guidelines with F344 rats (n=10/sex/group) fed chitosan oligosaccharide in the diet at concentrations of 0.04, 0.2, or 1% for 90 days, histopathological effects at 1% included Sertoli cell vacuolization, unilaterally decreased germ cell production, and luminal cell debris, which the authors speculated was the result of decreased zinc and vitamins A and E, which are known to induce testicular atrophy and inhibit spermatogenesis. The NOAEL and LOAEL for male reproductive organ effects are 0.2% and 1% in the diet, which is equivalent to 124 mg/kg/day (according to study authors) and approximately 620 mg/kg/day (extrapolating proportionally from the reported dose at 0.2%).

- NTP 2017
  - In a multigenerational prenatal and postnatal assessment of high molecular weight chitosan, pregnant ICR mice (number/dose not specified) were administered the test substance as a single intraperitoneal injection at doses of 0, 125, 500, or 2,000 mg/kg on gestation day (GD) 6. Animals were subject to a laparotomy or allowed to litter. Offspring from the same exposure group were mated and similarly subject to a laparotomy or allowed to litter. High dose P generation females exhibited mortality and diarrhea, and there was dose-dependent increases in vaginal bleeding, postimplantation loss, and lower spleen weights in P generation females at doses ≥ 500 mg/kg; P generation females also displayed a dose-dependent reduction in litter size. Reduced fetal body weights were reported in both generations at the high dose; however, there were no external, visceral, or skeletal malformation in offspring from either generation. F1 offspring from high dose exposed dams had high uterus, ovary, and thymus weights on postnatal day (PND) 21, and lower thymus weights on PND 56 (females only). F2 offspring from high dose exposed dams had decreased testis and ovary weights on PNDs 21 and 56.
    - *As this study was a single exposure study via intraperitoneal injections, which is not a standard route of exposure for GreenScreen evaluations, the results of this study were not weighed heavily.*

- Based on the weight of evidence, a Data Gap was assigned as the available data are insufficient to evaluate effects on mating and fertility and conclusively determine if there would, or would not, be adverse effects on reproductive parameters following exposure to chitosan. One oral multigeneration study in mice did not report any adverse effects, but did not specifically evaluate reproductive toxicity (i.e., alterations to reproductive systems, adverse effects on onset of puberty, gamete production, and transport, reproductive cycle normality, sexual behavior, fertility, parturition, pregnancy outcomes, or premature reproductive senescence (UN 2023)). A subchronic oral study in rats reported effects on spermatogenesis in males, but the authors speculated the effects may be due to nutritional deficiencies resulting from reduced vitamin absorption, and similar effects were not reported in other repeated dose studies. Finally, a multigeneration study in mice that exposed animals intraperitoneally found some effects on fetal weights, postimplantation loss, and litter size, but the study involved only a single exposure, and the intraperitoneal route is of limited relevance. GHS Guidance states studies involving routes such as intraperitoneal injection must be interpreted with caution and on their own would not normally be the basis for classification (UN 2023). ToxServices did not identify available data for the surrogates acetyl glucosamine or

glucosamine that would help to classify this endpoint. Overall, the available data are insufficient to evaluate effects on mating and fertility.

Developmental Toxicity incl. Developmental Neurotoxicity (D) Score (H, M, or L): DG
Chitosan was assigned a score of Low for developmental toxicity based on lack of sufficient data for this endpoint.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- KitoZyme 2011
  - *Oral:* Chitosan oligosaccharides were evaluated in four generations of ICR mice given 0.01, 0.1, and 1% in drinking water for up to 180 days. The authors estimated that 1% w/v was equivalent to 10 mg/kg/day chitosan oligosaccharides. The F1, F2, and F3 generations were given drinking water containing 0, 0.01, 0.1, or 1% chitosan oligomers for 180 days. Although reproductive and developmental endpoints were not specifically examined in this study, the authors did not report any apparent toxicity. Therefore, ToxServices identified a NOAEL of 10 mg/kg/day, the highest dose tested in mice. No additional details were provided for this study.
- NTP 2017
  - In a multigenerational prenatal and postnatal assessment of high molecular weight chitosan, pregnant ICR mice (number/dose not specified) were administered the test substance as a single intraperitoneal injection at doses of 0, 125, 500, or 2,000 mg/kg on gestation day (GD) 6. Animals were subject to a laparotomy or allowed to litter. Offspring from the same exposure group were mated and similarly subject to a laparotomy or allowed to litter. High dose P generation females exhibited mortality and diarrhea, and there was dose-dependent increases in vaginal bleeding, postimplantation loss, and lower spleen weights in P generation females at doses ≥ 500 mg/kg; P generation females also displayed a dose-dependent reduction in litter size. Reduced fetal body weights were reported in both generations at the high dose, however, there were no external, visceral, or skeletal malformation in offspring from either generation. F1 offspring from high dose exposed dams had high uterus, ovary, and thymus weights on postnatal day (PND) 21, and lower thymus weights on PND 56 (females only). F2 offspring from high dose exposed dams had decreased testis and ovary weights on PNDs 21 and 56.
    - *As this study was a single exposure study via intraperitoneal injections, which is not a standard route of exposure for GreenScreen evaluations, the results of this study were not weighed heavily.*
- Based on the weight of evidence, a Data Gap was assigned as the available data are insufficient to evaluate effects on development and conclusively determine if there would, or would not, be adverse effects on developmental parameters following exposure to chitosan. One oral multigeneration study in mice did not report any adverse effects, but did not specifically evaluate developmental toxicity (i.e., effects that interfere with normal development of the conceptus, such as death of the developing organism, structural abnormality, altered growth, and function deficiency (UN 2023)). In addition, a multigeneration study in mice that exposed animals intraperitoneally found some effects on fetal weights, postimplantation loss, and litter size, but the study involved only a single exposure, and the intraperitoneal route is of limited relevance. GHS Guidance states studies involving routes such as intraperitoneal injection must be interpreted with caution and on their own would not normally be the basis for classification (UN 2023). ToxServices did not identify available

data for the surrogate acetyl glucosamine or glucosamine that would help to classify this endpoint. Overall, the available data are insufficient to evaluate effects on development.

Endocrine Activity (E) Score (H, M, or L): DG
Chitosan was assigned a score of Data Gap for endocrine activity based on insufficient 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.
- U.S. EPA 2024
  - Chitosan was inactive for estrogen receptor agonism, antagonism, and binding using the CERAPP Potency Level (from literature) models in ToxCast (Appendix D).

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
Chitosan was assigned a score of Low for acute toxicity based on oral LD_{50} values > 2,000 mg/kg in rats and mice. GreenScreen® criteria classify chemicals as a Low hazard for acute toxicity when acute 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 experimental data for the target substance.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- NTP 2017
  - *Oral*: LD_{50} (mice, sex and strain not specified) = 16,000 mg/kg chitosan
- KitoZyme 2011
  - *Oral*: LD_{50} (female Sprague Dawley rats) > 2,000 mg/kg (highly pure medical grade chitosan derived from *Agaricus bisporus*)
  - *Oral*: LD_{50} (male and female Kunming mice) > 10,000 mg/kg (chitosan oligosaccharide, MW = 1.86 kDa)
- Zhang et al. 2012
  - *Oral*: LD_{50} (male Sprague-Dawley rats) > 4,640 mg/kg and < 10,000 mg/kg (water soluble chitosan) (National Standard of Acute Toxicity Test GB 15193.3-1994 CN, GLP unspecified)

Systemic Toxicity/Organ Effects incl. Immunotoxicity (ST-single) (Group II) Score (vH, H, M, or L): L
Chitosan was assigned a score of Low for systemic toxicity (single dose) based on a lack of specific target organ toxicity at doses of 2,000 mg/kg/ and greater in acute oral toxicity studies in rats. GreenScreen® criteria classify chemicals as a Low hazard for systemic toxicity (single dose) when there is no evidence of organ toxicity at oral doses of 2,000 mg/kg/ and greater and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on experimental data for the target substance.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.

  - *Screening:* Not present on any screening lists for this endpoint.
- KitoZyme 2011
  - *Oral*: In an acute oral toxicity study, female Sprague Dawley rats (6/group) were administered highly pure medical grade chitosan derived from *Agaricus bisporus* at 0 or 2,000 mg/kg via gavage and observed for 14 days. There were no mortalities, clinical signs of toxicity, changes to body weight, or adverse changes at macroscopic examination.
  - *Oral*: In an acute oral toxicity study, male and female Kunming mice (number/sex/dose not specified) were administered chitosan oligosaccharide preparation (MW = 1.86 kDa) at doses of 0, 1,000, 2,150, 4,640, and 10,000 mg/kg via gavage and were observed for 7 days. There were no mortalities or clinical signs of toxicity reported.

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

Chitosan was assigned a score of Low for systemic toxicity (repeated dose) based on oral LOAEL of 450 mg/kg/day in a 90-day study in rats. GreenScreen® criteria classify chemicals as a Low hazard for systemic toxicity (repeated dose) when oral LOAEL values are greater than 100 mg/kg/day in 90-day studies (CPA 2018b). The confidence in the score is high as it is based on experimental data for the target substance.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- NTP 2017
  - *Oral:* In a subchronic study, male and female Sprague Dawley rats were fed 1%, 3%, and 9% chitosan (82 kDa, 86.5% deacetylation) in the diet for 6 months (reported to be equivalent to 450, 1,500, and 5,200 mg/kg per day in male rats and 650, 1,800, and 6,000 mg/kg per day in female rats). Three sets of animals were treated (10 rats/sex/group); these groups consisted of a set of core study animals treated for up to 25 weeks (Group A) and two other sets of animals treated for up to 26 weeks (Groups B and C). Although the 9% dietary concentration is higher than standard 5% concentration used in NTP dietary studies, the authors considered the 9% concentration of chitosan to provide adequate nutrition for rats. There were no differences in body weights or food consumption between treated and control animals. Significant decreases in fat digestion occurred in males and females given 9% and in males given 3% chitosan. Significant decreases in cholesterol and triglycerides were also measured in male and female rats given 9% chitosan. Male and female rats given 9% chitosan had significantly decreased levels in serum vitamin A and serum and hepatic vitamin E levels, and increased levels of serum $1,25(OH)_2$ vitamin D. Males given 1 and 3% chitosan also had significantly decreased serum vitamin E levels and males given 3% chitosan had significantly decreased serum vitamin A levels. Serum levels of cholesterol, triglycerides, and phosphorous were significantly decreased in male and female rats given 9% chitosan. Fecal weights were significantly increased in males given 3 and 9% chitosan and in all female treatment groups. Fecal moisture was also significantly increased in males and females given 3 and 9% chitosan. Significantly decreased absolute and relative thymus weights were measured in males given 3 and 9% chitosan and in females given 9% chitosan, which the authors proposed to be possibly related to the effects on vitamins A and E and cholesterol and triglyceride levels. Females given 9% chitosan had significantly decreased liver weights and liver histology effects that included a significant decrease in periportal lipid accumulation. These liver changes also trended in females given 1 and 3% chitosan, but the changes were not significant. Males given 1 or 9% chitosan also had a decrease in

periportal lipid accumulation, but the decrease was not significant. The authors suggest that the high concentration of 9% chitosan may have possibly confounded the amount of fat being excreted, especially with higher fecal weights that can alter the calculated amount of fat excreted in the feces. However, the authors reported that the decrease in fat digestion is likely treatment related given the extent of fecal fat excretion and the decrease in liver periportal lipid accumulation. In the other sets of animals (Groups B and C) given the same treatments with chitosan, seizures were observed in 13 animals during or after blood collections at week 18 of the study. Five animals also died after the seizures. Seizures were not observed with the first set of core animals (Group A) that were treated with chitosan. The authors could not determine the cause of the seizures that occurred near the time of blood collections although the authors suggested a dietary concentration related increase in the occurrence. The authors identified a lowest-observed-effect level (LOEL) for chitosan of 1% (450 mg/kg) in male rats and 9% (6,000 mg/kg) in female rats, but they did not establish a NOAEL or LOAEL. However, the authors suggested that decreases in fat soluble vitamin levels (vitamins A and E) were significant enough to suggest nutritional inadequacies. Therefore, ToxServices determined the LOAEL to be 450 mg/kg/day in male rats, the lowest dose tested, since all male treatment groups had significantly decreased vitamin levels. ToxServices determined the LOAEL to be 6,000 mg/kg/day and the NOAEL to be 1,800 mg/kg/day in female rats since significantly decreased vitamin levels occurred for the female high dose group in the 6 month study.

- KitoZyme 2011
    - *Oral:* Sprague-Dawley rats (n=9/sex/group) were administered 0, 500, 1,000, or 2,000 mg/kg per day of chitosan oligosaccharide by gavage for 28 days in a non-GLP study that was not conducted according to current guidelines. Evaluations included body weight, feed consumption, clinical signs, mortality, urinalysis, hematology, organ weights, and histopathology. There were no differences reported in feed intake, body weight, clinical signs, or mortality between treated and control animals. Increased urinary mean leukocyte concentrations and significantly decreased albumin and total blood protein concentrations occurred in male rats administered 500 mg/kg/day. However, the findings were not dose related and occurred only in males and therefore, the study authors did not consider it to be toxicologically relevant. Hematological effects included significantly increased mean leukocyte concentration in males at 2,000 mg/kg/day and significantly decreased percentages of granulocytes in female rats at 1,000 mg/kg/day. The study authors did not consider these findings to be toxicologically relevant since the changes were within normal range. There was also a significant increase in mean platelet volume in male rats at 1,000 mg/kg/day was reported, but the authors of the GRAS notice did not consider it to be toxicologically significant because it was not dose related and found only in male rats. In addition, the GRAS notice authors did not consider decreased albumin and total blood protein concentrations in male rats at 500 mg/kg/day to be toxicologically relevant due to a lack of dose response. There were no effects on clinical chemistry, organ weights, or histopathology. The study authors identified a NOAEL of 2,000 mg/kg/day, which was the highest dose tested.
        - *Due to the 28-day duration of the study, the guidance values were tripled (i.e., 100 mg/kg/day * 3 = 300 mg/kg/day) as 28-days is approximately 1/3 the duration of 90-day studies.*
    - *Oral:* In a study conducted according to Japanese Ministry of Health Guidelines, F344 rats (n=10/sex/group) were fed chitosan oligosaccharide (100% deacetylation) in the diet at concentrations of 1, 0.04, 0.2, or 1% for 90 days. In animals given 1% chitosan

oligosaccharide, a significant reduction in weight gain and food consumption occurred for both sexes. Adverse clinical signs were observed only at the high dose, and included swelling of the snout, auricals and forelimbs, alopecia of the forelimbs, piloerection, and emaciation. Males and females given 1% chitosan oligosaccharide had significant increases in relative weights for the brain, heart, lungs, kidneys and adrenal glands. Males also had significant increases in relative weights for the testes, submaxillary gland, pituitary, and thyroid at the high dose. Adverse microscopic findings in the spleen and thymus along with abnormal findings in clinical chemistry parameters and urinalysis occurred in males given the high dose. Males given the high dose also had a significant increases in platelet, lymphocyte, and differential neutrophil counts. No adverse findings were reported for the for the 0.04 and 0.2% dietary dose levels. The authors reported that it was not clear if the adverse findings reported at the high dose were directly related to the chitosan oligosaccharide or are secondary to malnutrition from reduced food consumption in combination with sequestration of vitamins and minerals by the chitosan oligosaccharides. Some ocular effects, including unilateral corneal opacities in one male per treatment group, failure of mydriasis with synechia, increased light reflection by the retina, and distention of the eyeball in the animal with corneal opacity in the 1% group, and unilateral increases in light reflection by the retina in one male at 0.04%, one female in each at 0 and 0.2% groups, lens opacity in one female at 0.04%, enlargement of the left eye in one male at 0.04%, and opacity of the right eye in one female at 0.04%. The authors stated that there were no significant differences in macroscopic eye examination, and did not discuss the ophthalmological findings. Based on adverse findings at the 1% dietary level in the 90 day dietary study, the authors identified a NOAEL of 0.2% chitosan oligosaccharides, which they report is equivalent to 124 mg/kg/day in male rats and 142 mg/kg/day in female rats.

- *Oral:* In a non-GLP-compliant, non-guideline subchronic study, female Kunming mice (10/group) were administered diet containing 1.05% of one of four preparations of chitosan: 1.) high molecular weight chitosan (760 kDa), 2.) middle molecular weight chitosan (32.7 kDa), 3.) water-soluble chitosan (39.1 kDa), and 4.) chitosan oligomer (0.99 kDa), for 90 days. The dose was reported to be equivalent to 500 mg/kg/day. There were no mortalities and no clinical signs of toxicity or changes to body weights in treated animals. Relative thymus weight was significantly increased in animals exposed to the water-soluble chitosan, however, gross examination did not reveal any treatment-related abnormalities and there were no histopathological findings in any of the treatment groups. Animals exposed to the middle molecular weight chitosan had increased levels of iron in the liver and spleen, increased levels of zinc in the liver, spleen, and heart, and increased copper levels in the liver. Authors attributed these effects to chitosan's metal-chelating properties. Authors concluded the administration of the chitosan did not produce any explicit adverse effects. ToxServices identified a NOAEL of 500 mg/kg/day for this study.

- Based on the weight of evidence, a score of Low was assigned. The lowest oral LOAEL identified was 450 mg/kg/day in the 6-month study in male and female Sprague Dawley rats conducted by NTP (2017). The LOAEL of 450 mg/kg/day in male rats was the lowest dose tested; thus, it is not possible to determine from this study alone if adverse effects would occur below this dose level. However, the oral 90-day study in F344 rats identified a NOAEL 0.2% chitosan oligosaccharides (reported to be equivalent to 124 mg/kg/day in male rats and 142 mg/kg/day in female rats). Thus, NOAEL of 124/142 mg/kg/day is the highest NOAEL below the lowest LOAEL of 450 mg/kg/day. Based on these NOAELs and LOAELs, it can be concluded that no adverse effects would be expected to occur below the oral guidance value of 100 mg/kg/day for 90-day studies. Therefore, a high confidence score of Low was assigned for this endpoint.

Neurotoxicity (single dose, N-single) (Group II) Score (vH, H, M, or L): *L*
Chitosan was assigned a score of Low for neurotoxicity (single dose) based on a lack of clinical signs of neurotoxicity in standard acute oral toxicity studies in rats. GreenScreen® criteria classify chemicals as a Low hazard for neurotoxicity (single dose) when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is low as there were no specified neurotoxicity examinations.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- KitoZyme 2011
  - *Oral*: In an acute oral toxicity study, female Sprague Dawley rats (6/group) were administered highly pure medical grade chitosan derived from *Agaricus bisporus* at 0 or 2,000 mg/kg via gavage and observed for 14 days. There were no mortalities, clinical signs of toxicity, changes to body weight, or adverse changes at macroscopic examination.
  - *Oral*: In an acute oral toxicity study, male and female Kunming mice (number/sex/dose not specified) were administered chitosan oligosaccharide preparation (MW = 1.86 kDa) at doses of 0, 1,000, 2,150, 4,640, and 10,000 mg/kg via gavage and were observed for 7 days. There were no mortalities or clinical signs of toxicity reported.

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

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

Skin Sensitization (SnS) (Group II*) Score (H, M, or L): L
Chitosan was assigned a score of Low for skin sensitization based on negative results in all three validated test methods for skin sensitization (OECD Guidelines 442C, 442D, and 442E) for the monomeric unit N-acetyl glucosamine. GreenScreen® criteria classify chemicals as a Low hazard for skin sensitization when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on guideline studies for 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.
- Coreleader Biotech 2015
  - A bandage composed of a woven gauze of chitosan fiber and rayon fiber was negative for dermal sensitization in guinea pigs. No additional details were provided.
- CIR 2022
  - *Surrogate: N-Acetyl Glucosamine (CAS #7512-17-6):* Acetyl glucosamine was negative in an OECD Guideline 442C direct peptide reactivity assay (DPRA). Neat test substance (99.42% purity) had a mean percent depletion of cysteine and lysine of 1%.
  - *Surrogate: N-Acetyl Glucosamine (CAS #7512-17-6):* Acetyl glucosamine was negative in an OECD Guideline 442D KeratinoSens™ assay. Human epidermal keratinocytes were exposed to the test substance (99.42% purity) at 0.98-2,000 µm and analyzed for luciferase

activity after a 48-hour incubation period. The half maximal inhibitory concentration (IC_{50}) was ≥ 2,000 µM.

- *Surrogate: N-Acetyl Glucosamine (CAS #7512-17-6):* Acetyl glucosamine was negative in an OECD Guideline 442E human cell line activation test (h-CLAT) assay. THP-1 cells were incubated with the test substance (99.42% purity) at 1,395-5,000 µg/mL for 24 hours and analyzed by flow cytometry. The cell viability was >50% at all concentrations tested.
  - *Based on negative results in all three validated test methods for skin sensitization (OECD Guidelines 442C, 442D, and 442E), the surrogate N-acetyl glucosamine is predicted to be non-sensitizing.*
- *Surrogate: N-Acetyl Glucosamine (CAS #7512-17-6):* N-Acetyl glucosamine was negative for sensitization in a human repeat insult patch test (HRIPT) in 108 subjects with a formulated mask product containing 0.005% N-acetyl glucosamine when tested neat under occlusive conditions.
- *Surrogate: N-Acetyl Glucosamine (CAS #7512-17-6):* N-Acetyl glucosamine was negative for sensitization in a HRIPT in 105 subjects with a formulated liquid foundation product containing 2% N-acetyl glucosamine when tested neat under occlusive conditions.

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

Chitosan was assigned a score of Low for respiratory sensitization based on negative dermal sensitization potential following ECHA (2017)'s guidance. GreenScreen® criteria classify chemicals as a Low hazard for respiratory sensitization when adequate data are available and negative 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 2023
  - Chitosan does not contain any structural alerts for respiratory sensitization (Appendix E)
- 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 chitosan is not expected to be sensitizing to the skin (see skin sensitization section above), and a literature search did not find any human evidence of respiratory sensitization by chitosan, and as chitosan does not contain any structural alerts for respiratory sensitization (OECD 2023), chitosan is not expected to be a respiratory sensitizer.

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

Chitosan was assigned a score of Low for skin irritation/corrosivity based on lack of skin irritation in *in vitro* and human studies with the monomeric unit and an *in vivo* study in guinea pigs with the target substance with limited reporting details. GreenScreen® criteria classify chemicals as a Low hazard for skin irritation/corrosivity when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on experimental data for the target substance and 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.
- Rao and Sharma 1997
  - Skin irritation tests in guinea pigs did not reveal any undesirable toxic effects of chitosan. No additional details were provided.
- CIR 2022
  - *Surrogate: N-Acetyl Glucosamine (CAS #7512-17-6):* Undiluted N-acetyl glucosamine (16 mg, 99.42% purity) was negative for skin irritation in an *in vitro* test conducted according to OECD Guideline 439 with reconstructed human epidermis.
  - *Surrogate: N-Acetyl Glucosamine (CAS #7512-17-6):* In a 21-day cumulative patch test in human volunteers (n=12), 0.2 g of an eye cream containing 2% N-acetyl glucosamine was applied each day for 21 days (excluding weekends) under occlusive conditions. The average irritation score was 0.34/4 and study authors indicated very mild cumulative irritation.

Eye Irritation/Corrosivity (IrE) (Group II) Score (vH, H, M, or L): L
Chitosan was assigned a score of Low for eye irritation/corrosivity based on lack of eye irritation in *in vitro* studies with the monomeric unit and an *in vivo* study in rabbits with the target substance with limited reporting details. GreenScreen® criteria classify chemicals as a Low hazard for eye irritation/corrosivity when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is high as it is based on experimental data for the target substance and 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.
- Rao and Sharma 1997
  - Eye irritation tests in rabbits did not reveal any undesirable toxic effects of chitosan. No additional details were provided.
- CIR 2022
  - *Surrogate: N-Acetyl Glucosamine (CAS #7512-17-6):* N-Acetyl glucosamine (at 2% in a formulated product) was negative for ocular irritation in an EpiOcular™ test with stratified human keratinocytes. The ET_{50} (time causing a 50% reduction in tissue viability) was 17.2 hours, whereas the ET_{50} for the positive control was 16.3 minutes.
  - *Surrogate: N-Acetyl Glucosamine (CAS #7512-17-6):* N-Acetyl glucosamine (20% in a saline solution) was negative for eye irritation in a bovine corneal opacity and permeability (BCOP) test conducted according to OECD Guideline 437. The mean *in vitro* irritancy score was 0.42, whereas the scores for the negative and positive controls were 0.70 and 105.42, respectively.

Ecotoxicity (Ecotox)

Acute Aquatic Toxicity (AA) Score (vH, H, M, or L): *vH*
Chitosan was assigned a score of Very High for acute aquatic toxicity based on the lowest identified L/EC_{50} values of 0.37 mg/L in fish. GreenScreen® criteria classify chemicals as a Very High hazard for acute aquatic toxicity when acute aquatic toxicity values are less than 1 mg/L for any of the three trophic levels (CPA 2018b). The confidence in the score is low as the values used for classification is based on a tested trade name with incomplete compositional information and additional experimental data suggest the toxicity for chitosan may be lower.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* GHS – New Zealand – Hazardous to the aquatic environment – acute category 1.
- Sigma Aldrich 2023
  - 96 hr LC_{50} (*Oncorhynchus mykiss*, rainbow trout) = 1.73 mg/L
  - 48 hr EC_{50} (*Daphnia pulex*, water flea) = 13.69 mg/L
- U.S. EPA 2024b
  - 24 hr behavior IC_{50} (*Pyropia yezoensis*, red algae) = 0.1% (equivalent to 1,000 mg/L).
- U.S. EPA 2024b, Waller et al. 1993
  - Studies were performed for two trade names, KML V2 and KML V54. Chitosan is described as 2% active ingredient in trade name KML V2 and 5.4% active ingredient in trade name KML V54. No further information was provided for the two trade names regarding other chemical components. Concentrations in results are based on percent active ingredient in the formulation.
    - 48 hr LC_{50} (*Ictalurus punctatus*, channel catfish) = 0.37 mg/L (KML V2).
    - 48 hr LC_{50} (*I. punctatus*, channel catfish) = 0.92 mg/L (KML V54).
    - 48 hr LC_{50} (*Oncorhynchus mykiss*, rainbow trout) = 0.38 mg/L (KML V2).
    - 48 hr LC_{50} (*O. mykiss,* rainbow trout) = 0.5 mg/L (KML V54).
    - 48 hr LC_{50} (*Obliquaria reflexa,* three-horned wartyback) > 100 mg/L (KML V2).
    - 48 hr LC_{50} (*O. reflexa*, three-horned wartyback) > 100 mg/L (KML V54).
    - 48 hr LC_{50} (*Dreissena polymorpha,* zebra mussel) > 100 mg/L (KML V2).
    - 48 hr LC_{50} (*D. polymorpha,* zebra mussel) > 100 mg/L (KML V54).
- Wang et al. 2016
  - 72 hr yield EC_{50} (*Chlorella vulgaris*, green algae) = 3.5 mg/L
  - 48 hr immobilization EC_{50} (*Daphnia magnia*, water flea) 2.2 mg/L
  - 96 hr immobilization EC_{50} (*Limnodrilus hoffmeisteri*, red worm) = 6.9 mg/L
  - 96 hr LC_{50} *(Cyprinus carpio*, common carp) = 3 mg/L
- CCID 2024
  - Chitosan is classified to Category 1 in New Zealand based on a 48-hour LC_{50} of 0.037 mg/L in *Ictalurus punctatus* (channel catfish).
    - ToxServices notes that CCID references the Waller et al. (1993) paper summarized above, and incorrectly reports the LC_{50} as 0.037 mg/L rather than 0.37 mg/L.
- Based on the weight of evidence, a low confidence score of Very High was assigned. Acute aquatic toxicity values reported in the Waller et al. (1993) paper are < 1 mg/L active ingredient for fish, which corresponds to a score of Very High. These values are reported for trade names containing only 2%-5.4% chitosan, and no additional data were identified on the remaining composition of the trade names. Acute aquatic toxicity values in fish reported by Sigma Aldrich (2023) and Wang et al. (2016) are between 1 and 10 mg/L, which warrants a score of High. Based on the available data from Waller et al. (1993), ToxServices conservatively assigned a score of Very High, but reduced the confidence as the complete compositions of the trade names tested by Waller et al. (1993) are unknown and values from other studies indicate the toxicity of active chitosan may be lower.

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

Chitosan was assigned a score of Very High for chronic aquatic toxicity based on the lowest ChV of 0.019 mg/L in fish. GreenScreen® criteria classify chemicals as a Very High hazard for chronic aquatic toxicity when the chronic aquatic toxicity values are less than 0.1 mg/L for any trophic level (CPA 2018b). The confidence in the score is high because although though data are lacking for two of three trophic levels, experimental data for fish alone correspond to a Very High.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* GHS – New Zealand – Hazardous to the aquatic environment – chronic category 1.
- U.S. EPA 2024, Bullock et al. 2000
  - 14 day mortality NOEC (*O. mykiss,* rainbow trout) = 0.019 ppm (0.019 mg/L)
- U.S. EPA 2013
  - As limited chronic data were identified, ToxServices applied the acute-to-chronic (ATC) ratios to derive the chronic values. Chitosan belongs to the chemical class of cationic polymers with a natural-based backbone, which has ATC ratios of 18 and 14 for fish and daphnia, respectively.
    - ChV (fish) = lowest 96h EC_{50} /14 = 0.38 mg/L / 14 = 0.021 mg/L
    - ChV (daphnia) = lowest EC_{50}/5 = 2.2 mg/L / 14 = 0.157 mg/L
  - For green algae, data indicate that chronic toxicity toward green algae will be less than that for carbon-based backbone polymers. SAR analysis should employ the nearest analog method. As the available and estimated data for fish and daphnia already indicate a very high score, SAR analysis for green algae was not conducted.
- CCID 2024
  - Chitosan is classified to Category 1 in New Zealand based on a 14-day NOEC of 0.038 mg/L in *Oncorhynchus mykiss* (rainbow trout).

Environmental Fate (Fate)

Persistence (P) Score (vH, H, M, L, or vL): *vL*
Chitosan was assigned a score of Very Low for persistence based on an OECD Guideline 301B ready biodegradability test demonstrating ready biodegradability and meeting the 10-day window for a chitosan aerogel. GreenScreen® criteria classify chemicals as a Very Low hazard for persistence when they are readily biodegradable and meet the 10-day window (CPA 2018b). The confidence in the score is low as the source of the chitin, deacetylation, and molecular weight of the chitin can vary greatly, and these factors are likely to impact the biodegradation of the polymer.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- Gooday 1990
  - Chitin undergoes degradation by deacetylation to chitosan, which is further hydrolyzed by chitosanases.
- Bonilla et al. 2020
  - Chitosan was readily biodegradable in a test conducted according to OECD Guideline 301D. No additional details were provided.
- Radwan-Praglowska et al. 2017
  - Chitosan aerogels, prepared by heating chitosan flakes with aspartic acid, levulinic acid, adipic acid, or succinic acid in the presence of acetic acid with heat, were readily biodegradable in tests conducted according to OECD Guideline 301B. All of the materials reached 70-80% biodegradation within the first 10 days of the study, indicating that they met the 10-day window. Biodegradation after 28 days was 90-100%.
- Ratajska et al. 2003
  - Chitosan is biodegradable in aqueous media based on results of respirometric tests using sludge from wastewater from a cellulose plant. Biodegradation rate increased with

increasing temperature within the range of 23-36°C but further increase in temperature to 50°C resulted in slowing of the rate. The maximum amount of CO_2 released after 4 weeks was approximately 15% for low, medium, and high molecular weight polymers and after 10 weeks was approximately 35, 30, and 25% for low, medium, and high molecular weight polymers, respectively (all at 36°C).

- Although polymers are generally poorly biodegradable, as a biological polysaccharide, chitosan is likely to have the potential for microbial degradation. Accordingly, respirometric tests demonstrated up to 35% degradation (CO_2 release) in 10 weeks. Several chitosan aerogels were readily biodegradable according to OECD guidelines; however, it is unclear how the acid component of the gel may impact biodegradation. Chitosan is a biological polymer, and its composition varies depending on the source of the chitin from which it is derived, as well as the deacetylation process. In addition, the molecular weight of this polymer may vary greatly, ranging from 150,000 (low molecular weight) to 600,000 (high molecular weight). These factors are likely to impact the biodegradation of the polymer. Therefore, ToxServices assigned a low confidence score of Very Low for this endpoint.

Bioaccumulation (B) Score (vH, H, M, L, or vL): *vL*
Chitosan was assigned a score of Very Low for bioaccumulation based on expert judgement that chitosan is unlikely to be a concern for bioaccumulation. GreenScreen® criteria classify chemicals as a Very Low hazard for bioaccumulation when the log K_{ow} is less than 4 and the BCF/BAF is less than 100 (CPA 2018b). The confidence in the score is low due to the lack of measured data and as it is based on expert judgement.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- KitoZyme 2011
  - Bioaccumulation is unlikely as it is not expected to be significantly digested or absorbed.
  - Because chitosan is expected to undergo hydrolysis to produce chitosan oligomers, followed by further degradation to glucosamine, which is a natural constituent of aquatic organisms, it is unlikely to be of concern for bioaccumulation.
- U.S. EPA 2013
  - Polymers with molecular weight > 1,000 are typically of low concern for bioaccumulation.
  -

Physical Hazards (Physical)

Reactivity (Rx) Score (vH, H, M, or L): *L*
Chitosan was assigned a score of Low for reactivity based on its stability/physical hazard ratings of 0 from NFPA and HMIS. GreenScreen® criteria classify chemicals as a Low hazard for reactivity when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is low due to the lack of measured data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- Santa Cruz Biotechnology 2019
  - An SDS for chitosan (>98% purity) has a stability rating of 0 from the NFPA ("Normally stable, even under fire exposure conditions, and is not reactive with water") and physical hazard rating of 0 from HMIS ("Materials that are normally stable, even under fire conditions, and will not react with water, polymerize, decompose, condense, or self-react.

Non-explosives").

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

Chitosan was assigned a score of Low for flammability based on its flammability hazard rating of 0 from NFPA and HMIS. GreenScreen® criteria classify chemicals as a Low hazard for flammability when adequate data are available and negative and they are not GHS classified (CPA 2018b). The confidence in the score is low due to the lack of measured data.

- Authoritative and Screening Lists
  - *Authoritative:* Not present on any authoritative lists for this endpoint.
  - *Screening:* Not present on any screening lists for this endpoint.
- Santa Cruz Biotechnology 2019
  - An SDS for chitosan (>98% purity) reports a flammability score of 0 under HMIS ("Materials that must be preheated before ignition will occur. Includes liquids, solids and semi solids having a flash point above 200°F (93°C)") and NFPA ("Materials that require considerable preheating, under all ambient temperature conditions, before ignition and combustion can occur (e.g. mineral oil). Includes some finely divided suspended solids that do not require heating before ignition can occur. Flash point at or above 93°C (200°F)").

Use of New Approach Methodologies (NAMs)[9] in the Assessment, Including Uncertainty Analyses of Input and Output

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

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

As shown in Table 5, Type I (input data) uncertainties in chitosan's NAMs dataset include lack of experimental data for endocrine activity, and respiratory sensitization, lack of validated test methods for respiratory sensitization, and the availability of *in vitro* data for skin and eye irritation endpoints. Chitosan's Type II (extrapolation output) uncertainties include the lack of a defined applicability domain in ToxCast models, limitation of *in vitro* genotoxicity assays in mimicking *in vivo* metabolism, their focusing on one or only a few types of genotoxicity events, the limitation of OECD Toolbox in identifying structural alerts for respiratory sensitization without accounting for non-immunologic mechanisms of respiratory sensitization, and limitations of *in vitro* skin and eye irritation assays to differentiate between GHS Category classifications. Some of chitosan's type II uncertainties were alleviated by the use of *in vitro* test batteries and/or in combination of *in vivo* data.

Table 4: Summary of NAMs Used in the GreenScreen® Assessment, Including Uncertainty Analyses	
Uncertainty Analyses (OECD 2020)	
Type I Uncertainty: Data/Model Input	**Endocrine activity**: No experimental data are available. **Skin irritation**: One *in vitro* study available (OECD Guideline 439) and human studies with formulated products containing the monomer unit. **Eye irritation**: Only two *in vitro* studies available (OECD Guideline 437 and 492) conducted with the monomer unit. **Respiratory sensitization**: No experimental data are available and there are no validated test methods.
Type II Uncertainty: Extrapolation Output	**Genotoxicity:** The bacterial reverse mutation assay (as defined in OECD Guideline 471) only tests point-mutation inducing activity in non-mammalian cells, and the exogenous metabolic activation system does not entirely mimic *in vivo* conditions[10].

[9] 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).

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

	Endocrine activity: ToxCast models don't define applicability domain. **Skin sensitization:** The *in silico* and *in vitro* assays evaluating key events in the skin sensitization adverse outcome pathway (AOP) don't typically include metabolism or abiotic transformation to address chemicals that are pro-haptens or pre-haptens, respectively. Further, each test has their applicable domain such as limitations in test substance solubility or log K_{ow}.[11] **Skin irritation**: The OECD Guideline 439 test is only used to identify irritating substances (GHS Category 2) and non-irritating substances (no category), and does not allow the classification as a mild skin irritant (GHS Category 3)[12]. **Eye irritation**: The BCOP (OECD Guideline 437) test is not recommended for identifying GHS Category 2A or 2B irritants[13]. The RhCE test (OECD Guideline 492) cannot differentiate between Category 2 and Category 1, or between Category 2A and Category 2B. There is no single *in vitro* method that can replace an *in vivo* eye irritation test[14]. Therefore, this method is not recommended for identifying eye irritants (Category 2) or substances causing serious eye damage (Category 1) (ECHA 2017). **Respiratory sensitization**: The OECD Toolbox only identifies structural alerts, and does not define applicability domains. Additionally, the ECHA guidance (2017), on which the use of OECD Toolbox structural alerts is based, does not evaluate non-immunologic mechanisms for respiratory sensitization.	
Endpoint	**NAMs Data Available and Evaluated? (Y/N)**	**Types of NAMs Data (*in silico* modeling/*in vitro* biological profiling/frameworks)**
Carcinogenicity	N	
Mutagenicity	Y	*In vitro* data: Bacterial reverse mutation assay
Reproductive toxicity	N	
Developmental toxicity	N	
Endocrine activity	Y	*In silico* modeling: ToxCast models
Acute mammalian toxicity	N	
Single exposure systemic toxicity	N	
Repeated exposure systemic toxicity	N	

[11] https://www.oecd-ilibrary.org/environment/test-no-442c-in-chemico-skin-sensitisation_9789264229709-en; https://www.oecd-ilibrary.org/environment/test-no-442d-in-vitro-skin-sensitisation_9789264229822-en; https://www.oecd-ilibrary.org/environment/test-no-442e-in-vitro-skin-sensitisation_9789264264359-en
[12] https://www.oecd-ilibrary.org/docserver/9789264242845-en.pdf?expires=1614097324&id=id&accname=guest&checksum=D664A7EDCDE297919BE9A478941EBEC6
[13] https://www.oecd-ilibrary.org/docserver/9789264203846-en.pdf?expires=1614095760&id=id&accname=guest&checksum=1613168F64BDB3558225572BDD75FC8D
[14] https://www.oecd.org/env/ehs/testing/E492_2017.pdf

Single exposure neurotoxicity	N	
Repeated exposure neurotoxicity	N	
Skin sensitization	Y	*In vitro*/*in chemico* tests: OECD Guideline 442C, 442D, and 442E tests
Respiratory sensitization	Y	*In silico* modeling: OECD Toolbox structural alerts
Skin irritation	Y	*In vitro* tests: OECD Guideline 439 Test
Eye irritation	Y	*In vitro* tests: OECD Guideline 437 and 492 Tests
Acute aquatic toxicity	N	
Chronic aquatic toxicity	Y	Acute to chronic ratios
Persistence	Y	Non-animal testing: OECD 301 Biodegradation 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 Chitosan (CAS #9012-76-4)

TOXSERVICES
TOXICOLOGY RISK ASSESSMENT CONSULTING

GREEN SCREEN FOR SAFER CHEMICALS

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	Chitosan	9012-76-4	L	L	DG	DG	DG	L	L	L	*L*	DG	*L*	L	L	L	*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
Chitosan	2

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

Table 6

Chemical Name	Final GreenScreen® Benchmark Score
Chitosan	U

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	No	Yes	Yes	Yes	Yes							U
3												
4												

APPENDIX C: Pharos Output for Chitosan (CAS #9012-76-4)

9012-76-4
Poliglusam
ALSO CALLED 1118546-53-4, 1268699-64-4, 1403748-72-0, 1548444-29-6, 1869074-89-4, 1884679-12-2, 191045-06-4, 2-a...
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All Hazards View

Show PubMed Results | Request Assessment | Add to Comparison

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

Hazard Lists

Download Lists

ENDPOINT	HAZARD LEVEL	GREENSCREEN®	LIST NAME	HAZARD DESCRIPTION	OTHER LISTS
Acute Mammalian Toxicity	pC	NoGS	US EPA - OPP - Registered Pesticides	FIFRA Registered Pesticide	
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	+1
	vH	LT-P1	GHS - New Zealand	Hazardous to the aquatic environment - chronic category 1	

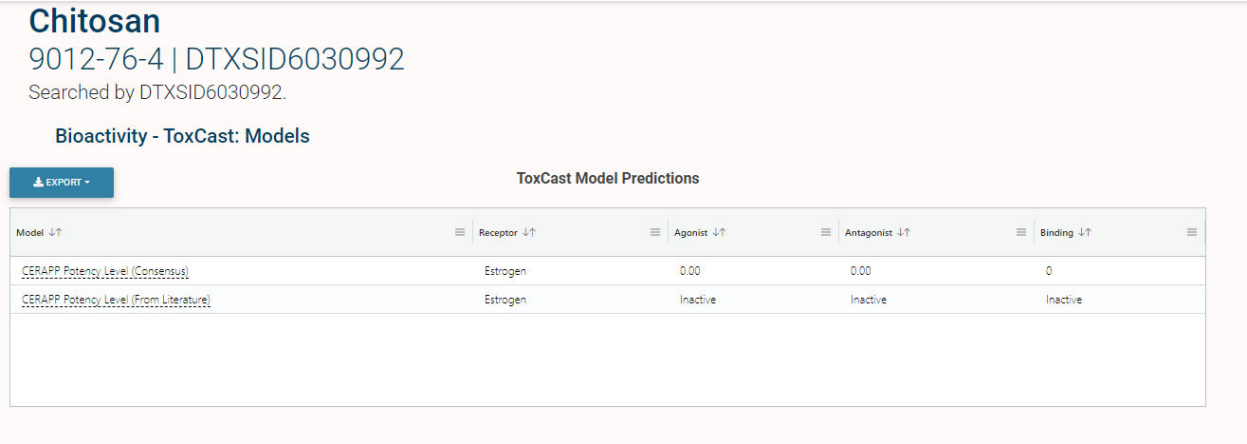
Restricted Substance Lists (2)

- Food Contact Chemicals Database (FCCdb): Food Contact Chemicals Database Version 5.0
- TSCA Chemical Substance Inventory (Active-Inactive): TSCA Chemical Substance Inventory - Active

Positive Lists (4)

- Inventory of Existing Cosmetic Ingredients in China (IECIC 2021): Cosmetic Ingredients
- German FEA - Substances Hazardous to Waters: Non-Hazardous to Water (Water Hazard Class 0 NWG)
- US EPA - DfE Safer Chemicals Ingredients list (SCIL): Antimicrobial Actives - Green Circle (Verified Low Concern)
- US EPA - DfE Safer Chemicals Ingredients list (SCIL): Preservatives-Antioxidants - Green Circle (Verified Low Concern)

APPENDIX D: ToxCast Model Results for Chitosan (CAS #9012-76-4)



Chitosan

9012-76-4 | DTXSID6030992

Searched by DTXSID6030992.

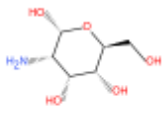
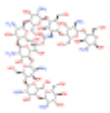
Bioactivity - ToxCast: Models

EXPORT

ToxCast Model Predictions

Model	Receptor	Agonist	Antagonist	Binding
CERAPP Potency Level (Consensus)	Estrogen	0.00	0.00	0
CERAPP Potency Level (From Literature)	Estrogen	Inactive	Inactive	Inactive

APPENDIX E: OECD Toolbox Respiratory Sensitization Results for Chitosan (CAS #9012-76-4)

Filter endpoint tree...	1	2
Structure		
Structure info		
Additional Ids		
CAS Number	9012-76-4	9012-76-4
CAS-SMILES relation	Low	Low
Chemical name(s)	Chitosan	Poliglusan
Identity	Sources:5	Sources:1
Molecular formula	C6H13NO5	C56H103N9O39
Predefined substance type	Mono constituent	Mono constituent
SMILES	N[C@@H]1[C@H](O)O[C@...	COC(=O)N[C@H]1[C@@H](...
Parameters		
Physical Chemical Properties		
Environmental Fate and Transport		
Ecotoxicological Information		
Human Health Hazards		
Profiling		
Endpoint Specific		
Respiratory sensitisation	No alert found	No alert found

APPENDIX F: Change in Benchmark Score

Table 5 provides a summary of changes to the GreenScreen® Benchmark™ for chitosan. The original GreenScreen® assessment was performed in 2024 under version 1.4 criteria and ToxServices assigned a Benchmark 2 (BM-2) score.

Table 5: Change in GreenScreen® Benchmark™ for Chitosan

Date	GreenScreen® Benchmark™	GreenScreen® Version	Comment
March 18, 2024	BM-2	v. 1.4	New GreenScreen® assessment.

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