Squid and Squid Byproducts

Crops

Identification of Petitioned Substance

Chemical Names:

Squid hydrolysate, Squid- processing byproduct hydrolysate, Enzymatically hydrolyzed animal protein, Hydrolyzed protein

Other Name:

Squid based fertilizer, Squid silage, Squid protein hydrolysate

Trade Names:

Shoreside Organic Fertilizer Liquid Squid 2-2-2, Biomarine Cold Processed Squid Fertilizer 2-3-1, Hydrosquid Liquid Organic Fertilizer & Micronutrient Solution

CAS Number:

9015-54-7 (protein hydrolysate)

Other Codes:

SID 135309854; EINECS 309-203-1

Summary of Petitioned Use

A petition received by the National Organic Program (NOP) requested the addition of "squid and squid byproducts" to the National List as soil amendments with listing similar to §205.601(j)(7) Liquid fish products. This section reads:

> § 205.601 Synthetic substances allowed for use in organic crop production. … the following synthetic substances may be used in organic crop production: Provided, that, use of such substances do not contribute to contamination of crops, soil, or water. (j) As plant or soil amendments. (7) Liquid fish products—can be pH adjusted with sulfuric, citric or phosphoric acid. The amount of acid used shall not exceed the minimum needed to lower the pH to 3.5.

The manufacturing processes are similar for both liquid fish products and squid and squid byproducts; however, the petition indicates a distinction between liquid fish products and squid and squid by products.

Squid differ anatomically and phylogenically from fish. Squid belong to the phylum *mollusca*, class *cephalopoda* which are bilaterally symmetric, tentacled and exclusively marine. Fish are gill bearing craniate animals lacking limbs and digits. They are endemic to both fresh and marine environments. Squid and squid byproducts in the form of a proteolytically rendered hydrolysate consisting mainly of the squid parts (byproducts) not normally used for human consumption are used as a soil amendment in crop production (Igelsias, 2014).

Characterization of Petitioned Substance

Composition of the Substance:

Squid is one of the world's most important commercial seafood products (Choi et al., 2015). Products such as sliced raw squid (sashimi), dried, seasoned and smoked products, and fermented products are popular in many food markets worldwide. Squid depending on the particular distribution market can be dried, packed whole and either chilled or frozen prior to use or further processing. Like many other seafood products, squid can be processed manually or mechanically as a value added food. Processing of this raw material consists minimally of evisceration and washing (Kim, 2013). The squid body, arm and tail (tentacles) comprise the primary raw material for food leaving squid byproducts (Fig. 1). Byproducts including squid ink, pen, skin, milt, liver and viscera makeup about 52% of the total body

weight (Joseph et al., 1987). These can be further processed into a number of useful products (Singh and Brown, 1980; Booman and Singh, 1986). Squid ink contains the pigment melanin, proteins, lipids, glycosaminoglycans, and muco-polysaccharides. It is commonly used in bread, confectionary, tofu, pasta, curry, potato chips, candies, snacks, kimchi, sauces and noodles. The squid pen has been identified as a source of chitin, the precursor of chitosan which has uses in medicine and food preparation as a thickener. Skin from the squid has been identified as a source of collagen. Squid liver is a good source of polyunsaturated edible oils. Viscera, including the head and the eye are not edible and may be processed further as food additives and fertilizers (Kim, 2013). Squid milt is a traditional food in Asian culture and may have a role in preventing cardiovascular disease. Female squid are usually sorted from males and marketed as whole squid since milt is difficult to process (Wang et al., 2008; Booman and Singh, 1986).

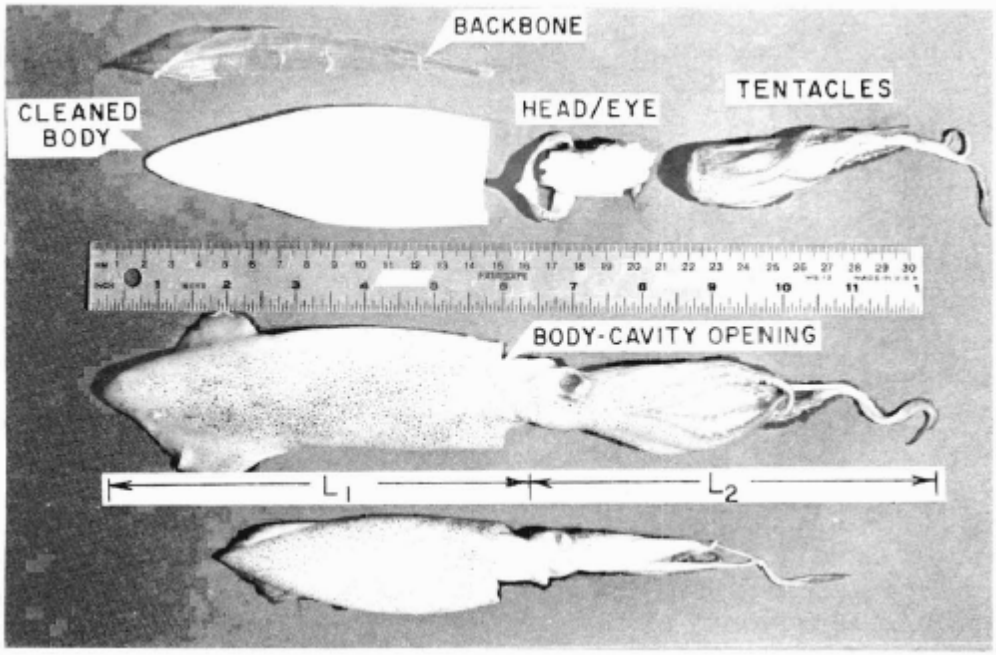

Fig. 1. Whole squid (bottom) and cleaned parts (above) from Singh and Brown, 1980.

Because of their high protein content, squid and squid byproducts are candidates for hydrolyzed soil amendments (Iglesias, 2014). Particularly since the harvest and processing of squid for use as food most often results in the production of large unrecoverable amounts of this material that otherwise would be considered a waste product. In the US, when market squid fisheries in California were first developing, ca. 1863, Sino-American fisherman already had begun drying their catch which was later sold for export to Asian countries as both a food staple and fertilizer (California Department of Fish and Game, 2006).

Source or Origin of the Substance:

Squid are littoral invertebrates first described by Lamarck in 1801. He classified them into the phylum *Mollusca*, class *Cephalopoda* and order *Loligo* (Lamarck, 1801). *Loligo* was later renamed *Doryteuthis*. The longfin squid, species *Doryteuthis* (*Loligo) pealli*, common to the northeastern Atlantic coast was described by Lesueur (Lesueur, 1821). Market squid (*Doryteuthis (Loligo) opalescens*) common to the American west coast were reclassified into the genus *Doryteuthis* in 2005 (Vecchione et al., 2005). About 300 species of squid are known and range throughout the world.

Prized as a human food, e.g. calamari, squid are commercially harvested using nets during seasonal "mating runs" (Williams, 1905). Most of the time squid are scattered along the ocean bottom in moderately deep water. Spawning squid assemble into large schools that come into shallow water. These schools break-up into smaller schools to mate and lay eggs. The eggs hatch within two or three

weeks. Adults die a few months after reproduction, while emerging larvae mature in 6-9 months. Fishermen target spawning squid because they die shortly after reproduction. Even without fishing, the entire population replaces itself annually (California Department of Fish and Game, 2006). Therefore, the stock is entirely dependent on successful spawning from each generation coupled with good survival of recruits to adulthood. Because the market squid fishery takes place above the spawning grounds, it is critical that fisheries management allows for an adequate number of eggs to be spawned prior to harvest. Allowing enough market squid to spawn before capture helps to ensure production for the next generation (California Department of Fish and Game, 2006).

A number of economically productive commercial squid fisheries have developed throughout the world. There are two main squid fisheries in the United States, one for market squid along the Pacific coast and one for long finned squid along the Atlantic coast. U.S. fisheries for squid on both coasts are managed to keep harvests at a level that ensures future abundance and sustainable operation of the fishery.

Established in 1863, and supplying dried product to Asia for use as a staple food and for fertilizer, California's market squid fishery expanded rapidly during the 1980s. Today, the fishery supplies the majority of market squid on the global market (Vojkovich, 1998; California Department of Fish and Game, 2006). Fishermen usually fish for market squid at night directly above the spawning grounds where females lay their eggs. Squid seiners typically work with light boats—smaller vessels with several high-powered lights pointed from various angles. The lights attract groups of spawning squid to surface waters. Once a group of squid comes to the surface, the light boat signals the seiner to deploy its net, encircling the light boat, in order to catch the squid located under the lights. The fishery takes place in northern California and southern California at different times of the year. The northern fishery season (mainly in Monterey Bay) traditionally occurs from April through November, and the southern fishery (mostly in the Channel Islands vicinity) begins in October and generally lasts through March. The U.S. Pacific squid fishery is managed by the California Department of Fish and Game, the National Oceanographic and Atmospheric Administration (NOAA) Fisheries, and the Pacific Fishery Management Council. Management includes seasonal catch limits, timed fishery closures and limitations on using lights to attract squid to ensure uninterrupted spawning. The California Department of Fish and Game and NOAA Fisheries also administer permit systems and cooperatively monitor the fishery to evaluate its impact on the resource. All U.S. harvest of market squid comes from California. The 2010 landings totaled close to 288.4 million pounds. The 2011 landings totaled more than 267.9 million pounds, and in 2012 landings totaled more than 214.8 million pounds. About one half of this was exported to Asian countries (NOAA, 2014).

The Atlantic long finned squid is part of a geographically defined and jointly managed fishery that includes Atlantic mackerel, squid and butterfish. The Atlantic mackerel, squid, and butterfish fishery operates primarily in the Mid-Atlantic region of the eastern coast of the U.S, from Massachusetts to North Carolina. The fishery uses predominantly single and paired mid-water trawl, bottom trawl, purse seine, and to a lesser extent, gillnet gear throughout the entire range. Atlantic mackerel, squid, and butterfish are managed in federal waters by NOAA Fisheries in conjunction with the Mid-Atlantic Fishery Management Council (NOAA, 2015). Commercial harvests of long finned squid totaled more than 28.1 million pounds in 2012, with the majority landed in Rhode Island, New York, New Jersey, and Massachusetts. Although landed less often than the long finned squid and incidentally caught, the ilex squid, *Illex ilecebrosus*, is also a part of the Atlantic fishery comprising less than half of the total harvest.

Properties of the Substance:

Squid processing byproducts constituting about 52% of the total animal weight are frequently discarded as waste (Table 1). The main edible portion is the cone shaped trunk of the body

(mantle) which forms about 48% of the total body weight. The fins, head, tentacles, skin and viscera are considered byproducts.

Table 1 Composition of various squid* body parts (%)		
	Mantle	48
BYPRODUCTS	Head and Tentacles**	25
	Fin	15
	Viscera	8
	Skin	3
	Pen	1

*average weight of whole squid—225 grams (~8 ounces)

**average combined composition of byproducts (head and tentacles, fin, viscera, skin and pen) is moisture—80%, protein—18 %, fat (ether soluble)—~1% and Ash—~1%

Adapted from Joseph et al., 1987

Squid has been dried for centuries for food and fertilizer. Squid contains natural proteases, amylases and lipases capable of respectively digesting proteins, polysaccharides and fats (Kim, 2013). Pulverized squid byproducts can self-digest (autolyze) over a wide pH range to form a natural hydrolysate. The storability of this material can be improved with the addition of acid processing (Lian et al., 2005).

Squid and squid byproducts hydrolysates contain crude protein, amino acids, crude fat, phospholipids and carbohydrates. Amino acids found in protein and fatty acids from crude fat are respectively good sources of bound nitrogen and phosphorus, desirable for crop production. Squid and squid byproducts hydrolysates contain approximately 55.2 % crude protein, 0.7 % fat, 23 % moisture, 16.6 % carbohydrate and 4.4% ash (Choi et al., 2014).

Evidence of the potential of squid byproduct hydrolysate as a sustainable resource in crop production was provided by significantly improved leaf growth in *Arabidopsis thaliana* after application of enzymatically produced squid byproduct hydrolysate as a model soil amendment. This hydrolysate was produced using the commercially produced proteolytic enzyme, Alcalase (0.82 grams/liter) at 55 degrees Celsius with a pH of 7.5 (Pina-Cortes et al., 2010).

Specific Uses of the Substance:

Squid are wild and carnivorous, eating small fish, crabs and shrimp. Their reproduction and growth cannot be monitored as are farmed and cultured products; however, fisheries and landings are carefully monitored to ensure that catches include only those post-reproductive squid. Wild aquatic animals are not eligible for organic certification. Thus, squid and squid byproducts although natural are not organic.

Squid skin gelatin hydrolysate produced with the proteolytic enzyme Alcalase 2.4L (Novozymes, Bagsvaerd, Denmark) can be used in food systems as a natural additive with antioxidant, foaming and emulsifying functional properties. This squid skin gelatin hydrolysate chelates

metal ions such as iron, and prevents lipid oxidation via free radical scavenging. These activities are comparable to the synthetic antioxidant butylated hydroxytoluene (BHT) which has fallen out of favor in the food production industry (Gimenez et al., 2009; Cassiday, 2015). Squid skin gelatin hydrolysate produced with the proteolytic enzyme Alcalase 2.4L also have medically significant antihypertensive, cytotoxic and anti-proliferative functional properties (Aleman et al., 2011).

Squid and squid byproducts can be chopped into a slurry. The beak can be removed using a commercial food finisher. The slurry can be diluted with water, spray dried and extracted with a solvent such as ethanol or isopropanol to remove odors and flavors. This dried product has excellent emulsifying and antioxidant properties and finds use in a number of food processing applications (Lee et al., 1973).

The nutritional profile of squid and squid byproduct hydrolysate makes it a good starter food for larval fish and shrimp (Lian et al., 2005).

Fish sauce was known in ancient Greece and Rome and more recently exported to Asia where annual production has surpassed 250,000 tons. Fish sauce is produced with endogenous digestive enzymes. Squid and squid byproducts are chopped and mixed with 20-40% sea salt and stored at ambient tropical temperatures. The resulting protein hydrolysate liquid is filtered, bottled and sold (Gildberg, 1993).

Squid and squid byproduct protein hydrolysates contain relatively high levels of the amino acids, proline and hydroxyproline. Both proline and hydroxyproline are necessary for germination of melon seeds. Proline and hydroxyproline are required for the production of glutamate and donor protons required in the pentose phosphate metabolic pathway. The pentose phosphate metabolic pathway in turn drives the production of the auxins and cytokinins that stimulate shoot outgrowth (Milazzo et al., 1999). Experimentally, addition of the amino acid analog thioproline, to melon growth medium does not stimulate shoot outgrowth, but either a combination of hydroxyproline and proline or squid protein hydrolysate does (Milazzo et al., 1999; Pena-Cortes et al., 2010).

Although slower in action compared to synthetic fertilizers, squid and squid byproduct hydrolysates are still effective, since nitrogen rich hydrolysate peptides and amino acids are readily digested by exoenzymes produced by soil bacteria and released to crops at a steady rate. Squid and squid byproduct hydrolysate produce a beneficial effect on turf grass increasing both growth and soil microbiological activity. Phosphate and nitrate leaching is slower and more readily controlled, since these nutrients are bound to biomolecules requiring bacterial action for release (Fetter et al., 2012; 2013). Seafood derived hydrolysates including squid and squid byproduct hydrolysates have been used both as foliar sprays and soil amendments for propagating cranberries, cherries and apples (Pigott, 1997).

Squid and squid byproducts can be composted but require a carbon source, such as autumn leaves, shredded paper, sawdust, grain hulls, peat, etc. Vermicomposting is also possible with squid and squid byproducts (Goldhor and Regenstein, 2007).

Approved Legal Uses of the Substance:

The disposal of squid and squid byproducts is regulated as solid waste by the US Environmental Protection Agency (EPA) (40 CFR parts 239, 256, and 257). Federal regulations describe requirements for establishing adequate state biosolids waste disposal programs that include controlling odors and potential runoff into groundwater. In this context, squid and squid byproducts as solid waste may be stored, processed or incorporated into compost for redistribution onto crop lands (EPA, 1999a). The EPA defines a soil amendment as:

> Any substance that is added to soil (other than the substances used primarily as fertilizer) that is thought to improve the physical characteristics of the soil, such as

> porosity to water and air. Soil amendments do not include commercial fertilizers, agricultural liming materials, unmanipulated animal manures, unmanipulated vegetable manures, pesticides and other materials exempted by regulations but can contain important fertilizer elements (EPA, 1999b).

Fertilizer is defined as:

> A substance that contains one or more plant nutrients specially designed to be used for its plant nutrient content and is claimed to promote plant growth. A fertilizer material is a fertilizer that
>
> 1) Contains important quantities of no more than one of the primary plant nutrients: nitrogen (N), phosphorus (P), and potassium (K), or,
> 2) Has 85% or more of its plant-nutrient content present in the form of a single chemical compound, or
> 3) Is derived from a plant or animal residue, byproducts or natural material deposits that have been processed in such a way that their content of plant nutrients has not been materially changed except by purification or concentration (EPA, 1999b)

The EPA regulates the extent to which nitrates from N-P-K[1] fertilizers may leach into and contaminate groundwater with the Safe Drinking Water Act of 1974. The maximum contamination level for nitrate is ten parts per million (EPA, 2007). Phosphates contribute to nutrient pollution. Many states have control criteria regarding nutrient pollution from phosphates as a result of fertilizer use. Fertilizers made from squid and squid byproducts, if over applied can potentially leach nitrogen and phosphate into the soil (Fetter et al., 2012).

The US Food and Drug Administration (FDA) defines "fish" to mean fresh or saltwater finfish, crustaceans, other forms of aquatic animal life (including, but not limited to, alligator, frog, aquatic turtle, jellyfish, sea cucumber, and sea urchin and the roe of such animals) other than birds or mammals, and all mollusks, where such animal life is intended for human consumption. The term "fishery products" means any human food product in which fish is a characterizing ingredient. However, molluscan shellfish means any edible species of fresh or frozen oysters, clams, mussels, or scallops, or edible portions of such species, except when the product consists entirely of the shucked adductor muscle (21 CFR 123.3(d)). This definition is not inclusive of the squid, in spite of its classification as a mollusk. Squid and squid byproduct hydrolysate have potential uses as food products. FDA is authorized by the Food Allergen Labeling and Consumer Protection Act of 2004 to identify allergens in food and establish thresholds for them (FDA, 2006). Squid and byproducts or derived products contain at least one allergen and may be subject to regulation by FDA under this authority (Miyazawa et al., 1996).

Squid and squid byproducts and their derivative products are considered "biological soil amendments of animal origin" and regulated by the US Food and Drug Administration (21CFR112.3). Biological soil amendment of animal origin means a biological soil amendment that consists, in whole or in part, of materials of animal origin, such as animal byproducts. FDA considers a biological soil amendment of animal origin treated if it has been processed to completion to adequately reduce microorganisms of public health significance including *Listeria monocytogenes* (*L. monocytogenes*), *Salmonella* species and *E. coli* O157:H7 to levels at which they cannot be detected using standard microbial laboratory detection methods (7CFR112.51-59). Depending upon farm size and income, documentation is required for use of any biological soil amendment of animal origin such as a certificate of conformance that 1) the process used to treat

[1] N-P-K = % nitrogen-% phosphate-% potassium, often used to describe the contents of fertilizer.

the biological soil amendment of animal origin is a scientifically valid process that has been carried out with appropriate process monitoring; and 2) the biological soil amendment of animal origin has been handled, conveyed and stored in a manner and location to minimize the risk of contamination by an untreated or in process biological soil amendment of animal origin (7CFR112.60).

Under the Saltonstall-Kennedy Grant program in fisheries research and development, the Department of Commerce, National Oceanic and Atmospheric Administration (NOAA) granted two-hundred thousand dollars in 2009 to expand the squid processing byproduct hydrolysate (SPBH) utilization potential to applications in aquaculture larval feed and organic fertilizer (Bryson et al., 2010).

Action of the Substance:

Squid and squid byproducts as petitioned are the starting ingredients in the production of enzymatically produced hydrolysates. The hydrolysates are proposed as soil amendments for organic crop production (NOP, 2015a). Squid and squid byproducts contain approximately 2.2% lipid, 86% moisture, 1.5% ash and 10.3% protein. Nitrogen, phosphorus and potassium are essential for plant growth. With soil bacterial action, protein becomes a good source of nitrogen for plants and lipids become a good source of phosphate. Ash itself is a good source of potassium for plants. Additional potash may be added to the hydrolysate to ensure a sufficient supply of soluble potassium (Lian et al., 2005). Enzymatically hydrolyzed pH balanced (pH 3.5-4.6) squid and squid byproducts hydrolysate base fertilizer are produced with N-P-K values ranging from 2-2-2 to 3.3-7.3-2 or more. However, most of the nitrogen in these products requires bacterial action and conversion to organic matter for availability to plants (Fetter et al., 2013).

Combinations of the Substance:

Squid and squid byproduct hydrolysate is produced by chopping the byproduct and heating to 55°C at a pH of 5.5 (Lian, 2005). Native proteases digest the mixture producing peptides and free amino acids. Bacterial proteases such as Alcalase can be added during processing to increase the rate and specificity of peptide cleavage and digestion of the hydrolysate (Pena-Cortes et al., 2010). After digestion, the hydrolysate is stabilized with an acid such as phosphoric, hydrochloric, sulfuric or citric acid to prevent microbial growth. Potassium salts (potash) may be added to provide an additional source of soluble potassium as a plant nutrient. Squid and squid byproduct hydrolysate can be 1) added to compost as part of a soil amendment, 2) combined with clay and applied to soil in granulated form or 3) combined with other fertilizers (Fetter et al., 2013; Lian, 2005; Pena-Cortes et al., 2010).

Status

Historic Use:

In the mid-1800s, Chinese fishermen living in the Monterey Bay area of California known as cannery row and Point Alones began to fish for squid using small boats, lanterns and purse seines. Their catch, although not popular in the US, had a vast market in China. Squid was fished at night and dried in the sunlight. Most of the dried squid was shipped overseas. The end use for the dried squid varied, but a major application was as fertilizer. At one point, the barrels of squid were heavily salted before they left California. Salt was extracted from the squid once at its receiving port, to avoid the high import taxes in China on salt (Dillian and White, 2009; Palumbi and Sotka, 2011).

In the absence of the Organic Foods Production Act of 1990 (OFPA) which was adopted as part of the 1990 Farm Bill to define organic food and assure consumers that foods marketed as organic

meet prescribed standards, states such as Oregon, California and Texas had established organic standards. The scheme from California included fish emulsion acidified with phosphoric acid for use as a naturally occurring fertilizer. Fish emulsions fell into a grey zone of synthetic natural materials when used in organic farming, since they were produced with phosphoric acid which was considered synthetic (Bones, 1992). The addition of fish emulsion as a historically used material in the OFPA was one of several inconsistencies that confronted the first crops standards committee and the National Organic Standards Board (USDA, 1993).

Organic Foods Production Act, USDA Final Rule:

The Organic Foods Production Act of 1990 (OFPA) does not specifically list squid and squid byproducts (7 U.S.C. § 6517(c)(1)(B)(i)). It does provide an exemption for the use of fish emulsions in organic production.

In the US law the term "fish" is often taken to mean finfish, mollusks, crustaceans, and all other marine animal and plant life with the exclusion of marine mammals and birds, e.g. Magnuson-Stevens Fisheries Conservation and Management Act (16 U.S.C. § 1801, § 1802, § 1853, § 4102, § 5002), US Coast Guard (46 U.S.C. § 2101) and others. Squid are considered mollusks (Williams, 1905).

The National Organic Program Final Rule, 7 CFR part 205, provides a definition of fertilizer which is:

> A single or blended substance containing one or more recognized plant nutrient(s) which is used primarily for its plant nutrient content and which is designed for use or claimed to have value in promoting plant growth.

The OFPA includes fertilizer as a soil amendment and prohibits (7 U.S.C. § 6508) the use of any fertilizer containing synthetic ingredients or any commercially blended fertilizers containing materials prohibited under an applicable State organic certification program.

The National Organic Program Final Rule, 7 CFR 205.203(e)(1) prohibits the use of any fertilizer or composted animal material containing a synthetic substance not included on the National List. It does permit a producer to manage crop nutrients and soil fertility by applying animal material included on the National List that is chemically altered by a manufacturing process provided that the material doesn't contribute to contaminating crops, soil or water by plant nutrients, pathogenic organisms, heavy metals, or residues of prohibited substances (7 CFR Part 205.203(d)(5)). Thus the rule permits the use of liquid fish products as plant or soil amendments that are pH adjusted with sulfuric, citric or phosphoric acid not exceeding the minimum needed to lower the pH to 3.5 (7 CFR 205.601(j)(7)).

International

Canada - Canadian General Standards Board Permitted Substances Lists

The Canadian Organic Production Systems General Principles and Management Standards definition of livestock prohibits fertilizers not on the permitted substance lists to be used in organic production. However, the Canadian organic production systems permitted substances lists includes animal and animal by products including fishery as agricultural inputs: soil amendments and crop nutrition for the production of composting feedstocks and also allows the use of fish products:

> Fish Products—Natural substances or those derived from natural substances without the addition of ethoxyquin (food preservative, antioxidant-prevents rancidity) or other chemically synthesized substances or chemical treatment except that liquid fish products as soil and plant amendments may be pH adjusted with (in preferential order) organic vinegar, organic citric acid, phosphoric acid or sulphuric acid. The amount of acid used

shall not exceed the minimum needed to reach pH 3.5. Shall not contain synthetic preservatives or fertilizing substances not listed in this standard.

The opal squid (*Loligo opalescens*) is resident to and commercially fished in Canada. It is one of seventeen species of Canadian squid. In Canadian fisheries, the definition of fish includes marine invertebrates such as the squid (Government of Canada, 1985).

CODEX Alimentarius Commission, Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (GL 32-1999) -
ftp://ftp.fao.org/docrep/fao/005/Y2772e/Y2772e.pdf

Fertilizers and soil conditioners may be used provided they are essential for obtaining or maintaining the fertility of the soil or fulfill specific nutrition requirements of crops, or specific soil conditioning and rotation purposes which cannot be satisfied by the Codex Principles of Organic Production. Codex provides guidance concerning substances for use in soil fertilizing and conditioning. The compositional requirements of processed animal products from fish industries need to be reviewed and recognized by the certification body or authority.

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

http://www.organic-world.net/news-eu-regulation.html

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/l_189/l_18920070720en00010023.pdf

In the European Union, fertilizers and soil conditioners and plant protection products are only permitted if they are compatible with the objectives and principles of organic production and have been authorized. Molluscan (squid) products and byproduct autolysates and hydrolysates from sustainable fisheries are authorized and may be used in organic production of feeds for non-herbivores. These products are not explicitly authorized for use in organic crop production, although fish meal is authorized.

Japan Agricultural Standard (JAS) for Organic Production –
http://www.ams.usda.gov/nop/NOP/TradeIssues/JAS.html

The Japanese Agricultural Standard for Organic Plants (Notification No. 1605 of the Ministry of Agriculture, Forestry and Fisheries of October 27, 2005) permits the use of food industry byproducts of fish origin if they are derived from natural sources without the use of chemical treatment. These can be applied to soil for providing plants with nutrition or improving soil properties, applied to plants for providing nutrition, derived from natural sources. Chemical treatment includes burning, calcining, melting, dry distilling, and saponification. Mollusks (squid) are included in the Japanese fisheries (Japan External Trade Organization, 2010).

International Federation of Organic Agriculture Movements (IFOAM) –
http://www.ifoam.org/standard/norms/cover.html

IFOAM permits the use of fish and shell products and food processing byproducts of animal origin that are free from significant contaminants or composted and confirmed free of significant contaminants for use as fertilizers and soil conditioners.

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

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?

Squid and squid byproduct hydrolysate is similar to a fish emulsion. The California Health and Safety Code § 26569.21(p)(3) describes fish emulsion as a natural nitrogen source which may contain phosphoric acid as a stabilizer; however, fortification with urea is prohibited. Fish emulsions were allowed synthetic substances in organic production because they were mostly used in organic farming before state and federal laws were passed and have minimal impact on human health and the environment (Bones, 1992). According to the US Food and Drug Administration, fish means fresh or saltwater finfish, crustaceans, other forms of aquatic animal life (including, but not limited to, alligator, frog, aquatic turtle, jellyfish, sea cucumber, and sea urchin and the roe of such animals) other than birds or mammals, and all mollusks, where such animal life is intended for human consumption. (FDA, 2014). This definition appears to include squid but 40 CFR 123.3 also contains a definition narrowing mollusks strictly to the bivalve order—molluscan shellfish means any edible species of fresh or frozen oysters, clams, mussels, or scallops, or edible portions of such species, except when the product consists entirely of the shucked adductor muscle. Scientifically, squids are cephalopod mollusks; however, for legal purposes and because they are part of a defined fishery, squid can be included "as other forms of aquatic life" in the US Food and Drug Administration fish definition (FDA, 2014).

<u>Evaluation Question #2:</u> 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)).

Squid byproducts consist of the heads, viscera, fins, and small tubes. The cleaned body and tentacles are usually reserved for food products. For many years, Sino-American fishermen in California simply dried their catch of squid in open fields. The material that was not used for food was incorporated into the soil or composted. The need for drying and the relatively large amounts of squid that became fertilizer at that time were likely due to high levels of both acid and alkaline proteolytic activity in post mortem squid. Even on ice, eviscerated squid becomes inedible within 2-3 days without freezing (Leblanc and Gill, 1982; Rodger et al., 1984; Sugiyama et al., 1989; Stanley and Hultin, 1984).

Modern squid and squid byproducts meal production consists of coarse grinding, cooking, pressing, drying, antioxidant stabilization and fine milling, dehydrating, partially de-oiling (Goldhor and Regenstein, 2007; Joseph et al., 1987)

Squid and squid byproducts can be ensiled. Ensiling takes advantage of the autolytic protease present in fish tissue, preserving the product until a hydrolysate can be produced or allow the digestion process to continue until the product can be used. Another name for fish silage is liquid fish protein. Fish sauce, a popular condiment takes advantage of this same process only salt is added as a preservative. Often spoilage and over digestion of silage occurs as a result of bacterial infection. This can be prevented with acidification or the addition of antioxidants (Jangaard, 1987; Goldhor and Regenstein, 2007).

Byproducts are the primary starting ingredients for production of squid and squid byproduct hydrolysate, although whole squid can be used if it is available. Studies have shown that squid byproduct hydrolysate produced auto-proteolytically by chopping byproducts in a Hobart meat chopper at a high speed for two minutes and incubating the macerated squid at 55^{o}C with stirring for 1-6 hours provides a good yield of nutrients (Lian et al., 2005). A pasteurization step at 85^{o}C may be included prior to acidification to reduce spoilage bacteria, yeast and mold (Goldhor et al., 1990). The international food and animal feed industries also provide market demand for squid byproduct hydrolysate, because it has high protein content, e.g. milk protein

replacement, fish feed, etc. (Merritt, 1982; Lian et al., 2008; Lee et al., 2008). Several studies have shown that added bacterially derived proteases can reduce production time, and improve the quality to satisfy this market demand (Kristinsson and Rasco, 2000; Kim, 2013). Several proteases have been evaluated for squid byproduct hydrolysates, but the *Bacillus* proteases called subtilisins, known for their application as additives in household detergents were found to be most effective (Zuidweg et al., 1972; Choi et al., 2015; D'Avila dos Santos, 2011; Chalamaiah et al., 2012; Alvarez, 2006; Kristinsson and Rasco, 2015; Choi et al., 2014; Nilsang et al., 2005; Bhaskar et al., 2008; Yuan et al., 2008; Ritchie and Mackie, 1982).

Alcalase® is an alkaline serine protease or subtilisin Carlsberg (CAS # 9014-01-1; EC 3.4.21.62) produced from fermentation of the bacterium *Bacillus licheniformis*. It is marketed by the Danish company Novozymes. Alcalase® is a non-glycosylated single polypeptide chain without disulfide bonds. Its molecular weight is 27 kilodaltons. Alcalase® is a member of the Serine S8 endoproteinase family and has broad specificity with a preference for a large uncharged residue in the carboxy terminus of the cleavage site (P1 site). It hydrolyzes native and denatured proteins, and is active under alkaline conditions with an isoelectric point: pI = 9.4, an extinction coefficient of 8.6 at 280 nm, a sedimentation coefficient (S20w) of 2.77[3] and a liquid density of 1.25 g/ml. The Dutch company Gist-Brocades has trademarked a similar enzyme under the name Maxatase® (Ee and Misset, 1997). The entire nucleotide sequence for subtilisin Carlsberg from *Bacillus licheniformis* has been determined and expressed in *Bacillus subtilis* (Jacobs et al., 1985). However, Alcalase as marketed is a native enzyme, commercially batch produced from *Bacillus licheniformis* (van Putten et al., 1996).

Alcalase is widely used for production of enzymatic squid byproduct hydrolysate. The stability of Alcalase is higher at high temperature and alkaline pH. Its optimized activity is also outside the temperature and pH range which is optimal for bacterial growth. (Mackie, I.M., 1982; El-Saied, 1979; Polgar, 1968). An enzymatic digest of squid byproducts at 55˚C, pH 7.5 with 0.82 grams/liter added Alcalase 2.4L produced a hydrolysate that as a soil amendment increased foliar diameter up to 90% in an Arabadopsis model system when compared to non-treated control plants (Pena-Cortes et al., 2010). Unless inactivated both the endogenous squid proteases and Alcalase continue to digest the hydrolysate. Eventually digestion would remove potentially nutritious components and make the hydrolysate susceptible to bacterial contamination. The addition of acid (phosphoric, citric, or sulfuric) at low concentration (≤ 3%) after hydrolysis has progressed to the appropriate stage prevents over-digestion and stabilizes the hydrolysate.

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

Squid and squid byproduct hydrolysates are similar in composition to fish emulsions (FDA, 2015). In an early California organic statute for organic production (California Health & Safety Code § 26569.21(pX3) West Supp. 1992), naturally occurring fertilizers were permitted, but some materials applied to crops prior to harvest were not easily classified under this law. Fish emulsions fell into this category. The description and purpose of fish emulsions was listed in code §26569.21(pX3) as a natural nitrogen source which may contain phosphoric acid as a stabilizer; however, fortification with urea is prohibited (Bones, 1992). This regulation was subsequently carried over and updated in the USDA National Organic Program to include phosphoric, sulfuric and citric acid not to exceed the amount necessary to reduce the pH to 3.5 (7 CFR 205.601).

Squid and squid byproduct hydrolysate need only contain squid byproducts and water. This is a natural process. The addition of a non-agricultural non-synthetic allowed substance such as a proteolytic enzyme derived from non-pathogenic fermented bacteria, e.g. Alcalase (subtilisin

Carlsberg) from *Bacillus megaterium* may still be considered a natural process. Of the acids permitted for acidification, citric acid sourced from an agricultural product is considered a nonagricultural product and its addition to the hydrolysate would still be natural. Both sulfuric and phosphoric acid are considered synthetic and not allowed for organic crop production, except for acidification of fish emulsion. Their addition to squid and squid byproduct hydrolysate for acidification is not a natural process.

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

Organic matter is usually lost when soils are placed under cultivation; with a new equilibrium being reached that is characteristic of the cultural practices and soil type. For most soils, organic matter can only be maintained at high levels by inclusion of a sod crop in the rotation, by no till or minimal tillage practices or by frequent addition of large quantities of organic matter (Stevenson, 1994). Squid and squid byproduct hydrolysate is used as fertilizer in crop production providing organic matter. Compounds such as proteins, peptides, amino acids, nucleic acids, lipid and polysaccharides are all constituents of squid and squid byproduct hydrolysate (Lian et al., 2005). Decomposition of these constituents constitutes a basic biological process in soil, where carbon is recirculated to the atmosphere as carbon dioxide and associated elements such as nitrogen, phosphorus, sulfur and micronutrients appear in forms that are required by higher plants. In the process, some of the carbon is redistributed into microbial tissues and some into stable humus (Stevenson, 1994).

Contamination by persistent organic pollutants (POP), e.g., PCBs (polychlorinated biphenyls), DDTs (DDT and its metabolites), CHLs (chlordane compounds), HCHs (hexachlorocyclohexane isomers) and HCB (hexachlorobenzene) has spread all over the world and the oceans may play a role as the final sink for them. Although concentrations of POPs are low in the ocean, POP can be detected in livers of squid from various coastal locations indicating pollution levels in the seawater from which they were collected. POP concentrations are higher in squid from coastal waters than those from open waters (Ueno et al., 2003). Thus, squid hydrolysate or any form of liquid fish product produced from squid or finfish containing high levels of POPs will also contain high levels of POPs that would be transferred to farms soils upon application (Witczak and Abdel-Gawad, 2012; Stancheva et al, 2011). These environmental toxins although found at detectable levels in squid are often converted to harmless derivatives and detoxified by the squid's own cytochrome P-450 hemoproteide or glutathione S-transferase systems or during subsequent prokaryote or eukaryote metabolism in the soil (Korte, 2001; Komives et al., 2009). Once in the soil POPs may be removed by phytoremediating crops such as pumpkins or zucchini which concentrate toxins such as DDT in their fruits and flowers (Pascal-Lorber and Laurent, 2011).

When squid caught off the Portuguese coast were analyzed for methyl mercury only low levels were found. Squid do not live very long and cannot concentrate mercury like higher level predators. Squid are a good source of phosphorus, magnesium, zinc and copper. They do not concentrate methyl mercury or lead to any great extent. Fertilizer produced from squid and squid byproducts is not expected to lead to methyl mercury contamination in soil (Cardoso et al., 2011; Lourenco et al., 2009).

Squid and squid byproduct fertilizer contains relatively high levels of nitrogen and phosphorus. Leaching and losses from lawns fertilized with squid and squid byproduct hydrolysate at high levels of application is similar to leaching and losses observed during the application of synthetic fertilizers (Fetter et al., 2012). Environmentally, squid and squid byproduct hydrolysate and synthetic N-P-K fertilizers are similar with respect to leaching. Leaching depends on application

rate, thus at the high levels of application there is greater potential for leaching of nitrogen and phosphorus.

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

Squid are not long-lived animals; therefore they do not concentrate marine pollutants such as methyl mercury or polychlorinated biphenyls (PCB). However, they have a potential to accumulate high concentrations of copper, zinc and cadmium in their digestive glands. Levels for copper, zinc, cadmium respectively have been reported respectively at 264, 140 and 32 parts per million (Tavakoli and Yoshida, 2005). Studies with other non-synthetic fertilizers containing elevated copper, zinc and cadmium levels provide evidence of increased availability of soil zinc, copper and cadmium to plants, when used at appropriate agronomic rates. Long term use of these soil amendments did not result in unsafe soil levels (Lipoth and Schoneau, 2007). Humus is also responsible for retaining and stabilizing metallic salts in the soil, since its colloidal nature is in part due to the structure of multi-metallic complexes, making it difficult to accurately determine relative toxicity or environmental impact of metals stabilized in soil (Evans, 1989; OECD, 2012a).

Squid have a naturally high protein content consisting mostly of tegument and contractile proteins such as myosin and connectin. Squid is very difficult to keep fresh because of the rapid post-mortem auto-proteolytic degradation of connectin (Kasamatsu et al., 2004). Some molluscan species produce powerful peptide toxins resulting from the degradation of proteins, e.g. conotoxin from the cone snail (Gilly et al., 2011). Hydrolysis of squid proteins does not lead to the formation of known toxic peptides. Anti-toxic activities such as antioxidation, antihypertension, tyrosinase and anti-whitening comparable to oyster sauce, fish sauce and other foods have been associated with various peptide cleavage products from squid hydrolysate (Choi et al., 2015, Lian et al., 2005).

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

Freshly caught squid and squid byproducts do not have an offensive odor. However, decaying fish products are recognized as one of the most common contributors to malodor formation among various sources in a living environment. Some squid and squid byproducts have the potential to emit strong offensive odors when decaying (Kim et al., 2009). Because the odor is so bad, it may not be possible to use some squid and squid byproducts hydrolysate fertilizers in a greenhouse (Hempe, 2008). Food products from squid and squid byproducts not including processed products (e.g. fish sauce, protein replacers) represent about forty eight percent of the squid. Thus, fifty two percent of squid and squid byproducts are discarded as waste. As more uses for this material are found, less of it is dumped back into the ocean or into landfills. Discharges of dead squid and wastewater boost ammonia concentrations and reduce oxygen content in the water posing a threat to marine life (Polakovic, 1999). A developed balanced economic approach to handling squid waste from a burgeoning calamari industry includes both federal and state grant funding for independent academic researchers to develop systems for securing waste streams preventing environmental damage. Organic fertilizer from squid and squid byproducts is one such outcome (Rhode Island Sea Grant News, 2010; Hempe, 2008).

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

572 Squid and squid byproduct hydrolysates left as liquids are acidified to prevent over-digestion,
573 spoilage and eutrophication. Acidification to pH 4.6 or less has been shown to significantly
574 reduce or stop the growth of many spoilage bacteria including *Clostridium botulinum*, a toxin
575 forming bacterium (FDA, 2015b). Further acidification to pH 3.85 effectively prevents the growth
576 of most proteolytic and spoilage yeasts, molds and bacteria. At pH 3.85 most squid autolysis and
577 the proteolytic action added proteases is also inhibited. The USDA NOP allows liquid fish
578 hydrolysate to be further acidified to not lower than pH 3.5 (7 CFR 205.601(j)(7)).

579 Raw materials used to produce squid and squid byproduct hydrolysates are generally digested
580 under optimal conditions and acidified to prevent bacterial growth, lipolysis and further
581 proteolysis. The acids used are chosen according to cost and functionality. Acid amounts vary
582 with batch production. Fertilizer may be stabilized with phosphoric acid which is relatively
583 inexpensive and adds phosphorus to the product (Goldhor and Regenstein, 2007).

584 The pKa of an acid determines its strength. pKa technically indicates the ability of the acid to lose
585 a proton decreasing the pH of an aqueous solution. Stronger acids have lower pKas. A list of the
586 acids used for production of fish hydrolysates is provided in Table 2. Smaller amounts of strong
587 acid with lower pKas are required to reduce the pH of a hydrolysate than weaker acids with
588 higher pKa. Therefore, a lower percentage of synthetic material is added to the product. It is the
589 preference of fish and squid and squid byproducts producers to reduce the pH of their product to
590 3.85 or as close to this value as possible. Three of the acids listed, phosphoric, sulfuric and citric
591 are able to be used for this purpose. It is practical to reduce the pH of squid and squid byproduct
592 hydrolysate with less than 1-10% volume of sulfuric, phosphoric or citric acid. Because squid and
593 squid byproduct hydrolysates are produced from aquatic animals, they cannot be represented as
594 organic or made with organic (§ 205.2), even though they contain respectively 5% or less or 30%
595 or less synthetic material (§ 205.301 (b)-(c)). Other naturally produced acids with slightly higher
596 pKas can be used to reduce the pH, e.g. formic, lactic acid and ascorbic (Jangaard, 1987).

597

Table 2 Strong acids used to produce squid and squid byproducts fertilizer*

Acid	HA	A^-	Ka	pKa	Acid Strength	Approved for use in Organic Crop Production**
Sulfuric	H_2SO_4	HSO_4^-	Ka>1	pKa<1	+++++	Yes
Phosphoric	H_3PO_4	$H_2PO_4^-$	7.52×10^{-3}	2.13	++++	Yes
Citric	$H_3C_6H_5O_7$	$H_2C_6H_5O_7^-$	8.4×10^{-4}	3.13	+++	Yes
Formic	HCOOH	$HCOO^-$	1.77×10^{-4}	3.75	++	No
Lactic	$HCH_3H_5O_3$	$CH_3H_5O_3^-$	1.38×10^{-4}	3.86	++	Only if non-synthetic
Ascorbic	$H_2C_6H_6O_6$	$HC_6H_6O_6$	7.9×10^{-5}	4.10	++	No

*Peters et al., 1974

**§205.601(j)(7) for liquid fish products

598

Because phosphoric acid and sulfuric are manufactured by chemically based processes, they are considered synthetic and must be included in the National List for use in organic crop production. Both acids are included in the National List entry for Liquid Fish Products. Citric acid is manufactured using biological fermentation, and considered a non-synthetic product. Squid and squid byproducts hydrolysate fertilizer acidified with citric acid is considered a non-synthetic product, since the proteolytic enzyme Alcalase is also considered a non-synthetic product.

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

Squid and squid byproduct hydrolysate fertilizer contains: peptides and amino acids resulting from the enzymatic digestion process; fats and oils; complex carbohydrates; and various micronutrients (fats and oils may be removed during the manufacturing process). These compounds containing carbon, nitrogen, phosphorus, sulfur and micronutrients associated with animal decomposition provide starting ingredients for humus formation which comprises much of the organic matter in soil. The decomposition of animal remains in soil constitutes a basic biological process where carbon (C) is recirculated to the atmosphere as carbon dioxide and associated elements (nitrogen, N; phosphorus, P; sulfur, S; and various micronutrients) appear in forms required by higher plants. In the process, some of the C is assimilated into microbial tissues (i.e. the soil biomass): part of it is converted into stable humus. Some of the native humus is mineralized concurrently. Consequently, total organic matter content is maintained at a steady-state level characteristic of the soil and management system (Stevenson, 1994).

Microbial activity as determined by adenosine triphosphate activity is significantly higher in soils fertilized with squid and squid byproducts hydrolysate than comparable soils fertilized with other organic fertilizers or synthetic mineral products (Fetter et al., 2013). Addition of organic matter significantly increased 1) soluble organic carbon in soil, particularly polysaccharide carbon and 2) stability of 1–2 mm macro-aggregates compared to similar mineral fertilization treatments.

Marked changes such as losses in organic matter may occur as a result of farming. Crop yields naturally decline if nutrients, particularly N, are not replaced by organic or fertilizer input such as squid and squid byproduct hydrolysate. Ultimately, soils may become unsustainable for farming. Organic matter content decline also results from soil cultivation which increases aeration and microbe dependent mineralization. Since productivity depends on intensity, organic matter in most agricultural soils can only be maintained at high levels by inclusion of a cover crops in the cropping sequence, by the frequent addition of large quantities of organic residues, such as squid and squid byproduct hydrolysate and/or by minimizing tillage operations and maximizing the amount of plant material returned to the soil (Weil, 1992; Fukuoka, 1975; Stevenson, 1994).

Vegetable mould production by earthworms is an important indicator of the soil and humus health (Sykes, 1949). Charles Darwin proposed that worms were in fact capable of changing the composition of the soil (Darwin, 1838). Darwin described the abilities of earthworms and fungi to chemically rework mineral soil into humus (Feller et al., 2003). Metal binding to earthworm metallothionein allows earthworms to safely carry these contaminants until they are excreted facilitating their transfer from the contaminated site. Earthworms accumulate heavy metals by increasing production of metallothioneins. Earthworms can live in soils with relatively high levels of metals such as zinc, cadmium and copper depending on the pH of the soil and its ability to exchange cations, as would be the case for humus. At lower pH levels that might result from

the addition of squid and squid byproduct hydrolysate, earthworms do not survive well in contaminated soil, however, as pH increases so does both earthworm survival and fecundity (Spurgeon et al., 2006).

Organic fertilizers, for example squid and squid byproduct hydrolysate breakdown slowly and provide nitrogen over a longer period of time than synthetic fertilizers. Slow release adds a requirement that fertilizer application must occur before critical plant developmental stages. Application timings must be worked out for crops to ensure the availability of appropriate soil nutrients. Squid and squid byproduct hydrolysate fertilizer is used for cranberry production. For cranberries, performance is as good as or better than synthetic fertilizers. In addition, the liquid fertilizer may be applied through an irrigation system (Henderson and Strombom, 1995).

Four to six weeks following bloom is a critical time for apple crop development. It is when vegetative growth takes place determining fruit set, return bloom, potential yield and fruit size. Because utilization is greater than root uptake, mineral nutrient depletion can occur even though soil reserves are adequate. Foliar fertilization is helpful, however, fish hydrolysates should not be used for this purpose in apple production, because they can cause reduction of fruit set and russeting, which is the formation of a rough brown netting that forms over the apple surface indicating the killing of the fruit epidermis (Schupp et al., 1993).

Evaluation Question #9: Discuss and summarize findings on whether the use of the petitioned 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) (i)).

Levels of persistent organic pollutants and heavy metals such as methyl mercury and cadmium have been associated with squid and squid byproducts. These values have been used analytically to track trace amounts of these contaminants in the seas. In some cases amounts are high enough to consider limiting consumption for food in some areas of the world. In most cases, levels are not high enough to overcome natural remediation from soil bacterial processes (Cardoso et al., 2012; Galitsopoulou et al., 2009; Ueno et al., 2003).

Squid and squid byproducts are processed to a hydrolysate form in the production of fertilizer. During processing, fats and oils are removed from the product. The fats and oils may be further processed into a value added product or may enter the waste stream. Such waste, if not properly treated, can lead to pollution, including increased biological oxygen demand, chemical oxygen demand, total suspended solids, pathogenic microflora and increased nutrient levels in local waters (Islam et al., 2004).

Formic acid may be used as a stabilizer for squid and squid byproduct fertilizer. Formic acid may be toxic to some crops, e.g. cranberries. Phosphoric acid stabilization of fish hydrolysate does not decrease productivity in cranberries making the product useful for addition to irrigation systems, avoiding the need for aerial fertilizer application (DeMoranville, C., 1989). The use of formic acid stabilization of squid and squid byproduct hydrolysate was not requested by the petitioner (NOP, 2015a).

Fertilizers produced with squid and squid byproducts and acidified with phosphoric acid are effective in providing essential nutrients to soils when compared to synthetic commercial fertilizers. However, it has been observed that they are no more environmentally friendly than other organic fertilizers or synthetic fertilizers, rather they have been found to have a similar risk of NO_3—N and PO_4—P leaching to that of liquid or granular synthetic fertilizers applied at rates up to 292 kilograms per hectare per year (Fetter, 2011). Leaching of PO_4—P can promote eutrophication, toxic algal blooms, loss of dissolved oxygen and fish kills in aquatic ecosystems. NO_3—N leaching into groundwater subsequently used as drinking water has been linked with thyroid disease, blue baby syndrome, and nitrosamine production (which can cause cancer).

Thus, application of this material must be carefully managed to ensure that leaching is controlled (Galaviz-Villa et al., 2010; Liu et al., 2011; Knobeloch et al., 2000; Knobeloch et al., 2011).

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

Because they are acidified, hydrolysates made from squid and squid byproducts do not readily support the growth of bacterial pathogens such as *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria* spp. Furthermore, compost produced with fish hydrolysates is also generally free of human pathogens, unless these bacteria are inoculated via other compost constituents. Under these conditions, bacteria will grow rapidly. However, after reaching its incubation temperature, compost containing fish emulsion has only low levels of *Enterobacteriaceae*, coliforms and *E. coli* (Miller, 2011).

A 38 kilodalton protein is a major allergen of squid. The protein is believed to be squid muscle tropomyosin. It binds human immunoglobulin E from sensitive patients. This allergen is cross reactive with a similar protein from shrimp. No other allergen has been reported from squid (Myazawa et al., 1996).

Enzyme inactivation to prevent rancidity and bacterial contamination requires a pH shift and the use of strong acids such as sulfuric or phosphoric acid (Reed, 1975). These acids in concentrated form can present a hazard to human health in cases of contact or ingestion. Once acids are incorporated into the product the hazard is eliminated.

Squid and squid byproducts may contain pathogenic bacteria (Elhadi et al., 2004). Further processing can reduce contamination; however, it is important for workers in plants producing these products to be protected with the appropriate breathing gear, such as masks, eye protection and protective clothing.

International squid fishing remains a concern of human rights advocacy. Particularly in Southeastern Asia and New Zealand, crews for fishing boats that routinely catch squid among other forage fish crops are assembled by human resources companies practicing debt bondage and captive enslavement, whereupon crew members are forced into highly restrictive contracts that do not provide fair compensation and under which the signee may be responsible and enslaved for damages amounting to more than the value of the contract if it is breached. Frequently, these fishing companies have been cited as using these contracts as leverage to permit abuse of the fishing boat crew members (Skinner, 2012; Urbina, 2015; US Department of State, 2015).

Illegal, unreported and unregulated (IUU) fishing is a significant problem that affects the marine ecosystem and those who depend on it for survival (Petrossian, 2015). Illegal and unreported catches represented 20–32% by weight of wild-caught seafood imported to the USA in 2011. The value is between $1.3 and $2.1 billion of $16.5 billion total for 2.3 million tons of edible seafood imports, including farmed products. An estimated ten to fifteen percent of squid caught by fisherman from China, ten to twenty percent from Chile, fifteen to thirty percent from Thailand and twenty to thirty five percent from India are illegal and unreported (Pramod et al., 2014).

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

Animal and plant materials for organic production include raw animal manure, composted plant and animal materials and uncomposted plant and animal materials (§ 205.203(c)). Producers can apply: 1) a crop nutrient or soil amendment included on the National List, 2) a mined substance

included on the national list, ash, and 3) chemically altered plant or animal materials included on the national list (§ 205.204(d)). Plant and soil amendments included on the National List are aquatic plant extracts, elemental sulfur, humic acids, lignin sulfonate, magnesium sulfate, micronutrients (soluble boron products, sulfates, carbonates, oxides or silicates of zinc, copper, iron, manganese, molybdenum, selenium and cobalt), liquid fish products, vitamins (B_1, C, and E), and sulfurous acid (§ 205.601(j)).

Squid and squid byproduct fertilizers are rich in nutrients, contain high levels of protein and are generally good microbial substrates once applied to and adsorbed by soil, equivalent alternatives include liquid fish product, other proteinaceous hydrolysates and protein enriched composted and vermi-composted fertilizers.

Subcritical or superheated water under pressure (443-673 degrees Kelvin and 0.792-30 Mega Pascals) is effective in hydrolyzing squid byproducts. Under pressure cooker like conditions water behaves like a solvent decomposing the squid byproducts into sugars, lipids and amino acids in both a solid and a liquid phase after only a few minutes time (Yoshia and Tavakoli, 2004). This process is improved by removing lipids and oils using supercritical CO_2 fluid extraction prior to hydrolysis. Squid and squid byproducts are dried, extracted with supercritical CO_2 to remove oils and then submitted to subcritical water hydrolysis to decompose proteins into amino acids. Subcritical water hydrolysis produces a hydrolysate rich in amino acids (≥ 20%) in as little as one minute (Uddin et al., 2010). Although not yet used on an industrial scale, high pressure treatment further retards microbial growth preventing spoilage (Gou et al., 2010).

Squid and squid byproducts hydrolysate is most similar in origin, composition and manufacturing process to liquid fish products (Sykes, 1949; Fukuoka, 1975; Goldhor, 2007). Liquid fish products are scheduled for sunset on 6/27/2017. The NOSB completed its most recent sunset review of liquid fish products at their fall 2015 meeting in Stowe, Vermont (NOP, 2015b). Table 3 includes a list of a variety of liquid and solid fish products certified for organic crop production by both the Organic Materials Review Institute (OMRI) and the Washington State Department of Agriculture (WSDA) Organic Food Program that could be used in place of the petitioned substance in its variety of forms. The list is non-exhaustive providing examples of many available and representative replacement products. OMRI lists input products that it has verified for use in organic production and are compliant with organic standards.. WSDA Organic Food Program also maintains a list of products that have been reviewed and determined to be compliant under USDA organic regulations.

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

Organic crop producers must maintain and improve soils and soil fertility. Self-sufficiency through fixation of atmospheric nitrogen, recycling of crop residues, careful management and application of manures and composts, and growing fertility building leguminous crops such as red clover, white clover, vetch, alfalfa, ryegrasses, peas, and beans alleviates the use or requirement for any soil amendment or fertilizer (ADAS, 2006). Cover crops, crop rotations and the application of plant and animal materials are essential to minimizing the loss of nutrients and maintaining soil fertility (§ 205.204).The producer must implement a crop rotation including but not limited to sod, cover crops, green manure crops, and catch crops that provide the following functions that are applicable to the operation: (a) Maintain or improve soil organic matter content; (b) Provide for pest management in annual and perennial crops; (c) Manage deficient or excess plant nutrients; and (d) Provide erosion control (§ 205.205). Liquid fish products are similar to squid and squid byproduct hydrolysate, i.e. aquatic animal product, processed with natural enzymes, acidified with 1-10% of phosphoric, sulfuric or citric acid, high in protein and

Table 3 List of fish products fertilizers and soil amendments approved for use in organic production by OMRI or WSDA*			
Product Name	Company	Date Listed	Expiration
15-1-1 Pure Protein Dry Primo Aminos	AZ Enterprises Inc. and Organic Ag Products LLC	8/27/2014	06/01/2016
AGGRAND Organic Series™ 4-3-3 Fertilizer	Amsoil Inc.	12/06/2011	03/01/2016
Agro Thrive LFP	AgroThrive, Inc.	2015*/ 07/31/2015	9/2/2015*/ 09/01/2016
Alaska All Purpose Fish Fertilizer 2-2-2	Lilly Miller Brands	2010*	9/25/2015*
Alaska Salmon Fish Fertilizer	Trident Seafoods Corporation	06/07/2006	09/01/2016
ALASKA® Fish Fertilizer 5-1-1	Lilly Miller Brands	04/21/2011	12/01/2016
Aqua Power™ 5-1-1	JH Biotech, Inc.	12/21/2011	09/01/2016
Avenger® Organics 2-3-1 Liquid Fish Fertilizer Concentrate	Cutting Edge Formulations, Inc.	09/13/2013	06/01/2016
Avenger® Organics 2-3-1 Liquid Fish Fertilizer Ready-To-Spray	Cutting Edge Formulations, Inc.	09/27/2013	06/01/2016
Beneficial Biologics Fish Logic 1-0.5-0.2	Beneficial Biologics	04/09/2014	03/01/2016
Biostimulante Orgánico	GreenCorp Biorganiks de Mexico, S.A. de C.V.	06/28/2013	09/01/2016
Bioplanet Plant's Choice	Bioplanet Mexico, SAPI de CV	05/20/2014	06/01/2016
Bluegill Heaven Fish Hydrolysate	HBR Enterprise LLC dba Bluegill Heaven	02/21/2014	03/01/2016
BioGan 6-5-0 (Fish Bone Meal)	BioOregon Protein, Inc.	2013*	9/3/2015
Brown's Fish Hydrolysate	C.R. Brown Enterprises	2010*/ 04/15/2010	8/31/2015*/ 06/01/2016
Clear Organic Fish Fertilizer 1.5-2-0	Clear Springs Food, Inc.	2009*/ 10/22/2010	9/22/2015*/ 12/01/2016
Clear Organic Fish Fertilizer 1-1-0	Clear Springs Food, Inc.	2009*	9/22/2015*
Denali Organic Fish Fertilizer	Denali Organics	2013*	9/2/2015*
Down to Earth Bio-Fish™ 7-7-2	Down To Earth Distributors, Inc.	09/27/2012	03/01/2016
Down To Earth Fish Bone Meal 3-16-0	Down To Earth Distributors, Inc.	02/04/2008	03/01/2016
Down to Earth Fish Meal 8-6-0	Down To Earth Distributors, Inc.	11/11/2011	03/01/2016
Down to Earth Liquid All Purpose 4-1-3	Down To Earth Distributors, Inc.	03/18/2014	03/01/2016
Drammatic "C" Liquid Fish Plant Food 4.3-3-0.3	Dramm Corp.	01/09/2014	12/01/2015
Drammatic® "E" 2-5-0	Dramm Corp.	03/18/2014	12/01/2015
Drammatic® "E" Energized Plant Food 2-5-0.2	Dramm Corp.	02/15/2007	12/01/2015
Drammatic® "K" Earth Friendly Fertilizer 2-5-1	Dramm Corp.	12/02/2009	12/01/2015
Drammatic® "K" Liquid Fish with Kelp Plant Food 2-5-0	Dramm Corp.	02/12/2014	12/01/2015
Drammatic "K"	Dramm Corporation	2011*	9/2/2015*
Drammatic "O"	Dramm Corporation	2011*	9/2/2015*

Table 3 (cont.) List of fish products fertilizers and soil amendments certified for use in organic production by OMRI or WSDA*			
Product Name	Company	Date Listed	Expiration
Drammatic® "O" Earth Friendly Fertilizer 2-5-1	Dramm Corp.	12/04/2009	12/01/2015
Drammatic® "O" Liquid Fish Plant Food 2-5-0	Dramm Corp	02/12/2014	12/01/2015
Drammatic® "O" Liquid Fish Plant Food 2-5-0.2	Dramm Corp.	12/06/2002	12/01/2015
Drammatic® "S" Liquid Fish Plant Food 2-2-0.2	Dramm Corp.	09/08/2009	12/01/2015
Earthbank Fish Compost	Earthbank Resource Systems, Ltd.	02/18/2005	03/01/2016
Eco-Hydro Fish 1.5-3-0.2	Eco-Nutrients, LLC	2009*	10/14/2015*
Eco-Nutrients 1.5-3-0.2 Eco-Hydro Fish All Purpose Liquid Fertilizer	Eco-Nutrients, LLC	02/16/2009	12/01/2016
Energized Fish Protein 15-1-1	Pro-Active Agriculture	07/21/2014	09/01/2016
Fish Hydrolysate 2-3-1 Liquid Fertilizer	Southern Organics & Supply	04/09/2014	03/01/2016
Fish Protein 15 Nitrogen	Rongcheng Hongde Marine Bio-Tech Ltd.	02/11/2014	03/01/2016
Fish On! 2-4-0.2	Native Nutrients, LLC	2014*	9/3/2015*
Fish Plus Organic	BioWest Ag Solutions	2011*	9/2/2015*
Fish Rich™ Organic Plant & Garden Fertilizer 2-2-2	Bell Aquaculture	02/07/2012	03/01/2016
Fish-O-Mega® 4-1-1	BioFlora Systems	02/14/2001	09/01/2016
FLN 3-1-1	BWF Banducci, Inc.	2014*	9/22/2015*
FON FISH (9.5-4-0.5) Dry Organic Fertilizer	APELSA Guadalajara SA de CV	02/25/2014	09/01/2016
FP-LL15	Biological Nutrient Technologies	2013*	9/9/2015*
Fresh Fish Power All Purpose Soil & Plant Food	Red Worm Power	10/23/2014	06/01/2016
FUSA Organic Nutrients Enzyme USA Bio NPK Fish Hydrolysate	Fertilizers USA LLC	03/05/2015	06/01/2016
Gardner & Bloome G&B Organics High Growth Fertilizer 4-0-2 Fish & Kelp, with Molasses	KSI	03/03/2014	06/01/2016
Gardner & Bloome G&B Organics Organic All Purpose Fertilizer 3-2-3 Fish & Kelp, with Molasses	KSI	05/01/2014	06/01/2016
Genesis Fish-N	Genesis Agri Products, Inc.	2013*	10/29/2015*
Geoponics FertaFlow Organic Fish Fertilizer	Geoponics Corp.	07/15/2014	09/01/2016
GrowAmin Organic	Grow Green	2015*	9/15/2015
Grassoline™ All Purpose Fish Fertilizer 2-0-0	Vancouver Island Renuable Resources LTD	03/18/2014	06/01/2016
Grassoline™ All Purpose Fish Fertilizer 2-0-0	Vancouver Island Renuable Resources LTD	03/18/2010	06/01/2016
Grassoline™ All Purpose Fish Fertilizer 2-3-0	Vancouver Island Renuable Resources LTD	05/04/2011	06/01/2016
Grassoline™ All Purpose Fish Fertilizer 2-3-0 Stabilized	Vancouver Island Renuable Resources	10/29/2014	06/01/2016
Green Fish	Agroinsumos del Mar S.A. de C.V.	03/13/2001	06/01/2016

Table 3 (cont.) List of fish products fertilizers and soil amendments certified for use in organic production by OMRI or WSDA*			
Product Name	Company	Date Listed	Expiration
Gro-Wild Root Boost	Schafer Fisheries, Inc.	07/25/2011	09/01/2016
GrowBetter™ Fish plus Kelp Fertilizer	Jongs Organic Fertilizers, Inc.	01/02/2013	09/01/2016
Grower Preferred Organics™ Pride of the Sea™ 3-1-1 Stabilized Fish Solubles	Dune Company Mexicali, S. de R.L. de C.V.	08/27/2012	09/01/2016
Grower Preferred Organics™ Pride of the Sea™ 4-1-1 Stabilized Fish Solubles	Dune Company Mexicali, S. de R.L. de C.V.	08/27/2012	09/01/2016
Grower Preferred Organics™ Pride of the Sea™ 5-1-1 Stabilized Fish Solubles	Dune Company Mexicali, S. de R.L. de C.V.	08/27/2012	09/01/2016
Grower's Secret Organic All-Purpose Fish Fertilizer Flourish 3-3-1	Grower's Secret, Inc.	07/02/2013	12/01/2015
Indian River Organics Liquid Fish Fertilizer	Eco-Friendly Products,	06/19/2013	09/01/2016
Jaws Liquid Fish Fertilizer	Source to Source, a TAP family company	04/17/2013	06/01/2016
Kellogg Garden Organics Organic Plus Fish & Kelp Fertilizer with Molasses	Kellogg Garden Products	01/17/2014	06/01/2016
Lighthouse Fish Bone 5-16-0	Wilbur Ellis Co.	2004*	11/5/2015*
Lighthouse Fish Meal 9-4-0.	Wilbur Ellis Co	2004*	11/5/2015*
LIQUID SUNSHINE S.E. 5-1-1	BWF Banducci Inc	10/18/2010	03/01/2016
Mar y Tierra 4-1-1	Mar Y Tierra Fertilizantes Orgánicos	10/12/2011	03/01/2016
Mar y Tierra 4-5-1	Mar Y Tierra Fertilizantes Orgánicos	02/24/2012	03/01/2016
Mar y Tierra 5-1-1	Mar Y Tierra Fertilizantes Orgánicos	10/22/2010	03/01/2016
Nature's Intent Granulated Fish BoneMeal 4-13-0	Pacific Calcium	2012*	9/9/2015*
Neptune's Harvest Fish & Seaweed Fertilizer	Neptune's Harvest Fertilizers / Ocean Crest Seafood	03/18/2003	09/01/2016
Neptune's Harvest Fish Fertilizer	Neptune's Harvest Fertilizers / Ocean Crest Seafood	03/24/1997	09/01/2016
New Era Farm Service Nutra-Mix I 3.0-1.0-3.0	New Era Farm Service	03/13/2006	09/01/2016
Nutritec PHYTAFISH® 4-1-1 Fertilizante Orgánico Líquido Soluble	US Mex Nutrition Technologies SA de CV	12/20/2010	06/01/2016
Nutritec PHYTAFISH® 4-6-1 Fertilizante Orgánico Líquido Soluble	US Mex Nutrition Technologies SA de CV	08/26/2014	06/01/2016
Nutritec VIGILANTE® 6-1-1 Fertilizante Orgánico Líquido Soluble	US Mex Nutrition Technologies SA de CV	02/03/2011	06/01/2016
OmegaGrow 4-1-1 Liquid Fish Plant Nutrient	Omega Protein, Inc.	05/12/2015	12/01/2016

Table 3 (cont.) List of fish products fertilizers and soil amendments certified for use in organic production by OMRI or WSDA*			
Product Name	Company	Date Listed	Expiration
OmegaGrow™ 5-1-1 Liquid Fish Plant Nutrient	Omega Protein, Inc.	10/21/1999	12/01/2016
OmegaGrow™ Plus 3-1-1 Liquid Fish Plant Nutrient	Omega Protein, Inc.	08/19/2003	12/01/2016
Organic BioLink® Liquid N-Five 5-1-1	Westbridge	06/28/2011	12/01/2016
Organic Gem™ Liquid Fish Fertilizer 2.9-3.5-0.3	Advanced Marine Technologies	12/20/1999	03/01/2016
Organics by Rapid Growth Nutrients Rapid-HYDRO 2-4-0.2 All-Purpose Water Soluble Concentrated All-Purpose Liquid	Rapid Growth Nutrients, LLC	12/12/2012	03/01/2016
Organique Exquis 3-1-1	BWF Banducci Inc	12/21/2011	03/01/2016
Organo Fish 4-1-1	Crop Services International, Inc.	11/29/2010	12/01/2015
Pacific Gro Liquid Oceanic Fertilizer All Purpose	Pacific Gro, LLC	09/09/2014	06/01/2016
Pacific Gro™ Standard Liquid Fish Hydrolysate	Pacific Gro, LLC	04/07/2011	06/01/2016
Pacific Gro™ Zfish	Pacific Gro, LLC	09/08/2011	06/01/2016
Pacific Natural® Fresh Fish Fertilizer	Great Pacific BioProducts, Ltd.	05/04/2001	06/01/2016
Phytamin® All-Purpose	California Organic Fertilizers Inc.	03/04/2010	09/01/2016
Phytamin® Fish	California Organic Fertilizers Inc.	03/04/2010	09/01/2016
Phytamin® Fish Concentrate	California Organic Fertilizers Inc.	03/04/2010	09/01/2016
Phytamin® Fish Gold	California Organic Fertilizers Inc.	02/03/2011	09/01/2016
Phytamin® Fish Plus	California Organic Fertilizers Inc.	04/16/2010	09/01/2016
Primo Aminos 15-2-0.5 100% Water Soluble Fertilizer from Fish Protein	Summerland Distribution Group Inc	04/16/2015	06/01/2016
Pro-Pell-It! Fish Bone Meal 4-17-0	Marion Ag Service, Inc.	2015*	9/2/2015*
Pro-Pell-It! Fish Meal 9-4.5-0	Marion Ag Service, Inc.	2013*	9/2/2015*
Pure Protein 2-2-0.2	AZ Enterprises, Inc. / Organic Ag Products	2011*	9/3/2015*
Pure Protein Dry	AZ Enterprises, Inc. / Organic Ag Products	2011*	9/3/2015*
PURE PROTEIN Dry 15-1-1 Primo Hydrolysate	AZ Enterprises Inc. and Organic Ag Products LLC	04/12/2011	06/01/2016
SaferGro® Aqua Power™ 5-1-1	JH Biotech, Inc.	11/16/2012	09/01/2016
SeaMix-O	North American Kelp	06/07/2011	03/01/2016
Secure Organics 4-1-1	BWF Banducci Inc	12/17/2009	03/01/2016
Secure Plus 4-6-1	BWF Banducci Inc	02/24/2012	03/01/2016
SF Organics 2-2-0	Schafer Fisheries, Inc.	03/14/2006	09/01/2016
SF Organics 2-5-0	Schafer Fisheries, Inc.	08/09/2005	09/01/2016
SHARK™ Fish Fertilizer	Full Measure Industries, LLC	03/29/2012	06/01/2016
Simply Fish Soil Amendment	West Coast Fish Culture (Lois Lake) Ltd.	10/28/2015	12/01/2016
Solu-Fish™ 2-4-1 Hydrolyzed Fish Fertilizer	Compostwerks LLC	05/12/2014	03/01/2016

Table 3 List of fish products fertilizers and soil amendments certified for use in organic production by OMRI or WSDA*			
Product Name	Company	Date Listed	Expiration
Stemilt World Famous Liquid Fish	Stemilt World Famous Compost	2011*	9/16/2015*
Super 6-1-1 Plus Kelp	BWF Banducci Inc	03/04/2014	03/01/2016
Superior O-M-G® 3-2-0 X-tra Liquid Fertilizer	Superior Soil Supplements	06/14/2011	09/01/2016
Superior O-M-G® 4-3-4 Nature's Balance Liquid Fertilizer	Superior Soil Supplements	06/14/2011	09/01/2016
Superior O-M-G® 5-1-2 Soil Formula Liquid Fertilizer	Superior Soil Supplements	06/14/2011	09/01/2016
Tierra Fértil 4-6-1	Mar Y Tierra Fertilizantes Orgánicos	10/22/2010	03/01/2016
Tierra Fértil 5-7-1	Mar Y Tierra Fertilizantes Orgánicos	10/22/2010	03/01/2016
Triton Fertilizante Orgánico Pescado Y Algas Marinas	Tecniprocesos Biologicos, S.A. de C.V.	02/27/2002	03/01/2016
True 1-5-0	True Organic Products, Inc.	2014*	10/14/2015*
TRUE 413	True Organic Products, Inc.	06/11/2012	06/01/2016
True 511	True Organic Products, Inc.	06/11/2012	06/01/2016
TRUE 512	True Organic Products, Inc.	06/11/2012	06/01/2016
True Fish Hydrolysate 4.8-3-0.4	True Organic Products, Inc.	04/08/2015	06/01/2016
True Fish Meal	True Organic Products, Inc.	10/23/2012	06/01/2016
Vital Earth's O.G. Seabird Guano Liquid Grow 4-3-4	Vital Earth's	02/11/2014	09/01/2016

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composed primarily of organic matter. Production of liquid fish products, or other high protein natural fertilizers and their use for soil amendments is an alternative practice to producing squid and squid byproduct hydrolysate for this purpose. Farmers in Weston, Massachusetts growing cilantro with liquid fish products as a soil amendment significantly improved their yields compared to conventional amendments such as manure, leaf compost and synthetic fertilizer (Mangan et al., 2001).

In particular, the addition of organic matter from liquid fish products affects soil structure. Soil structure is the arrangement of soil particles into secondary particles or aggregates. Gelatinous organic materials composed of polysaccharides and produced by living bacteria and other microorganisms surround soil particles and hold them together as aggregates through a cementing or encapsulation action (Stevenson, 1994). Stable soil aggregates positively influence plant growth through their effects on: 1) aeration, 2) water penetrance and retention, 2) mechanical impedance to roots and 4) emergence of shoots. Organic matter application improves soil response to compressive as well as tensile stresses and the friability of organically amended soil is less affected by soil compaction than soil dressed only with mineral fertilizers (Abdollahi, 2014). The lack of organic matter causes the physical condition of soil to deteriorate preventing water from entering or draining from soil. Germinating seeds cannot obtain oxygen required for respiration and shoots cannot break through the soil surface crust. Yields may be reduced, even though adequate nutrients are available (Stevenson, 1994).

Organic content is greatest in non-cultivated soil compared to cultivated soils where soil structure and microbial communities have been disturbed. Squid and squid byproduct hydrolysates can be applied in liquid form in irrigation water reducing or eliminating the need for tilling. Alternative practice including the use of an irrigation system for application of liquid soil amendments must address the advantages of a no-till system where quality is based on 1) resulting organic matter content and 2) ability of the soil amendment to increase ATP activity and beneficial soil microflora. Effectiveness is correlated closely with soil aggregate formation and microbial colonization. Squid and squid byproduct hydrolysates improve soil organic content to 4.5-5.5% or greater and ATP activity level to 0.16 to 0.20 nano-mole/milligram (Williams and Petticrew, 2009).

Fertile soil is soil that is rich in humus. Whenever the humus content is depleted (as in growing a wheat crop), the humus must be replaced with more humus manufactured by the biological process, e.g. by vegetable growth (as in grass) and by its decay, when ploughed, accelerated and activated by the earthworms and by the microorganisms of animal dung and urine. A healthy soil means a soil re-fertilized by natural organic manures and residues which will grow a healthy plant, which must have a considerable bearing on human health (Sykes, 1946).

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