

Lignin Sulfonate

Aquaculture – Aquatic Animals

Identification of Petitioned Substance

4	Chemical Name:	24	Phyto-Plus® Plant Stimulator – Baicor L.C.
5	Lignin sulfonate	25	(OMRI, 2013)
6		26	SHADOW – LignoTech USA, Inc. (OMRI, 2013)
7	Other Names:	27	Orzan – ITT Rayonnier (Sugar and Spotts, 1986)
8	Lignosulfonate		
9	Lignosulfuric acid		
10	Lignosulfonic acid	28	CAS Numbers: 8062-15-5 (lignin sulfonic acid)
11	LST 7	29	Lignosulfonate salts:
12	Ligninsulfonic acid	30	8061-51-6 (lignin sulfonic acid, sodium salt)
13	Poly(lignosulfonic acid)	31	9009-75-0 (sodium lignosulfonate)
14	Protectol W	32	8061-54-9 (magnesium lignosulfonate)
15	Sulfite lignin	33	8061-53-8 (ammonium lignosulfonate)
16	(NLM, 2013a)	34	8061-52-7 (calcium lignosulfonate)
17	There are also various salts of lignin sulfonate	35	(U.S. EPA, 2010a)
18	listed in the CAS Numbers section.		
19			Other Codes:
20	Trade Names:		705707 (USEPA PC Code [U.S. EPA 2010b])
21	Lignosite® – Georgia-Pacific (Georgia-Pacific		160226 (EPA Reference ID)
22	West, Inc., 2000)		705705, 705708–705714 (U.S. EPA PC Code [U.S.
23	BorrePlex – LignoTech USA, Inc. (OMRI, 2013)		EPA, 2010b], various lignosulfonate salts)
			1522 (CODEX Alimentarius Commission INS
			Number, calcium lignosulfonate)

Summary of Petitioned Use

The petitioner is requesting the addition of lignin sulfonate, a synthetic substance, to the National List of Allowed and Prohibited Substances (hereafter referred to as the National List) for use as a synthetic substance allowed for use in organic aquatic animal production. Lignin sulfonate currently is allowed for use as a synthetic substance in organic crop production as a plant or soil amendment (chelating agent) and as a dust suppressant (7 CFR 205.601[j][4]), or as a floating agent in postharvest handling (7 CFR 205.601[i][1]).

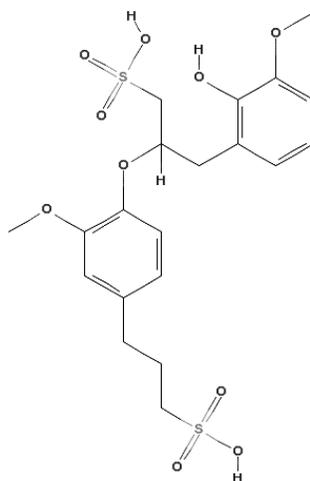
Characterization of Petitioned Substance

Composition of the Substance:

Lignin is a constituent of woody plants that functions as a strengthening element. Lignin sulfonate is a derivative of lignin, where the lignin has been sulfonated in a wood pulping process (Zhor and Bremner, 1999). Lignin sulfonate has the chemical formula $C_{20}H_{26}O_{10}S_2$ (NLM, 2013b) and its structure is presented in Figure 1. However, lignin sulfonate may be composed of a variety of sulfonated aromatic alcohols which make up lignin polymers, including *p*-coumaryl alcohol, sinapyl alcohol, and coniferyl alcohol (FAO, 2008). The lignin molecule is complex and not well understood in spite of its prevalence (Pure Lignin, 2010). The sulfonate complex may be in association with calcium, magnesium, ammonium, or sodium (U.S. EPA, 2010b).

The National List identifies lignin sulfonate as a synthetic substance allowed for use in organic crop production (7 CFR 205.601). Because the lignin sulfonate molecule is negatively charged, it typically complexes with various cations to form lignin sulfonate salts. Four specific lignin sulfonate salts are included in the Generic Materials

60 List published by the Organic Materials Review Institute (OMRI): sodium lignosulfonate, magnesium
61 lignosulfonate, ammonium lignosulfonate, and calcium lignosulfonate (OMRI, 2013). Although these salts are
62 not specifically named on the National List, this Technical Report provides relevant information about them
63 when available.
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66 **Figure 1. Chemical Structure of Lignin Sulfonic Acid, CAS 8062-15-5 (NLM, 2013b)**
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69 **Source or Origin of the Substance:**

70 Lignin exists naturally in all woody plants and is integrated into the plant cell wall, functioning as a
71 structural and strengthening component. Lignin sulfonates are produced from lignin in the process of
72 sulfite chemical pulping. This process involves cooking softwood chips under pressure in sulfur dioxide-
73 containing cooking liquors. Sulfonated lignin is collected as a liquid byproduct in the spent liquor when
74 the pulping process is complete, and the pulp is used for paper production. The lignin sulfonates that
75 result are further purified through fermentation to remove excess sugars, and the resulting liquid is heated
76 to remove alcohol resulting from fermentation (Zhor and Bremner, 1999; Westvaco Corp., 1987; U.S. EPA,
77 1990; Gundersen and Sjoblom, 1999).
78

79 **Properties of the Substance:**

80 A molecular weight of 490.5 g/mol has been reported for lignin sulfonate (NLM, 2013b). Other reports on
81 the lignin sulfonates as a group state that they may have molecular weights ranging from 1,000 to 20,000
82 daltons or higher (up to 100,000 daltons in some cases) depending on their composition (Zhor and
83 Bremner, 1999; Hawley, 1981). Weight-average molecular weights of calcium lignosulfonate are reported
84 to range from 40,000 to 65,000 (unitless). Calcium lignosulfonate is soluble in water and insoluble in
85 organic solvents (FAO, 2008).
86

87 Other physical and chemical properties of lignin sulfonate are presented in Table 1. The properties
88 presented in Table 1 describe lignin sulfonate generally, unless a specific salt is otherwise indicated.
89

90 **Specific Uses of the Substance:**

91 The specific petitioned use of lignin sulfonate is as an animal feed additive, specifically a non-nutritive feed
92 binder for use in aquaculture. In general, feed binders are added to fish feed pellets or particles to keep the
93 feed whole during transport and handling and to maintain stability of the feed in water prior to
94 consumption by the fish (Gatlin, 2010; Lende, 2013). Lignin sulfonate used as a binder for feed pellets
95 allows for additional steam to be used during the manufacture of wet feed pellets because of its binding
96 capacity and reduces the amount of fine particle debris leaving the pellets (Gatlin, 2010; Lende, 2013).
97 Lignin sulfonate is typically added at 1-2% of the total pellet mass and at levels up to 4% of the dry weight
98 of the pellets (Lende, 2013).
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Table 1. Chemical Properties of Lignin Sulfonate

Chemical or Physical Property	Value
Color	Light tan to dark brown*†† (CAMEO, Undated-a; CAMEO, Undated-b)
Physical State	Solid (powder) (Hawley, 1981)
Odor	Odorless (CAMEO, Undated-a)
Melting Point	Decomposes at >200°C (>392°F) (Hawley, 1981)
Boiling Point	Exists in a solid state
Solubility	Soluble in water† (FAO, 2008)
Stability	Soil half-life up to one year depending on chemical species and soil flora*† (Selin et al., 1975)
Reactivity	Lignin sulfonates are corrosive to aluminum and aluminum alloys in the absence of calcium carbonate (CPWA, 2005)
Oxidizing or Reduction Action	React as acids to neutralize bases. Usually do not react as reducing or oxidizing agents (CAMEO, Undated-a)‡
Flammability/Flame Extension	Flash point data not available although probably combustible (CAMEO, Undated-a)‡
Explosibility	Dust explosions may occur if fine particles are mixed with air in the presence of an ignition source. Ignition temperature is approximately 250–300°C (482–572°F)* (Georgia-Pacific West, Inc., 2000)

*Sodium lignosulfonate

‡Ammonium lignosulfonate

†Calcium lignosulfonate

103 Lignin sulfonate is used in organic crop production as a plant or soil amendment (dust suppressant,
104 chelating agent) and as a floating agent for postharvest handling of products (e.g., pears). Lignin sulfonate
105 acts as a dust suppressant due to its large size and affinity for binding with other polar and nonpolar
106 compounds. The smaller dust compounds adsorb to the lignin sulfonate and form a larger, heavier
107 complex that is not as friable, which suppresses the dust (CWPA, 2005).

108
109 Chelating agents are large, organic molecules that are used to envelop highly-reactive trace metal ions.
110 Lignin sulfonates can be used for the complexation of metal ions for the purpose of environmental
111 remediation of heavy metals. Garcia-Valls et al. (2001) cited the biodegradability and relatively
112 inexpensive nature of the lignin sulfonates as factors supporting their use in remediation. Lignin
113 sulfonates have also been used in bioremediation at dairy operation facilities and meat and seafood
114 canneries. Lignin sulfonates were applied to waste streams at the facilities to help remove dairy and
115 seafood waste. The lignin sulfonates formed complexes with the waste materials, which then precipitated
116 out of wash solutions. This process allowed for diversion of some food waste from waterways. The
117 resulting food waste/lignin sulfonate complex was then used in animal feed (USDA, 1969). Lignin
118 sulfonate is also used as a chelating agent to bind soil amendments (e.g., nutrients such as sulfate,
119 phosphorus, calcium, and nitrogen; and biologically relevant metals including copper, iron, manganese,
120 zinc, and others (7 CFR 205.601(j)(6)) for slow release (USDA, 1969).

121
122 When used as a floating agent, lignin sulfonate is added to dump water for fruit processing to increase the
123 density of the water. Dump water is used to fill containers that receive fruit; the fruit is “dumped” into
124 totes containing the water to limit damage during processing. This is necessary for fruits such as pears that
125 may be heavier than water. Increasing the density of the dump water with lignin sulfonate allows the
126 pears to float and, therefore, the pears are less likely to be damaged during processing (Agar and Mitcham,
127 2000).

128
129 Calcium lignosulfonate may be used as an inert ingredient or adjuvant (mixing agent/sticking aid) in
130 pesticide products under 21 CFR §172.715, except for those exempt under Section 25(b) of the Federal

131 Insecticide, Fungicide, and Rodenticide Act (FIFRA)¹ (OMRI, 2013). Calcium lignosulfonate is also used as
132 an encapsulating agent or carrier for fat-soluble carotenoids, vitamins, and other functional ingredients in
133 foods such as fruit-based beverages, hard candies, vitamin drinks, and dairy products (Toledo and
134 Kuznesof, 2008). As discussed in the Composition of the Substance section, other lignin sulfonate salts
135 exist (U.S. EPA, 2010b) and are included on the OMRI Generic Materials List (OMRI, 2013), but little
136 specific information is available about them.

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

139 Lignin sulfonate is currently included on the National List as a synthetic substance allowed for use in
140 organic production (7 CFR 205.601). Lignin sulfonate may be used in organic crop production as a plant or
141 soil amendment (dust suppressant, chelating agent, or floatation agent) or as a floatation agent in
142 postharvest handling (see 7 CFR 205.601[j][4] and 7 CFR 205.601[l][1]). The OMRI generic materials list
143 includes the following lignin sulfonate salts: sodium lignosulfonate, magnesium lignosulfonate,
144 ammonium lignosulfonate, and calcium lignosulfonate (OMRI, 2013). These lignosulfonate salts are not
145 specifically identified on the National List.

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147 Under 21 CFR 573.600(a) and (b), the FDA Food Additives Permitted in Feed and Drinking Water of
148 Animals, lignin sulfonate (including the ammonium, calcium, magnesium, or sodium salts of lignin
149 sulfonate) may be used in a liquid form (moisture not to exceed 50% by weight) or a dry form (moisture
150 not to exceed 6% by weight) in animal feeds. As specified at 21 CFR 573.600(b), lignin sulfonate may be
151 used in an amount calculated on a dry weight basis:

152

- 153 (1) As a pelleting aid in the liquid or dry form in an amount not to exceed 4 percent of the finished
154 pellets.
- 155 (2) As a binding aid in the liquid form in the flaking of feed grains in an amount not to exceed 4
156 percent of the flaked grain.
- 157 (3) As a surfactant in molasses used in feeds, as a liquid lignin sulfonate, in an amount not to
158 exceed 11 percent of the molasses.
- 159 (4) As a source of metabolizable energy, in the liquid or dry form, in an amount not to exceed 4
160 percent of the finished feed.

161

162 Calcium lignosulfonate (CAS No. 8061-52-7) and sodium lignosulfonate (CAS No. 8061-51-6) may be used
163 as inert ingredients in pesticide products. These inert ingredients are on EPA's former inert ingredients list
164 (List 4B) which, in combination with List 4A, is used as a reference for allowed inert ingredients in organic
165 pesticide products. In 2006, EPA changed its classification system for inert ingredients, but lists 4A and 4B
166 are still referenced by the USDA organic regulations. The NOP still allows inert ingredients on these lists
167 to be used as inert ingredients in pesticide products unless the ingredients were removed following EPA
168 reassessment. Calcium lignosulfonate and sodium lignosulfonate have not been removed from the EPA
169 List 4B and are therefore allowed for use as inert ingredients in pesticide products (USDA, 2009; U.S. EPA,
170 2010).

171

172 According to OMRI, ammonium lignosulfonate is an allowed form of lignin sulfonate, with restrictions, for
173 use as a nutrient chelate in organic fertilizer products. According to OMRI, formulated fertilizer products
174 that contain ammonium lignosulfonate may not make nitrogen claims on the label and/or the contribution
175 of ammonium lignosulfonate to the total nitrogen content of the formulated product must be less than 1%
176 based on 7 CFR 205.601(j)(4), 7 CFR 205.601(l)(1), and OMRI, 2013 .

177

178 Lignin sulfonate is exempt from the requirement of a tolerance when used as an inert ingredient in pre-
179 and post-harvest agricultural production and as an inert ingredient applied to animals(40 CFR 180.910, 40
180 CFR 180.930) . Tolerances are acceptable levels of pesticide residues on food products that are set by the
181 U.S. EPA and enforced by the USDA and FDA. According to 40 CFR 180.900, "An exemption from a
182 tolerance shall be granted when it appears that the total quantity of the pesticide chemical in or on all raw

¹ FIFRA is the federal law that regulates pesticide products in the United States and is administered by the U.S. EPA.

183 agricultural commodities for which it is useful under conditions of use currently prevailing or proposed
184 will involve no hazard to the public health.”

185

186 **Action of the Substance:**

187 Lignin sulfonate and other feed binders are added to feed mixtures to improve the durability and water
188 stability of the feed by binding or sticking the composite feed together (New, 1987). Lignin sulfonate is
189 known to have chelating or sticking properties based on its use as a dust control agent, chelating agent, and
190 mixing agent/sticking aid in pesticide products (OMRI, 2013). To make pelleted fish feed, a finely ground
191 feed mixture containing proteins of various types, carbohydrates, fats, vitamins, and trace minerals is
192 prepared (FAO, 1980). Lignin sulfonate is added to the mixture before the mixture is added to the
193 conditioning chamber and steam is applied (Uniscope, 2007). When the pellet mixture is passed through
194 the conditioning chamber, water is added at a rate of 4–6% by weight, usually as steam, which causes the
195 lignin sulfonate to become soluble and form an adhesive coating (FAO, 1980; Gatlin, 2010; Uniscope, 2007).
196 The steam may also gelatinize existing starches in the feed, which contributes to feed stability (Gatlin,
197 2010). The feed is compressed by the pellet mill and forced through holes in a ring-type die, and the
198 pressure forces the lignin sulfonate further into the pellet (FAO, 1980; Uniscope, 2007).

199

200 **Combinations of the Substance:**

201 Lignin sulfonate is formulated into feed pellets where it is combined with proteins of various types,
202 carbohydrates, fats, vitamins, and trace minerals. Two of the synthetic substances allowed for use in
203 organic livestock production as identified in 7 CFR 205.603(d) are typically used in fish feed: trace minerals,
204 used for enrichment or fortification when FDA approved; and vitamins, used for enrichment or
205 fortification when FDA approved. Lignin sulfonate sold as a feed binder is not combined with other
206 materials, but is intended for combination with the pellet mixtures (FAO, 1980; Gatlin, 2010).

207

208 Status

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210 **Historic Use:**

211 Lignin sulfonate has been used in organic agricultural production as a dust suppressant, chelating agent
212 for micro- and macronutrient fertilizer applications, floatation agent in pear production, and as an
213 emulsifier and stabilizer for pesticide applications. A previous technical report for lignin sulfonate was
214 created as part of the 1995 petition process to add lignin sulfonate to the National List (USDA, 1995). A
215 second technical report was prepared in 2011 for the Sunset Review of lignin sulfonate as an allowed
216 synthetic substance used as a floatation agent, chelating agent, or dust suppressant (USDA, 2011).

217

218 Lignin sulfonate has been investigated for its potential as a chelating agent in the environmental
219 remediation of heavy metals and in the remediation of food processing wastes (Garcia-Valls et al., 2001;
220 USDA, 1969). Lignin sulfonate also has been used as an encapsulating agent for vitamins and other
221 ingredients in food products (Toledo and Kuznesof, 2008).

222

223 Lignosulfonates have a variety of industrial applications. One of the main applications of lignosulfonates
224 is in the concrete industry, where they may be used as dispersing agents and to delay the setting of
225 concrete. In other industries, they may be used as additives in oil well drilling, dispersants for dyestuffs,
226 cleaning agents, and as a partial substitute for phenol in the manufacture of adhesives. Sodium
227 lignosulfonate is potentially useful in inhibiting corrosion and scale formation in recirculating cooling
228 water systems (Ouyang et al., 2006).

229

230 **Organic Foods Production Act, USDA Final Rule:**

231 Lignin sulfonate is included within the scope of the Organic Foods Production Act of 1990 by implied
232 inclusion in the group, “...an active synthetic ingredient in the following categories: copper and sulfur
233 compounds...”

234

235 Lignin sulfonate is explicitly identified on the National List in 7 CFR Part 205, as follows:

236

- 237 • 205.601(j)(4) – Lignin sulfonate – chelating agent, dust suppressant.

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- 205.601(l) – As floating agents in postharvest handling; (1) Lignin sulfonate

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International:

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

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

(A). Lignin sulfonate is listed by inference as part of the group, “copper and sulfur compounds” in the OFPA, Section 2118 (c)(1)(B)(i).

(B). Lignin sulfonate is a synthetic inert ingredient that is not classified by EPA as an inert of toxicological concern. Lignin sulfonate (and the lignosulfonate salts) are exempt from the requirement of a tolerance under 40 CFR parts 180.910 and 180.930.

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

Lignin sulfonates are produced from the process of sulfite chemical pulping. Sulfite pulping involves cooking softwood chips under pressure in sulfur dioxide-containing cooking liquors. When the cooking process is complete, sulfonated lignin is collected as a liquid byproduct in the spent liquor, while the pulp is used for paper production. The lignin sulfonates that result from the spent liquor of the sulfite pulping process must be further purified to remove excess sugars. This is done by fermentation of the liquor followed by heating to remove the alcohol generated by fermentation. The resulting lignin sulfonate polymers can have high molecular weights ranging from less than 1,000 to more than 100,000 daltons (Zhor and Bremner, 1999; Westvaco Corp., 1987).

Lignin sulfonates may also be obtained from the Kraft pulping process; these are referred to as Kraft lignins. Kraft pulping is similar to sulfite pulping, but involves treating the wood at high temperature and pressure in a water solution containing sodium sulfide and sodium hydroxide. This process dissolves lignin into a soluble salt which dissolves in the pulping liquor. The lignin is removed by precipitation from the liquor using carbon dioxide (CO₂). The Kraft lignins must then be sulfonated after extraction by a

292 reaction of the material with bisulfate or a sulfite compound (Gundersen and Sjoblom, 1999; U.S. EPA,
293 1990).

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295 A third pulping process, acid sulfite pulping, is similar to Kraft pulping, but different chemicals are used.
296 Sulfurous acid, used in place of sodium hydroxide, is combined with sodium, magnesium, calcium, or
297 ammonium bisulfite. After the cooking is complete, the pulp is separated from the spent liquor, which
298 may then be treated to obtain various chemical materials (U.S. EPA, 1990).

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300 **Evaluation Question #3: Discuss whether the petitioned substance is formulated or manufactured by a**
301 **chemical process, or created by naturally occurring biological processes (7 U.S.C. § 6502 (21)).**
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303 Lignin exists naturally in all woody plants as a structural and strengthening component. Because lignin is
304 integrated into the plant cell wall, there are no natural processes that liberate lignin other than natural
305 decomposition of wood by microorganisms. Lignin sulfonates are produced from the application of
306 pressure and heat to wood in the presence of sulfur dioxide or by the addition of alkali and various acids
307 and sulfates, as described for Evaluation Question #2. This process adds the sulfonate moiety (part of a
308 molecule) to the lignin, thus modifying it chemically, and the sulfonate moiety remains in the finished
309 product. Purification of lignin sulfonate obtained from sulfite pulping is accomplished through
310 fermentation to remove excess sugars. Although fermentation is a natural process, the heating of wood
311 pulp under high pressure with the addition of acids is not a naturally-occurring process (U.S. EPA, 1990;
312 Gundersen and Sjoblom, 1999).

313
314 Lignin produced from the Kraft pulping process, as described in the response to Evaluation Question #2, is
315 removed from the pulping liquor using CO₂ and is then sulfonated after extraction. Sulfonation is carried
316 out by reacting lignin with bisulfate or another sulfite compound, which remains as part of the resulting
317 lignin sulfonate product (Gundersen and Sjoblom, 1999; U.S. EPA, 1990). Acid sulfite pulping is similar to
318 Kraft pulping, but sulfurous acid is used in combination with sodium, magnesium, calcium, or ammonium
319 bisulfite, and the product is further treated to yield the remaining lignin sulfonate (U.S. EPA, 1990). These
320 are not naturally-occurring processes, and the materials used in the processes are not naturally occurring.
321 For all processes, the remaining chemical moiety attached to lignin is sulfonate, which does not naturally
322 occur in the lignin of woody plants and is the only material created in processing that remains in the final
323 product.

324
325 **Evaluation Question #4: Describe the persistence or concentration of the petitioned substance and/or its**
326 **by-products in the environment (7 U.S.C. § 6518 (m) (2)).**
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328 Lignin sulfonates are used to modify feed for aquatic animals that are raised under controlled conditions.
329 These controlled conditions can be generally described as open or closed aquaculture systems. In an open
330 system, fish are raised in netted pens that are in direct hydraulic exchange with surrounding water bodies.
331 Closed aquaculture systems are closed off or away from surface water bodies, at least directly, and utilize
332 water recycling practices (Cottee and Petersan, 2009). Closed systems may be more sensitive to
333 environmental impacts due to their much smaller size, but open systems can have broader to larger scale
334 environmental systems. It is much easier to control the environmental impacts of closed systems compared
335 with open systems, and long-term impacts to open systems may be more difficult to detect.

336 Lignosulfonates discharged into water bodies from open systems may cause foaming and discoloration.
337 Due to their high biological oxygen demand (BOD) during breakdown in water, lignosulfonates will
338 remove dissolved oxygen from waterways in amounts that may be harmful to aquatic organisms in both
339 open and closed systems (CPWA, 2005). This may be of concern if high amounts of lignin sulfonate are
340 used in feed, or if large amounts of feed go to waste in ponds or tanks used to raise fish. However, the
341 maximum amount of lignin sulfonate typically used in feed is 4% by weight, and 10% of the feed is
342 typically not eaten by the fish (Craig and Helfrich, 2002). For larger aquaculture systems, in which
343 considerable amounts of feed must be distributed in tanks or ponds where fish are raised, this could lead to
344 larger deposits of lignin sulfonate from uneaten feed. Fish waste and waste feed, which also has a high
345 BOD upon decomposition, will be present at much higher levels in aquaculture systems than lignin
346 sulfonate, especially in closed systems. Management of nutrient balances would likely be included in the

347 regular maintenance of wastewater systems in aquaculture production as required by EPA (U.S. EPA,
348 2012a; U.S. EPA, 2006). Little additional information is available in the published literature on the
349 degradation pathways for lignosulfonates in water, and no information was found on environmental
350 accumulation or bioaccumulation of lignosulfonates.

351 The half-life of lignosulfonates in soil, as evidenced by the loss of sulfates or sulfonic acid groups, is up to
352 one year. Soil microorganisms, enzymatic reactions, and ultraviolet (UV) radiation contribute to the
353 breakdown of lignosulfonates. The structure of lignosulfonates may be altered by wood-rotting fungi, with
354 polymerization being the main pathway, based on a decrease in phenolic hydroxyl groups (Selin et al.,
355 1975). Laccase, an enzyme produced by fungi, increased degradation of lignosulfonates to low-molecular
356 weight fractions under laboratory conditions (Cho et al., 2004). In addition to microbial decomposition,
357 enzymatic oxidative reactions contribute to the early breakdown process of lignosulfonates. Light (UV
358 radiation) also hastens the degradation of lignosulfonates by creating radicals capable of degrading the
359 lignosulfonate molecular structure. This breakdown process yields lower molecular weight lignosulfonate
360 fragments and CO₂. The CO₂ may account for 15–20% of the original organic carbon. As lignosulfonates
361 degrade, they become more susceptible to further degradation by microbial populations. Due to the
362 complex and heterogeneous structures of lignosulfonates, the breakdown products are complex and
363 variable (Lauten et al., 2010). Lignin sulfonate is not used as a fuel source when it is broken down by
364 microorganisms, but rather incorporated into biomass through biosynthesis which broadens the scope of
365 potential reaction pathways (Lauten et al., 2010). Given that lignin is a naturally-occurring component of
366 wood, once the sulfonic acid groups have been cleaved, the lignin that remains would likely be broken
367 down in a manner very similar to that of rotting wood.

368

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

372

373 Lignin sulfonates are soluble in water, and it is likely that long-term addition of feeds containing lignin
374 sulfonate would increase their concentration in a given body of water, particularly in closed systems that
375 are not regulated properly. Lignin sulfonate and its breakdown products have the potential to adversely
376 affect environmental systems in several ways. Lignin sulfonate and its breakdown products may cause
377 acute or chronic toxicity to fish that ingest the feed although toxicity has only been observed at doses much
378 higher than would exist in feed (Weber and Ramesh, 2005, as cited in Munro and Baines, 2009). Decreased
379 dissolved oxygen (as well as decreased pH) may result from lignin sulfonate decomposition in aquatic
380 systems. Lower pH levels can increase the availability of some metals in aquatic systems, leading to higher
381 exposures in fish.

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383 One study on lignosulfonates has shown toxicity to fish. A 48-hour LC₅₀ of 7,300 parts per million (ppm)
384 was observed by Roald (1977) in rainbow trout (*Oncorhynchus mykiss*). As lignosulfonates break down in
385 water, they consume dissolved oxygen in the water due to their high BOD, which may adversely affect
386 aquatic organisms through a decrease in available oxygen for respiration (CPWA, 2005; Jones and
387 Mitchley, 2001). Lignin sulfonates readily dissolve in water (forming acids) and may decrease the pH of
388 waters they contaminate. The severity of this effect will depend on the amount discharged and the
389 characteristics of the receiving water body (CPWA, 2005).

390

391 Due to the acidifying potential of lignin sulfonate, large spills of lignin sulfonate in water may decrease pH.
392 If those same bodies of water are contaminated with mercury or other metals, the mercury may be more
393 likely to enter the aquatic food chain (Reddy et al., 1995; USGS, 2009). One study in guppies (*Poecilia*
394 *reticulata*) showed that while lignosulfonate in aerobic sediments helped to bind mercury and make it less
395 bioavailable, lignosulfonate in anaerobic sediments stimulated the methylation of mercury, a more toxic
396 metabolite of mercury (Gillespie, 1972). Methylation of mercury is also increased by decreasing pH, and
397 pH is lowered when large amounts of lignin sulfonates are in water bodies (CPWA, 2005; Jones and
398 Mitchley, 2001). For these reasons, mercury in waterways used to raise fish may be more likely to be taken
399 up by fish, potentially leading to adverse health effects and human consumption (USGS, 2009). Findings of
400 methyl mercury concentrations in farmed fish are contradictory; some studies have observed no effects

401 when fish are fed on formulated compound feeds, while others have observed elevated concentrations
402 when fish are fed marine-based feeds, indicating that the feed type may play a role, as well (Schultz et al.,
403 2012).

404
405 Sodium lignosulfonate is relatively low in toxicity based on results of tests in laboratory animals, including
406 rats, rabbits, and guinea pigs (Luscombe and Nicholls, 1973; Marcus and Watts, 1974). However, high
407 doses of sodium lignosulfonate have been found to cause adverse health effects in laboratory animals. Rats
408 that were given drinking water containing purified sodium lignosulfonate at a 10 g/100 ml concentration
409 for 16 weeks had skin lesions at the bases of their tails, decreased weight gain, increased leukocyte counts,
410 and increased kidney, spleen, and liver weights (Luscombe and Nicholls, 1973). In a study with guinea
411 pigs and rabbits administered sodium lignosulfonate at a 1% concentration for a two- to six-week period, a
412 high percentage of the animals developed ulcerative colon disease (Marcus and Watts, 1974). A median
413 lethal oral dose of greater than 40 g/kg has been reported for rats (Luscombe and Nicholls, 1973). This
414 dose corresponds to the U.S. EPA Toxicity Category IV for oral exposure (greater than 5000 mg/kg), which
415 is the lowest toxicity category (U.S. EPA, 2007).

416
417 Results of a 28-day oral toxicity study in which calcium lignosulfonate was incorporated into the diet of
418 rats at target doses of 0, 500, 1500, or 4000 mg/kg body weight per day showed no observed adverse effects
419 other than chronic inflammation of the rectum at the highest dose level (4000 mg/kg-day). The no-
420 observed-adverse-effect level (NOAEL), the concentration below which no adverse effects were observed,
421 from this study was identified as 1300 and 1350 mg/kg-day for males and females, respectively (Weber
422 and Ramesh, 2005, as cited in Munro and Baines, 2009). By comparison, 4000 mg/kg-day is 4 parts per
423 thousand, or roughly ten times higher than the highest concentration of lignin sulfonate used in fish feed,
424 which is 4 parts per 100, or 4%.

425
426 In a 90-day study with Wistar rats dosed with calcium lignosulfonate in the diet at target doses of 0, 500,
427 1000, and 2000 mg/kg-day, the rats showed no adverse clinical signs or organ weight changes following
428 complete pathological evaluations. Test results for primary immune response in the rats were normal. The
429 only observed adverse effect was a dose-related increase in the incidence of histiocytosis (abnormal
430 increase in the number of immune cells) of the mesenteric lymph nodes in male and female rats, with no
431 observed histiocytosis in other lymph tissues. The Joint FAO/WHO Committee reviewed the finding of
432 histiocytosis and concluded that it does not represent an adverse effect, based on findings with other
433 compounds with similar properties (Munro and Baines, 2009).

434
435 No evidence of genotoxicity (the ability of a chemical to damage DNA or other genetic material) was found
436 for calcium lignosulfonate in *Salmonella typhimurium* and *Escherichia coli* assays, and in a test for
437 chromosomal aberration in Chinese hamster cells. No developmental effects were found in a study with
438 calcium lignosulfonate and pregnant female Wistar rats. The no-observed-effect level (NOEL) for
439 reproductive effects was identified as 1000 mg/kg-day, the highest dose tested (Thiel et al., 2006b, as cited
440 in Munro and Baines, 2009).

441
442 As discussed in a previous technical report on lignin sulfonate (USDA, 2011), the petitioner for lignin
443 sulfonate (Western Chemicals) addressed concerns with dioxin as a contaminant from the process of paper
444 pulping. Dioxin is a highly-toxic contaminant that is considered a likely human carcinogen (U.S. EPA,
445 2010c). The petitioner noted that dioxins are generally associated with the Kraft pulping process and that
446 dioxins are produced as part of pulp bleaching. The original petitioner for lignin sulfonate for crop
447 production noted that the Georgia-Pacific Corp. generates its lignosulfonates using the bisulfite pulping
448 process (USDA, 2011). The petitioner also explained that the sulfite liquor, which contains the
449 lignosulfonates, is removed from the pulp before the bleaching process; thus, it is not likely that dioxins
450 would be found in the lignosulfonates generated from this process (USDA, 2011). In addition, the
451 petitioner reported that they had analyzed their lignosulfonate products for dioxins and furans and have
452 not detected the contaminants in their lignosulfonate products above the level of detection (USDA, 2011).

453
454 Georgia-Pacific Lignosite® 100, 260, 431-H, and 458 have been classified as biodegradable, with a hazardous
455 decomposition product of sulfur dioxide (SO₂), which can adversely affect the respiratory system (Ash and

456 Ash, 2004; U.S. EPA, 2011a). Selin et al. (1975) commented on the recalcitrant (resistant to degradation)
457 nature of lignosulfonates, but also showed that many wood-decomposing microbes could break down
458 lignosulfonates. Lignin sulfonates bind strongly to smaller particles in the soil or water. Once adsorbed to
459 the soil, lignosulfonates will likely be degraded by soil microorganisms. Lignosulfonates may persist for
460 six months to one year in the soil before they break down (CPWA, 2005; Selin et al., 1975).

461

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

464

465 Environmental concentrations of lignin sulfonate in aquaculture are a function of multiple factors,
466 including feed decomposition, feeding rates, utilization efficiency of feed, water circulation and exchange
467 in a closed or open system, and the fate of lignin sulfonate given local conditions and the management of
468 the system (Craig and Helfrich, 2002; Gatlin, 2010; New, 1987). Additionally, the potential for
469 environmental contamination varies by life cycle scenarios, including manufacture, use, misuse, and
470 disposal (Gatlin, 2010). The potential for contamination will also vary depending on whether a closed or
471 open aquaculture system is used (Cottee and Petersan, 2009).

472

473 Manufacture: Spent sulfite liquors from the paper pulping process contain lignin sulfonate. Before the
474 widespread use of lignin sulfonates, these liquors were treated as waste from the sulfate pulping process
475 and discarded or burned for energy recovery. Given that lignin sulfonate is recovered from waste
476 products of the paper pulp industry after manufacture, the likelihood of environmental contamination
477 from its manufacture is reduced. However, there is still the possibility that spills of effluent from paper
478 pulping operations could contribute large amounts of lignin sulfonate to soils, nearby waterways, or
479 environmentally-sensitive areas. The same possibility of spills exists in manufacturing facilities that
480 produce feed pellets for aquatic organisms, where lignin sulfonate may be stored in bulk. As discussed in
481 the responses to Evaluation Questions #4 and #5, large spills of lignin sulfonate could acidify soils or
482 bodies of water, decrease the available oxygen in bodies of water, or make mercury contamination of fish
483 more likely in already contaminated waterways.

484

485 Use: Lignin sulfonates used as feed binders will be deposited in water bodies or closed aquaculture
486 systems based on their usage pattern in fish feed. If the lignin sulfonate binder works as petitioned, then
487 the fish feed is unlikely to break down before the fish consume the feed. The maximum concentration of
488 lignin sulfonate in feed is 4% (Lende, 2013) and a maximum of 50% of that amount will likely be available
489 in aquatic systems based on assumptions of the amounts of food eaten and waste produced by fish (Craig
490 and Helfrich, 2002). The primary concerns regarding lignin sulfonates in waterways are their high BOD
491 upon decomposition and potential to acidify the aquatic environment. In closed systems, this change will
492 only have internal effects on the system until the water is disposed. In open systems, lignin sulfonate
493 would be available to circulate through the aquatic ecosystem, broadening the exposure area but
494 decreasing levels through dilution. High BOD leads to removal of dissolved oxygen (DO) from the water
495 (CPWA, 2005). DO is a very important water quality indicator, and low DO levels can trigger stress
496 responses in fish (Francis-Floyd, 2012). In addition a decrease in pH, resulting from lignin sulfonate
497 decomposition, may also cause stress in aquatic organisms and make metals such as mercury more
498 bioavailable (Francis-Floyd, 2012; Gillespie, 1972).

499

500 Misuse: Misuse of lignin sulfonate feeds by overfeeding could result in higher loads of the feed and,
501 therefore, larger amounts of lignin sulfonate deposited to bodies of water or tanks and ponds used to raise
502 fish. Dry decomposition of lignin sulfonate can release SO₂, which is an eye and airway irritant although it
503 is unclear if this occurs in aquatic systems (Sullivan and Krieger, 1992; Georgia-Pacific West, Inc., 2000).
504 Excessive amounts of feed containing lignin sulfonate or spills of feed into bodies of water may have the
505 same effects. Dust explosions may occur if finely-divided lignin sulfonate is mixed with air in the presence
506 of an ignition source. Excessive accumulation of dust should be avoided to help prevent explosions
507 (Georgia-Pacific West, Inc., 2000).

508

509 Disposal: Water from closed aquaculture systems containing lignin sulfonate may need to be processed in
510 a treatment system before its disposal. Any discharge of solids such as uneaten feed, waste, or animal

511 remains must be minimized according to EPA aquaculture regulations (U.S. EPA, 2012b). For open
512 systems, wastewater discharge must be limited (U.S. EPA, 2012b). As described in a fruit processing
513 manual where lignin sulfonate is used in the process water, the goal for treatment of these waters before
514 disposal is a reduction in BOD to a level that does not impact aquatic life (McLellan and Padilla-Zakour,
515 2005). The U.S. EPA has published Aquatic Life Criteria for DO in salt water in coastal areas from Cape
516 Cod, MA to Cape Hatteras, NC (U.S. EPA, 2000). The criteria state that DO cannot be below 5 mg/L for
517 “long periods,” the level at which larval stages of many marine fish and shellfish are harmed (U.S. EPA,
518 2000). Untreated water could adversely affect aquatic organisms by competing for DO or lowering pH.
519

520 Under the Clean Water Act (Section 402), aquaculture projects are required to obtain National Pollutant
521 Discharge Elimination System (NPDES) permits. NPDES permits are used to track and control discharges
522 into navigable waters and are meant to protect the waters’ ability to support aquatic life or permit
523 recreation (U.S. EPA, 2012b). This includes any discharges into a “defined managed water area which uses
524 discharges of pollutants into that designated area for the maintenance or production of harvestable
525 freshwater estuarine or marine plants or animals” (U.S. EPA, 2012a). In addition, any concentrated aquatic
526 animal production facilities, which are considered direct dischargers, require an NPDES permit if they
527 produce more than 9090 kg (about 20,000 lbs.) harvest weight of cold water fish or produce more than
528 100,000 pounds of warm water fish annually (U.S. EPA, 2012a). The permits must be reviewed to
529 determine whether the discharge may impair the waters’ ability to support aquatic life, and would be
530 denied if they are found to impair aquatic life (U.S. EPA, 2012a).
531

532 In the case of spills or accidents involving lignin sulfonate from closed systems, the area should be washed
533 with water to dilute the spill (Georgia-Pacific West, Inc., 2000). To the extent possible, care should be taken
534 to ensure that wastewater with high levels of lignin sulfonate does not flow directly to water bodies from
535 closed systems. For these types of spills, waste disposal methods include customary procedures for
536 industrial waste treatment (Georgia-Pacific West, Inc., 2000). Spills or releases of lignin sulfonate are not
537 subject to the reporting requirements of the Federal Superfund Amendments and Reauthorization Act
538 (SARA) of 1986 (Georgia-Pacific West, Inc., 2000).
539

540 Other: Lignin sulfonates have been investigated for their potential role as chelating agents for the
541 complexation of metal ions, including copper, magnesium, and mercury, for the purpose of environmental
542 remediation. The biodegradability and relatively inexpensive nature of the lignin sulfonates were cited as
543 factors that support their use in remediation (Garcia-Valls et al., 2001).
544

545 **Evaluation Question #7: Describe any known chemical interactions between the petitioned substance**
546 **and other substances used in organic crop or livestock production or handling. Describe any**
547 **environmental or human health effects from these chemical interactions (7 U.S.C. § 6518 (m) (1)).**
548

549 By virtue of their intended use, chemical interactions of lignin sulfonates with other substances such as
550 components of fish feed (proteins, fats, carbohydrates, ash, phosphorous, water, and trace amounts of
551 vitamins and minerals) would be expected (Craig and Helfrich, 2002). Lignin sulfonates are generally
552 nonreactive by nature, as evidenced by their uses in dust suppressants and emulsifiers. When used as
553 emulsifiers, lignin sulfonates keep chemical mixtures dispersed in solution by limiting interaction between
554 the two constituents of the mixture (Gundersen and Sjoblom, 1999). When lignin sulfonates come into
555 contact with small soil particles through their use as dust suppressants or chelating agents, the soil
556 particles are adsorbed to the lignin sulfonate due to the presence of polar and non-polar areas on the
557 surface of the lignin molecule. These interactions do not generally result in chemical change but are mostly
558 limited to a physical binding and adsorption. The exception to this is the observed corrosion of aluminum
559 and its alloys by lignin sulfonates when the material is used as a dust suppressant (CPWA, 2005). Alum
560 (aluminum sulfate) is used in aquaculture applications to clear muddy ponds (Williams, 2000). As shown
561 in road dust applications, mixing the lignin sulfonate with calcium carbonate in slurry neutralizes the
562 acidic lignin sulfonate and decreases the reaction with aluminum (CPWA, 2005). It may be possible to
563 prevent corrosion and acidification of the water with a similar treatment in aquaculture applications
564 although no specific methods were found. Appropriate water testing is urged when using alum in
565 aquaculture to prevent acidification of water (Williams, 2000).

566
567 Information on human health effects from chemical interactions with lignin sulfonate was not available.
568 As a chelating agent, lignin sulfonates could serve to reduce environmental health effects and potential
569 toxicity to humans from exposure to the free metal ions (Garcia-Valls et al., 2001). The biodegradability
570 and relatively inexpensive nature of the lignin sulfonates were cited as factors supporting their use in
571 remediation (see Specific Uses and Historic Use sections and the response to Evaluation Question #6).

572
573 **Evaluation Question #8: Describe any effects of the petitioned substance on biological or chemical**
574 **interactions in the agro-ecosystem, including physiological effects on soil organisms (including the salt**
575 **index and solubility of the soil), crops, and livestock (7 U.S.C. § 6518 (m) (5)).**
576

577 Aquaculture is performed in either open or closed systems. In open systems, finfish are raised in netted
578 pens that are in direct hydraulic exchange with surrounding water bodies. Open aquaculture systems
579 could also be used for shellfish such as clams, oysters, or shrimp, which may use other methods for
580 exclusion of the farmed fish from the environment. Closed systems are closed off or away from surface
581 water bodies, at least directly, and utilize water recycling practices (Cottee and Petersan, 2009). In both
582 systems, wasted feed and animal wastes are two outputs that may adversely affect the agro-ecosystem
583 although the adverse effects of waste are not all attributable to lignin sulfonate. The majority of waste
584 material from aquaculture waste is not lignin sulfonate based on the fact that the highest concentration in
585 fish feed is 4% by weight. These wastes are rich in nutrients that may affect BOD much more than the
586 lignin sulfate additive.

587
588 Open aquaculture systems are more likely than closed systems to affect the environment at large because
589 the water used in those systems is in continuous direct exchange with the surrounding aquatic ecosystem
590 without waste management. However, the impact on farmed fish in open systems may be lessened by
591 dilution with the surrounding water body. There are restrictions in place for open systems, such as the
592 requirement of an NPDES permit for certain operations such as aquaculture projects or concentrated
593 aquatic animal production facilities (U.S. EPA, 2012a). Under the NPDES system, aquaculture projects are
594 defined as open systems that use “discharges of pollutants into that designated area for the maintenance or
595 production of harvestable freshwater estuarine or marine plants or animals” (U.S. EPA, 2012b).
596 Concentrated aquatic animal production facilities meet the following criteria: They produce more than
597 9,090 kg (about 20,000 lbs.) harvest weight of cold water fish or produce more than 100,000 pounds of
598 warm water fish annually (U.S. EPA, 2012b).

599
600 Regardless of the restrictions, impacts from open systems, including discharges of fish waste and feed
601 waste, reduced DO, and decreased pH, may be more difficult to mitigate (Cottee and Petersan, 2009).
602 Sediments high in nutrients resulting from deposition of open system waste may also decrease DO as they
603 decompose and affect aquatic life in areas outside of the farming operation (Kutti et al., 2007). One report
604 found that oxygen consumption in the sediment below a fish farm could be as much as 15 times higher
605 than in natural areas (Iwama, 1991, as cited in Cottee and Petersan, 2009). The DO and pH impacts may be
606 exacerbated by lignin sulfonate breakdown. The impacts from open systems may adversely affect wild fish
607 and the surrounding ecosystems due to nutrient imbalances and the production of large amounts of waste
608 (Cottee and Petersan, 2009). Due to the likely effects on sediments, bottom-feeding fish and benthic (living
609 at the bottom of a water body) organisms in open systems may be most intensely affected, while effects on
610 pelagic (open water) fish and other organisms that live in the water column may be less severe. It is
611 important to note that while adverse effects on aquatic systems may result from aquaculture practices, the
612 total impact on those systems from lignin sulfonate in pelleted fish feed would be relatively small
613 compared with overall waste and feed waste. In addition, the use of lignin sulfonate as a feed pellet binder
614 slows the decomposition of feed and might prevent some waste from entering aquatic ecosystems.

615
616 In closed aquaculture systems, animal waste may have an increased impact on the quality of water in the
617 system due to reductions in DO levels, increases in carbon dioxide, and increases in levels of ammonia,
618 nitrate, nitrite, and suspended solids (Cottee and Petersan, 2009). The use of lignin sulfonate in feed in
619 those systems may lead to additional reductions in DO and decreases in pH. Fish respond to low levels of
620 DO and decreased pH with a stress response, which may lead to infections of the blood and other tissues

621 (Cottee and Petersan, 2009). Disposal of wastewater from closed systems, if performed according to U.S.
622 EPA guidelines for aquaculture and NPDES permit requirements, would not be expected to adversely
623 impact the environment (U.S. EPA, 2012a; U.S. EPA, 2012b). U.S. EPA aquaculture guidelines encourage
624 individuals to recycle aquaculture waste and to avoid the discharge of waste into U.S. waters generated by
625 rinsing or washing of nets (U.S. EPA, 2006).

626
627 According to U.S. EPA guidelines for aquaculture NPDES permits, solid wastes from aquaculture
628 production, including waste feed and fecal matter, are required to be captured from liquid wastewater and
629 disposed (U.S. EPA, 2006). These solids might be disposed on land (e.g., as fertilizer, compost) or in
630 evaporation ponds or reed drying beds (U.S. EPA, 2006). All of these methods aim to limit waste discharge
631 and encourage recycling of nutrients in a manner that limits environmental impacts (U.S. EPA, 2006).

632
633 Although it is not likely, if wastewater is not disposed of properly and discharged to soils, it could have
634 adverse effects. When lignin sulfonate decomposes in soil, sulfates, sulfonic acid groups, and CO₂ are
635 liberated (Selin et al., 1975). The breakdown of a large amount of lignin sulfonate in the soil (resulting in
636 the release of additional CO₂) could acidify the soil because CO₂ forms carbonic acid in the presence of
637 water. These changes due to the addition of large amounts lignin sulfonate (which would be unlikely
638 based on the low percentage of lignin sulfonate in fish feed) could adversely affect plants and soil
639 organisms. These effects have not been quantified in the published literature. These impacts would not be
640 of issue if the wastes are disposed of according to U.S. EPA regulations.

641
642 **Evaluation Question #9: Discuss and summarize findings on whether the use of the petitioned**
643 **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)**
644 **(i).**

645
646 As discussed in the responses to Evaluation Questions #4 through #8, lignin sulfonates may contaminate
647 waterways through buildup of waste feed over time in open systems or disposal of large amounts of water
648 from closed aquaculture systems. Lignin sulfonates readily dissolve in water (forming acids) and decrease
649 the pH of waters they contaminate. Contamination from improper disposal of aquaculture waste
650 containing lignin sulfonate or accidental spills would lead to decreased DO in the waterways due to the
651 high BOD of aquaculture wastes and lignin sulfonate. As discussed in the response to Evaluation Question
652 #7, lignin sulfonate is a minor component of aquaculture waste but could still lead to decreased DO in
653 aquaculture systems. Decreased DO and lower pH can adversely impact all aquatic life, as evidenced by
654 increased stress responses in fish (CPWA, 2005; Francis-Floyd, 2012). Increased stress responses can lead to
655 higher prevalence of diseases in both the farmed fish and any aquatic organisms in open systems.

656
657 The severity of effects on the aquatic environment due to lignin sulfonate in feed will vary depending on
658 the amount discharged and the characteristics of the receiving water body. Lignin sulfonate used in open
659 systems is more likely to adversely impact the environment, while closed systems may be managed in a
660 way that limits environmental impacts. Certainly, the adverse impacts of aquaculture will exist regardless
661 of whether lignin sulfonate is used as a pellet binder in aquatic feeds. The low concentration (4%) of lignin
662 sulfonate in feed indicates that it will play a minor role in the overall environmental impact of aquaculture
663 waste. Although it is likely low, the precise environmental impact of lignin sulfonate used in aquaculture
664 has not been studied and is as yet unknown for both open and closed systems.

665
666 **Evaluation Question #10: Describe and summarize any reported effects upon human health from use of**
667 **the petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (i), 7 U.S.C. § 6517 (c) (2) (A) (ii) and 7 U.S.C. § 6518**
668 **(m) (4).**

669
670 No data were found that indicate there would be direct adverse effects on human health from use of lignin
671 sulfonate in the formulation of fish feed. The U.S. EPA issued an exemption from the requirement of a
672 tolerance for lignin sulfonates when they are used as inert ingredients pre- and post-harvest in agricultural
673 production under 40 CFR 180.910. This exemption is based on the conclusion that there is a "...reasonable
674 certainty that no harm will result..." to the general public, infants, and children from aggregate exposures
675 (includes all dietary, drinking water, and nonoccupational exposures) to lignosulfonates, as defined by the

676 Federal Food, Drug, and Cosmetic Act (FFDCA) and the Food Quality Protection Act of 1996 (FQPA) (U.S.
 677 EPA, 2011b). Although this is not a guideline based on aquaculture use, the “incorporation” of lignin
 678 sulfonate into fish that will become food likely retains a low level of concern. There is no published
 679 evidence of lignin sulfonate accumulating in fish that are fed pellets containing lignin sulfonate.
 680

681 **Evaluation Question #11: Describe all natural (non-synthetic) substances or products which may be**
 682 **used in place of a petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (ii)). Provide a list of allowed**
 683 **substances that may be used in place of the petitioned substance (7 U.S.C. § 6518 (m) (6)).**
 684

685 Many substances are used as feed binders for increasing water stability of aquaculture feeds (New, 1987).
 686 Substances used to make feed more stable include: casein, gelatin, collagen, chitosan, guar gum, locust
 687 bean gum, agar, carrageenan, corn starch, tapioca starch, potato starch, wheat gluten, sodium alginate,
 688 hemicellulose, bentonite, banana waste, and (New, 1987). Chitosan, carageenan, and collagen have been
 689 evaluated as feed binders but are not commonly used (Lende, 2013). Some of the substances listed above
 690 may be available in organic forms, such as organic corn, tapioca, or potato starch; and some of the
 691 compounds listed may be used in organic handling (7 CFR 205.605 and 7 CFR 205.606) , such as bentonite,
 692 guar and locust bean gum.

693 The most widely used of these substances as feed binders are starches (corn, tapioca, or potato), bentonite,
 694 lignin sulfonate, and hemicellulose (Lende, 2013). Table 2 below shows the water stability of test pellets
 695 made with many different types of binders including rice dust, soybean flour, and corn solids in a standard
 696 feed formula. In this series of experiments in which a number of binders were tested (FAO, 1980), rice mill
 697 dusts (a starch from the milling of rice) had the longest solids retention time (i.e., the duration that feed
 698 pellets remain solid before breaking down) of all the binders tested.
 699

700 **Table 2. Water Stability of Test Pellets in a Standard Formula***
 701

Test Pellet	Percentage of Solids Retained
5% gelatinized corn solids (dry)	85.0
5% bentonite	88.6
Control formula - unground	90.0
5% guar meal	90.2
5% gelatinized corn solids - wet	92.0
Control formula ground through 2 mm screen	93.0
5% rice mill dust, 75 microns diameter	93.0
5% soybean flour	94.0
5% lignin sulfonate	94.0
5% rice mill dust sifted through 180 microns	96.2
10% rice mill dust sifted through 180 microns	98.2
20% rice mill dust sifted through 180 microns	98.5

*Measured as percentage of solids retained on a screen after 10 minutes in quiet water (FAO, 1980).

702 Starches (from wheat, rice, or corn) are used as binders in aquatic feeds that are steam pelleted or extruded,
 703 where gelatinization of the starches by water and heat dissolves the starch granules and distributes the
 704 gelatinized starch to bind the pellet (Tucker and Robinson, 1990). For pellet feeds, depending on the
 705 temperature and pressure under which the pellets are produced, the starch may cause the pellets to float or
 706 sink, a characteristic that is set depending on the variety of aquatic livestock being fed (e.g., floating pellets
 707 are preferred for salmonids, sinking pellets are better for catfish) (Stickney, 2009). Due to the heat and
 708 pressure of the extrusion process, it is usually not necessary to add binders like lignin sulfonate to floating
 709 feeds that contain starch because the starch binds the feed sufficiently (Tucker and Robinson, 1990).
 710

711 Pelletized feeds may require additional non-nutritive binders such as lignin sulfonate, bentonite, or
 712 cellulose to provide additional stability and decrease the amount of feed dust (Tucker and Robinson, 1990).
 713 Pregelatinized starches have been characterized as relatively expensive (Smiley, 2012), but starches and
 714 sugars are noted as some of the most economical sources of energy for fish diets (Craig and Helfrich, 2002).
 715

716 Bentonite is a type of clay that is added to fish feed and consists mainly of colloidal aluminum silicate with
 717 varying amounts of iron, alkalies, and alkaline earths (21 CFR 184.1155[a]; FDA, 1977). Bentonite is added
 718 to compressed, dry fish feeds at no more than 2% as a binding agent and lubricant for feed pellet mills
 719 (Lende, 2013). Bentonite is naturally occurring and is used in the food industry to clarify liquids and as an
 720 ingredient in coatings and adhesives for food packaging (FDA, 1977). Bentonite is considered generally
 721 recognized as safe (GRAS) by the U.S. FDA (21 CFR 184.1155[c]). As shown in Table 2, bentonite had the
 722 shortest solids retention time with the exception of corn solids (FAO, 1980).
 723

724 Another alternative to the use of lignin sulfonate in pelleted feeds is hemicellulose. Hemicellulose is a
 725 nondigestible starch that is used as a non-nutritive binder in steam pelleted feeds. Hemicellulose extract is
 726 allowed for use in animal feed if it meets the following conditions as specified in 21 CFR 573.520:

- 727 a) The additive is produced from the aqueous extract obtained by the treatment of wood with water
 728 at elevated temperatures (325 degrees-535 degrees F) and pressure (80 to 900 pounds per square
 729 inch) and contains primarily pentose and hexose sugars.
- 730 b) The additive may be used in a liquid or dry state with the liquid product containing not less than
 731 55 percent carbohydrate and the dry product containing not less than 84 percent carbohydrate.
- 732 c) The additive is used as a source of metabolizable energy in animal feed in accordance with good
 733 manufacturing and feeding practices.
 734

735 Table 3 provides a list of selected companies that manufacture feed binders or nonorganic fish feed using
 736 alternate binding materials.
 737

738 **Table 3. Selected Manufacturers of Feed Binders or**
 739 **Nonorganic Fish Feed with Alternate Binding Materials**
 740

Ingredient	Company	Address
Rice starch (premanufactured feed)	Wet Thumb Aquatics	52700 Base St., New Baltimore, MI 48047 www.wetthumbaquatics.com
Bentonite (for adding to feed)	Bentonite Performance Minerals, LLC	3000 N. Sam Houston Pkwy. East Houston, TX 77032 www.bentonite.com
Hemicellulose (premanufactured feed)	M-G Feed Inc.	P.O. Box 697 Weimar, TX 78962; m-ginc.com

741 **Evaluation Question #12: Describe any alternative practices that would make the use of the petitioned**
 742 **substance unnecessary (7 U.S.C. § 6518 (m) (6)).**
 743
 744

745 Types of aquatic feed formulations include pellets, crumbles, and finely ground meals, and most contain
 746 no more than 10% moisture (Gatlin, 2010). Most diets are sold as dry products although some semi-moist
 747 diets are made for early life stages or carnivorous fish (Gatlin, 2010). Moist diets with moisture contents of
 748 35% to 70% will always require a binder, whereas semi-moist feed with 25% to 35% moisture can be made
 749 into pellets by selecting feed ingredients that also act as binders, such as certain starches (Halver and
 750 Hardy, 2002). The use of starches to bind the pellets is an alternative to non-nutritive binders such as lignin
 751 sulfonate, and the types of starches may vary, but include rice, corn, wheat, tapioca, and potato starch
 752 (New, 1987). However, if nutritive binders are not included in the feed recipe, then non-nutritive binders
 753 must be used (Halver and Hardy, 2002).
 754

755 Compression pelleting of feeds is the most common type of feed manufacture for sinking pellets, while
 756 cooking extrusion is the most common form for floating pellets, and is also very costly (Gatlin, 2010). In

757 compression pelleting, starch gelatinizes during the preconditioning and pelletizing processes, which
758 increases cohesion and durability of the pellets, which may preclude the need for additional binders
759 (Gatlin, 2010). However, sometimes a pellet binder is still included to increase the durability of feed pellets
760 (Gatlin, 2010). With the extrusion process, the feed is still heated during preconditioning, but it is then
761 heated to a higher temperature at higher moisture content than with compression pelleting, causing the
762 pellets to expand when they exit the extrusion barrel, reducing their density (Gatlin, 2010).

763
764 Water stability of feeds can be improved by using finely ground raw materials as well as die plates that
765 produce smaller pellets (New, 1987). However, these techniques and others aimed at increasing feed water
766 stability may considerably increase the cost of processing the feed (New, 1987). One report suggests that
767 the need for extremely well-bound feed is up to the farmer. If the diet is consumed within a few minutes of
768 feeding or if more frequent feeding can be accommodated, then the need for extremely well-bound feed
769 may be decreased (New, 1987). New techniques are available for formulating feeds – including
770 microbinding, microencapsulation, or microcoating – that may eliminate the need for some types of
771 binders (Lovell, 1998).

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