

Chitosan

Crops

Identification of Petitioned Substance

Chemical Names:	18	ELEXA-4
(C ₆ H ₁₁ NO ₄) _n	19	BIOREND
poly-D-glucosamine	20	
Poly(beta-(1,4)-2-amino-2-deoxy-D-glucose);		CAS Numbers:
Poly(beta-(1,4)-D-glucosamine)		9012-76-4
Other Name:		Other Codes:
deacetylated chitin		UNII: 23R93M6Y64
water-soluble chitin (some forms)		UNII: 5GV09YMO52
poliglusam/polyglusam		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.

54 The petitioner describes many different uses of chitosan throughout the petition. It is difficult to provide a
 55 useful summary comparison of chitosan versus all other alternatives because the number of input
 56 materials, organic practices, disease-causing organisms, and crops to evaluate is large. Where possible,
 57 examples are used which illustrate a few comparisons between chitosan and other materials, focusing on
 58 the disease-causing organisms that appear on the part of the label included with the petition. However,
 59 these examples do not relate to all possible crops and diseases that could be covered under this petition.
 60 The 2004 technical report on chitosan, written in support of the NOSB's 2005 review, is still relevant. The
 61 following technical report confirms many of the same conclusions included in that report.
 62
 63

Characterization of Petitioned Substance

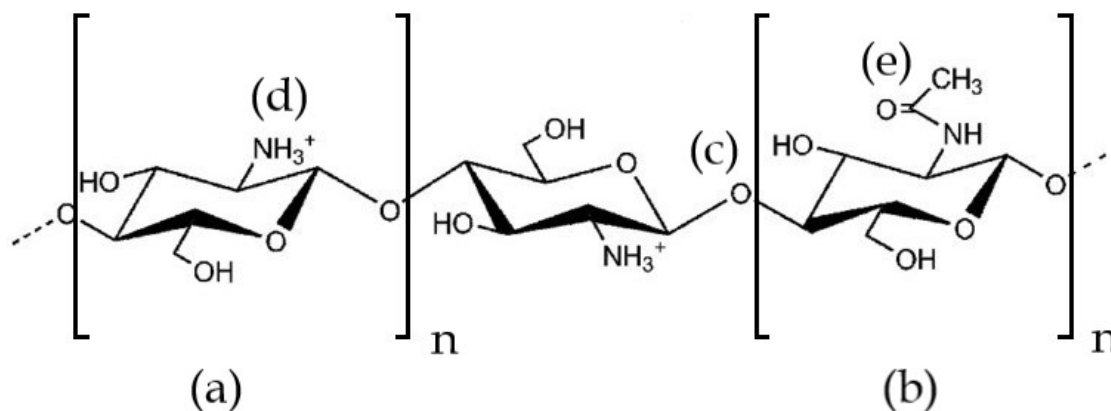
Composition of the Substance:

66 Chitosan is a copolymer composed of two different chemical subunits (monomers) that repeat in no
 67 particular order: glucosamine (2-acetamido-2-deoxy-d-glucopyranose) and N-acetylglucosamine (2-amino-
 68 2-deoxy-d-glucopyranose) (Pillai, Paul and Sharma 2009). It is derived from chitin, which is structurally
 69 similar to cellulose (Dutta, Dutta and Tripathi 2004) and only differs slightly in the individual monomers
 70 bonded together to form the polymer. While cellulose is composed of glucose monomers linked together,
 71 chitin is mostly composed of N-acetylglucosamine monomers. Cellulose and chitin are the two most
 72 abundant biopolymers found on earth (Pillai, Paul and Sharma 2009; Chawla, Kanatt and Sharma 2014).
 73
 74

75 There is no degree of deacetylation that officially defines when chitin becomes chitosan, but the lower limit
 76 described in literature is 40–60 percent (Hussain, Iman and Maji 2014). Typically chitin contains 85–95
 77 percent N-acetylglucosamine and 5-15 percent glucosamine (Pillai, Paul and Sharma 2009). Chitosan is a
 78 synthetic made by removing acetyl groups from chitin. Commercial chitosan usually contains at least
 79 65 percent glucosamine and less than 35 percent N-acetylglucosamine (Pillai, Paul and Sharma 2009).
 80

81 The degree of deacetylation can vary, and so any given quantity of chitin or chitosan will typically contain
 82 both types of monomers. Below a pH of approximately 6, chitosan becomes positively charged by
 83 acquiring hydrogen ions (see Figure 1 below). This pH also coincides with chitosan becoming soluble in
 84 aqueous solutions, such as in a solution of acetic acid and water (Pillai, Paul and Sharma 2009).
 85

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



87
 88 *Glucosamine monomers (a) are bonded to additional glucosamine monomers or N-acetylglucosamine
 89 monomers (b) via 1-4 glycosidic linkages (c). Glucosamine monomers possess an amine group (d), ionized
 90 due to low pH. N-acetylglucosamine monomers possess the acetamide (amino-acetyl) group (e). Drawing
 91 adapted from (Nilsen-Nygaard, et al. 2015).

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

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

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

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

143

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,¹ 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 ^a
Physical state and appearance	Solid, powder
Color	White to light cream
Molecular weight	5 X 10 ³ to 10 ⁵ daltons
Specific gravity	1.4 kg/m ³
Bulk density	0.4 g/cm ³
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 ^aSource: (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

159 Depending on which acid is used, chitosan is soluble in aqueous solutions below a pH of around 6 (Pillai,
 160 Paul and Sharma 2009). Above a pH of 6, the amino groups become deprotonated, which leads to overall
 161 insolubility in water. Chitosan is soluble in organic acids such as acetic, formic, and lactic acids; however, it
 162 is insoluble in some mineral acids such as sulfuric and phosphoric acid. Chitosan is insoluble in most
 163 organic solvents, such as dimethylformamide and dimethyl sulfoxide, but is soluble in N-methyl
 164 morpholine-N-oxide (NMMO) (Pillai, Paul and Sharma 2009).
 165

166 Both the amino (NH₂) and hydroxyl (OH) groups of chitosan are useful as sites for chemical substitutions
 167 to create functional derivatives (Dutta, Dutta and Tripathi 2004). These “pendant groups,” or side chains,
 168 can create new or alter existing physicochemical properties of chitosan. For example, the amino groups can
 169 be reacted with aldehydes to form a substance with reduced hydrophobicity and improved solubility (N-
 170 alkyl chitosan) (Bellich et al. 2016; Dutta, Dutta and Tripathi 2004). N-carboxybutyl chitosan is a derivative
 171 that has enhanced antibacterial properties. Chemical modifications to chitosan are typically intended to
 172 improve physicochemical characteristics – such as solubility under specific conditions or its compatibility
 173 with other biopolymers – or to enhance biological properties such as chitosan's antibacterial activity
 174 (Bellich et al. 2016).
 175

¹ 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
183 Chitosan chelates many metals, including nickel (Ni^{2+}), zinc (Zn^{2+}), cobalt (Co^{2+}), iron (Fe^{2+}), magnesium
184 (Mg^{2+}), and copper (Cu^{2+}) (Dutta, Dutta and Tripathi 2004; Kong et al. 2010). These metals include plant
185 micronutrients as well as substances that are essential to the proper functioning of bacterial cell walls
186 (Kong et al. 2010). Some chitosan-metal complexes themselves have bactericidal properties, such as Zn^{2+} ,
187 Zr^{2+} , and Ag^{1+} (Zalloum and Mubarak 2013). Several models of how chitosan chelates metals have been
188 proposed, and more than one manner of chelation may occur at the same time (Gerente et al. 2007). Most
189 models consider the amino groups present in chitosan as the basis for the chelation of transition metals. For
190 example, the amino groups from the same chitosan chain may be involved in donating electrons to create
191 the chelation complex (forming a bridge), or separate adjacent chains can be involved. Another model
192 suggests that hydroxyl groups may participate where the metal is simultaneously bonded to two amino
193 groups and two hydroxyl groups. Alternatively, the metal may be bonded to a hydroxyl group, an amino
194 group, and two water molecules. The pH of the solution may influence the way in which chitosan chelates
195 specific metals. Not only that, but pH also affects the total capacity for chitosan to chelate metals. At lower
196 pH, the positively charged amino groups interfere with the ability of chitosan to chelate metal cations
197 (Gerente et al. 2007). Chitosan preferentially adsorbs divalent metals in the following order: $\text{Cu(II)} > \text{Hg(II)}$
198 $> \text{Zn(II)} > \text{Cd(II)} > \text{Ni(II)} > \text{Co(II)} \sim \text{Ca(II)}$; and trivalent metals: $\text{Eu(III)} > \text{Nd(III)} > \text{Cr(III)} \sim \text{Pr(III)}$ (Rhazi, et
199 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 (TGAIs), 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
296 In 1986, the EPA first approved the use of chitosan under section 3(c)(5) of the Federal Insecticide,
297 Fungicide, and Rodenticide Act (FIFRA) as a plant growth regulator in or on wheat. At the same time, the
298 EPA also exempted chitosan from the requirement of a tolerance under 40 CFR §180.1072 (National
299 Archives 1986). Subsequent EPA approvals included applications to other crops (National Archives 1989,
300 National Archives 1995).

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

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

313 *Food and Drug Administration (FDA)*

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

317
318
319 Several Generally Recognized as Safe (GRAS) notices have been filed with the FDA. Three notices relate to
320 general food use. At the notifier’s request, the FDA ceased to evaluate these three notices (US FDA 2020b;
321 US FDA 2020c; US FDA 2020d). GRN Notice 397 states that chitosan from the fungus *Aspergillus niger* is
322 considered GRAS when used as “a secondary direct food ingredient in alcoholic beverage production at
323 levels between 10 and 500 grams per hectoliter (100 liters)” (US FDA 2020e).

324
325 The Association of American Feed Control Officials (AAFCO), which has a memorandum of
326 understanding with the FDA (US FDA 2019), lists chitosan as a flocculant, allowed for precipitating
327 proteins during animal feed processing (AAFCO 2020).

328 *United States Department of Agriculture (USDA)*

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

331 **Action of the Substance:**

332
333 Chitosan has multiple modes of action. When used as a pesticide, it acts directly on target pathogens with
334 toxic as well as growth inhibitory effects. It also has effects on plants themselves, stimulating plant
335 immunity. Chitosan’s effect on both plants and pathogens is not universal. The following is a summary of
336 available information.

339 *Antibacterial Modes of Action*

340 Researchers do not fully understand how chitosan inhibits bacterial growth, and Gram-negative² 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/Antichromista³ 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

² 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.

³ Kingdom Chromista includes organisms previously considered fungi, such as the oomycete, *Phytophthora infestans* (Maneveltdt and Keats 2004).

382 pathogens (biotrophs⁴) 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
389 By contrast to such localized responses, plants also exhibit systemic (non-localized) defenses. Two better-
390 known systemic defenses are systemic acquired resistance (SAR) and induced systemic resistance (ISR)
391 (Choudhary, Prakash and Johri 2007; Dong 2004; Agrios 2005; van Loon, Bakker and Pieterse 1998). SAR is
392 associated with the accumulation of salicylic acid (SA) within the plant, which leads to the production of
393 pathogenesis related substances, such as antifungal enzymes and phytoalexins that inactivate pathogens.
394 SAR can be triggered by a plant recognizing a pathogenic organism and tends to negatively affect plant
395 growth. ISR, on the other hand, is often triggered by beneficial microbes and can positively affect plant
396 growth. Jasmonic acid (JA) is associated with the ISR response, but exactly how ISR protects plants is not
397 well understood. Plants do not necessarily accumulate the same types of anti-pathogen substances as occur
398 with SAR but instead appear to respond more quickly when infection is attempted – in other words, ISR
399 “primes” plants for subsequent defenses. Ethylene and nitric acid are linked to additional plant defense
400 pathways (Choudhary, Prakash and Johri 2007; Dong 2004; Agrios 2005; van Loon, Bakker and Pieterse
401 1998).

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

411
412 *Nematode Control Mode of Action*

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

424
425 **Combinations of the Substance:**

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

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

⁴ 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

Status

448

Historic Use:

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

455

Plant Disease Control

456
457 Chitosan is reported as an active ingredient in 22 EPA registered products (National Pesticide Information
458 Center 2020). Of these, three are labelled as plant growth regulators for seed germination, three are
459 Technical Grade Active Ingredients (TGAIs), and five are antimicrobials for textiles. The remaining nine
460 products make disease prevention claims for a wide range of crops and application methods (National
461 Pesticide Information Center 2020).

462

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

473

Use as a Biostimulant

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

484

Unintentional Side Effects

485
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

(c) Guidelines for prohibition and exemptions. –

505

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

508

(B) the substance –

509

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

511

512 Similarly, chitosan is not mentioned explicitly within the USDA organic regulations in 7 CFR Part 205;
513 however, it is present on 2004 EPA List 4, and therefore included by incorporation under §205.601(m) and
514 205.603(e):

515

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

518

519

520

International

521

522

Canadian General Standards Board Permitted Substances List

523

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

528

529

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

531

532 Chitosan is not included within the Codex guidelines; however, chitin nematicides of natural origin are
533 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

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

541

542

543 According to the EC Expert Group for Technical Advice on Organic Production (EGTOP), chitosan is a
544 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 nematocide 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 cleansers? (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 (NaOH), sodium carbonate (Na₂CO₃), sodium bicarbonate (NaHCO₃), potassium hydroxide (KOH),
597 potassium carbonate (K₂CO₃), calcium hydroxide (Ca(OH)₂), sodium sulfite (Na₂SO₃), sodium bisulfite
598 (NaHSO₃), calcium bisulfite (CaHSO₃), trisodium phosphate (Na₃PO₄) and sodium sulfide (Na₂S). 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 borohydrate 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 (HNO₃), sulfuric acid (H₂SO₄), acetic acid (CH₃COOH), and formic
606 acid (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



614

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

619

620 Decolorizing

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

624

625 *Biological Extraction and Isolation of Chitin*

626 While slower than chemical methods, shells can also be deproteinated and demineralized with enzymes or
627 fermenting with microorganisms (Younes and Rinaudo 2015). Biological extraction has the advantage in
628 that it better preserves the structure and size of chitin molecules. However, enzymatic methods can leave
629 4–15 percent residual protein. These can be removed through subsequent chemical treatment (such as with
630 sodium hydroxide). This process requires less solvent and less energy. Crude protease formulations (e.g.,
631 derived from fish viscera) are efficient and more economical than purified forms (Younes and Rinaudo
632 2015).

633

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

636

637 *Chemical Conversion of Chitin to Chitosan*

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

640

641 Chemical Deacetylation

642 While acids can be used to deacetylate chitin monomers, they also hydrolyze the glycosidic bonds that
643 connect monomers together. Therefore, alkaline materials are necessary to produce chitosan (Younes and
644 Rinaudo 2015). Dilute sodium hydroxide is used to separate and extract chitin from shells, but a more
645 concentrated solution is required to effectively deacetylate chitin to form chitosan due to the way that the
646 acetyl groups are positioned relative to the sugar ring. Potassium or sodium hydroxide solutions of 40–
647 60 percent concentration are used, with a ratio of 1:10 weight by volume (w/v) solids-to-solvent (Chawla,
648 Kanatt and Sharma 2014). Chitin can be converted into chitosan using either a high-heat process or a lower
649 temperature process. Repeating these processes produces chitosan with more complete deacetylation
650 (Islam, Bhuiyan and Islam 2017; Younes and Rinaudo 2015). Additional reagents such as sodium
651 borohydride (NaBH₄) can be used to prevent degradation of the polymer into smaller molecular weight
652 pieces (Younes and Rinaudo 2015). Chitosan processes typically produce material that is 56–99 percent
653 deacetylated (Islam, Bhuiyan and Islam 2017).

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 Biological deacetylation

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

674 Production of Chitosan Oligomers

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

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

684
685 Commercially available forms of chitosan are manufactured through a chemical process. Several different
686 steps during the manufacturing process create synthetic materials. During the process of extracting chitin,
687 sodium hydroxide is used to hydrolyze bonds that connect chitin to proteins. The chitin that is extracted is
688 no longer chemically bound to proteins, arguably leaving it in a form that does not exist in nature when
689 found in shells. Once isolated, chitin undergoes deacetylation to form chitosan. Again, this step chemically
690 modifies chitin and is mediated by synthetic chemicals. Some forms of chitosan are additionally modified
691 to form salts and other derivatives (Dutta, Dutta and Tripathi 2004; Pillai, Paul and Sharma 2009; Islam,
692 Bhuiyan and Islam 2017).

693
694 Following NOP Guidance 5033-1 Guidance, Decision Tree for Classification of Materials as Synthetic or
695 Nonsynthetic (NOP 2016) leads to a determination that chitosan is **synthetic**. Chitosan can be extracted
696 from a natural source (shells), but the material has been transformed into a different substance via a
697 chemical change. Alternatively, one could consider chitin (which may or may not be considered synthetic,
698 depending on how one views the deproteination step in chitin extraction) to be the source for chitosan. In
699 this case, the chitin has undergone a chemical change in conversion to chitosan, which (with rare
700 exceptions) is not mediated by a biological process.

701 Evaluation Question #4: Describe the persistence or concentration of the petitioned substance and/or its 702 by-products in the environment (7 U.S.C. § 6518 (m) (2)).

703
704 Chitosan’s primary means for breakdown is through microbial action, where it eventually breaks down
705 into small amino sugars, such as glucosamine monomers (Roberts, Bol and Jones 2007; Wieczorek, Hetz
706
707
708

709 and Kolb 2014). These sugars can undergo different fates, including direct uptake as nutrients by plants
710 and other microorganisms and further breakdown into carbon dioxide or mineralization in the soil to form
711 substances like ammonia and nitrate (Roberts, Bol and Jones 2007; Wieczorek, Hetz and Kolb 2014). This
712 involves hydrolysis of the polymer to produce glucosamine monomers and subsequent glucosamine
713 degradation. Chitosan naturally occurs in fungi, and microorganisms have evolved to produce different
714 chitosanase enzymes that break the substance down as a source of carbon and nitrogen (Sawaguchi et al.
715 2015). With that said, chitin is much more common in nature, being found in nearly all fungi and in many
716 (if not most) invertebrates (Hirano 2012; Wieczorek, Hetz and Kolb 2014; Sato et al. 2010). Chitin appears to
717 break down more quickly than chitosan in soils and is able to do so through a different biodegradation
718 pathway (Wieczorek, Hetz and Kolb 2014).

719
720 The time that microbes take to break chitosan down depends on the characteristics of the soil. In one study,
721 chitosan in silty soils with a relatively larger microbial biomass broke down much faster than chitosan in
722 sandy soils with less microbial biomass (Sawaguchi et al. 2015). Another study indicated that
723 decomposition of fungal cell walls (including both chitin and chitosan) was not affected by the specific
724 microbial community structure, but was slower in clay soils (Hu et al. 2020). One study indicated that
725 chitosan breaks down completely within 30 days at 5 percent concentration (w/w) chitosan/soil, but only
726 60 percent was broken down after 180 days in another case (1 percent w/w chitosan/soil) (Sawaguchi et al.
727 2015; Sato et al. 2010). The biodegradation kinetics of chitosan is also dependent on the polymer chain
728 length of chitosan and the distribution of acetyl groups, making it difficult to identify a broadly applicable
729 decomposition time (Islam, Bhuiyan and Islam 2017; Sato et al. 2010; Sawaguchi et al. 2015; Wieczorek,
730 Hetz and Kolb 2014).

731
732 Based on EPA product labels, chitosan is applied at a rate of between 0.003 pounds of chitosan per acre,
733 and 2.5 pounds per acre, with multiple applications suggested and typically on a 7–14 day cycle (see
734 *Specific use of the Substance* above). Based on the generally biodegradable nature of chitosan, naturally
735 occurring biodegradation pathways, and low application rates, chitosan and its breakdown products are
736 unlikely to accumulate within the agro-ecosystem.

737
738 **Evaluation Question #5: Describe the toxicity and mode of action of the substance and of its**
739 **breakdown products and any contaminants. Describe the persistence and areas of concentration in the**
740 **environment of the substance and its breakdown products (7 U.S.C. § 6518 (m) (2)).**

741
742 Chitosan has multiple modes of actions, which are described in *Action of the Substance* (above). While
743 chitosan is antimicrobial, this activity requires recurring applications to maintain. Furthermore, chitosan
744 can also stimulate microbial growth. Studies showing temporary changes to microbial community
745 structures used much larger concentrations of chitosan than are proposed within the petitioned use in
746 order to elicit an effect (Sato et al. 2010; Sawaguchi et al. 2015; Wieczorek, Hetz and Kolb 2014).

747
748 Applying chitosan as a pesticide is not expected to increase its concentration beyond background levels
749 existing in nature (US EPA 2007a). Amino sugars like those that are found in chitin and chitosan make up
750 1–10 percent of the dry weight of Gram-positive bacteria, and 5–10 percent of fungi (Roberts, Bol and Jones
751 2007).

752
753 Chitosan's breakdown products are small molecules such as glucosamine, carbon dioxide, and ammonia
754 that act as nutrients for many organisms, including bacteria, fungi, and plants.

755
756 **Evaluation Question #6: Describe any environmental contamination that could result from the**
757 **petitioned substance's manufacture, use, misuse, or disposal (7 U.S.C. § 6518 (m) (3)).**

758
759 EPA and FDA data were not found that indicated that environmental contamination risks were assessed
760 for the manufacture or disposal of chitosan. One EPA report (US EPA 2007a) indicated that the use of
761 chitosan as an active ingredient in pesticide products did not pose a risk to non-target organisms, and that
762 it was unlikely to be an ecological risk. The EPA's Biopesticides and Pollution Prevention Division chose to
763 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 hydroxychloride (EGTOP 2016).

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

774
775 The manufacture of chitosan involves the use of relatively large amounts of corrosive chemicals, notably
776 sodium hydroxide and hydrochloric acid (see *Evaluation Question #2*). In use, these chemicals are
777 neutralized during hydrolysis reactions and also go on to produce benign salts such as calcium chloride
778 (CaCl_2) and sodium chloride (NaCl) during the isolation of chitin and synthesis of chitosan (Chawla,
779 Kanatt and Sharma 2014; EGTOP 2016). Muñoz et al. (2018) estimated that for one chitosan supplier in
780 India, production of 1 kg of chitin requires 33 kg shells, 8 kg hydrogen chloride (HCl), 1.3 kg sodium
781 hydroxide (NaOH), and 167 L of fresh water. To produce 1 kg of chitosan for the Indian supplier, 1.4 kg of
782 refined chitin were needed, as well as an additional 5.18 kg NaOH and 250 L of water. They also evaluated
783 a European supplier's production process and found that it required even larger amounts of chemicals and
784 water as well as four times as much energy – though this chitosan was destined for medical use. Yan and
785 Chen (2015) estimated that on average, one metric tonne of water is required to produce one kilogram of
786 chitosan.

787
788 Both sodium hydroxide and chlorine are products of the energy-intensive chloralkali process. In cases
789 where electricity for the chloralkali process is powered by fossil fuels, approximately two tons of CO_2 is
790 produced for every ton of Cl_2 and NaOH (Marini et al. 2014). Hydrochloric acid is produced via several
791 different routes, but these typically involve chlorine gas produced from the chloralkali process mentioned
792 above (Mansfield, Depro and Perry 2000). In 2015, hydrochloric acid was the fifth most released toxic
793 chemical in the United States, though this is likely related to coal-fired power plant emissions (US EPA
794 2017b). Depending on the specific equipment used in the process (mercury cell, diaphragm cell, or
795 membrane cell), different wastes and emissions are produced during the chloralkali process used to make
796 sodium hydroxide and chlorine (used to eventually produce hydrochloric acid) (US DOE 2000):

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

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

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

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

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
912 According to numerous sources, chitosan is nearly non-toxic to humans and most other animals, and its
913 degradation products do not cause side effects in the body (US EPA 2007a; Islam, Bhuiyan and Islam 2017;
914 Pillai, Paul and Sharma 2009; Hirano 2012; Friedman and Juneja 2010; Baldrick 2010). The EPA considers
915 chitosan to have an acute oral and acute eye irritation toxicity classification of IV, or practically non-toxic
916 and not an irritant (US EPA 2007a). The lethal dose necessary to kill 50 percent of the test population (LD₅₀)
917 ranges from 1500 mg/kg body weight orally in rats to over 16,000 mg/kg bodyweight in mice (Baldrick
918 2010). Many studies where large, repeated doses of chitosan oligomers (short fragments) were fed to rats,
919 no toxic effects were observed (Baldrick 2010).

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

928

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

937

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

941

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

949

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

956

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

961

962 *Non-Synthetic Microbials, Botanicals, and Oils*

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

967

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

971

972 The effectiveness of these microbials and botanicals is at least in part due to their role as elicitors.

973 Nonsynthetic elicitors of plant defenses from microorganisms include (Malik, Kumar and Nadarajah 2020):

974 • **Bacterial components** – harpin (HrpZ), flagellin, cold shock proteins, elongation factor (EF-Tu),
975 lipopolysaccharides (LPS), peptidoglycans, oligogalacturonides, lipopeptides, dimethylsulfide,
976 pseudobactin, type-III secreted effector (T3E), tri-N-alkylated benzylamine derivative (NABD), 2,4-
977 diacetylphloroglucinol (DAPG), pyocyanine and pyochelin, exopolysaccharides, and N-acyl-L-
978 homoserine lactone

979 • **Fungal components** – chitin/chitosan, B-glucans, cerebrosides A & C, ergosterol, xylanase, HR-
980 inducing protein, PemG1, PebC1, oligosaccharides, and ethylene-inducing xylanase (EIX)

981 These substances can elicit plant defenses in a variety of plants, including potatoes, peppers, tomatoes,
982 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 laminarins 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
989 Nonsynthetic oils, such as from soybean, sunflower, corn, and neem, can be used successfully to treat
990 powdery mildew (Agrios 2005). Oils are recommended for control of powdery mildew by Colorado State
991 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
1002 The following synthetic materials are currently allowed for plant disease control and exist within
1003 commercially available products: aqueous potassium silicate (CAS #-1312-76-1); coppers, fixed; copper
1004 sulfate; hydrogen peroxide; lime sulfur; oils, horticultural; peracetic acid; potassium bicarbonate; elemental
1005 sulfur; and polyoxin D zinc salt. Refer to previous Technical Reports on these materials for comparisons of
1006 the effect, form, function, quality, and quantity of these substitutes and for literature, including product
1007 and practice descriptions, performance, and test data.

1009 **Evaluation Question #12: Describe any alternative practices that would make the use of the petitioned 1010 substance unnecessary (7 U.S.C. § 6518 (m) (6)).**

1011
1012 As opposed to substances applied for highly specific purposes, it is unlikely that there are any specific
1013 alternative practices that would render the use of chitosan or any other general-use disease control
1014 substances unnecessary, unless one is willing to accept crop losses caused by plant disease. However,
1015 cultural practices are important because fungicides can be limited in their ability to stop some pathogens,
1016 such as Botrytis (McGourty 2008). Good farming practices, including building suppressive soils, creating
1017 unfavorable conditions for pathogens, managing disease vectors such as aphids, sterilizing soils with heat
1018 as appropriate, planting resistant cultivars, and managing fertility all contribute to reducing the need to
1019 use disease control substances (Agrios 2005; Choudhary, Prakash and Johri 2007). For specific crops such as
1020 grapes, infections of powdery mildew can be treated by removing leaves and using water sprayed directly
1021 on plant surfaces. Downy mildew can be managed by thinning crops and using air-blast sprayers
1022 (McGourty 2008).

1025 **Report Authorship**

1026
1027 The following individuals were involved in research, data collection, writing, editing, and/or final
1028 approval of this report:

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1036

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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