

# Laminarin

## Crops

### Identification of Petitioned Substance

**Chemical Names:**

Laminarin

**CAS Numbers:**

9008-22-4

**Other Names:**

Laminaran, leucosin

**Other Codes:**

EC Number 232-712-4; MDL Number

MFCD00466918; US EPA PC Code 123200

**Trade Names:**

Vacciplant

### Summary of Petitioned Use

Laminarin was petitioned in 2013 for inclusion on the National List of synthetic substances allowed for use in organic crop production at §205.601. In 2014, the NOSB Crops Subcommittee issued a proposal in which the majority considered laminarin to be a nonsynthetic substance and therefore not requiring addition to the National List. The NOSB has requested a limited-scope technical report to further assess the synthetic/nonsynthetic classification of laminarin, and to evaluate the presence of the synthetic residuals from the manufacturing process, sodium and sulfate ions. Accordingly, only a subset of questions is included in this technical report.

### Characterization of Petitioned Substance

**Composition of the Substance:**

Laminarin is a polysaccharide derived from seaweed, a polymer of simple sugars linked together by glycosidic bonds. More specifically, it is a class of storage  $\beta$ -glucans comprised of  $\beta$ -(1-3)-linked glucose residues with some  $\beta$ -(1-6)-intrachain linkages, and some 6-O-branching in the main chain. D-mannitol occurs at 2-3% of reducing termini and these chains are referred to as M chains, while those with glucose at the reducing termini are known as G chains. The exact molecular structure of laminarin varies depending on frond age and environmental factors such as water temperature, salinity and nutrient salts, waves, sea current and immersion depth. Its chemical formula is  $C_{18}H_{32}O_{16}$  (University of Hertfordshire 2014; ChEBI 2015; Kadam, Tiwari and O'Donnell 2015).

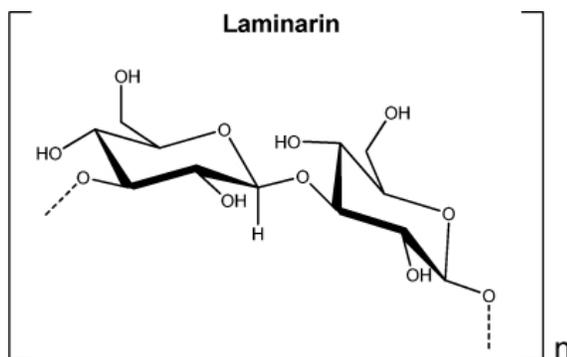


Figure 1. Units of glucose in laminarin of brown seaweeds. (Vera, et al. 2011)

**Source or Origin of the Substance:**

Laminarin is a naturally occurring polysaccharide food reserve found in the fronds of brown seaweed.

*Laminaria digitata* is the petitioner's stated source and the most predominant source; other species include

42 those of *Laminaria*, *Fucus*, *Saccharina*, *Undaria*, and *Ascophyllum* genera of brown algae (Kadam, Tiwari and  
 43 O'Donnell 2015). Brown algae may contain up to 35% laminarin on a dry weight basis, depending on  
 44 species, season, habitat and extraction method.

#### 45 **Properties of the Substance:**

46 Laminarin is a low molecular weight, bioactive polysaccharide. It does not have gelling or thickening  
 47 properties like other algal polysaccharides, namely alginate and carrageenan. Its physical and chemical  
 48 properties are outlined in Table 1.

49 Table 1. Properties of Laminarin. (Kada, Tiwari and O'Donnell 2015; Laboratoires Goëmar SA 2013; Sigma-  
 50 Aldrich 2015; Pesticide Action Network 2014)

Color	White
Physical State	Powder. Can also be formulated in granules or liquid form.
Odor	Low odor
pH	6.25±0.02 at 23.2°C (1% w/v)
Turbidity	Clear to slightly hazy
Solubility	Generally water soluble, though forms with low molecular branching are insoluble in cold water. Avg. solubility 301,500 mg/L

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 54  
 55 **Specific Uses of the Substance:**  
 56 Laminarin was petitioned for addition to the National List for use as a pre-harvest pesticide on various  
 57 fruits and vegetables to stimulate the plants' natural disease-defense mechanisms (Laboratoires Goëmar SA  
 58 2013). Its ability to stimulate plant defenses is well documented (Aziz, et al. 2003; Vera, et al. 2011;  
 59 Klarzynski, et al. 2000; Kadam, Tiwari and O'Donnell 2015). Laminarin has also been shown to enhance the  
 60 biological control of crop pests by attracting parasitic wasps (Sobhy, et al. 2014). One patent describes its  
 61 use as a seed germination and plant growth accelerator (Yvin, et al. 1998).

62  
 63 Laminarin is also reported to have numerous biofunctional activities which are utilized in the  
 64 pharmaceutical, cosmetic and food industries. Specifically, laminarin may be used as an anti-inflammatory,  
 65 anticoagulant and antioxidant, as well as for its antitumor and anti-cell death activities (Kadam, Tiwari and  
 66 O'Donnell 2015).

#### 67 **Approved Legal Uses of the Substance:**

68 EPA regulation of pesticide chemical residues in food at 40 CFR 180.1295 establishes an exemption from  
 69 the requirement of a tolerance for residues for laminarin in or on all food commodities when it is applied  
 70 pre-harvest as a biochemical pesticide to stimulate natural defense mechanisms in plants.

#### 71 **Action of the Substance:**

72  
 73 Laminarin enhances plant protection against disease pathogens by activating the plant's natural defense  
 74 mechanisms (Aziz, et al. 2003). When a pathogen infects a plant, the spread of disease is determined by  
 75 reactions to molecular signals generated by both the pathogen and the plant. Plants recognize elicitor  
 76 molecules from the pathogen which triggers the activation of various defense responses. Laminarin,  
 77 though not from a pathogen, has been characterized as this type of elicitor (Aziz, et al. 2003). Its  
 78 application to a plant causes a local oxidative burst, which then activates the salicylic (SA) signaling  
 79 pathway in the plant at a systemic level. Activation of this pathway leads to an increase in the expression of  
 80 genes which encode proteins involved in plant defense. These include pathogenesis-related (PR) proteins  
 81 with antifungal and antibacterial activities; defense enzymes which affect the accumulation of compounds  
 82 having antiviral, antifungal and antibacterial activities; and enzymes which aid in the synthesis of terpenes,  
 83 terpenoids and/or alkaloids with antimicrobial activities (Vera, et al. 2011). All of these induced responses  
 84 function to enhance the plant's protection against pathogens and deter their spread throughout the plant.  
 85 In grapevine cells, laminarin has been observed to induce a rapid calcium influx and alkalization of the  
 86 extracellular medium, both typical responses to elicitors, as well as to induce a transient production of  
 87

88 hydrogen peroxide, an active oxygen species (AOS) that signals the SA pathway as described above (Aziz ,  
89 et al. 2003).

90  
91 Klarzynski et al. (2000) reported that laminarin applied to tobacco plants elicits defense reactions, including  
92 stimulating the activities of phenylalanine ammonia lyase, caffeic acid O-methyl transferase, and  
93 lipoxygenase, and promoting the accumulation of salicylic acid and anti-microbial pathogenesis-related  
94 proteins.

#### 95 **Combinations of the Substance:**

96 The formulated product (Vacciplant) affiliated with the NOSB petition for laminarin is EPA registered and  
97 contains 3.51% laminarin as the active ingredient. The active ingredient, laminarin, contains some  
98 residuals of other substances from its manufacturing process as will be discussed in Evaluation Question 2.  
99 The Material Safety Data Sheet (MSDS) for the final formulated product (Vacciplant) also discloses use of a  
100 surfactant (<10%) and two different preservatives (each <1%) that are used to formulate the product. The  
101 identity of these formulants is not given; however, the MSDS states that both preservatives are permitted  
102 for use as food additives.

103  
104  
105 The patent describing use of laminarin as a seed germination and plant growth accelerator notes optional  
106 formulation with an agriculturally acceptable carrier as well as deficiency-correcting elements, other active  
107 pesticides, growth hormones, lipoamino acids, betains, dispersants and emulsifiers (Yvin, et al. 1998).

108

### 109 **Status**

110

#### 111 **Historic Use:**

112 Laminarin was first isolated in 1885 by Schmiedeberg (Kadam, Tiwari and O'Donnell 2015). Most patents  
113 for its use as a pesticide, however, are from the past 15 years. The EPA granted an exemption from the  
114 requirement of a tolerance for laminarin in 2010 (EPA 2010). The commercial product containing laminarin  
115 (Vacciplant) has not been evaluated for compliance by the Organic Materials Review Institute, and it is  
116 unknown whether it is currently in use in organic production or is approved by other material review  
117 organizations or certification agencies (OMRI 2015).

118

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

120 Laminarin is not listed in the Organic Foods Production Act of 1990 (OFPA), and it is not specifically listed  
121 in the NOP organic regulations at 7 CFR Part 205. However, aquatic plant extracts (other than hydrolyzed)  
122 are listed as synthetic substances allowed for use in organic crop production at §205.601(j)(1). They are  
123 listed as plant or soil amendments with the following annotation: "Extraction process is limited to the use  
124 of potassium hydroxide or sodium hydroxide; solvent amount used is limited to that amount necessary for  
125 extraction." The substance is not listed for pest control, but nonsynthetic substances that are not prohibited  
126 at §205.602 are permitted for organic crop production per §205.105 provided they also meet the  
127 requirements of §205.206 Crop pest, weed and disease management practice standard.

128

#### 129 **International**

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##### 131 **Canada - Canadian General Standards Board Permitted Substances List**

132 <http://www.tpsgc-pwgsc.gc.ca/ongc-cgsb/internet/bio-org/index-eng.html>

133 <http://www.tpsgc-pwgsc.gc.ca/ongc-cgsb/internet/bio-org/documents/032-0311-2008-eng.pdf>

134 Laminarin is not specifically listed on the Permitted Substances List at CAN/CGSB-32.311-2006. However,  
135 aquatic plants and aquatic plant products appear under Crop Production Aids and Materials, Table 4.3 of  
136 the Permitted Substances List, with the following annotation: "Shall not contain synthetic preservatives,  
137 such as formaldehyde. Natural (nonsynthetic) extracts are allowed. Extraction with synthetic solvents is  
138 prohibited except for potassium hydroxide or sodium hydroxide, provided the amount of solvent used  
139 does not exceed the amount necessary for extraction. The manufacturer shall prove the need to use sodium  
140 hydroxide."

141

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

144 [http://organicrules.org/538/1/Codex\\_Alimentarius\\_organic\\_food\\_gl32\\_booklet04\\_en.pdf](http://organicrules.org/538/1/Codex_Alimentarius_organic_food_gl32_booklet04_en.pdf)

145 Seaweeds and seaweed products are listed in the CODEX Alimentarius Commission Guidelines for the  
146 Production, Processing, Labeling, and Marketing of Organically Produced Food which states that a “Need  
147 [for said substance is] recognized by the certification body or authority.” Laminarin is not specifically  
148 listed.

149  
150 **European Economic Community (EEC) Council Regulation, EC No. 834/2007 and 889/2008**

151 <http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32008R0889&from=EN>

152 Seaweed and seaweed products are listed in Commission Regulation EC No. 834/2007 and EC No.  
153 889/2008 under Annex I: Fertilisers and soil conditioners referred to in Article 3(1). Article 3(1) describes  
154 the use of materials to meet the nutritional needs of plants. Seaweed and seaweed products’ listing in  
155 Annex I includes the following annotation: “As far as directly obtained by: (i) physical processes including  
156 dehydration, freezing and grinding; (ii) extraction with water or aqueous acid and/or alkaline solution; (iii)  
157 fermentation.”

158  
159 Neither laminarin nor seaweed/seaweed products are listed in EC Regulation No. 889/2008 Annex II:  
160 Pesticides – plant protection products referred to in Article 5(1). Article 5(1) states that only products  
161 referred to in Annex II may be used in organic production to protect plants from pests and diseases.  
162 However, the Expert Group for Technical Advice on Organic Production (EGTOP) determined that,  
163 provided the kelp is harvested in a sustainable way, the use of laminarin is in line with the objectives,  
164 criteria, and principles of organic farming and recommended that it should therefore be included in EC  
165 Regulation No. 889/2008, Annex II (EGTOP 2011).

166  
167 **Japan Agricultural Standard (JAS) for Organic Production**

168 [http://www.maff.go.jp/e/jas/specific/pdf/834\\_2012-3.pdf](http://www.maff.go.jp/e/jas/specific/pdf/834_2012-3.pdf)

169 Marine Products appear in the Japanese Agricultural Standard for Organic Processed Foods under Article  
170 4: Items, Criterion 3, with the annotation “(except for ionizing radiated foods and those produced by the  
171 recombinant DNA technology).” Laminarin is not specifically listed.

172  
173 **International Federation of Organic Agriculture Movements (IFOAM)**

174 [http://www.ifoam.bio/sites/default/files/page/files/ifoam\\_norms\\_version\\_august\\_2012\\_with\\_cover.pdf](http://www.ifoam.bio/sites/default/files/page/files/ifoam_norms_version_august_2012_with_cover.pdf)

175 The IFOAM Norms for Organic Production and Processing list Seaweed and seaweed products in  
176 Appendix 2: FERTILIZERS AND SOIL CONDITIONERS, and Algal preparations in Appendix 3: CROP  
177 PROTECTANTS AND GROWTH REGULATORS both with the same annotation: “As far as obtained by: (i)  
178 physical processes including dehydration, freezing and grinding; (ii) extraction with water or potassium  
179 hydroxide solutions, provided that the minimum amount of solvent necessary is used for extraction; (iii)  
180 fermentation.”

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

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

188  
189 Laminarin can be extracted from brown algae by a number of different methods. The processes normally  
190 involve grinding, precipitation in an acid or base medium, ultrafiltration, and dialysis (Yvin, et al. 1998).  
191 While there are documented processes for extracting laminarin in hot water, there are also studies  
192 suggesting that extraction at a lower pH facilitates filtration. A number of methods are discussed below,  
193 and a summary of reported extraction methods for laminarin published by Kadam, Tiwari and O’Donnell  
194 (2015) is shown in Table 2.

195

196 The petition for laminarin to be added to the National List provides a 5-stage manufacturing process  
 197 beginning with the ocean harvest of *Laminaria digitata* seaweed. It is extracted in water that has been pH  
 198 adjusted to 2 using sulfuric acid in order to avoid the simultaneous extraction of other compounds such as  
 199 alginates. The extract is then filtered through a Seitz filter. This type of filter is commonly used to clarify  
 200 solutions and remove bacteria (Cortez Vieira and Ho 2008). The solution undergoes a second physical  
 201 separation by tangential flow filtration (TFF), in which laminarin is separated from most impurities,  
 202 yielding laminarin in water. The pH is then re-adjusted to between 6 and 7 via the addition of sodium  
 203 hydroxide. The petition does not provide specifications for the filtration, nor does it describe how much  
 204 sodium hydroxide is needed to neutralize the solution following TFF. The petition also does not describe  
 205 any further processing steps such as drying. The product is sold in liquid form.

206  
 207 A research study by Klarzynski et al. (2000) utilized laminarin obtained from the petitioning company that  
 208 had been extracted by an alternative process from that described above. It involved the harvest of *L. digitata*  
 209 and extraction with hot water for 2 hours, after which the aqueous extracts were fractionated by  
 210 ultrafiltration followed by additional filtration, and the resulting retentate was desalted and freeze dried  
 211 (Klarzynski, et al. 2000).

212  
 213 Black et al. (1951) described a number of methods that are commonly referenced in the literature for  
 214 obtaining laminarin. They performed extraction from different species of brown algae using different  
 215 extracting agents and conditions. They found two predominant types of laminarin in varying proportions  
 216 depending on species: relatively pure, insoluble laminarin and the impure, soluble form. The insoluble  
 217 form, which has a lower level of molecular branching (Kadam, Tiwari and O'Donnell 2015), was more  
 218 prevalent in *L. cloustoni* than *L. digitata*. To extract it, the seaweed was dried, ground and mixed with  
 219 extracting liquids such as hydrochloric or sulfuric acid, and left to stand for 16 hours. Next, the solution  
 220 was heated to dissolve precipitated laminarin, and the seaweed fronds were centrifuged and then washed  
 221 with the extracting liquid and air-dried. The centrifugate and washings were then stirred and left standing  
 222 after which any precipitated laminarin was centrifuged, washed with alcohol and dried.

223  
 224 The more highly branched, soluble fraction of laminarin was found to be more predominant in *L. digitata*. It  
 225 was precipitated from a dilute acid solution using alcohol. Sulfuric acid was found to be as effective as  
 226 hydrochloric acid in extracting laminarin, as was a calcium chloride solution, at pH 2.4. Their results  
 227 showed that the amount of laminarin extracted was independent of the pH, but filtering difficulties were  
 228 encountered at higher pH. A pH of 2.4 was identified as optimal – the highest pH that resulted in easy  
 229 filtration and also avoided hydrolytic action of dilute acid on laminarin (Black, et al. 1951).

230  
 231 The EGTOP (2011) report on Plant Protection Products describes extraction from the kelp by dipping it in  
 232 an acid, aqueous solution at 60°C, followed by purification by filtration with specific membranes.

233  
 234 Kim et al. (2006) extracted laminarin polysaccharides from fresh *L. japonica* using hot water. The aqueous  
 235 extracts were fractionated by ultrafiltration using 0.1-µm membranes, followed by additional membrane  
 236 filtration after which the filtrate was freeze dried (Kim, et al. 2006). A number of Chinese patents also cite  
 237 the extraction of laminarin in water, with or without ultrasonic action, followed by precipitation using  
 238 ethanol, and drying.

239  
 240 One patent describes a method for obtaining laminarin and another brown algae polysaccharide, fucoidan,  
 241 by collecting exudate from live harvested seaweed (Andersen, Hjelland and Yang 2012). Again, the  
 242 separation may be done either by precipitating the laminarin with alcohol or by ultrafiltration.

243  
 244 Table 2. Summary of reported methods for extraction of laminarin. (Adapted from Kadam, Tiwari and  
 245 O'Donnell 2015)

Seaweed	Method
<i>Laminaria saccharina</i>	Extracted with 0.09 M HCl at 4 °C for 2 h, after four successive extractions supernatant was precipitated by addition of absolute ethanol
<i>L. saccharina</i>	Extracted with 0.09 M HCl at 4 °C for 2 h, after four successive extractions supernatant was neutralized with 1 m NaOH

<i>Sargassum linifolium</i>	Extracted with HCl or oxalic acid solutions, neutralized with saturated sodium carbonate solution
<i>L. saccharina</i> <i>Laminaria digitata</i>	Extracted with 0.3% H <sub>2</sub> SO <sub>4</sub> at 50 °C for 1 h
<i>L. saccharina</i>	Extracted with 0.09 M H <sub>2</sub> SO <sub>4</sub> (1:14 w/v) at 70 °C for 2.5 h and precipitated by absolute ethanol
<i>L. saccharina</i>	Extracted with 0.09 M HCl (1:14 w/v) at 70 °C for 2.5 h and precipitated by absolute ethanol
<i>Saccharina longicuris</i>	Extracted with 1% CaCl <sub>2</sub> 85 °C for 4 h, further filtrate mixed with 2% NaCl and 95% ethanol at 1:2 ratio and dialysis using a 15 kDa cut-off membrane for 48 h
<i>Laminaria sp.</i>	Pretreated with ethanol, extracted with water at 50 °C for 2 h and precipitated using ethanol
<i>Laminaria japonica</i>	Extracted with dried seaweed to water ratio of 1:50 at 60 °C for 1 h
<i>Eisenia bicyclis</i>	Defatted algal fronds extracted twice with 0.1 M HCl at 60 °C for 2 h and precipitated with four volumes of 96% ethanol
<i>Sargassum fusiforme</i>	Extracted twice with 0.1 N HCl at room temperature for 2 h

246  
247 The petition states that the acidic conditions created by the addition of sulfuric acid do not modify the  
248 chemical structure of laminarin, and neither does the addition of dilute sodium hydroxide to neutralize the  
249 final solution. This claim is supported by a study which evaluated the extraction of water-insoluble 1-3-β-  
250 D-glucan from *Saccharomyces cerevisiae* using various different acids including hydrochloric, acetic, formic  
251 and phosphoric acids. Their analysis of the glucan products resulting from the different extractions showed  
252 strong correspondence to the hydrochloric acid-extracted glucan and laminarin, indicating that the primary  
253 structure and side-chain branching are not substantially altered regardless of the acid used for extraction  
254 (Müller, et al. 1997). The same study, along with several other studies, also reported that the type of  
255 extracting acid along with temperature can affect the molecular weight of the laminarin extracted. Deville  
256 et al. (2004) reported that laminarin extracted from *Laminaria saccharina* using hydrochloric acid had a  
257 higher molecular weight than that extracted using sulfuric acid under identical time and temperature  
258 conditions (Deville, et al. 2004). This does not necessarily indicate a change in the chemical structure of the  
259 laminarin with extraction, but rather that different amounts or lengths of the molecular chain are extracted.  
260

261 Black et al. (1951) reported that extraction of laminarin at high pH resulted in high retention of liquid in the  
262 seaweed residue, making centrifugation difficult and filtration almost impossible. Aqueous extraction, they  
263 claimed, removes soluble alginates which contaminate the laminarin precipitate. This supports the  
264 petitioner's claim that the sulfuric acid is used as a processing aid to facilitate filtration.

265  
266 **Evaluation Question #3: Discuss whether the petitioned substance is formulated or manufactured by a**  
267 **chemical process, or created by naturally occurring biological processes (7 U.S.C. § 6502 (21)).**  
268

269 Laminarin occurs naturally in brown algae species. As discussed in Evaluation Question 2, the acid-base  
270 extraction of laminarin does not change its chemical structure. Similarly, the material is not altered into a  
271 form that does not occur in nature. The response to this question will thus focus on the issue of synthetic  
272 residues from the use of sulfuric acid and sodium hydroxide in the petitioned extraction process, and  
273 synthetic residues in general considering the various extraction methods reported in the literature.  
274

275 Evaluation Question 2 covers differing methods for extracting laminarin from brown algae. The petitioned  
276 method uses an acid-base extraction in which sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) is added to tap water to lower the pH to  
277 2. Sulfuric acid is a strong acid which dissociates in water to HSO<sub>4</sub><sup>-</sup> (and possibly, to a lesser extent, SO<sub>4</sub><sup>2-</sup>)  
278 and H<sup>+</sup> (or H<sub>3</sub>O<sup>+</sup>) ions (Silberberg 1996). Sodium hydroxide (NaOH) is added after filtration and dissociates  
279 into Na<sup>+</sup> and OH<sup>-</sup> ions. As an acid-base reaction, the H<sup>+</sup> ions from the sulfuric acid react with the OH<sup>-</sup> ions  
280 from the sodium hydroxide to produce water. The remaining sulfate and sodium ions are what are known  
281 as spectator ions; that is, they remain present in the solution but do not affect the reaction (Silberberg 1996).  
282 Several extraction methods cited in the literature use dilute concentrations of 3% H<sub>2</sub>SO<sub>4</sub> and 0.09 M H<sub>2</sub>SO<sub>4</sub>.  
283 The latter represents 8.82 g of sulfuric acid per 1 liter of water. Assuming an initial pH of 7, approximately

284 960 ppm SO<sub>4</sub> from sulfuric acid may be added in lowering the water's pH to 2, and around 230 ppm NaOH  
285 added to then neutralize the solution. The minority NOSB opinion (2014) calculated that 624 ppm sulfate  
286 (SO<sub>4</sub>) and 299 ppm sodium (Na) are added to the extracting solution. They noted that the actual  
287 concentrations of sulfate and sodium added are difficult to determine exactly due to variable initial pH of  
288 the water and the unknown buffering capacity of the water-algae solution, and expect that their reported  
289 numbers are likely underestimates.

290  
291 All of the calculations and expected concentrations above are based on theoretical chemical reactions and  
292 do not take into account the tangential flow filtration (TFF) described in the petitioned process. TFF is used,  
293 among other applications, to concentrate, desalt and separate molecules, including biomolecules of  
294 different sizes (Desmech.com 2005). Thus, this step could facilitate the removal of molecules that are  
295 smaller than the laminarin polysaccharide, such as sulfate, from the solution. Because of this and the above  
296 mentioned variables, it is not possible to determine without additional information how much sulfate  
297 remains in the solution following filtration, nor how much sodium may remain following neutralization.  
298 According to the manufacturing process described in the petition, it is expected that some synthetic sulfate  
299 (SO<sub>4</sub><sup>2-</sup> as well as some HSO<sub>4</sub><sup>-</sup>) and sodium are present in ionic form in the final solution with the extracted  
300 laminarin. However, because the final product is in aqueous form, the sodium and sulfate ions would not  
301 be expected to react or precipitate as solid sodium sulfate.

302  
303 It should be noted that the active ingredient laminarin, with any residual synthetics, is formulated to make  
304 up 3.51% of the final pesticide product's composition. This substantially lowers the concentration of sulfate  
305 and sodium that would be added to a system through application of this material, as would the product's  
306 recommended application rates of 14 fluid ounces per acre, per application, as stated on the product's EPA  
307 registered label (EPA 2012).

308  
309 Other extraction processes describe the use of hydrochloric acid rather than sulfuric acid, which would  
310 yield synthetic residues of sodium and chloride ions if left in aqueous form, or synthetic table salt if dried.  
311 Again, concentrations would be dependent on the concentration and amount of extractant used, buffering  
312 capacity of the solution, and any filtration or other purification steps employed. The extractions using  
313 solvents or water in which the laminarin is precipitated with ethanol are not expected to have significant  
314 residual impurities, as ethanol is evaporable.

315  
316 In all extraction scenarios, the literature does not suggest that the residual ions resulting from the acid-base  
317 reactions lend any technical or functional effect in the laminarin ingredient once it is completely extracted.

318  
319 **Evaluation Question #6: Describe any environmental contamination that could result from the**  
320 **petitioned substance's manufacture, use, misuse, or disposal (7 U.S.C. § 6518 (m) (3)).**

321  
322 There are two sources of potential environmental contamination from the manufacture and use of  
323 laminarin – the cultivation, harvest and disposal of kelp, and the residual acid/base byproducts from  
324 laminarin extraction. However, in general, the literature suggests that these have minimal impact on the  
325 environment overall.

326  
327 The petition states that *L. digitata* is used as the raw kelp source (Laboratoires Goëmar SA 2013); therefore,  
328 this report focuses on the environmental contamination potential from its harvest. *L. digitata* is harvested  
329 primarily from Brittany (France), and Iceland (Seaweed Industry Association 2014). In France, where the  
330 vast majority of *L. digitata* is produced, seaweed harvesting is an important piece of the economy, so  
331 management schemes have been developed to ensure sustainable harvesting. Harvest is regulated by the  
332 French government and the National Syndicate of Marine Algae, which consists of a group from the kelp  
333 industry, fishermen and scientific advisors. Only a few dozen boats are licensed to harvest *L. digitata*, and  
334 each has a maximum annual quota of 1,000-1,500 tons. There are also regulations for timing of harvest to  
335 allow for growth, reproduction and regeneration of the kelp beds. Harvest takes place from a boat, using a  
336 spiral curved hook that twists the kelp and rips it from the kelp bed. Smaller, younger kelp plants are too  
337 small to be caught by the hook, which allows for subsequent year's growth (Seaweed Industry Association  
338 2014). There have been reports, however, of a decline in harvest tonnage in the last decade, for which

339 various explanations have been explored, including environmental factors, climate change, and the  
340 intensity of harvest. Davoult, et al. (2011) studied the effects of temperature, light intensity, and storm  
341 intensity on standing stock density. They also analyzed harvest efforts, including number of fishing boats  
342 and days at harvest. The authors concluded that environmental parameters did not directly control the  
343 population dynamics of *L. digitata*; rather, the decline in harvest tonnage was attributed to the decline in  
344 the number of kelp fishing boats and the number of days at sea.

345  
346 There are also implications for seaweed biomass disposal after extracting the laminarin and other  
347 substances (e.g., alginates). Rojan and Anisha (2011) note that there is potential for macroalgae biomass to  
348 be used as fermentation feedstocks to make ethanol and methane. However, they note that kelp biomass  
349 byproducts from laminarin and alginate extraction would be of significantly lower value as feedstock,  
350 because up to 50% of the fermentable compounds would be depleted by the extraction process. Other uses  
351 for kelp byproducts include fertilizers, animal feed and fish feed (McHugh 2003). No literature was  
352 available to describe the specific fate of *L. digitata* waste after laminarin extraction in France.

353  
354 When using laminarin as an active pesticide ingredient, it is expected to biodegrade by 65% after two  
355 weeks of application. Since it is applied early in the crop's life cycle, and because it is considered  
356 toxicologically innocuous, residue tolerances are not needed (EPA 2010). According to the EPA  
357 Biopesticide Registration Action Document (2010), laminarin is known to occur naturally in the  
358 environment and is not known to have detrimental effects. Due to its lack of persistence in the  
359 environment, non-toxic mode of action, and lack of adverse effects on non-target organisms, its use is not  
360 considered to contaminate the environment. Disposal consists of sweeping up and containing in a suitable  
361 container. Dust inhalation should be avoided (Sigma-Aldrich 2015).

362  
363 The other potential source of contamination from the manufacture and use of laminarin is sodium and  
364 sulfate ions (by-products from the acid base reaction; see Question 3 above for more information). In the  
365 environment, the sulfate ions are incorporated into living organisms as a source of sulfur, and thus are  
366 included in the sulfur cycle. In ion form, they will spread into the "aquasphere" and be incorporated into  
367 the sulfur cycle or react with other terrestrial elements. While some sulfates may be deposited eventually,  
368 the majority will be incorporated into the sulfur cycle. Of the living organisms exposed to high doses of  
369 sodium sulfate, algae were shown to be the most sensitive ( $EC_{50}^1$  120h = 1,900 mg/l or ppm), while fish  
370 appeared to be the least sensitive ( $LC_{50}^2$  96h = 7,960 mg/l or ppm). Currently, sulfate in drinking water is  
371 unregulated, but as a guideline the EPA has set a secondary maximum contaminant level of 250 mg/L (250  
372 ppm) based on esthetic effects such as odor and taste (EPA 2012).

373  
374 Sodium ions are ubiquitous in the environment, both from human-made sources (road de-icers, water  
375 treatment chemicals, and sewage effluent) and natural sources (minerals, seawater). While very high doses  
376 of sodium chloride (table salt; 1,570 mg sodium/kg body weight) have been found to cause reproductive  
377 effects in pregnant rats, sodium is considered an essential component of the body and adequate levels are  
378 required for good health (EPA 2003). The EPA (2003) advises sodium concentrations in drinking water  
379 between 30 and 60 mg/L for esthetic effects (i.e., taste), while most American adults consume between  
380 4,000-6,000 mg of sodium/day.

381  
382 The EPA typically requires any component of a pesticide formula greater than or equal to 0.1% to be  
383 declared on the Confidential Statement of Formula (CSF), including impurities from acid-base reactions  
384 such as those described in this technical report. There can be no exceptions for listing on the CSF where  
385 'Impurities of Toxicological Significance' are concerned (Pfieifer 2015). Based on theoretical calculations in  
386 Question 3, sulfate ions could conceivably comprise 0.0034% of a final commercial laminarin product, and  
387 sodium consists of .001%. Therefore, these residual by-products from the acid-base reaction would not  
388 likely be declared on the CSF, even as impurities.

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<sup>1</sup>  $EC_{50}$  is the concentration of substance that produces 50% of the maximal effect within a certain time period (Merck Veterinary Manual 2012).

<sup>2</sup>  $LC_{50}$  is the concentration of a substance that is lethal to 50% of the organisms in a toxicity test. The most common exposure period is 96 hours (Boyd 2005).

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