UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

SUBJECT: Addendum to the science review in support of the addition of chitosan (Poly-D-Glucosamine) to the list of minimum risk pesticides (MRPs) contained in 40 CFR 152.25(f)

Active Ingredient Type:	Biochemical
PC Code:	128930
CAS Number:	9012-76-4
Active Ingredient Tolerance Exemption:	40 CFR 180.1072
Docket Number:	EPA-HQ-OPP-2019-0701

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This memorandum contains confidential business information that has been removed to a confidential appendix

In October of 2018, Tidal Vision Products, LLC submitted a petition requesting the addition of chitosan (poly-d-glucosamine) to the list of minimum risk pesticides (MRPs) contained in 40 CFR 152.25(f) under the authority of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), section 25(b). Specifically, the request was to add "the substance commonly called chitosan, with a Chemical

Abstracts Service (CAS) # of 9012-76-4" to the list of pesticide active ingredients (AI) on the list of MRPs. In April of 2019, Tidal Vision Products, LLC amended the petition to include a request to add chitosan to the inert ingredients listed in 40 CFR 152.25(f).
To support the petition and its amendment, Tidal Vision Products, LLC submitted a letter outlining the seven criteria that were considered in the addition of chemicals to the MRP list and how chitosan fulfills each of the seven criteria. In 2019, the EPA reviewed this information and produced an assessment of the application of these criteria to chitosan (US EPA, 2019), which was used to support a proposed rule to add chitosan to the active and inert ingredients listed in 40 CFR 152.25(f) and exempted from regulation under FIFRA.

The petition to include chitosan on the list of MRPs specifically requests addition of chitosan with CAS # 9012-76-4 to the list, which is the chitosan polymer produced from deacetylation of chitin, an insoluble chemical commonly referred to "dry" chitosan. This is the form of chitosan that the Agency understood to be the active ingredient in registered pesticide products containing chitosan at the time of their registration. However, in the industries utilizing chitosan, the term "chitosan" refers not only to this form but can also refer to derivatives of chitosan, including soluble salts of chitosan formed through the reaction of "dry" chitosan with various acids[1]. Through further investigation, the Agency believes that some of the registered products containing "dry" chitosan as active ingredients along with solubilizing acids form chitosan salts[2]. Therefore, since chitosan salts may have increased bioavailability resulting in higher toxicity, a thorough review of the safety of chitosan as a pesticide potentially added to the MRP active ingredients list must include not only the "dry" chitosan with CAS # 9012-76-4, but also chitosan salts that may potentially be formed through combination of "dry" chitosan with acids included on the MRP inert list. Other acids could be added to the MRP inert list in the future and would likely need to be assessed in a similar manner. It should be noted that the Agency's characterization of chitosan and its salts herein does not include nanoparticle formulations or non-acid-base derivatives.

This review presents an addendum to the previous review on chitosan to include information on chitosan salts as it relates to each of the seven criteria considered in adding chitosan to the MRP lists.

BACKGROUND

Pesticidal uses of chitosan according to labels of currently registered products include uses to control nematodes and plant diseases, or as a plant growth regulator, on agricultural sites such as food and non-food crops, residential, greenhouse, and nursery crops; in addition, there are non-agricultural use sites like turf, ornamentals, and trees. The EPA has also registered products containing chitosan for antimicrobial uses to control odor causing, spoilage, and discoloration microbes on textiles and surfaces. The percentage of chitosan in end use products ranges from 0.05% to 85%, and chitosan is present at <5% in most products. Agricultural application rates range from 0.11-2.5 lbs active ingredient (AI)/acre (A) for foliar sprays, 0.13-0.90 lbs/A for soil drench, 0.24-2.5 lbs AI/A for chemigation, and 0.11- 0.33 lbs AI/10 gallons or 3-50 g/100 lbs of seed for seed treatments according to instructions on end-use product (EP) labels. It is understood that if chitosan and its salts are added to the list of MRP active and inert ingredients, these uses and application rates could be expanded; however, it is noted that uses for currently registered agricultural products is extensive.

[1] See, for example, https://www.waterboards.ca.gov/water_issues/programs/stormwater/docs/advtreat/naturalsitesolutions.pdf
[2] See, for example, http://chemtexlaboratories.com/bacshield/wp-content/uploads/2011/09/Bac-Shield-MSDS.pdf; https://s3-us-west-1.amazonaws.com/agrian-cg-fs1-production/pdfs/Consensus_MSDS4.pdf; https://fda.report/DailyMed/a71b8b28-1cca-7056-e053-2a95a90aee5f

Information on the chemical properties and formation of "dry" chitosan are described in the previous assessment (US EPA, 2019). To form "wet" chitosan or chitosan salts, the general process involves solubilization of "dry" chitosan with an acid. These salts may form when the deacetylated amine groups in chitosan are protonated upon acidification of the mixture, resulting in the chitosan becoming a polyelectrolyte. This protonation is a common step in chitosan solubilization, and it is the Agency's understanding that this will result in the formation of conjugate acid-base pairs between the chitosan (base) and the acid used for solubilization (e.g., acetate). These chitosan salts could be formed by mixing chitosan with acids and/or other chemicals currently listed as acceptable for use as inert ingredients in minimum risk pesticide products under 40 CFR 180.950(e). These salts include but are not limited to chitosan acetate, chitosan lactate, chitosan ascorbate, chitosan citrate, chitosan sulfate, chitosan sorbate, and chitosan hydrochloride. The EPA has included available data on chitosan salts which are expected to be present in some currently registered end-use products in the assessment below. If additional acids are included on the MRP inert ingredient lists and utilized in end-use products, the resulting salts would need to be similarly assessed. Refer to Appendix A below for a table of the acids currently on the MRP inert list which could potentially be mixed with chitosan to form chitosan salts.

FACTORS CONSIDERED FOR AMENDING 40 CFR 152.25 TO EXEMPT SUBSTANCES FROM REGULATION UNDER FIFRA (61 Federal Register 8876, March 6, 1996)

As discussed in US EPA (2019), the following seven factors described at 61 Federal Register (FR) 8876 (March 6, 1996) are considered when substances are added to the list of substances exempted from FIFRA requirements as pesticides:

1. Whether the pesticidal substance is widely available to the general public
2. If it is a common food or a constituent of a common food
3. If it has a nontoxic mode of action
4. If it is recognized by the US Food and Drug Administration as safe
5. If there is no information showing significant adverse effects
6. If its use pattern will result in significant exposure
7. If it is likely to be persistent in the environment

Additionally, this FR notice clarified that the factors are not meant to be absolute criteria and certain ones may be unsupported for some substances. However, taken as a whole, the EPA has operated under the belief that these factors indicate that substances proposed for this list will not pose a risk that warrants regulation under FIFRA.

APPLICATION OF THE CRITERIA TO CHITOSAN AND CHITOSAN SALTS

1. Widely available to the general public

The availability of chitosan to the general public was covered in the previous review. While that review focused on chitosan, many of the sources cited also covered chitosan derivatives, including chitosan salts (see Bellich et al., 2016; Hamad et al., 2016; NTP, 2017). It is the Agency's understanding that chitosan and its salts are widely available to the general public, and no information has been provided since the previous review that would refute that understanding.

2. Common food or constituent of a common food

The discussion of chitosan as a common food or constituent of a common food was covered in the previous review. Similar to its availability to the general public, that review focused on chitosan; however, one source from that review also described the presence of chitosan derivatives in food packaging (see Sinha et al., 2012).

3. Nontoxic mode of action

The nontoxic mode of action for chitosan has been addressed in the previous review. There are no known adverse effects reported for humans and other non-target organisms following agricultural, biopharmaceutical, biomedical, cosmetic, textile, and food additive applications of products that contain chitosan (US EPA, 2019). The chitosan quaternary salts appear to be non-toxic based on studies identified in the public literature. Like the parent compound chitosan, the chitosan salt derivatives exhibit antibacterial modes of action that include but are not limited to disruption of the bacterial cell membranes and cell wall integrity; moreover, the salt derivatives enhance the antifungal property of chitosan (Britto et. al., 2011).

4. Recognized by the US FDA as safe

The status of chitosan as a generally-recognized-as-safe (GRAS) substance was discussed in the previous review. According to a search of the available FDA databases (accessed 09/30/2021), none of the chitosan salts considered in this document are designated as GRAS.

5. No information showing significant adverse effects (to humans and nontarget organisms)

A. Effects of chitosan and chitosan salts on human health

The safety profile of chitosan was covered in the previous review. In addition, further review of the literature establishes that chitosan toxicity varies based on molecular weight as well as degree of deacetylation. However, there is no consensus within the literature on size classifications with 'high', 'medium', and 'low' molecular weight nomenclature varying and overlapping between different studies while also not matching the classifications of vendors. Chitosan forms can vary from 3-3,600 kilodaltons and 40-100% deacetylation (NTP, 2017). Currently, the Agency does not have enough information to determine how the molecular weight or degree of deacetylation will influence the potential toxicity of chitosan salts that could be formed with acids currently on the inert list or others that may be added in the future. Use of sources of chitosan that are acceptable for food or medicinal use could limit the scope of these uncertainties.

In the open scientific literature, there is limited information on the toxicity of the chitosan salts familiar to the Agency and the chitosan salts that could be formed using the current MRP inert ingredient list. Toxicology data for these chitosan salts in the literature is limited to acute and repeat dose oral studies in rats with chitosan lactate and chitosan acetate. Additionally, intranasal application of chitosan hydrochloride in rats demonstrated no toxicity to the nasal epithelium, and ocular administration in rabbits was non-irritating. This limited data set suggests that these substances are of low toxicity. However, the Agency acknowledges that many salts can potentially be produced, and toxicology data are not available for all of these substances. Nonetheless, a standard method for solubilizing chitosan utilizes acetic acid for which the available data correlate to the chitosan human health toxicology database.

Chitosan and its salts are anticipated to have similar mammalian toxicological profiles. In non-guideline 28-day oral toxicity studies in male and female rats (Lagarto et. al., 2015), no adverse effects were observed at the highest dose tested (No-observed-adverse-effect-level (NOAEL) = 1000 mg/kg/day (chitosan and chitosan lactate); NOAEL = 700 mg/kg/day (chitosan acetate)). In these studies, body weight, clinical chemistry, hematology, organ weights and histopathology parameters were assessed. Ophthalmological and neurological evaluations and clinical examinations were not reported. Clinical chemistry and hematological effects were observed but were not considered adverse by the study authors due to a lack of histopathological correlation. The Agency could not verify the study authors' conclusions because the histopathology data were not reported.

Guideline acute toxicity studies on an unknown salt of chitosan (6.2% dilution of chitosan in a "weak acid") showed low oral (MRID 45886507, Toxicity Category IV), dermal (MRID 45895201, Toxicity Category IV) and inhalation toxicity (MRID 45886508, Toxicity Category IV). The test substance was considered mildly irritating to the eye (MRID 45886510, Toxicity Category III) and slightly irritating to the skin (MRID 45886509, Toxicity Category IV) and was not a skin sensitizer (MRID 45886511). Chitosan, chitosan lactate and chitosan acetate were not lethal at the highest dose tested (2,000 mg/kg) in nonguideline oral toxicity studies (Lagarto et. al., 2015). In another non-guideline study, an aqueous solution of 1% chitosan hydrochloride produced no irritation in the eyes of rabbits (Di Colo et. al., 2004).

In a review of chitosan hydrochloride (July 2021), the European Commission Directorate-General for Health and Food Safety determined that there were no health concerns for its supported uses. Chitosan hydrochloride was not considered to be a substance of concern nor was it considered to have an inherent capacity to cause endocrine disrupting, neurotoxic or immunotoxic effects. It was concluded that the chemical has neither an immediate or delayed harmful effect on human or animal health nor an unacceptable effect on the environment when used in accordance with the supported uses. These uses include applications to a variety of food crops, ornamental plants and seeds in outdoor and indoor settings.

B. Effects of chitosan and chitosan salts on nontarget organisms

1. Non-target Organism Toxicity

There are currently no guideline non-target terrestrial organism toxicity studies for chitosan or chitosan salts available in the EPA database. As part of the petition to add chitosan and chitosan salts to the list of minimum risk pesticides, Tidal Vision submitted data from aquatic toxicity studies with chitosan acetate. Results from these studies are summarized in Table 1 below.

TABLE 1. Nontarget Organism Toxicity Summary for Potassium Carbonate (40 CFR § 158.2060)

Study/Guideline	Results	Toxicity* Category/Description	Reference
Freshwater fish (*Pimephales promelas*) acute toxicity USEPA Office of Water (2002), EPA-821-R-02-012	96-hour LC_{50} = >4 mg/L[1, 3] NOAEC = 4 mg/L	-	Tidal Vision USA, 2019a
Freshwater fish (*Oncorhynchus mykiss*) acute toxicity US EPA Office of Water (2002), EPA-821-R-02-012	96-hour LC_{50} = >4 mg/L[1, 3] NOAEC = 4 mg/L	-	Tidal Vision USA, 2019a

Freshwater fish (*Oncorhynchus mykiss*) acute toxicity US EPA Office of Water (2002), EPA-821-R-02-012	96-hour LC_{50} = 3.54 mg/L[2] NOAEC = 2.5 mg/L	Moderately toxic	Tidal Vision USA, 2019b
Freshwater fish (*Oncorhynchus mykiss*) 7-day toxicity (850.1075) Lazorchak and Smith (2007)	LOAEC (survival and growth) = >4 mg/L[1, 3] NOAEC = 4 mg/L	-	Tidal Vision USA, 2019a
Aquatic invertebrate (*Ceriodaphnia dubia*) acute toxicity US EPA Office of Water (2002), EPA-821-R-02-012	48- hour LC_{50} = 2.62 mg/L[1] NOAEC = 1.0 mg/L	Moderately toxic	Tidal Vision USA, 2019a
Aquatic invertebrate (*Ceriodaphnia dubia*) 7-day toxicity US EPA Office of Water (2002), EPA-821-R-02-013	LOAEC (reproduction) = 0.5 mg/L[1, 4] NOAEC = 0.25 mg/L	-	Tidal Vision USA, 2019a
Aquatic invertebrate (*Ceriodaphnia dubia*) 7-day toxicity US EPA Office of Water (2002), EPA-821-R-02-013	LOAEC (reproduction) = 0.3 mg/L[2, 4] NOAEC = <0.3 mg/L	-	Tidal Vision USA, 2019b

[1]Data derived from study with product (Tidal Clear 1%) containing 1% chitosan acetate. Toxicity values are based on the concentration of chitosan acetate contained in the product.
[2]Data derived from study with product (Tidal Clear 2%) containing 2% chitosan acetate. Toxicity values are based on the concentration of chitosan acetate contained in the product.
[3]Chitosan acetate toxicity cannot be categorized because treatments used did not cause adequate mortalities to calculate an LC_{50} value.
[4]Chitosan acetate toxicity cannot be categorized because the study did not calculate an LC_{50} value.

In the open scientific literature, much of the terrestrial organism data on chitosan salt effects are for plants. Data from plant studies indicate that chitosan salts provide beneficial effects, including resistance to dehydration (Iriti et al., 2009) and disease (Hadwiger, 2020), as well as improved growth (Algam et al., 2020) and fruit yield (Sajid et al., 2020).

The available scientific literature on chitosan effects to non-target aquatic species include data from studies in aquaculture and in toxicology. When used as a dietary supplement, dry chitosan has been demonstrated to enhance immune function in fish (Siwicki et al. 1994) and promote growth in shrimp (Rochana et al., 2020). Contrastingly, chitosan as an acid solubilized form (chitosan acetate) exhibits a high degree of toxicity to fish when the compound is present in water. A study by Bullock et al. (2000), exposed rainbow trout to 0.019, 0.038, 0.075, 0.75 mg/L chitosan acetate for 7 days in a preliminary toxicity test followed by a 14-day trial whereby trout were exposed to 0.019 and 0.038 mg/L chitosan acetate. The seven-day trial resulted in 0%, 7%, 73%, and 80% mortalities at the 0.019, 0.038, 0.075, 0.75 mg/L treatment levels, respectively. There were no mortalities in the water or 1% acetic acid controls. In the 14-day trial, 47% fish died at the 0.038 mg/L exposure concentration while the 0.019 mg/L concentration resulted in no mortalities.

Research on the toxic mechanisms of chitosan acetate indicates that toxicity in rainbow trout is induced by respiratory distress from hypoxia, which is proposed to be caused by damage or obstruction of branchial epithelium in the gill (Valenzuela et al., 2003). Additionally, mucus secretion caused by hypoxia may increase the diffusion distance for oxygen (Valenzuela et al., 2003). A study by Chou et al. 2020 proposes a similar toxicity mechanism (disruption of membrane epithelial cells) for low molecular weight chitosan hydrochloride when exposed to larval zebrafish. Given the proposed mode of chitosan acetate toxicity, the degree to which chitosan chemistry (free chitosan vs. acid solubilized) affects

chitosan toxicity is uncertain. Data demonstrating the beneficial effects of the free form of chitosan are from dietary studies where exposure primarily occurs through the gut as opposed to the gill, where chitosan acetate is proposed to elicit its toxic effects in fish.

In addition to studies with rainbow trout and zebrafish, chitosan salt aquatic toxicity studies are available for several other species. Research by Wang et al. (2016), determined the acute toxicity of chitosan acetate to carp (*Cyprinus carpio*), *Daphnia magna*, oligochaetes (*Limnodrilus hoffmeisteri*), and algae (*Chlorella vulgaris*). In these experiments, a 96-hour LC_{50} in carp and a 48-hour EC_{50} (immobilization) in *Daphnia* were observed at 3.0 mg/L and 2.2 mg/L, respectively. The 72-hour EC_{50} for cell yield inhibition in algae was 3.5 mg/L. In oligochaetes, the immobilization EC_{50} was observed at 6.9 mg/L after 72 hours of exposure. Based on results from experiments with carp and *Daphnia*, chitosan acetate would be classified as moderately toxic.

2. Aquatic Exposure and Risk Characterization

Chitosan acetate is classified as moderately toxic to fish and aquatic invertebrates though some studies suggest a lower level of toxicity (Table 1). Toxicity data used for the assessment were the lowest observed LC_{50} values for fish (3.54 mg/L) and aquatic invertebrates (2.62 mg/L) (Table 1).

The Pesticide in Water Calculator[3] (PWC version 2.001) was used to calculate chitosan acetate estimated exposure concentrations (EECs) for fish in surface waters. Model inputs (Table 2) were based on chitosan acetate physical/chemical properties and information from the label of an EPA registered product with the highest maximum application rate (resulting in 1.8 lbs chitosan acetate/A). Because the product label is for terrestrial spray applications to a broad range of agricultural crops, risk was determined for the crop scenario (cotton) which resulted in the highest EEC. Environmental exposure was calculated for the maximum application rate at three different application frequencies (1, 6, and 12 applications), each with 1-day application intervals, per season or growing period. These applications are not an assumed maximum seasonal rate because the maximum number of applications and maximum yearly/seasonal rate are not provided on the label. Applications of the total amount of chitosan applied are also based on calculated levels of the salt form (chitosan acetate) contained in the product. Refer to **Appendix B** for the assumptions and calculations used to derive the concentration of chitosan acetate for a theoretical pesticide product. Information specific to any products used in the analysis has been removed to a **Confidential Appendix**. Environmental fate values for chitosan acetate were calculated using EPA's Estimation Programs Suite (EPISuite Version 4.11). The specific models used within the EPISuite program were KOCWIN version 2 (absorption coefficient estimate) and the Level III Fugacity Model (water half-life estimate). Because a simplified molecular-input line-entry system (SMILES) is currently available for chitosan but not chitosan acetate, environmental fate parameters were estimated for chitosan acetate using the chitosan SMILES as a surrogate. The chitosan acetate soil half-life was derived from a study in the scientific literature (Sawaguchi et. al., 2015), which was used in the previous chitosan assessment (US EPA, 2019) associated with this addendum. Refer to **Appendix C** for additional model details, including input parameters and estimated outputs.

Table 2. Summary of Model Inputs for PWC Estimates

Input	Value
Maximum Application rate (kg/ha)	2.02*
Cropped Area Fraction	1

3 https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment#PWC

Table 2. Summary of Model Inputs for PWC Estimates	
Input	**Value**
Koc (ml/g)	1E+010 (EPISuite 4.11)**
Water Half-Life (days) @ 25 °C	15 (EPISuite 4.11)
Benthic Half-Life	Not available
Photolysis Half-Life	Not available
Hydrolysis Half-Life	Not available
Soil Half-Life (days) @ 25 °C	10 (Sawaguchi et. al., 2015)
Foliar Half-Life	Not available
Molecular Weight (g/mole)	1526.48 (PubChem)
Vapor Pressure (torr)	Not available
Solubility (mg/l)	Not available
Henry's Constant	Not available

*Estimated chitosan acetate concentration in formulated product is based on percent deacetylated amine groups and amine group protonation at pH 6.0. Refer to Appendix B for calculation details.
**Estimated Koc value from EPISuite/KOCWIN estimation program is based on the molecular connectivity index (MCI) method.

Results of the exposure modeling and toxicity effects data were used to evaluate the likelihood of adverse ecological effects to aquatic species. To calculate risk quotients (RQs), the 1-day EEC that resulted from either 1, 6, or 12 chitosan acetate applications was divided by the lowest acute toxicity value for fish and invertebrates, resulting in a conservative estimate of risk. The RQs were compared to the Agency's limit of concern (LOC), which is 0.5 and 0.05 for non-listed and listed aquatic species, respectively ("listed" refers to federally listed threatened and endangered species). The RQs calculated using PWC for run-off and spray drift exposure to nontarget listed and non-listed aquatic organisms from use of chitosan acetate are provided in Table 2 below.

Table 3. Acute RQs for fish and aquatic invertebrates exposed to the residues of chitosan aetate.					
Number of Applications*	**Listed Status**	**EEC (mg/L)**	**LC_{50} Value (mg/L)****	**RQ**	**LOC**
Fish**					
1	Non-listed	5.80E-07	3.54	<0.01	0.5
	Listed	5.80E-07	3.54	<0.01	0.05
6	Non-listed	7.85E-07	3.54	<0.01	0.5
	Listed	7.85E-07	3.54	<0.01	0.05
12	Non-listed	9.67E-07	3.54	<0.01	0.5
	Listed	9.67E-07	3.54	<0.01	0.05
Aquatic Invertebrates**					
1	Non-listed	5.80E-07	2.62	<0.01	0.5
	Listed	5.80E-07	2.62	<0.01	0.05
6	Non-listed	7.85E-07	2.62	<0.01	0.5
	Listed	7.85E-07	2.62	<0.01	0.05
12	Non-listed	9.67E-07	2.62	<0.01	0.5
	Listed	9.67E-07	2.62	<0.01	0.05

**Application rate ate is based on highest application (1.8 lb AI/A or 2.02 kg AI/ha) specified by label (ARMOUR-Zen, EPA File Symbol: 75747-3).
**Fish LC_{50} value derived from study with pesticide formulation (Tidal Clear 2%) containing 2% chitosan acetate. Invertebrate LC_{50} value derived from study with pesticide formulation (Tidal Clear 1%) containing 1% chitosan acetate

The RQs are below the LOC for non-listed and listed species at the modeled maximum chitosan acetate application rate across each of the application frequencies. Even though chitosan acetate is moderately

toxic to fish and aquatic invertebrates, the modeled exposure based on repeated uses is low due to high estimated soil partitioning (Koc) and environmental degradation. Therefore, adverse effects to aquatic organisms are not anticipated.

3. Uncertainties

There are several uncertainties in the assumptions used to calculate risk. The study by Bullock et al. (2000), discussed in section B.1 above, was not used as part of the quantitative risk analysis because it did not address specific data requirements, such as reporting of an LC_{50} value, typically required for BPPD environmental risk assessments. However, it is uncertain as to why mortalities in the Bullock et al. study occurred at lower concentrations than the studies submitted to the Agency by Tidal Vision. Given the proposed mechanism (respiratory distress from hypoxia caused by damage or obstruction of branchial epithelium) by which chitosan acetate elicits toxicity in fish, chitosan acetate toxicity may be influenced by organism age because older fish have larger, more developed gills than juvenile fish. The study by Bullock et al. 2000 used adult fish with a mean weight of 120.5 grams as opposed to juvenile fish weighing (<) 3.0 grams as indicated in the EPA toxicity testing guideline (OCSPP 850.1075). Additionally, toxicity data used in this assessment were from static renewal tests that took place over a 96-hr period with test solution renewal at 48 hours. Studies by Bullock et al., on the other hand, used a flowthrough test system whereby solutions were continually renewed over a 7-day period. Water quality parameters, such as dissolved oxygen, were not reported in the studies by Bullock et al., so it cannot be determined whether water quality or other aspects of the conduct of these studies had an impact on the observed toxicity differences between the toxicity studies used in this assessment and those performed by Bullock et al.

The EECs used in this assessment are based on a registered label use rate. The label does not specify the maximum number of yearly or seasonal applications. The current assessment does not account for risks associated with additional applications, higher application rates, or direct applications to water.

Due to the limited environmental fate data available for chitosan and chitosan salts, environmental fate parameters, including Koc and water half-life, used to calculate chitosan acetate EECs are based on computational estimates. Chitosan Koc estimates from the KOCWIN model provides a high Koc value. If measured Koc values for chitosan acetate are lower than modeled values used in the current assessment, then exposure and ultimately risk could be underestimated. However, one of chitosan's current uses is as a flocculent, and it has been shown to reduce erosion-induced soil loss (Orts et al. 2000), so chitosan acetate is not anticipated to enter aquatic habitats at significant levels after application. Lastly, the similarity between chitosan acetate and other salt forms constitutes an uncertainty when assessing risk to aquatic organisms.

6. Use pattern results in significant exposure

Exposure to chitosan was discussed in the prior assessment. In consideration of the salts that could be formed using the current MRP list, it is unlikely that the additional agricultural uses that may be allowed will significantly expand the potential for exposure of humans to chitosan, since current pesticidal uses are already extensive. As stated previously, the Agency believes that some of the registered pesticide products contain chitosan salts or "wet" chitosan, rather than "dry" chitosan, and pesticidal uses of chitosan likely do not contribute more exposure than other uses of chitosan. However, it should be noted that there is a lack of information in the scientific literature identifying if absorption, metabolism and elimination of chitosan salts are equivalent to those of chitosan.

Approved non-pesticidal uses of chitosan salts include biomedical applications such as enhanced wound healing products (e.g., bandages and creams) and cartilage repairing formulations (Straccia, 2015; Rizwan, 2019; Bellich, 2016). The salts are also used in water treatment as flocculation and coagulation agents.

According to the available scientific literature, chitosan salts are also being evaluated in a variety of fields, including pharmacology, biomedicine and polymer science (Bellich, 2016). It is unknown to the Agency if these applications are currently in use or have been approved by any regulatory entities.

7. Not likely to persist in the environment

The persistence of chitosan in the environment was covered in the previous review. Information from the previous review indicate that chitosan may readily degrade in soils. In a study using sandy and silty soils (Sawaguchi et. al., 2015), it was observed that chitosan added to silty soil dissipated by 50% after 10 days incubation at 25°C and was non-detectable after 30 days. As of now, there are no data or information on the persistence of chitosan in aquatic environments however its use as a flocculent suggests persistence in aquatic environments will be low.

CONCLUSIONS

As part of the consideration for the addition of chitosan to the MRP list, the Agency recognizes the necessity to review and include chitosan salts as these can be created during formulation of some chitosan pesticide products. As such, available Agency data were reviewed, and an extensive literature search and data analysis was performed for several chitosan salts. Data and information were the most abundant for chitosan acetate. As such, the data for chitosan acetate was used as a surrogate for evaluating chitosan salts as a group.

The human health assessment database is limited both in terms of studies performed and representative chitosan salts tested. There are also uncertainties in the potential toxicological effects of differences in chitosan molecular weights and degrees of deacetylation which could be mitigated by use of sources of chitosan that are acceptable for food or medicinal use. However, the Agency's overall analysis of the available data suggests that these substances are of low toxicity. No risks of concern have been identified.

There is no evidence that chitosan salts would have any adverse effects on non-target terrestrial organisms. While the use of dry chitosan in fish feed suggests low risk to aquatic taxa, studies identified in the scientific literature indicate chitosan acetate is highly toxic to rainbow trout. Guideline studies available in the Agency's database, on the other hand, indicate that chitosan acetate is moderately toxic to fish and aquatic invertebrates. Studies used in this assessment were selected because they reported the necessary information (e.g., LC_{50} values) for risk calculations and adhered to Agency guidelines. Calculated risks (RQs) based on non-target organism toxicity data and aquatic exposure modeling are below the Agency's limit of concern by several orders of magnitude.

As discussed in more detail above, there are several uncertainties in the exposure and toxicity assumptions used to calculate risk. Of important consideration, is that the Koc value used in the exposure modeling is based on a computational estimate because measured values are currently

unavailable. The chitosan Koc estimate from the KOCWIN model indicates a high tendency for chitosan to bind to soil, and thus a reduced tendency to enter the water column after application to crops. If measured Koc values for chitosan acetate are lower than modeled values used in the current assessment, than exposure and ultimately risk (RQs) are being underestimated. However, chitosan acetate is not anticipated to enter aquatic habitats at significant levels following application to crops because chitosan has been shown to reduce erosion-induced soil loss, which indicates that chitosan acetate will likely have a greater tendency to remain in soil rather than enter the water column following application. Risks to nontarget listed and unlisted organisms are acceptable when chitosan acetate is used in accordance with currently EPA-registered label directions. However, this assessment does not cover all potential future use scenarios for chitosan acetate as an MRP ingredient.

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Appendix A

Acids currently on the MRP Inert List that could be formulated into chitosan salts

Acids		
Acetate	Dicaprylate	Myristate
Acetyl tributyl citrate	Dimyristate	Octanoate
Alginate	Dioleate	Oleate
Benzoate	Distearate	Palmitate
Bicarbonate	Dodecanoate	Ricinoleate
Butanedioate	Fumarate	Silicate
Carbonate	Humate	Sorbate
Citrate	Lactate	Stearate
Decanoate	Malate	Sulfate

Appendix B

Chitosan Salt Concentration Estimate

Relevant parameters for estimating chitosan salt concentrations in a theoretical pesticide formulation are listed below. Information specific to any products used in the analysis has been removed to a **Confidential Appendix**.

- Chitosan concentration
- Acetic acid concentration
- pH of concentrated chitosan before neutralization
- Final pH
- pKa of chitosan's primary amine is 6.5
- Since we are attempting to estimate the highest salt amount, we will assume 95% deacetylation in our calculations
- Percentage of deacetylated amine groups that will be protonated at the formulation's pH
- Pesticidal use rates in lbs active ingredient/acre
- We will assume that acetic acid exists in excess of chitosan and as such, acetate will not be a limiting reagent in the reaction
- Chitosan's protonated amine groups will function as a basic cationic polyelectrolyte (Chawla *et al.* 2014; Pardo-Castaño *et al.* 2019) which will form acid-base conjugate pairs with the anion of the acid, resulting in chitosan salts. We will assume that 100% of the deacetylated, protonated amine groups will form salt

Henderson-Hasselbalch equation with work-up

$$pH = pK_a + \log_{10}\left(\frac{[A-]}{[HA]}\right)$$

$$x = 6.5 + \log_{10}\left(\frac{[A-]}{[HA]}\right)$$

$$(10^{x - 6.5})*[HA] = [A-]$$

Rest of computations

$$[HA] + [A-] = 1$$

$$[A-] = 1 - [HA]$$

$$(10^{x - 6.5})*[HA] = 1 - [HA]$$

$$(10^{x - 6.5})*[HA] + [HA] = 1$$

Solve for $[HA]$

$[HA] = Y$

Y * 100 = Y% deacetylated, protonated amine groups

Assumption of 95% chitosan deacetylation

Assumption that 100% of deacetylated, protonated amine groups will form the salt

0.95 * 1.00 * Y% = Z% of the chitosan salt in the formulation

Z% * application rate (lbs chitosan/acre) = **lbs chitosan-salt per acre.**

Additional considerations

- Chitosan is a polymer with repeating subunits. Each of those subunits has an amine group that can be deacetylated, protonated, and undergo an acid-base reaction to form the salt.
- If the formation of a salt on a single subunit of the polymer were to then increase the affinity of other subunit amines for the salt on that same polymer through an increase in the K_a (association constant), it is conceivable that multiple acetate ions would associate with the same chitosan polymer. This would leave an insufficient amount of acetate to form the salt with all of the protonated amines and make our calculations incorrect.
    - There is no available information suggesting a changing association constant with salt interaction
    - Even if this were to occur, it would decrease overall salt-form concentration and as such, our current estimate would account for the upper limit of chitosan salt application

Appendix C

Water Modeling of Chitosan Acetate Applied to Cotton and the USEPA Standard Pond

Estimated Environmental Concentrations for chitosan acetate are presented in Table 1 below for the USEPA standard pond with the NCcottonSTD field scenario. A graphical presentation of the year-to-year acute values is presented in Figure 1. These values were generated with the Pesticide Water Calculator (PWC), Version 2.001. Critical input values for the model are summarized in Tables 2 and 3 below.

This model estimates that about 2.1% of chitosan acetate applied to the field eventually reaches the water body. The main mechanism of transport from the field to the water body is by spray drift (60.2% of the total transport) followed by erosion (39.9%).

In the water body, chitosan acetate dissipates with an effective water column half-life of 26.6 days. (This value does not include dissipation by transport to the benthic region; it includes only processes that result in removal of chitosan acetate from the complete system.) The main source of dissipation in the water column is metabolism (effective average half-life = 26.6 days).

In the benthic region, chitosan acetate is stable. The vast majority of chitosan acetate in the benthic region (100%) is sorbed to sediment rather than in the pore water.

Table 1. Estimated Environmental Concentrations (ppb) for chitosan acetate.

1-day Avg (1-in-10 yr)	0.9672E-003
4-day Avg (1-in-10 yr)	0.4741E-003
21-day Avg (1-in-10 yr)	0.4456E-003
60-day Avg (1-in-10 yr)	0.4297E-003
365-day Avg (1-in-10 yr)	0.4120E-003
Entire Simulation Mean	0.2371E-003

Table 2. Summary of Model Inputs for chitosan acetate.

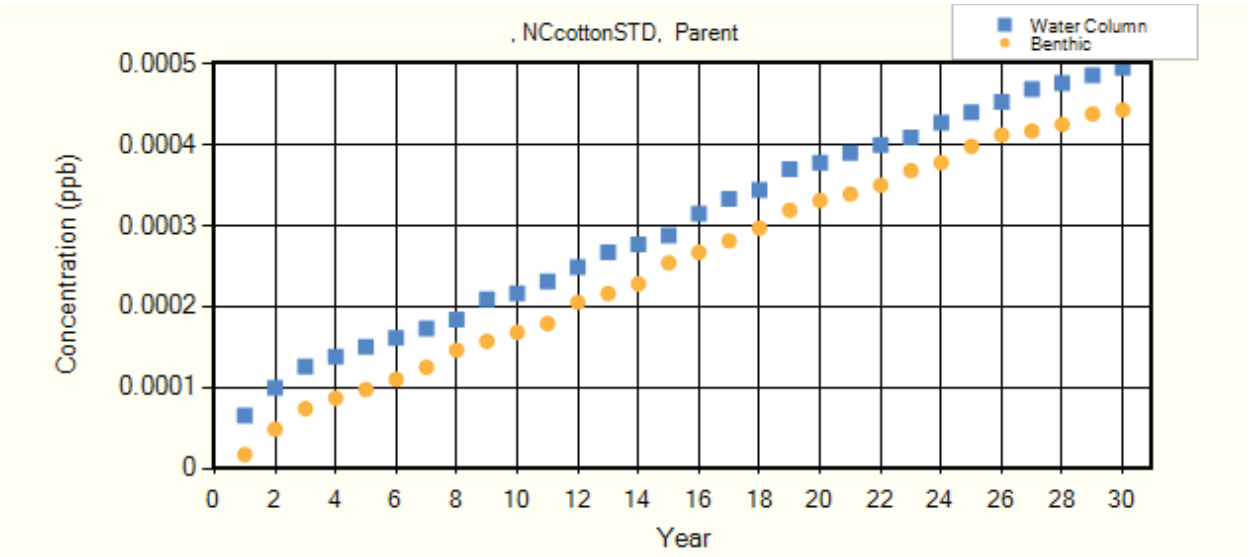
Scenario	NCcottonSTD
Cropped Area Fraction	1
Koc (ml/g)	1E+010 (EPISuite 4.11)
Water Half-Life (days) @ 25 °C	15 (EPISuite 4.11)
Benthic Half-Life (days) @ °C	
Photolysis Half-Life (days) @ °Lat	
Hydrolysis Half-Life (days)	
Soil Half-Life (days) @ °C	10 (Sawaguchi et. al., 2015)

Foliar Half-Life (days)	
Molecular Weight	1526.48 (PubChem)
Vapor Pressure (torr)	
Solubility (mg/l)	
Henry's Constant	

Table 3. Application Schedule for chitosan acetate.

Date (Days Since Emergence)	Type	Amount (kg/ha)	Eff.	Drift
0	Above Crop (Foliar)	2.02	0.95	0.125
1	Above Crop (Foliar)	2.02	0.95	0.125
2	Above Crop (Foliar)	2.02	0.95	0.125
3	Above Crop (Foliar)	2.02	0.95	0.125
4	Above Crop (Foliar)	2.02	0.95	0.125
5	Above Crop (Foliar)	2.02	0.95	0.125
6	Above Crop (Foliar)	2.02	0.95	0.125
7	Above Crop (Foliar)	2.02	0.95	0.125
8	Above Crop (Foliar)	2.02	0.95	0.125
9	Above Crop (Foliar)	2.02	0.95	0.125
10	Above Crop (Foliar)	2.02	0.95	0.125
11	Above Crop (Foliar)	2.02	0.95	0.125

Figure 1. Yearly Highest 1-day Average Concentrations


, NCcottonSTD, Parent
Water Column
Benthic
Concentration (ppb)
0.0005
0.0004
0.0003
0.0002
0.0001
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Year