

# Chitosan

## Crops

### Identification of Petitioned Substance

Chemical Names:  
(C<sub>6</sub>H<sub>11</sub>NO<sub>4</sub>)<sub>n</sub>  
poly-D-glucosamine  
Poly(beta-(1,4)-2-amino-2-deoxy-D-glucose);  
Poly(beta-(1,4)-D-glucosamine)

18 ELEXA-4  
19 BIOREND  
20

CAS Numbers:  
9012-76-4

Other Name:  
deacetylated chitin  
water-soluble chitin (some forms)  
poliglusam/polyglusam

Other Codes:  
UNII: 23R93M6Y64  
UNII: 5GV09YMO52  
UNII: 7SRJ3W89J8  
UNII: 82LKS4QV2Y  
UNII: SBD1A2I75N

Trade Names:  
ARMOUR-Zen  
Armour-Zen 15%  
LifeForce Roots  
NUPRO  
EXCEED PLANT DEFENSE BOOSTER  
USAG2020  
RAISAN

### Summary of Petitioned Use

In 2004, the National Organic Program (NOP) received a petition to add synthetic chitosan to the National List of Allowed and Prohibited Substances (hereafter known as the “National List”) for use as an adhesive adjuvant, used in conjunction with fungicides (Washington State University). In 2005, the National Organic Standards Board (NOSB) recommended that chitosan be added to § 205.601 for use in organic crop production with the annotation “as an adjuvant only” (NOSB 2005a). At that time, the board expressed that chitosan was an adjuvant that was needed as a “sticker” for certain crop pesticide uses when visible residues were not acceptable (NOSB 2005b). The board stated that they did not want chitosan to be used as a plant growth regulator. Following the NOSB’s recommendation, the NOP did not take steps to separately add chitosan to the National List, but instead clarified that it was already allowed as an adjuvant under the §205.601(m) allowance for inert ingredients (NOP 2007). Chitosan remains permitted for use as an inert ingredient when formulated with an allowed active pesticidal ingredient.

In 2019, the NOP received a second petition to add synthetic chitosan to the National List – this time for plant disease control. The petitioner stated that chitosan is an alternative to sulfur-based pesticides, which can be phytotoxic to plants. A partial label, which lists several organisms that cause powdery mildew, downy mildew, and gray mold (*Botrytis* bunch rot) on a variety of crops, is included in the petition (Bio-Gro, Inc. 2019). Chitosan is also approved by the Environmental Protection Agency (EPA) to control other plant pathogens, but these are not specifically listed on the portion of the product label submitted by the petitioner. The complete product label registered with the EPA lists at least 150 disease-causing organisms and more than 200 crops (US EPA 2016). The petition did not clearly indicate a specific use for which chitosan is essential for organic production. Instead, it suggested that chitosan is an alternative to currently available materials and organic management practices, offering benefits related to toxicity and environmental safety.

The petitioner describes many different uses of chitosan throughout the petition. It is difficult to provide a useful summary comparison of chitosan versus all other alternatives because the number of input materials, organic practices, disease-causing organisms, and crops to evaluate is large. Where possible, examples are used which illustrate a few comparisons between chitosan and other materials, focusing on the disease-causing organisms that appear on the part of the label included with the petition. However, these examples do not relate to all possible crops and diseases that could be covered under this petition. The 2004 technical report on chitosan, written in support of the NOSB's 2005 review, is still relevant. The following technical report confirms many of the same conclusions included in that report.

#### Characterization of Petitioned Substance

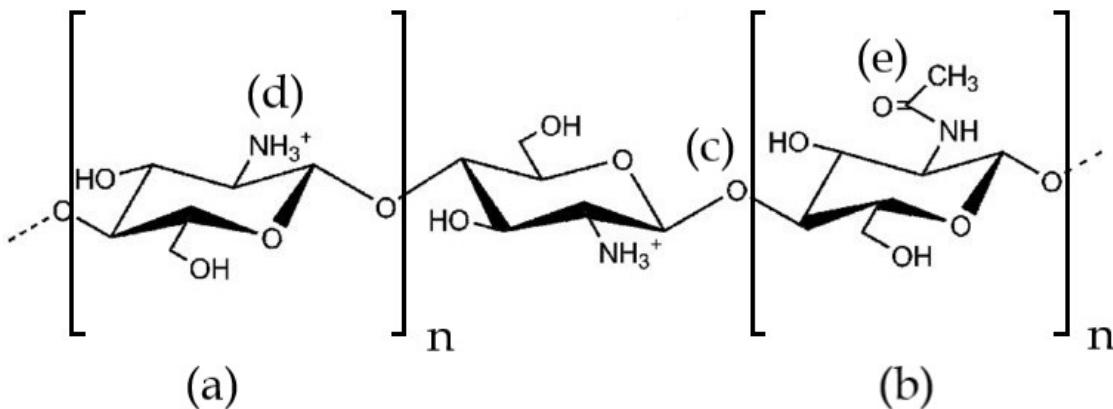
##### Composition of the Substance:

Chitosan is a copolymer composed of two different chemical subunits (monomers) that repeat in no particular order: glucosamine (2-acetamido-2-deoxy-d-glucopyranose) and N-acetylglucosamine (2-amino-2-deoxy-d-glucopyranose) (Pillai, Paul and Sharma 2009). It is derived from chitin, which is structurally similar to cellulose (Dutta, Dutta and Tripathi 2004) and only differs slightly in the individual monomers bonded together to form the polymer. While cellulose is composed of glucose monomers linked together, chitin is mostly composed of N-acetylglucosamine monomers. Cellulose and chitin are the two most abundant biopolymers found on earth (Pillai, Paul and Sharma 2009; Chawla, Kanatt and Sharma 2014).

There is no degree of deacetylation that officially defines when chitin becomes chitosan, but the lower limit described in literature is 40–60 percent (Hussain, Iman and Maji 2014). Typically chitin contains 85–95 percent N-acetylglucosamine and 5–15 percent glucosamine (Pillai, Paul and Sharma 2009). Chitosan is a synthetic made by removing acetyl groups from chitin. Commercial chitosan usually contains at least 65 percent glucosamine and less than 35 percent N-acetylglucosamine (Pillai, Paul and Sharma 2009).

The degree of deacetylation can vary, and so any given quantity of chitin or chitosan will typically contain both types of monomers. Below a pH of approximately 6, chitosan becomes positively charged by acquiring hydrogen ions (see Figure 1 below). This pH also coincides with chitosan becoming soluble in aqueous solutions, such as in a solution of acetic acid and water (Pillai, Paul and Sharma 2009).

**Figure 1: Structure of chitosan under acidic conditions\***



\*Glucosamine monomers (a) are bonded to additional glucosamine monomers or N-acetylglucosamine monomers (b) via 1-4 glycosidic linkages (c). Glucosamine monomers possess an amine group (d), ionized due to low pH. N-acetylglucosamine monomers possess the acetamide (amino-acetyl) group (e). Drawing adapted from (Nilsen-Nygaard, et al. 2015).

92 Chitosan is a polymer, which means that it can exist in a range of molecular sizes (usually measured by  
93 weight). The molecular weight of a chitosan sample can affect its properties. For example, low-molecular-weight  
94 chitosan is more effective as a plant growth stimulator than high-molecular-weight chitosan  
95 polymers (Nwe, Furuike and Tamura 2013). Not only does the molecular weight of chitosan affect its  
96 properties, but so too does the degree (and distribution) of deacetylation. For example, as the degree of  
97 acetylation increases, chitosan becomes more amorphous (less crystalline) and better able to chelate metal  
98 ions (Chawla, Kanatt and Sharma 2014). See *Action of the Substance* section below for more information on  
99 the effect chelation may have on microbial growth.  
100

101 **Source or Origin of the Substance:**

102 Commercial forms of chitosan are usually produced from chitin. Chitosan is also a naturally occurring  
103 polymer, having been directly extracted and isolated from certain groups of fungi, including the  
104 Basidiomycota and Zygomycetes (Tajdini et al. 2010). Chitosan is also produced from chitin through both  
105 chemical and biological processes. According to Chowla et al. (2014), the United States, Japan, Norway,  
106 Thailand, India, Australia, and Poland are the largest producers of chitosan.  
107

108 Commercially available chitosan is produced from chitin through synthetic means (see *Evaluation Question*  
109 #2). Chitin is abundant on Earth; organisms produce an estimated 100 billion metric tonnes of chitin each  
110 year (Yan and Chen 2015).  
111

112 Most commercial chitin (and chitosan) is derived from shrimp, prawn, and crab wastes (Chawla, Kanatt  
113 and Sharma 2014; Dutta, Dutta and Tripathi 2004; Islam, Bhuiyan and Islam 2017; Younes and Rinaudo  
114 2015). Chitin makes up 20–30 percent of crustacean shells (Chawla, Kanatt and Sharma 2014). Isolating  
115 chitin from shrimp is easier than from other animals due to their thinner shells, but still requires chemical  
116 treatments to separate the chitin from other biological materials (Younes and Rinaudo 2015). At least one  
117 commercial chitosan source has been developed using mycelium wastes from fungal fermentation  
118 processes (Bellich et al. 2016); however, chitosan derived from fungi is not common (Chawla, Kanatt and  
119 Sharma 2014).  
120

121 **Properties of the Substance:**

122 Chitin and chitosan share many properties but differ in a few significant ways. Like cellulose in plants and  
123 collagen in vertebrate animals, chitin acts as a structural polymer that organizes into ordered strands and  
124 provides support within a wide variety of organisms (Navard 2012; Pillai, Paul and Sharma 2009). It forms  
125 structures that strengthen cell walls, insect skeletons, crustacean shells, and internal mollusk body parts  
126 (Navard 2012; Chawla, Kanatt and Sharma 2014). Chitin is insoluble in solvents in part due to hydrogen  
127 bonding that leads to a semi-crystalline structure (Pillai, Paul and Sharma 2009). While the insolubility of  
128 chitin is a benefit for stability in biological systems, the same quality makes it difficult to adapt for use by  
129 humans.  
130

131 Chitosan exhibits many similar properties to chitin, but with the benefit of being more soluble in mildly  
132 acidic aqueous solutions and more reactive (Islam, Bhuiyan and Islam 2017). Numerous industries have  
133 taken interest in chitosan over the last few decades because of the ability to fine-tune its physicochemical  
134 properties for specific purposes (Bellich et al. 2016). Chitosan is a versatile and promising material for  
135 developing bioplastics (including films), healthcare products, food additives, pesticides, fruit coatings, seed  
136 treatments, wastewater treatments, and other products. The substance's versatility is due to its properties  
137 as a structural polymer as well as its ability to form cations, to chelate, and to be chemically modified in a  
138 number of ways. These properties, combined with its biocompatibility, biodegradability, and antimicrobial  
139 effect, has made it an attractive molecule for product development. Bellich et al. (2016) notes that more than  
140 1,100 papers were published about chitosan in the 1980s, 5,700 in the 1990s, and more than 23,000 in the  
141 2000s.  
142

144 Chitosan is unique in that it is basic, unlike cellulose and most other biopolymers (which are acidic)  
 145 (Chawla, Kanatt and Sharma 2014). As a cationic polyelectrolyte,<sup>1</sup> chitosan can bond with anionic  
 146 molecules in ways that other biopolymers do not. This property leads to some of chitosan's antimicrobial  
 147 qualities (see *Action of the Substance* below). Generally, chitosan has high positive charge density owing to  
 148 protonation of the amino groups formed from deacetylation (Islam, Bhuiyan and Islam 2017). These amino  
 149 groups become protonated (and positively charged) at a pH below around 6, corresponding to the  
 150 material's pKa value (see Table 1 below). Properties such as the solubility of chitosan depend not just on  
 151 the degree of deacetylation, but also on how the acetylated and deacetylated groups are distributed  
 152 through the polymer (Younes and Rinaudo 2015). The distribution of these chemical groups is determined  
 153 by the manufacturing process used (See *Evaluation Question #2*).  
 154

155 **Table 1: Properties of Chitosan**

Property	Value <sup>a</sup>
Physical state and appearance	Solid, powder
Color	White to light cream
Molecular weight	5 X 10 <sup>3</sup> to 10 <sup>5</sup> daltons
Specific gravity	1.4 kg/m <sup>3</sup>
Bulk density	0.4 g/cm <sup>3</sup>
Solubility	Soluble in dilute acids and 1,1,1 trichloroethane (TCA)
pKa	6.3 (amino groups)
Degradation temperatures	86–230°F (30–110°C) (dehydration); 256–644°F (180–340°C) (polymer decomposition); 878°F (470°C) (chemical breakdown leading to loss of mass)
Stability	Stable under standard storage conditions
Reactivity	Decomposes under highly acidic or highly basic conditions, or applications of strong oxidizing agents.
Nitrogen content	5–8 percent

156 <sup>a</sup>Source: (Thermo Fisher Scientific 2018; Sigma Aldrich 2020; Pillai, Paul and Sharma 2009; Szymańska and  
 157 Winnicka 2015; Parchem Fine & Specialty Chemicals 2020; Islam, Bhuiyan and Islam 2017)

158 Depending on which acid is used, chitosan is soluble in aqueous solutions below a pH of around 6 (Pillai,  
 159 Paul and Sharma 2009). Above a pH of 6, the amino groups become deprotonated, which leads to overall  
 160 insolubility in water. Chitosan is soluble in organic acids such as acetic, formic, and lactic acids; however, it  
 161 is insoluble in some mineral acids such as sulfuric and phosphoric acid. Chitosan is insoluble in most  
 162 organic solvents, such as dimethylformamide and dimethyl sulfoxide, but is soluble in N-methyl  
 163 morpholine-N-oxide (NMMO) (Pillai, Paul and Sharma 2009).

164 Both the amino (NH<sub>2</sub>) and hydroxyl (OH) groups of chitosan are useful as sites for chemical substitutions  
 165 to create functional derivatives (Dutta, Dutta and Tripathi 2004). These "pendant groups," or side chains,  
 166 can create new or alter existing physicochemical properties of chitosan. For example, the amino groups can  
 167 be reacted with aldehydes to form a substance with reduced hydrophobicity and improved solubility (N-  
 168 alkyl chitosan) (Bellich et al. 2016; Dutta, Dutta and Tripathi 2004). N-carboxybutyl chitosan is a derivative  
 169 that has enhanced antibacterial properties. Chemical modifications to chitosan are typically intended to  
 170 improve physicochemical characteristics—such as solubility under specific conditions or its compatibility  
 171 with other biopolymers—or to enhance biological properties such as chitosan's antibacterial activity  
 172 (Bellich et al. 2016).

173

174

175

<sup>1</sup> A polyelectrolyte is a polymer where individual monomers (repeating molecular sub-units) can become ionized (charged), leading to a molecule with many charged regions.

176 Owing to its polymeric structure, chitosan can exist in a variety of sizes, typically from 10,000 to  
177 1,000,000 daltons (Pillai, Paul and Sharma 2009). Low-molecular-weight chitosan can permeate cell  
178 membranes while high-molecular-weight chitosan cannot (Tokura et al. 1997). It appears that molecular  
179 weight plays a role in chitosan's different modes of actions – in some cases acting as a growth inhibitor for  
180 bacteria, while in other cases having the opposite effect and acting as a bacterial growth promoter (Bellich  
181 et al. 2016).

182 Chitosan chelates many metals, including nickel ( $Ni^{2+}$ ), zinc ( $Zn^{2+}$ ), cobalt ( $Co^{2+}$ ), iron ( $Fe^{2+}$ ), magnesium  
183 ( $Mg^{2+}$ ), and copper ( $Cu^{2+}$ ) (Dutta, Dutta and Tripathi 2004; Kong et al. 2010). These metals include plant  
184 micronutrients as well as substances that are essential to the proper functioning of bacterial cell walls  
185 (Kong et al. 2010). Some chitosan-metal complexes themselves have bactericidal properties, such as  $Zn^{2+}$ ,  
186  $Zr^{2+}$ , and  $Ag^{1+}$  (Zalloum and Mubarak 2013). Several models of how chitosan chelates metals have been  
187 proposed, and more than one manner of chelation may occur at the same time (Gerente et al. 2007). Most  
188 models consider the amino groups present in chitosan as the basis for the chelation of transition metals. For  
189 example, the amino groups from the same chitosan chain may be involved in donating electrons to create  
190 the chelation complex (forming a bridge), or separate adjacent chains can be involved. Another model  
191 suggests that hydroxyl groups may participate where the metal is simultaneously bonded to two amino  
192 groups and two hydroxyl groups. Alternatively, the metal may be bonded to a hydroxyl group, an amino  
193 group, and two water molecules. The pH of the solution may influence the way in which chitosan chelates  
194 specific metals. Not only that, but pH also affects the total capacity for chitosan to chelate metals. At lower  
195 pH, the positively charged amino groups interfere with the ability of chitosan to chelate metal cations  
196 (Gerente et al. 2007). Chitosan preferentially adsorbs divalent metals in the following order:  $Cu(II) > Hg(II)$   
197  $> Zn(II) > Cd(II) > Ni(II) > Co(II) \sim Ca(II)$ ; and trivalent metals:  $Eu(III) > Nd(III) > Cr(III) \sim Pr(III)$  (Rhazi, et  
198 al. 2002).

200

201 **Specific Uses of the Substance:**

202

203 *Petitioned Use*

204 Chitosan is petitioned for use as a plant disease control agent. Traditionally, plant diseases are caused by  
205 pathogenic microorganisms and environmental conditions (Agrios 2005). The petitioner requested that  
206 chitosan be added so that the material may be used for plant disease control on a variety of crops (Bio-Gro,  
207 Inc. 2019). The petitioner bases the request on chitosan's antimicrobial properties as well as its role in plant  
208 defense signaling pathways (see *Action of the Substance* below). Additionally, chitosan's disease control  
209 properties extend to those caused by nematodes. For chitosan's use as an inert ingredient under  
210 §205.601(m), see the 2004 Technical Evaluation Report (USDA 2004).

211

212 As of March 26, 2020, there were 22 EPA-registered pesticide products containing chitosan as the active  
213 ingredient, including two products from the petitioner (National Pesticide Information Center 2020).  
214 Several of the currently active registrations are repackaged products, also registered under a different  
215 name. These products fall into four general use groups: fungal disease controls, seed treatments/plant  
216 growth regulators, nematode controls, and antimicrobial textile treatments. Additionally, four of the  
217 registered products are technical grade active ingredients (TGAI), used to manufacture other products.  
218 Fungal disease control and nematode control are the main foci in this technical report (TR).

219

220 *Fungal Disease Controls*

221 Nine EPA-registered products containing chitosan as the active ingredient are labelled for fungal disease  
222 control (National Pesticide Information Center 2020; US EPA 2016; US EPA 2020; US EPA 2009; US EPA  
223 2019a; US EPA 2019b; US EPA 2019c; US EPA 2019d) – see the list of Trade Names shown in *Identification of*  
224 *Petitioned Substance* above. Some of these are repackaged products. Application methods vary, including  
225 root or tuber dips, chemigation, in-furrow, foliar sprays, and soil drenches. Application rates also vary,  
226 from 0.003 pounds per acre to 2.5 pounds per acre, with multiple applications suggested, typically on a 7–  
227 14-day cycle. Essentially all types of crops are listed on the labels, including nuts; berries; pome, stone, and  
228 citrus fruits; grains; tubers; and vegetable crops. Two products list the mode of action as antibacterial and  
229 antifungal, directly affecting spore germination and mycelial growth. The remaining products all cite the

230 mode of action as elicitation of systemic plant defenses (National Pesticide Information Center 2020; US  
231 EPA 2016; US EPA 2020; US EPA 2009; US EPA 2019a; US EPA 2019b; US EPA 2019c; US EPA 2019d).  
232

### 233 Nematode Controls

234 Two EPA-registered products containing chitosan as the active ingredient are labelled for use as a  
235 nematode control (National Pesticide Information Center 2020; US EPA 2019b; US EPA 2018a). One of these  
236 products is also listed for fungal disease control, while the other is only listed as a nematicide. The  
237 nematicide-only product additionally contains Quillaja extract as an active ingredient (National Pesticide  
238 Information Center 2020; US EPA 2019b; US EPA 2018a).

239

### 240 Growth Regulation and Seed Treatments

241 Chitosan is known to act as a plant growth promoter. Mondal et al. (2012) found that foliar applications of  
242 chitosan from 0–125 ppm increased photosynthesis, nitrogen-related enzyme activity, total dry matter, and  
243 plant growth in okra. Soaking rice seed in chitosan was found to significantly increase rice yields, and rice  
244 treated with chitosan oligomers were found to have increased expression of a variety of genes related to  
245 carbon metabolism, photosynthesis, and cell redox homeostasis (Chamnanmanoontham et al. 2015).

246

247 Chitosan's effects as a plant growth promoter are variable, however, and depend on chitosan's chemical  
248 characteristics and the plant species involved. Khan, Prithiviraj, and Smith (2002) found small chitosan  
249 oligomers caused an 8–10 percent increase in maize photosynthesis but had little to no effect on soybean.  
250 On the other hand, larger chitosan molecules caused a decrease in photosynthesis for both maize and  
251 soybean. Despite these changes in photosynthesis, no differences in plant growth or development were  
252 observed after 10 days in comparison with control plants.

253

254 Three EPA-registered products containing chitosan as the active ingredient are labelled for use as a seed  
255 treatment (National Pesticide Information Center 2020; US EPA 2019e; US EPA 2012; US EPA 2015). These  
256 products are labelled as promoting seed germination and root development, and not explicitly for disease  
257 control. They also include as active ingredients indole-3-butyric acid and salicylic acid (National Pesticide  
258 Information Center 2020; US EPA 2019e; US EPA 2012; US EPA 2015).

259

### 260 Postharvest Protection

261 Chitosan can be used to form a protective film for postharvest protection. It is also antimicrobial and  
262 induces systemic resistance against rot pathogens (Romanazzi, Feliziani and Sivakumar 2018; Malerba and  
263 Cerana 2016). Studies have shown that chitosan can be effective against some postharvest diseases,  
264 including: anthracnose on citrus fruits; *Alternaria kikuchiana* and *Physalospora piricola* on pears; *Botrytis*  
265 *cinerea* on grapes; blue mold on apples; and strawberries artificially inoculated with *Cladosporium spp.* and  
266 *Rhizopus spp.* (Betchem, Johnson and Wang 2019). ARMOUR-Zen and ARMOUR-Zen 15% are currently the  
267 only EPA registered products that include post-harvest crop application uses on their labels (US EPA 2016;  
268 US EPA 2020). These products list the following postharvest applications: apples, cherries, citrus, pears,  
269 potatoes, and sweet potatoes; for the following diseases: blue mold, dry rot, gray mold, green mold, mucor  
270 rot, silver scurf, and sour rot.

271

### 272 Antimicrobial Textile Treatments

273 Four EPA-registered products containing chitosan as an active ingredient are labelled for use as an  
274 antimicrobial agent on textiles and surfaces (National Pesticide Information Center 2020).

275

### 276 Other Uses

277 Chitosan exhibits unique properties that are well suited to fill numerous roles (Dutta, Dutta and Tripathi  
278 2004; Islam, Bhuiyan and Islam 2017; Pillai, Paul and Sharma 2009; Sawaguchi et al. 2015). Some of these  
279 potential uses, particularly in the biomedical field, are limited by the difficulty in creating chitosan forms  
280 that are soluble in the appropriate solvents (Pillai, Paul and Sharma 2009). Chitosan is also used in the  
281 following industries:

- 282     • Agriculture (within coatings of slow-release fertilizer pellets)  
283     • Cosmetics (hair treatments, lotions, lipstick, and toothpaste)  
284     • Water engineering (to remove metal ions and petroleum pollutants)

- 285     • Pulp and paper (to strengthen recycled paper and increase moisture resistance)  
286     • Textiles (to remove dyes from effluent and produce fibers)  
287     • Food processing (as a carrier for food dyes and nutrients, as fruit coatings, and as antimicrobial  
288        food preservatives)  
289     • Photography (for developing color photographs)  
290     • Chromatography (to separate nucleic acids, phenol, and chlorophenol)  
291     • Biomedical (for a wide range of uses and research purposes including artificial membranes, wound  
292        dressings, sutures, tissue engineering, and drug delivery systems).

293     **Approved Legal Uses of the Substance:**

294     *Environmental Protection Agency (EPA)*

295     In 1986, the EPA first approved the use of chitosan under section 3(c)(5) of the Federal Insecticide,  
296     Fungicide, and Rodenticide Act (FIFRA) as a plant growth regulator in or on wheat. At the same time, the  
297     EPA also exempted chitosan from the requirement of a tolerance under 40 CFR §180.1072 (National  
298     Archives 1986). Subsequent EPA approvals included applications to other crops (National Archives 1989,  
299     National Archives 1995).

300     Currently, 40 CFR §180.1072 exempts chitosan from the requirement of a tolerance as follows: (a) "when  
301        used as a seed treatment in or on barley, beans, oats, peas, rice, and wheat;" and (b) "when used as a  
302        pesticide in the production of any raw agricultural commodity."

303     As an inert ingredient, chitosan is on the 2004 EPA List 4 (no longer maintained by EPA) (US EPA 2017a).  
304     Chitosan is neither on the Inert Ingredients Approved for Use in Minimum Risk Pesticide Products list, nor  
305     is it a substance with minimum risk tolerance exemptions under 40 CFR 180.950 (US EPA 2018b). Products  
306     containing chitosan as an inert ingredient are therefore **not** exempt from FIFRA. However, chitosan is listed  
307     on the EPA's Safer Chemical Ingredient List (US EPA 2019f). It is notated with a green circle indicating that  
308        "The chemical has been verified to be of low concern based on experimental and modeled data."

309     *Food and Drug Administration (FDA)*

310     The FDA regulations do not include specific reference to chitosan. They do permit the use of N-acetyl-D-  
311        glucosamine (chitin) as a "bulk drug substance that can be used to compound drug products in accordance  
312        with section 503A of the Federal Food, Drug, and Cosmetic Act" (US FDA 2020a).

313     Several Generally Recognized as Safe (GRAS) notices have been filed with the FDA. Three notices relate to  
314        general food use. At the notifier's request, the FDA ceased to evaluate these three notices (US FDA 2020b;  
315        US FDA 2020c; US FDA 2020d). GRN Notice 397 states that chitosan from the fungus *Aspergillus niger* is  
316        considered GRAS when used as "a secondary direct food ingredient in alcoholic beverage production at  
317        levels between 10 and 500 grams per hectoliter (100 liters)" (US FDA 2020e).

318     The Association of American Feed Control Officials (AAFCO), which has a memorandum of  
319        understanding with the FDA (US FDA 2019), lists chitosan as a flocculant, allowed for precipitating  
320        proteins during animal feed processing (AAFCO 2020).

321     *United States Department of Agriculture (USDA)*

322     Under the USDA organic regulations, chitosan is allowed as an inert ingredient in crop and livestock  
323        pesticide products (see *Summary of Petitioned Use*).

324     **Action of the Substance:**

325     Chitosan has multiple modes of action. When used as a pesticide, it acts directly on target pathogens with  
326        toxic as well as growth inhibitory effects. It also has effects on plants themselves, stimulating plant  
327        immunity. Chitosan's effect on both plants and pathogens is not universal. The following is a summary of  
328        available information.

339 *Antibacterial Modes of Action*  
340 Researchers do not fully understand how chitosan inhibits bacterial growth, and Gram-negative<sup>2</sup> and  
341 Gram-positive bacteria do not appear to respond the same way. Additionally confounding matters,  
342 chitosan can also have the opposite effect—it can cause accelerated growth in the same bacteria, depending  
343 on the molecular weight, and possibly the dosage, of the chitosan used (Tokura et al. 1997; Bellich et al.  
344 2016). Generally, chitosan's antibacterial effects are weaker than its antifungal effects (Kong et al. 2010).  
345  
346 Due to the variety of results found from different studies, it is likely that chitosan has multiple antibacterial  
347 modes of action. The most relevant mode of action depends on factors such as the type of bacteria involved  
348 and the properties of the chitosan used. One source observed that Gram-negative bacteria have more  
349 negatively charged (anionic) cell surfaces (Chung et al. 2004). Chitosan is a cation at a pH around 6 and  
350 below. One hypothesis is that under these conditions, positively charged chitosan binds to negatively  
351 charged cell surface molecules. The result of this interaction is increased cell permeability (Younes and  
352 Rinaudo 2015). Another hypothesis is that smaller chitosan molecules (low molecular weight) move  
353 through the cell wall and inhibit gene transcription by binding to DNA (Younes and Rinaudo 2015; Islam,  
354 Bhuiyan and Islam 2017). However, some are skeptical of this hypothesis because even low-molecular-  
355 weight chitosan is still a large molecule (Bellich et al. 2016). Another hypothesis is that chitosan chelates  
356 essential nutrients, making them biologically unavailable to bacteria (Kong et al. 2010). For Gram-negative  
357 bacteria, the antimicrobial effectiveness of chitosan is improved as the degree of deacetylation is increased,  
358 the molecular weight is decreased, and the environmental pH is decreased (Younes and Rinaudo 2015).  
359  
360 For at least some Gram-positive bacteria, larger-molecular-weight chitosan is more effective (Zheng and  
361 Zhu 2003). Chitosan may form a polymer layer around the Gram-positive bacteria that prevents movement  
362 of solutes in and out these cells (Younes and Rinaudo 2015).  
363  
364 *Antifungal/Antichromista<sup>3</sup> Modes of Action*  
365 As with its antimicrobial effect, the mode of action of chitosan on fungi and oomycetes is not fully  
366 understood. Researchers hypothesize that chitosan functions in two ways; chitosan can initiate systemic  
367 resistance in plants and may act directly between host and pathogen to block the growth of the pathogen  
368 itself (Younes and Rinaudo 2015). For example, chitosan blocks both the production and germination of  
369 fungal spores (Kong et al. 2010).  
370  
371 *Systemic Resistance*  
372 While some pesticides mitigate disease by killing or inhibiting a pathogenic organism, others do so by  
373 triggering immune responses in the host plant. The EPA classifies chitosan and other induced resistance  
374 promoters as biochemical pesticides (US EPA 2007a). These substances must have a non-toxic mode of  
375 action to the target pest and have demonstrated minimal toxicity to humans and the environment (Leahy et  
376 al. 2014). It is worth noting that despite chitosan's classification as such a material, it also has toxic modes  
377 of action to the target pest as described above.  
378  
379 Plants have numerous ways of resisting pathogens. For example, some plants exhibit a "hypersensitive"  
380 response to pathogen infection, whereby localized cells undergo programmed cell death when they sense a  
381 specific chemical elicitor from the invader (Govrin and Levine 2000). This limits the ability of some

<sup>2</sup> Microbiologists often separate bacteria into two general groups: those whose cell walls retain crystal violet stain (Gram-positive), and those that do not (Gram-negative). Gram-positive bacteria contain a cell wall and an inner cell membrane (Sylvia, et al. 2005). The cell walls of Gram-positive bacteria contain a large amount of peptidoglycan, a material very similar in structure to chitosan (Lodish, et al. 1995). Gram-negative bacteria have both outer and inner cell membranes, as well as a cell wall in between. The outer cell membrane of Gram-negative bacteria is permeable to large molecules due to protein channels called porins (Lodish, et al. 1995). Pathogenic Gram-negative bacteria tend to be more significant than Gram-positive bacteria because their cell walls can be toxic and their outer membrane can be resistant to many chemicals.

<sup>3</sup> Kingdom Chromista includes organisms previously considered fungi, such as the oomycete, *Phytophthora infestans* (Maneveldt and Keats 2004).

382 pathogens (biotrophs<sup>4</sup>) to continue to invade because they are dependent upon the biological machinery  
383 found in living cells. Additionally, the process of cell death results in the production of substances that can  
384 be toxic to pathogens. This type of plant defense is localized and often dependent on genetic recognition of  
385 a specific chemical elicitor. This same plant response can be taken advantage of by necrotrophic pathogens  
386 such as *Botrytis cinerea* and *Sclerotinia sclerotium*, which can survive on living or dead plant tissue (Govrin  
387 and Levine 2000).

388 By contrast to such localized responses, plants also exhibit systemic (non-localized) defenses. Two better-  
389 known systemic defenses are systemic acquired resistance (SAR) and induced systemic resistance (ISR)  
390 (Choudhary, Prakash and Johri 2007; Dong 2004; Agrios 2005; van Loon, Bakker and Pieterse 1998). SAR is  
391 associated with the accumulation of salicylic acid (SA) within the plant, which leads to the production of  
392 pathogenesis related substances, such as antifungal enzymes and phytoalexins that inactivate pathogens.  
393 SAR can be triggered by a plant recognizing a pathogenic organism and tends to negatively affect plant  
394 growth. ISR, on the other hand, is often triggered by beneficial microbes and can positively affect plant  
395 growth. Jasmonic acid (JA) is associated with the ISR response, but exactly how ISR protects plants is not  
396 well understood. Plants do not necessarily accumulate the same types of anti-pathogen substances as occur  
397 with SAR but instead appear to respond more quickly when infection is attempted—in other words, ISR  
398 “primes” plants for subsequent defenses. Ethylene and nitric acid are linked to additional plant defense  
399 pathways (Choudhary, Prakash and Johri 2007; Dong 2004; Agrios 2005; van Loon, Bakker and Pieterse  
400 1998).

401 Chitin elicits effects in plants normally associated with both SAR and ISR plant responses, including:  
402 chitinase and glucanase activation, phytoalexin biosynthesis, and biosynthesis of jasmonic acid (Hadrami  
403 et al. 2010). Chitosan can trigger plants to initiate systemic defense responses that include the biosynthesis  
404 of chitin-degrading enzymes (chitinases), proteinase inhibitors, and phytoalexins. Chitosan also elicits the  
405 production of callose (a type of structural polysaccharide), and it causes plants to increase lignin synthesis,  
406 typically part of the plant wound-healing process (Younes and Rinaudo 2015; Hadrami et al. 2010).  
407 Application of chitosan to wounded tomato plants resulted in significant increases in jasmonic acid  
408 (associated with ISR) over control plants (Doares, et al. 1995).

409  
410 *Nematode Control Mode of Action*  
411 Nematodes in the genus *Meloidogyne*, such as *M. incognita*, cause economically significant, damaging root  
412 galls on a variety of plants (Radwan et al. 2012). In potted plant experiments, Radwan et. al found that  
413 chitin and chitosan both significantly reduced root galls (51 percent and 70 percent respectively) on tomato  
414 plants. As the molecular weight of chitosan decreases, its effectiveness in reducing damage caused by  
415 nematodes increases (Khalil and Badawy 2012). Chitosan enhances the effect of the nematode parasitizing  
416 fungus *Pochonia chlamydosporia*, used as a bio-control against root-knot nematodes (Escudero et al. 2017).  
417 Under laboratory conditions, chitosan increases sporulation and the mycelial growth of beneficial *P.*  
418 *chlamydosporia* and causes an increase in the production of a protease used by the fungus to parasitize  
419 plant-damaging root-knot nematodes. However, these effects are not observed in agricultural soils. Instead,  
420 chitosan appears to promote the colonization of *P. chlamydosporia* in plant roots, which in turn makes the  
421 fungus a more effective biocontrol (Escudero et al. 2017).

422  
423 **Combinations of the Substance:**  
424 Chitosan is not a precursor to, or component of, substances on the National List—with the exception that it  
425 is an allowed inert in pesticide formulations (see *Summary of Petitioned Use*). Chitosan is present in the cell  
426 walls of many fungi (see *Source or Origin of the Substance*), and therefore is likely present in allowed  
427 nonsynthetic substances incorporating fungal ingredients.

428  
429 Chitosan is less hydrophobic than chitin and therefore less stable. In order to increase its stability, chitosan  
430 may be stabilized with chemicals such as epichlorohydrin, diisocyanate, 1,4-butanediol diglycidyl ether, or

<sup>4</sup> Some plant pathogens, such as viruses and some bacteria and fungi require a living host. In contrast with these biotrophs, necrotrophs live on dead tissue (Agrios 2005).

433 glutaraldehyde, oxalic acid, citric acid, and tripolyphosphate (Younes and Rinaudo 2015). Chitosan is  
434 sometimes complexed with other polymers such as alginate (Younes and Rinaudo 2015).  
435  
436 The composition of complete formulations for all EPA registered chitosan products were not reviewed as  
437 part of the writing of this TR. Two of the EPA-registered plant growth regulator pesticide products  
438 containing chitosan as an active ingredient are also formulated with indole-3-butyric acid and salicylic acid  
439 (US EPA 2012; US EPA 2015). Indole-3 butyric acid is a precursor to the plant hormone auxin (Velasquez et  
440 al. 2016), while salicylic acid is a signaling molecule involved with systemic acquired resistance (Agrios  
441 2005). Two other EPA-registered pesticide products containing chitosan as the active ingredient instruct  
442 users to add a buffer (acid) to lower the pH of the product below 6.5 (US EPA 2019a; US EPA 2019d). Due  
443 to chitosan's limited solubility above this pH, adding an acid might be necessary if running the product  
444 through equipment such as sprayers. One chitosan product disclosed citric acid as an inert ingredient on a  
445 safety data sheet (Loveland Products 2017).  
446  
447

448	Status
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449  
450 **Historic Use:**  
451 Available chitosan sources are synthetic (See *Evaluation Question #3*), and the substance is not present as an  
452 allowed active ingredient on the National List (nor has it been previously). Chitosan is allowed as an inert  
453 ingredient in pesticides for organic production (see *Summary of Petitioned Use*); however, information  
454 regarding its prevalence as an inert ingredient (broadly) is not currently available. At this time, it is used as  
455 an inert ingredient within at least 13 OMRI-Listed crop products and one livestock product (OMRI 2020).  
456

457 **Plant Disease Control**  
458 Chitosan is reported as an active ingredient in 22 EPA registered products (National Pesticide Information  
459 Center 2020). Of these, three are labelled as plant growth regulators for seed germination, three are  
460 Technical Grade Active Ingredients (TGAs), and five are antimicrobials for textiles. The remaining nine  
461 products make disease prevention claims for a wide range of crops and application methods (National  
462 Pesticide Information Center 2020).

463 Chitosan has been studied for use to control numerous disease-causing organisms on a wide array of crops.  
464 For example, chitosan has been found effective at controlling *Plasmopara viticola* (grape downy mildew)  
465 (Romanazzi et al. 2016); *Monilinia laxa* (brown rot), *Botrytis cinerea* (gray mold), *Rhizopus stolonifera*  
466 (Rhizopus rot) and *Alternaria alternata* (Alternaria rot) (Feliziani et al. 2013); and *Erysiphe cichoracearum*  
467 (powdery mildew) (Soliman and El-Mohamedy 2017). Malerba and Cerana (2018) summarized several  
468 studies published in 2017 on chitosan's effectiveness against plant pathogens. These studies demonstrated  
469 that chitosan was effective at reducing *Phytophtora infestans*, *Alternaria solani*, and *Meloidogyne* spp.;  
470 *Fusarium graminearum*; *Fusarium solani*, *Rhizoctonia solani*; *Colletotrichum* spp.; *Exobasidium vexans*; and  
471 *Heterodera glycines*.  
472

473 **Use as a Biostimulant**  
474 In one study, chitosan foliar sprays increased yields of strawberries by 42 percent; however, whether this  
475 was due to decreased disease pressure or through biochemical growth promotion was not elucidated  
476 (Rahman et al. 2018). Malerba and Cerana (2016) summarized several studies that demonstrated that  
477 application of chitosan improves plant growth characteristics. As a foliar spray or soil treatment, it  
478 increased yields in some plants such as tomatoes and okra, while improving plant growth in oregano,  
479 peppers, and coffee. As a seed treatment, it improved germination in orchid plants and spike formation in  
480 wheat. Chitosan also improved grapevine rooting and increased the number of internodes (Malerba and  
481 Cerana 2016). While chitosan was found to increase yield in some studies on tomatoes, other studies did  
482 not find similar results (see *Unintentional Side Effects* below).  
483

484  
485 **Unintentional Side Effects**  
486 The specific crops, diseases, and chitosan forms (degree of deacetylation, concentrations, and molecular  
487 weights) for which scientific literature covers this subject is too large to summarize within this technical

488 report. While the literature shows that chitosan is effective as both a disease control and as a growth  
489 regulator for specific applications, it can also create unintentional side effects. For example, in one study on  
490 tomatoes, chitin and chitosan significantly decreased powdery mildew on tomato plants (Dafermos,  
491 Kasselaki and Goumas 2012). However, chitin treatments also decreased plant yield, and chitosan-treated  
492 plants did not have greater yields of fruit than infected, untreated plants (Dafermos, Kasselaki and Goumas  
493 2012). Chitosan's role in disease control is often related to its ability to elicit systemic acquired resistance  
494 (SAR) in plants. Elicitation of SAR can, in some cases, be linked with decreased plant fitness – hence their  
495 evolution as inducible defenses that are not always left activated within plants (Heidel et al. 2004). In other  
496 words, being primed for defense can (in some cases) come at a cost depending on the availability of  
497 resources and disease pressure.

498

#### 499 **Organic Foods Production Act, USDA Final Rule:**

500 Chitosan is not listed anywhere in the Organic Foods Production Act of 1990 (OFPA) by name; however, it  
501 is included by incorporation in Section 2118(c)(1)(B)(ii) (7 U.S.C. 6517) because it is present on the 2004 EPA  
502 List 4:

503 "National List:  
504

505 (c) Guidelines for prohibition and exemptions. –  
506

507 (1) Exemption for prohibited substances in organic production and handling operations. – The  
508 National List may provide for the use of substances in an organic farming or handling operation  
509 that are otherwise prohibited under this title only if –  
510

511 (B) the substance –  
512

513 (ii) is used in production and contains synthetic inert ingredients that are not classified by the  
514 Administrator of the Environmental Protection Agency as inerts of toxicological concern..."

515 Similarly, chitosan is not mentioned explicitly within the USDA organic regulations in 7 CFR Part 205;  
516 however, it is present on 2004 EPA List 4, and therefore included by incorporation under §205.601(m) and  
517 205.603(e):  
518

519 "(m) As synthetic inert ingredients as classified by the Environmental Protection Agency (EPA),  
520 for use with nonsynthetic substances or synthetic substances listed in this section and used as an  
521 active pesticide ingredient in accordance with any limitations on the use of such substances."

#### 522 **International**

523

##### 524 *Canadian General Standards Board Permitted Substances List*

525 Chitosan is not included within the Canadian Organic Standards as an allowed material; however, chitin is  
526 included under CAN/CGSB 32.311-2015 PSL Table 4.2 *Soil amendments and crop nutrition, Chitin* and 4.3  
527 *Crop production aids and materials, Chitin*. Additionally, chitin is included by incorporation under PSL Table  
528 4.3 *Crop production aids and materials, Formulants* because chitin (not chitosan) is present on the Pest  
Management Regulatory Agency (PMRA) List of Formulants (CGSB 2018).

529

##### 530 *CODEX Alimentarius Commission, Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (GL 32-1999)*

531 Chitosan is not included within the Codex guidelines; however, chitin nematicides of natural origin are  
532 described within *Table 2: Substances for plant pest and disease control* (FAO 2007).

533

##### 534 *European Economic Community (EEC) Council Regulation, EC No. 834/2007 and 889/2008*

535 Chitosan hydrochloride is allowed for pest and disease management under the European Union organic  
536 regulations but may not be used as an herbicide. EC Regulation No. 889/2008 Article 5 allows substances  
537 found on Annex II when Article 12 materials are insufficient. The entry for "Basic Substances" in Annex II  
538 states that "only those basic substances as defined by Article 23 of Regulation (EC) No 1107/2009 (2) which  
539 are food as defined in Article 2 of Regulation (EC) No 178/ 2002 and have plant or animal origin.  
540 Substances not to be used as herbicides."

541

542 According to the EC Expert Group for Technical Advice on Organic Production (EGTOP), chitosan is a  
543 basic substance, and therefore allowed for pest and disease management as an active ingredient (EGTOP

544 2016). While this document indicates that the terms “chitosan” and “chitosan hydrochloride” are  
545 interchangeable in Europe, for the purposes of this report, these are considered two different chemical  
546 substances as they have different chemical structures. The manufacturing process for chitosan  
547 hydroxychloride requires additional steps compared with the process used to manufacture chitosan  
548 (Signini and Filho 1999).

549  
550 *Japan Agricultural Standard (JAS) for Organic Production*  
551 Chitosan is not included as an allowed synthetic for organic crop production under the Japan Agricultural  
552 Standard (MAFF 2017).

553  
554 *International Federation of Organic Agriculture Movements (IFOAM) – Organics International*  
555 Chitin (but not chitosan) processed without the use of acid hydrolysis is allowed as a nematicide under the  
556 IFOAM NORMS for Organic Production and Processing per Appendix 3 (IFOAM 2017).

### 557 558 559 Evaluation Questions for Substances to be used in Organic Crop or Livestock Production

560  
561 **Evaluation Question #1: Indicate which category in OFPA that the substance falls under:** (A) Does the  
562 substance contain an active ingredient in any of the following categories: copper and sulfur  
563 compounds, toxins derived from bacteria; pheromones, soaps, horticultural oils, fish emulsions, treated  
564 seed, vitamins and minerals; livestock parasiticides and medicines and production aids including  
565 netting, tree wraps and seals, insect traps, sticky barriers, row covers, and equipment cleaners? (B) Is  
566 the substance a synthetic inert ingredient that is not classified by the EPA as inerts of toxicological  
567 concern (i.e., EPA List 4 inerts) (7 U.S.C. § 6517(c)(1)(B)(ii))? Is the synthetic substance an inert  
568 ingredient which is not on EPA List 4, but is exempt from a requirement of a tolerance, per 40 CFR part  
569 180?

570  
571 Chitosan is a production aid per 7 USC 6517(c)(1)(B)(i).

572  
573 **Evaluation Question #2: Describe the most prevalent processes used to manufacture or formulate the**  
574 **petitioned substance. Further, describe any chemical change that may occur during manufacture or**  
575 **formulation of the petitioned substance when this substance is extracted from naturally occurring plant,**  
576 **animal, or mineral sources (7 U.S.C. § 6502 (21)).**

577  
578 Chitosan is typically produced using chemical methods. To produce chitosan, chitin is extracted and  
579 isolated from crustacean shells. This can be done with chemicals, but extraction/isolation can also be  
580 partially achieved through fermentation. Fermentation alone produces chitin that is less pure. Once chitin  
581 is obtained, chitosan is typically produced through chemical deacetylation. Again, this process can also be  
582 performed using biological means (enzymes), but this process works best when acting on material that has  
583 undergone chemical treatment.

#### 584 585 Chemical Extraction and Isolation of Chitin

586 To isolate chitin from crustacean shells, chitin undergoes deproteination and demineralization, often with  
587 an additional decolorization step. The petitioner describes the demineralization process first, followed by  
588 deproteination (Bio-Gro, Inc. 2019), but the process can also be done in reverse (Younes and Rinaudo 2015).

589  
590 **Deproteinating**  
591 Crustacean shells are primarily a matrix of proteins, minerals, chitin, and carotenoids (Chawla, Kanatt and  
592 Sharma 2014; Dutta, Dutta and Tripathi 2004; Younes and Rinaudo 2015). Aspartyl or histidyl residues  
593 within proteins are covalently bonded to chitin. In order to separate the protein fraction from chitin, these  
594 bonds must be separated through hydrolysis. While sodium hydroxide is the preferred alkalizing agent for  
595 deproteinating shells during chitin isolation, the following substances may also be used: sodium hydroxide  
596 ( $\text{NaOH}$ ), sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), sodium bicarbonate ( $\text{NaHCO}_3$ ), potassium hydroxide ( $\text{KOH}$ ),  
597 potassium carbonate ( $\text{K}_2\text{CO}_3$ ), calcium hydroxide ( $\text{Ca}(\text{OH})_2$ ), sodium sulfite ( $\text{Na}_2\text{SO}_3$ ), sodium bisulfite  
598 ( $\text{NaHSO}_3$ ), calcium bisulfite ( $\text{CaHSO}_3$ ), trisodium phosphate ( $\text{Na}_3\text{PO}_4$ ) and sodium sulfide ( $\text{Na}_2\text{S}$ ). Large

599 quantities of alkalizing agents are used, with ratios of solids-to-alkaline solvent of 1:4 to 1:20. Nitrogen gas  
600 or sodium borohydride is used in some cases to prevent additional oxidation reactions from degrading the  
601 material (Chawla, Kanatt and Sharma 2014; Dutta, Dutta and Tripathi 2004; Younes and Rinaudo 2015).  
602

### 603 Demineralizing

604 For demineralization, dilute hydrochloric acid is preferred (up to 10 percent concentration), but the  
605 following may also be used: nitric acid ( $\text{HNO}_3$ ), sulfuric acid ( $\text{H}_2\text{SO}_4$ ), acetic acid ( $\text{CH}_3\text{COOH}$ ), and formic  
606 acid ( $\text{HCOOH}$ ) (Younes and Rinaudo 2015; Chawla, Kanatt and Sharma 2014; Dutta, Dutta and Tripathi  
607 2004). In a process typically lasting 2–3 hours (but varying between 15 minutes and several days), acid  
608 decomposes calcium carbonate (and other mineral salts) in the shells into carbon dioxide and water-soluble  
609 calcium chloride as shown below (Younes and Rinaudo 2015; Chawla, Kanatt and Sharma 2014; Dutta,  
610 Dutta and Tripathi 2004). Even though it is diluted, substantial amounts of acid are used in this process; the  
611 solids-to-solvent ratio is 1:15 (Chawla, Kanatt and Sharma 2014).  
612



613 When deproteinization and demineralization is conducted with dilute solutions of bases and acids  
614 respectively, chitin is preserved in its acetylated state (Younes and Rinaudo 2015). For example, using  
615 multiple baths of hydrochloric acid and sodium hydroxide, chitin was isolated with 96–100 percent  
616 acetylation from several animal sources.  
617

### 618 Decolorizing

619 Crustacean shells contain organic pigments called carotenoids (Chawla, Kanatt and Sharma 2014). Solvents  
620 such as acetone, and bleaching agents such as sodium hypochlorite are used in commercial applications to  
621 remove these pigments from deproteinated and demineralized chitin (Chawla, Kanatt and Sharma 2014).  
622

### 623 Biological Extraction and Isolation of Chitin

624 While slower than chemical methods, shells can also be deproteinated and demineralized with enzymes or  
625 fermenting with microorganisms (Younes and Rinaudo 2015). Biological extraction has the advantage in  
626 that it better preserves the structure and size of chitin molecules. However, enzymatic methods can leave  
627 4–15 percent residual protein. These can be removed through subsequent chemical treatment (such as with  
628 sodium hydroxide). This process requires less solvent and less energy. Crude protease formulations (e.g.,  
629 derived from fish viscera) are efficient and more economical than purified forms (Younes and Rinaudo  
630 2015).  
631

632 Biological extraction and isolation of chitin is not known to be used outside of laboratories at the current  
633 time (Younes and Rinaudo 2015).  
634

### 635 Chemical Conversion of Chitin to Chitosan

636 To transform chitin into chitosan, the acetyl groups present on the N-acetylglucosamine monomers must  
637 be removed through deacetylation either chemically or biologically.  
638

### 639 Chemical Deacetylation

640 While acids can be used to deacetylate chitin monomers, they also hydrolyze the glycosidic bonds that  
641 connect monomers together. Therefore, alkaline materials are necessary to produce chitosan (Younes and  
642 Rinaudo 2015). Dilute sodium hydroxide is used to separate and extract chitin from shells, but a more  
643 concentrated solution is required to effectively deacetylate chitin to form chitosan due to the way that the  
644 acetyl groups are positioned relative to the sugar ring. Potassium or sodium hydroxide solutions of 40–  
645 60 percent concentration are used, with a ratio of 1:10 weight by volume (w/v) solids-to-solvent (Chawla,  
646 Kanatt and Sharma 2014). Chitin can be converted into chitosan using either a high-heat process or a lower  
647 temperature process. Repeating these processes produces chitosan with more complete deacetylation  
648 (Islam, Bhuiyan and Islam 2017; Younes and Rinaudo 2015). Additional reagents such as sodium  
649 borohydride ( $\text{NaBH}_4$ ) can be used to prevent degradation of the polymer into smaller molecular weight  
650 pieces (Younes and Rinaudo 2015). Chitosan processes typically produce material that is 56–99 percent  
651 deacetylated (Islam, Bhuiyan and Islam 2017).  
652

654  
655 In the “heterogenous” process, concentrated sodium hydroxide is used at high temperature to deacetylate  
656 chitin over the course of a few hours. For example, a 40–50 percent sodium hydroxide solution can be used  
657 at 320°F (160°C) for 1–3 hours to deacetylate chitin to form chitosan (Islam, Bhuiyan and Islam 2017). This  
658 process leads to a high degree of deacetylation (85–90 percent) in regions that are amorphous, but can leave  
659 remaining crystallized regions acetylated (Nemtsev, et al. 2002; Younes and Rinaudo 2015).

660  
661 In the “homogenous” process, sodium hydroxide is added to chitin, then they are frozen and thawed to  
662 room temperature (Nemtsev et al. 2002). This process causes the chitin to swell and form a viscous alkaline  
663 fluid. The alkaline-chitin fluid is then left at room temperature (or can be heated) to continue to  
664 deacetylate. The chitosan/alkali solution forms a gel that is disintegrated mechanically, washed to remove  
665 alkali, and dried (Nemtsev et al. 2002). The degree of deacetylation of chitosan produced from the  
666 homogenous method is dependent on the concentration of sodium hydroxide added, residence time, and  
667 temperature conditions, but averages 48–55 percent (Younes and Rinaudo 2015).

668  
669 Biological deacetylation  
670 The enzyme chitin deacetylase can be used to convert chitin to chitosan while using less energy and  
671 reducing the need for concentrated alkaline chemicals (Younes and Rinaudo 2015). Due to chitin’s  
672 insolubility and therefore inaccessibility to enzymes, chitin deacetylase enzymes work best on chitin that is  
673 already partially deacetylated (Younes and Rinaudo 2015). As of 2011, this process has not been used  
674 commercially due to limited enzyme availability (Suresh, Sachindra and Bhaskar 2011).

675  
676 *Production of Chitosan Oligomers*  
677 Chitosan polymers can be degraded into smaller pieces called oligomers. Chitosan oligomers exhibit  
678 increased solubility at neutral pH ranges (Chawla, Kanatt and Sharma 2014). Oligomers are primarily  
679 produced through enzymatic treatments and through chemical processes, though Cobalt-60 gamma rays  
680 radiation and physical processes can be used as well. Chemical treatments involve hydrolysis of the  
681 glycosidic bonds between monomers using hydrochloric acid or through a more complex reaction using a  
682 combination of nitrous acid and hydrogen peroxide (Chawla, Kanatt and Sharma 2014).

683  
684 **Evaluation Question #3:** Discuss whether the petitioned substance is formulated or manufactured by a  
685 chemical process, or created by naturally occurring biological processes (7 U.S.C. § 6502 (21)).  
686

687 Commercially available forms of chitosan are manufactured through a chemical process. Several different  
688 steps during the manufacturing process create synthetic materials. During the process of extracting chitin,  
689 sodium hydroxide is used to hydrolyze bonds that connect chitin to proteins. The chitin that is extracted is  
690 no longer chemically bound to proteins, arguably leaving it in a form that does not exist in nature when  
691 found in shells. Once isolated, chitin undergoes deacetylation to form chitosan. Again, this step chemically  
692 modifies chitin and is mediated by synthetic chemicals. Some forms of chitosan are additionally modified  
693 to form salts and other derivatives (Dutta, Dutta and Tripathi 2004; Pillai, Paul and Sharma 2009; Islam,  
694 Bhuiyan and Islam 2017).

695  
696 Following NOP Guidance 5033-1 Guidance, Decision Tree for Classification of Materials as Synthetic or  
697 Nonsynthetic (NOP 2016) leads to a determination that chitosan is **synthetic**. Chitosan can be extracted  
698 from a natural source (shells), but the material has been transformed into a different substance via a  
699 chemical change. Alternatively, one could consider chitin (which may or may not be considered synthetic,  
700 depending on how one views the deproteinization step in chitin extraction) to be the source for chitosan. In  
701 this case, the chitin has undergone a chemical change in conversion to chitosan, which (with rare  
702 exceptions) is not mediated by a biological process.

703  
704 **Evaluation Question #4:** Describe the persistence or concentration of the petitioned substance and/or its  
705 by-products in the environment (7 U.S.C. § 6518 (m) (2)).  
706

707 Chitosan’s primary means for breakdown is through microbial action, where it eventually breaks down  
708 into small amino sugars, such as glucosamine monomers (Roberts, Bol and Jones 2007; Wieczorek, Hetz

and Kolb 2014). These sugars can undergo different fates, including direct uptake as nutrients by plants and other microorganisms and further breakdown into carbon dioxide or mineralization in the soil to form substances like ammonia and nitrate (Roberts, Bol and Jones 2007; Wieczorek, Hetz and Kolb 2014). This involves hydrolysis of the polymer to produce glucosamine monomers and subsequent glucosamine degradation. Chitosan naturally occurs in fungi, and microorganisms have evolved to produce different chitosanase enzymes that break the substance down as a source of carbon and nitrogen (Sawaguchi et al. 2015). With that said, chitin is much more common in nature, being found in nearly all fungi and in many (if not most) invertebrates (Hirano 2012; Wieczorek, Hetz and Kolb 2014; Sato et al. 2010). Chitin appears to break down more quickly than chitosan in soils and is able to do so through a different biodegradation pathway (Wieczorek, Hetz and Kolb 2014).

The time that microbes take to break chitosan down depends on the characteristics of the soil. In one study, chitosan in silty soils with a relatively larger microbial biomass broke down much faster than chitosan in sandy soils with less microbial biomass (Sawaguchi et al. 2015). Another study indicated that decomposition of fungal cell walls (including both chitin and chitosan) was not affected by the specific microbial community structure, but was slower in clay soils (Hu et al. 2020). One study indicated that chitosan breaks down completely within 30 days at 5 percent concentration (w/w) chitosan/soil, but only 60 percent was broken down after 180 days in another case (1 percent w/w chitosan/soil) (Sawaguchi et al. 2015; Sato et al. 2010). The biodegradation kinetics of chitosan is also dependent on the polymer chain length of chitosan and the distribution of acetyl groups, making it difficult to identify a broadly applicable decomposition time (Islam, Bhuiyan and Islam 2017; Sato et al. 2010; Sawaguchi et al. 2015; Wieczorek, Hetz and Kolb 2014).

Based on EPA product labels, chitosan is applied at a rate of between 0.003 pounds of chitosan per acre, and 2.5 pounds per acre, with multiple applications suggested and typically on a 7–14 day cycle (see *Specific use of the Substance* above). Based on the generally biodegradable nature of chitosan, naturally occurring biodegradation pathways, and low application rates, chitosan and its breakdown products are unlikely to accumulate within the agro-ecosystem.

**Evaluation Question #5: Describe the toxicity and mode of action of the substance and of its breakdown products and any contaminants. Describe the persistence and areas of concentration in the environment of the substance and its breakdown products (7 U.S.C. § 6518 (m) (2)).**

Chitosan has multiple modes of actions, which are described in *Action of the Substance* (above). While chitosan is antimicrobial, this activity requires recurring applications to maintain. Furthermore, chitosan can also stimulate microbial growth. Studies showing temporary changes to microbial community structures used much larger concentrations of chitosan than are proposed within the petitioned use in order to elicit an effect (Sato et al. 2010; Sawaguchi et al. 2015; Wieczorek, Hetz and Kolb 2014).

Applying chitosan as a pesticide is not expected to increase its concentration beyond background levels existing in nature (US EPA 2007a). Amino sugars like those that are found in chitin and chitosan make up 1–10 percent of the dry weight of Gram-positive bacteria, and 5–10 percent of fungi (Roberts, Bol and Jones 2007).

Chitosan's breakdown products are small molecules such as glucosamine, carbon dioxide, and ammonia that act as nutrients for many organisms, including bacteria, fungi, and plants.

**Evaluation Question #6: Describe any environmental contamination that could result from the petitioned substance's manufacture, use, misuse, or disposal (7 U.S.C. § 6518 (m) (3)).**

EPA and FDA data were not found that indicated that environmental contamination risks were assessed for the manufacture or disposal of chitosan. One EPA report (US EPA 2007a) indicated that the use of chitosan as an active ingredient in pesticide products did not pose a risk to non-target organisms, and that it was unlikely to be an ecological risk. The EPA's Biopesticides and Pollution Prevention Division chose to waive requirements for an ecological risk assessment for the material due to its negligible toxicity, ubiquity

764 in nature, and its biodegradability (US EPA 2007a). The European Union's Expert Group for Technical  
765 Advice on Organic Production (EGTOP) had no concerns over the environmental impacts of the  
766 manufacture or use of a chitosan salt, chitosan hydroxylchloride (EGTOP 2016).

767 Muñoz et al. (2018) conducted the first known life cycle assessment (LCA) of chitosan produced at two  
768 different sites. Wastewater from the various isolation and deacetylation steps is ultimately treated and  
769 discharged into the ocean. Protein sludge extracted during the isolation process is used as fertilizer or  
770 animal feed, and calcium salts are either disposed of in landfills or used as road-building materials. As the  
771 chitosan production chains analyzed by Muñoz et al. are global, ingredients are moved large distances to  
772 go from one processing facility to another, often on different continents.

773  
774 The manufacture of chitosan involves the use of relatively large amounts of corrosive chemicals, notably  
775 sodium hydroxide and hydrochloric acid (see *Evaluation Question #2*). In use, these chemicals are  
776 neutralized during hydrolysis reactions and also go on to produce benign salts such as calcium chloride  
777 ( $\text{CaCl}_2$ ) and sodium chloride ( $\text{NaCl}$ ) during the isolation of chitin and synthesis of chitosan (Chawla,  
778 Kanatt and Sharma 2014; EGTOP 2016). Muñoz et al. (2018) estimated that for one chitosan supplier in  
779 India, production of 1 kg of chitin requires 33 kg shells, 8 kg hydrogen chloride (HCl), 1.3 kg sodium  
780 hydroxide (NaOH), and 167 L of fresh water. To produce 1 kg of chitosan for the Indian supplier, 1.4 kg of  
781 refined chitin were needed, as well as an additional 5.18 kg NaOH and 250 L of water. They also evaluated  
782 a European supplier's production process and found that it required even larger amounts of chemicals and  
783 water as well as four times as much energy – though this chitosan was destined for medical use. Yan and  
784 Chen (2015) estimated that on average, one metric tonne of water is required to produce one kilogram of  
785 chitosan.

786  
787 Both sodium hydroxide and chlorine are products of the energy-intensive chloralkali process. In cases  
788 where electricity for the chloralkali process is powered by fossil fuels, approximately two tons of  $\text{CO}_2$  is  
789 produced for every ton of  $\text{Cl}_2$  and NaOH (Marini et al. 2014). Hydrochloric acid is produced via several  
790 different routes, but these typically involve chlorine gas produced from the chloralkali process mentioned  
791 above (Mansfield, Depro and Perry 2000). In 2015, hydrochloric acid was the fifth most released toxic  
792 chemical in the United States, though this is likely related to coal-fired power plant emissions (US EPA  
793 2017b). Depending on the specific equipment used in the process (mercury cell, diaphragm cell, or  
794 membrane cell), different wastes and emissions are produced during the chloralkali process used to make  
795 sodium hydroxide and chlorine (used to eventually produce hydrochloric acid) (US DOE 2000):

- 796  
797
- 798 • **Energy usage:** 2,565–3,350 kilowatt hours per ton of chlorine
  - 799 • **Emissions:** chlorine gas, carbon dioxide, carbon monoxide, hydrogen, freon and mercury
  - 800 • **Effluents:** mercury, sulfuric acid, and ion exchange wash water
  - 801 • **Wastes/byproducts:** lead, mercury (elemental, mercuric chloride, mercuric sulfide), asbestos, spent  
802 graphite, spent filters, used membranes, anode materials, cathode materials, magnesium  
803 hydroxide, calcium carbonate, barium sulfate, and calcium sulfate

804  
805 The overall quantity of chitosan currently produced is low. While large amounts of water, sodium  
806 hydroxide, and hydrochloric acid are used in the production of chitosan, this usage is still small compared  
807 to overall global consumption. Approximately 10,000 metric tonnes of refined chitin are produced each  
808 year as well as 2000 metric tonnes of chitosan (Yan and Chen 2015; Muñoz, et al. 2018). The worldwide  
809 yearly production of sodium hydroxide is estimated to be 70 million metric tons (CIEC 2018), and that of  
810 hydrogen chloride is approximately 20 million tons (Ando et al. 2010).

811  
812 In contrast to the chemicals used to isolate and synthesize the material, chitosan itself is produced from  
813 chitin, a food related marine biowaste. Using wastes to produce chitosan contributes to recycling (EGTOP  
814 2016; Islam, Bhuiyan and Islam 2017).

815  
816 **Evaluation Question #7: Describe any known chemical interactions between the petitioned substance**  
817 **and other substances used in organic crop or livestock production or handling. Describe any**  
818 **environmental or human health effects from these chemical interactions (7 U.S.C. § 6518 (m) (1)).**

819 Chitosan is incompatible with alkaline materials that may be present in organic crop production. At  
820 alkaline pH, chitosan becomes insoluble (see *Properties of the Substance*). This interaction is unlikely to  
821 produce environmental or human health effects, but it could create problems for agricultural equipment.  
822 Conversely, acids will tend to improve chitosan's solubility. Acidifying chitosan with substances such as  
823 acetic acid, and then drying it out may form chitosan salts such as chitosan hydrochloride (Signini and  
824 Filho 1999). In Europe, chitosan hydrochloride is allowed as a food additive and as a crop pesticide.  
825

826 Being a large polymer with numerous reactive sites, it is difficult to characterize all the possible chemical  
827 interactions involving chitosan. Chitosan can undergo numerous chemical reactions, including  
828 esterification, etherification, cross-linking, copolymerization, acetylation, quaternization, alkylation, and it  
829 chelates metals (Pillai, Paul and Sharma 2009; Hirano 2012). Chitosan will spontaneously form an ionically  
830 cross-linked gel when exposed to tripolyphosphates (Bellich et al. 2016). Sodium and ammonium  
831 tripolyphosphates are 2004 EPA List 4 materials, and potassium tripolyphosphate is a List 3 material (US  
832 EPA 2004). Chitosan can also interact and form complexes with DNA, alginates, carrageenan, and xanthan  
833 gum (Bellich et al. 2016); examples can also be found on List 3 and List 4. Information was not found that  
834 indicated whether these substances have environmental effects at the concentrations used for pesticidal  
835 purposes beyond their intended effects within pesticide formulations. Many of these materials, such as  
836 chitosan-alginate complexes and chitosan gels, are either used for or under research for medical purposes  
837 and are well tolerated by humans (Abruzzo et al. 2013; Irimia et al. 2018; Pillai, Paul and Sharma 2009;  
838 Bellich et al. 2016).

839

840

841 **Evaluation Question #8: Describe any effects of the petitioned substance on biological or chemical  
842 interactions in the agro-ecosystem, including physiological effects on soil organisms (including the salt  
843 index and solubility of the soil), crops, and livestock (7 U.S.C. § 6518 (m) (5)).**

844

845 The EPA (2007) determined that the use of chitosan for disease control is unlikely to have lasting effects on  
846 soil microorganisms. Studies on chitosan were not found that evaluated its effects on soil parameters such  
847 as temperature, pH levels, salt concentration, or soil solubility. Studies were found that indicate that  
848 chitosan can limit the ability of some microorganisms to absorb nutrients (Bellich et al. 2016; Zheng and  
849 Zhu 2003; Tokura et al. 1997). Conversely, chitosan can also act as a chelator. Sharp (2013) suggested that  
850 because of this, it might be used as a material to improve the anionic exchange capacity of soils, to limit the  
851 leaching of anionic nutrients and improve nutrient delivery to plants.

852

853 Laboratory experiments show that concentrations similar to those used for EPA-registered products can  
854 kill pathogens (Bhattacharya 2013). Labels from EPA-registered chitosan products include numerous  
855 application methods such as seed treatments, foliar sprays, root dips, and soil drenches. At maximum  
856 application rates, chitosan solutions have a concentration of 0.3 percent and are applied at up to 2.5 pounds  
857 of active ingredient per acre.

858

859 When the direct antifungal effects of chitosan were evaluated on a suite of ten common pathogens of  
860 tomato plants, application of 0.5g/L (0.05 percent) inhibited growth by a collective average of 8 percent  
861 (Jabnoun-Khiareddine, et al. 2015). As the concentration was increased, the effect on growth increased  
862 linearly, with a concentration of 4 g/L (0.4 percent) resulting in an average inhibition of around 71 percent.  
863 Another study found that 0.05 percent, 0.10 percent, and 0.20 percent concentrations of chitosan inhibited  
864 growth of *Fusarium solani* by 42–59 percent respectively after 96 hours, and reduced spore germination by  
865 55–95 percent (Bhattacharya 2013).

866

867 Chitosan can also cause increases in soil microorganisms, especially those associated with its breakdown.  
868 When applied in relatively large dosages (approximately 0.25–5 percent weight chitosan/soil weight),  
869 bacterial community structures can change temporarily (tending to simplify) during the time when  
870 chitosan is being degraded (Sawaguchi et al. 2015; Sato et al. 2010; Wieczorek, Hetz and Kolb 2014).  
871 Sawaguchi et al. (2015) hypothesized that chitosinase enzyme producing bacteria dominated the soil  
872 microbial community while chitosan was available. Furthermore, Sawaguchi et al. identified that  
873 *Streptomyces* and *Kitasatospora* spp. were involved in chitosan degradation within the soils. After chitosan

874 was degraded, bacterial communities diversified again. Even at these relatively high soil concentrations,  
875 effects on soil microorganisms are temporary.

876  
877 When applied to the soil, chitosan solutions become less concentrated. For example, as an in-furrow  
878 treatment, EPA-registered chitosan products have concentrations in the soil of roughly 0.01 percent or less,  
879 assuming that the soil that is treated is 1 cm deep and the product is applied in a band 10 cm wide. Studies  
880 on chitosan's effect on soil microbial communities used one-time applications at concentrations many times  
881 larger (0.25 percent and 5 percent) than that used for pesticidal purposes (0.01 percent) (Sawaguchi et al.  
882 2015; Sato et al. 2010; Wieczorek, Hetz and Kolb 2014). Information was not found that evaluated the effects  
883 on microbial communities as a whole at the relatively small concentrations that are used when chitosan is  
884 applied as a pesticidal ingredient. Studies tend to focus on either chitosan-degrading bacteria or plant  
885 pathogens, or examine the effects of relatively high concentrations of chitosan applications. A direct  
886 comparison to the petitioned use based on this data should be considered with caution. However, based on  
887 the results of these larger applications, similar (but smaller) temporary changes to soil microbial  
888 communities should be expected.

889  
890 **Evaluation Question #9:** Discuss and summarize findings on whether the use of the petitioned  
891 substance may be harmful to the environment (7 U.S.C. § 6517 (c) (1) (A) (i) and 7 U.S.C. § 6517 (c) (2) (A)  
892 (i)).

893 Based on the information summarized in *Evaluation Questions #4-8*, the application of chitosan as an active  
894 ingredient in pesticidal products is unlikely to be harmful to the environment. It occurs naturally in  
895 quantities exceeding what would be used in organic crop production, and it degrades into substances that  
896 are non-toxic and readily used as nutrients (see *Evaluation Questions #4* and #5). Its effect is primarily as an  
897 elicitor of plant defense responses, which causes temporary changes to plant physiology (see *Evaluation  
898 Question #5* and *Action of the Substance*). While chitosan may be able to alter microbial communities, these  
899 effects appear to be transient and may require larger application quantities (see *Evaluation Question #8*).  
900 Manufacturing chitosan could recycle a small amount of marine food waste, though the process is  
901 currently heavily dependent on chemicals whose production is harmful to the environment (see *Evaluation  
902 Question #6*). In the life cycle assessment conducted by Muñoz et al. (2018), they identified that the primary  
903 impacts of chitosan manufacturing related to the use of NaOH, HCl, and energy. Of note, other synthetic  
904 materials currently allowed on the National List at §205.601, such as chlorine materials, soaps, and aquatic  
905 plant extracts, also make use of these chemicals or other industrially related substances.

906  
907  
908 **Evaluation Question #10:** Describe and summarize any reported effects upon human health from use of  
909 the petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (i), 7 U.S.C. § 6517 (c) (2) (A) (i)) and 7 U.S.C. § 6518  
910 (m) (4)).

911 According to numerous sources, chitosan is nearly non-toxic to humans and most other animals, and its  
912 degradation products do not cause side effects in the body (US EPA 2007a; Islam, Bhuiyan and Islam 2017;  
913 Pillai, Paul and Sharma 2009; Hirano 2012; Friedman and Juneja 2010; Baldrick 2010). The EPA considers  
914 chitosan to have an acute oral and acute eye irritation toxicity classification of IV, or practically non-toxic  
915 and not an irritant (US EPA 2007a). The lethal dose necessary to kill 50 percent of the test population (LD<sub>50</sub>)  
916 ranges from 1500 mg/kg body weight orally in rats to over 16,000 mg/kg bodyweight in mice (Baldrick  
917 2010). Many studies where large, repeated doses of chitosan oligomers (short fragments) were fed to rats,  
918 no toxic effects were observed (Baldrick 2010).

919  
920 The rate at which it degrades appears to vary depending on the degree of deacetylation (Baldrick 2010).  
921 More highly deacetylated chitosan (greater than 85 percent percent) can last up to several months when  
922 implanted in the body, while chitosan with less deacetylation degrades as quickly as three days in some  
923 cases. Enzymes capable of degrading chitosan exist both in animals as well as gut bacteria (Baldrick 2010).  
924 Chitosan is present in foods and has an exemption from the requirement of a tolerance. When ingested,  
925 chitosan is broken down into low-molecular weight substances and excreted in urine (Pillai, Paul, &  
926 Sharma, 2009).

927  
928

As of 2007, there were no reports of human or domestic animal incidents from the use of products containing chitosan (US EPA 2007a). Chitosan is considered essentially non-toxic and biocompatible, meaning it is not rejected by the human body and is biodegradable to normal body constituents (Islam, Bhuiyan and Islam 2017; Pillai, Paul and Sharma 2009; Dutta, Dutta and Tripathi 2004). No other FDA, EPA, or European Food Safety Authority (EFSA) guidelines or other scientific literature was found that contradicted this information or indicated that chitosan causes any negative human health effects. It is either currently used for or has been considered for a wide range of cosmetics, pharmaceuticals, and biomedical purposes (see *Specific Use of the Substance*, above).

**Evaluation Question #11: Describe all natural (non-synthetic) substances or products which may be used in place of a petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (ii)). Provide a list of allowed substances that may be used in place of the petitioned substance (7 U.S.C. § 6518 (m) (6)).**

Chitosan is petitioned for use as a plant disease control and has broad applications in terms of the number of crops and target pathogens. Due to this, the potential list of alternative disease control materials involved for comparison is large. It is therefore not practical to provide a complete and detailed comparison of the effect, form, function, quality, and quantity of all these materials within this report. There are undoubtedly cases where some disease control substances are better suited to certain crops, sites, pathogens, and rotations than others. Unlike for materials with highly specific petitioned uses, the matrix for comparison between chitosan and other materials is very large.

Currently, there are more than 200 products listed by OMRI for use as plant disease control (OMRI 2020). Some of these products contain synthetic active ingredients allowed for use in USDA organic production such as copper, oils, sulfur, aqueous potassium silicate, potassium bicarbonate, polyoxin-D salt, and hydrogen peroxide. Also included in the list by OMRI are products containing nonsynthetic active ingredients such as bacteria, various microorganism extracts, botanical substances, oils, and natural acids (OMRI 2020).

The current petition is to add synthetic chitosan to the National List, and the petitioner emphasized control of plant diseases such as powdery mildew, downy mildew, and botrytis. To limit the discussion, alternative substances that are commercially available for these diseases are identified and discussed below.

#### Non-Synthetic Microbials, Botanicals, and Oils

Commercially available microbial treatments for powdery mildew and botrytis include *Bacillus amyloliquefaciens* D747 (Double Nickel®) and *Bacillus subtilis* QST 713 (Serenade®, Rhapsody®, Cease®). These are approved for organic production by OMRI (OMRI 2020). Extract of giant knotweed (Regalia) is registered for powdery mildew and botrytis control (Quarles 2013; Su et al. 2012).

Chitin has the ability to generate plant defense responses (Hadrami et al. 2010), and OMRI does list a small number of chitin-based products. However, there is currently only one EPA-registered chitin end-use pesticide product (Clandosan 618, not OMRI listed), which is limited to nematode control.

The effectiveness of these microbials and botanicals is at least in part due to their role as elicitors. Nonsynthetic elicitors of plant defenses from microorganisms include (Malik, Kumar and Nadarajah 2020):

- Bacterial components – harpin (HrpZ), flagellin, cold shock proteins, elongation factor (EF-Tu), lipopolysaccharides (LPS), peptidoglycans, oligogalacturonides, lipopeptides, dimethylsulfide, pseudobactin, type-III secreted effector (T3E), tri-N-alkylated benzylamine derivative (NABD), 2,4-diacetylphloroglucinol (DAPG), pyocyanine and pyochelin, exopolysaccharides, and N-acyl-L-homoserine lactone
- Fungal components – chitin/chitosan, B-glucans, cerebrosides A & C, ergosterol, xylanase, HR-inducing protein, PemG1, PebC1, oligosaccharides, and ethylene-inducing xylanase (EIX)

These substances can elicit plant defenses in a variety of plants, including potatoes, peppers, tomatoes, brassicas, corn, tobacco, bean, grapes, and wheat. Microorganisms producing such substances include

983 *Pseudomonas* spp., *Bacillus* spp., *Trichoderma* spp. and likely many others. Other substances that can elicit  
984 plant defense responses include laminarin from brown algae (Malik, Kumar and Nadarajah 2020).  
985 Microorganisms such as *Cladosporium herbarum* or *Penicillium* spp. can also be used to antagonize  
986 pathogens (Agrios 2005). These species are represented within some of the OMRI Listed products  
987 mentioned above.

988 Nonsynthetic oils, such as from soybean, sunflower, corn, and neem, can be used successfully to treat  
989 powdery mildew (Agrios 2005). Oils are recommended for control of powdery mildew by Colorado State  
990 University Extension (Cranshaw and Baxendale 2013).

992 *Currently Allowed Synthetic Alternatives*

994 There are several synthetic materials available for plant disease control. Aqueous potassium silicate can be  
995 used as a fungicide. The EPA lists its mode of action as a desiccant (2007b), while the 2014 technical report  
996 lists its function as serving "to replenish plant's innate resistance" (USDA 2014a). Elemental sulfur,  
997 horticultural oils, and potassium bicarbonate can be used to control fungi such as powdery mildew  
998 (McGourty 2008). Copper pesticides are also allowed for use and act as broad-spectrum fungicides (USDA  
999 2014b). They work best for powdery mildew, downy mildew, and botrytis, although they have some effects  
1000 on other diseases (Quarles 2019).

1001 The following synthetic materials are currently allowed for plant disease control and exist within  
1002 commercially available products: aqueous potassium silicate (CAS # 1312-76-1); coppers, fixed; copper  
1003 sulfate; hydrogen peroxide; lime sulfur; oils, horticultural; peracetic acid; potassium bicarbonate; elemental  
1004 sulfur; and polyoxin D zinc salt. Refer to previous Technical Reports on these materials for comparisons of  
1005 the effect, form, function, quality, and quantity of these substitutes and for literature, including product  
1006 and practice descriptions, performance, and test data.

1007 **Evaluation Question #12: Describe any alternative practices that would make the use of the petitioned  
1008 substance unnecessary (7 U.S.C. § 6518 (m) (6)).**

1009 As opposed to substances applied for highly specific purposes, it is unlikely that there are any specific  
1010 alternative practices that would render the use of chitosan or any other general-use disease control  
1011 substances unnecessary, unless one is willing to accept crop losses caused by plant disease. However,  
1012 cultural practices are important because fungicides can be limited in their ability to stop some pathogens,  
1013 such as Botrytis (McGourty 2008). Good farming practices, including building suppressive soils, creating  
1014 unfavorable conditions for pathogens, managing disease vectors such as aphids, sterilizing soils with heat  
1015 as appropriate, planting resistant cultivars, and managing fertility all contribute to reducing the need to  
1016 use disease control substances (Agrios 2005; Choudhary, Prakash and Johri 2007). For specific crops such as  
1017 grapes, infections of powdery mildew can be treated by removing leaves and using water sprayed directly  
1018 on plant surfaces. Downy mildew can be managed by thinning crops and using air-blast sprayers  
1019 (McGourty 2008).

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1025 **Report Authorship**

1026 The following individuals were involved in research, data collection, writing, editing, and/or final  
1027 approval of this report:

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1037 All individuals are in compliance with Federal Acquisition Regulations (FAR) Subpart 3.11 – Preventing  
1038 Personal Conflicts of Interest for Contractor Employees Performing Acquisition Functions.  
1039  
1040

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