Trace Minerals

Aquaculture - Aquatic Animals

Identification of Petitioned Substance

This technical report discusses 14 specific minerals petitioned for use in organic aquatic animal production as premix or individual additions to feeds. The scope of mineral compounds petitioned, which are listed in Table 1, is based on those defined as "required nutrients" by the National Research Council's (NRC's) Nutrient Requirements of Fish and Shrimp (NRC, 2011; Aquaculture Working Group, 2012). Included in this definition are macrominerals (i.e., calcium, phosphorus, magnesium, sodium, potassium, and chloride) in addition to microminerals (i.e., cobalt, chromium, copper, iodine, iron, manganese, selenium, and zinc). Herein, information is provided about the petitioned minerals individually and collectively per the availability of information. Individual minerals potentially exist in a variety of biologically active forms; for the purposes of this discussion, an example of a predominant chemical derivative likely to be found in mineral supplements was chosen for each mineral species. Calcium and iron are well known examples of dietary minerals, and are allowed for use in organic crop production as plant or soil amendments (micronutrients) when soil deficiency is documented by testing (7 CFR 205.601(j)(6)) and in handling (calcium carbonate, ferrous sulfate; 7 CFR 205.605). As such, calcium and iron are presented as specific examples in portions of this report.

Table 1. Petitioned Minerals for Use In Aquatic Animal Feed

Mineral Name	Chemical Form Example	CAS Number	Trade/Other Names	Other Codes
Calcium	Calcium carbonate ($CaCO_3$)	471-34-1	Calcite	EINECS: 207-439-9
Cobalt	Cobalt(II) chloride ($CoCl_2$)	7646-79-9	Cobalt muriate	EINECS: 231-589-4
Chromium	Chromium(III) chloride ($CrCl_3$)	10025-73-7	Chromic chloride	EINECS: 233-038-3
Copper	Copper (II) sulfate ($CuSO_4$)	7758-98-7	Cupric sulfate	EINECS: 231-847-6
Iodine	Potassium iodide (KI)	7681-11-0	ThyroShield®, Radiban®	EINECS: 231-659-4
Iron	Iron(II) sulfate hepta-hydrate ($FeSO_4 \bullet 7H_2O$)	7782-63-0	Ferrous sulfate heptahydrate	EINECS: 231-753-5
Magnesium	Magnesium sulfate ($MgSO_4$)	7487-88-9	Epsom salt	EINECS: 231-298-2
Manganese	Manganese(II) sulfate monohydrate ($MnSO_4 \bullet H_2O$)	10034-96-5	N/A	EINECS: 232-089-9
Phosphorus	Tricalcium phosphate [$Ca_3(PO_4)_2$]	7758-87-4	Calcium phosphate tribasic	EINECS: 231-840-8
Potassium	Potassium chloride (KCl)	7447-40-7	Sylvite, muriate of potash	EINECS: 231-211-8
Selenium	Sodium selenite (Na_2SeO_3)	10102-18-8	N/A	EINECS: 233-267-9
Sodium and chlorine	Sodium chloride (NaCl)	7647-14-5	Halite, table salt	EINECS: 231-598-3
Zinc	Zinc sulfate heptahydrate ($ZnSO_4 \bullet 7H_2O$)	7446-20-0	Goslarite, white vitriol	EINECS: 231-793-3

Macrominerals and microminerals are generally grouped based on their relative abundance in the environment and dietary levels required for the healthy development of plants and animals. Because macrominerals such as calcium and sodium are required in higher doses for intensive biological processes (e.g., bone development and energy conversion), the risk of toxicity is minimal. However, trace minerals such as copper and zinc are needed in much smaller quantities for optimal activity, and carry heightened risks for toxicity if dietary excesses of these elements occur. A graphical representation of human upper intake levels (ULs) is presented in Figure 1 below to convey the difference between the nutritional requirements (and potential for toxicity) of macro- vs. microminerals (Driskell, 2009). Please see Evaluation Question #10 for additional information regarding ULs and human toxicity.

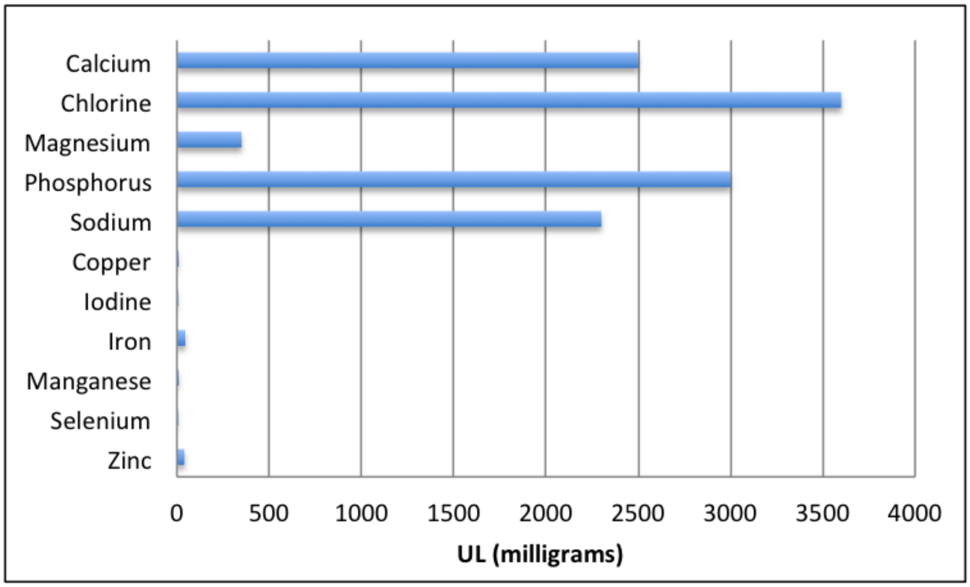

Figure 1. Distribution of human upper intake levels (ULs) for macro- and microminerals

Summary of Petitioned Use

The petitioner, the Aquaculture Working Group, is requesting the addition of minerals, including macrominerals (i.e., calcium, phosphorus, magnesium, sodium, potassium, chlorine) and microminerals (i.e., cobalt, copper, chromium, iodine, iron, manganese, selenium, and zinc) to the National List for the fortification of feeds used in organic aquatic animal production. Specifically, the petitioner is seeking the addition of trace minerals, including those listed in Table 1, to the National List as Synthetic Substances Allowed for Use in Organic Aquatic Animal Production. Petitioned trace minerals are included as ingredients in feed pellets for aquatic animals at approximately 0.1–0.2% of the feed pellet mass, and not directly dissolved in growing water (Aquaculture Working Group, 2012).

The National Organic Program (NOP) final rule currently allows the use of trace minerals in organic livestock production for enrichment or fortification, under 7 CFR 205.603(d)(2), Synthetic substances allowed for use in organic livestock production, when FDA approved.

Characterization of Petitioned Substance

Composition of the Substance:

Mineral premixes used to fortify animal feed are composed of four to six essential minerals, inorganic chemical compounds that must be obtained through the diet or supplemental means in order to meet nutritional requirements. Additional minerals may be individually supplemented in the feed depending on the organism being fed and dietary availability of the specific minerals (NRC, 2011). Minerals are typically categorized based on the dietary requirement for a given organism: macrominerals (i.e., calcium, phosphorus, magnesium, sodium, potassium, chloride) are required in relatively high doses (1.7–15 g/kg diet), while microminerals (i.e., cobalt, copper, chromium, iodine, iron, manganese, selenium, and zinc) are typically required in the diet and animal body at much lower doses (0.1–200 mg/kg diet). Mineral supplements generally deliver the metal ion of interest as a salt containing a corresponding counterion (an ionic species of opposite charge). As examples, anionic iodide may coordinate to cationic potassium to form potassium iodide, while a number of metal cations (e.g., iron, manganese, zinc, etc) coordinate the polyatomic anion sulfate (SO_4^{2-}) to form the sulfate salt. Structures of example derivatives of the petitioned minerals are presented in Figure 2.

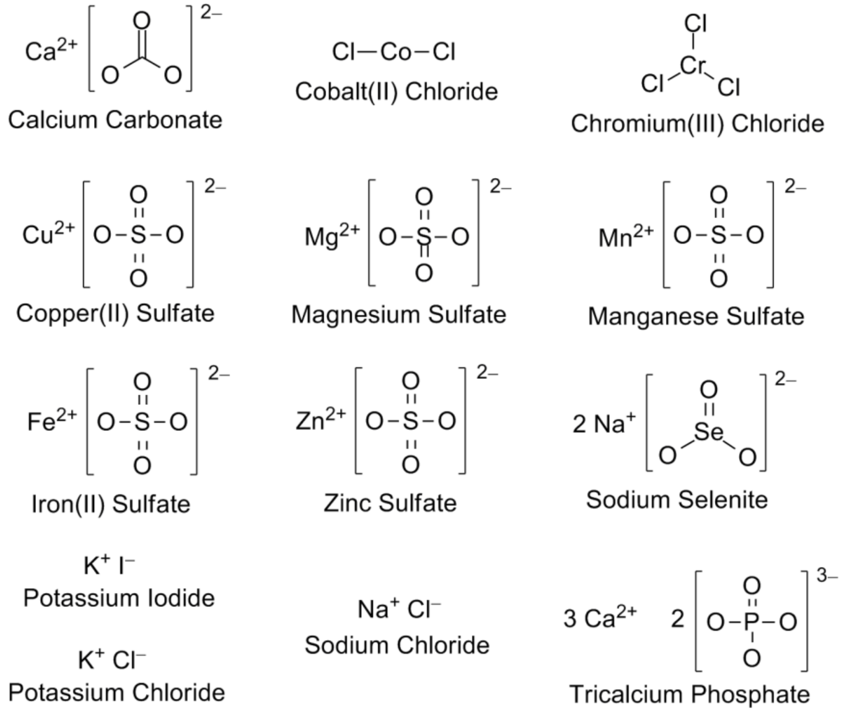

Figure 2. Structures and Formulas of Common Mineral Compounds

Source or Origin of the Substance:

Chemical synthesis is the most common industrial method for mineral production. Commercial methods generally involve the treatment of a mineral source (e.g., rocks, metal hydroxides, or scrap metal) with strong acids or other suitable reagents. For example, the treatment of magnesite ore, a naturally occurring feedstock, with intense heat followed by sulfuric acid generates magnesium sulfate, which is commonly used for magnesium fortification. Alternatively, potassium iodide for iodine fortification is produced in a straightforward process involving the reaction of potassium hydroxide with molecular iodine (I_2) followed by chemical reduction of the intermediate iodate. For details regarding the production methods for all petitioned mineral compounds, please see Evaluation Question #2.

Properties of the Substance:

Synthetic trace minerals are generally obtained in ionic form with an associated counterion. Electropositive metal species cobalt, copper, chromium, manganese, iron, and zinc are mono-, di-, and/or trivalent cations existing in an intimate ion pair with counter anions such as sulfate (SO_4^{2-}) or chloride (Cl^-). The selenium metal within sodium selenate and selenite is overall neutral, but exists as a salt of the anionic selenium oxide and cationic sodium metal. Finally, electronegative iodine generally exists in anionic form with associated cations, such as the potassium cation or ethylenediammonium cation. As a result of their ionic nature, many trace mineral compounds readily dissolve in aqueous solutions, and dietary excesses not metabolized by the organism are generally excreted. Trace minerals exist in anhydrous (free of water) form in the absence of moisture; however, many trace mineral complexes are hygroscopic, or have the ability to absorb water molecules from the surrounding environment to form hydrates. As a class of substances, trace minerals have low vapor pressures (i.e., minerals are non-volatile).

Calcium

Calcium carbonate, a common form of the calcium used as a mineral supplement, exists as a white powder or crystals with a melting point of 800 °C. Pure calcium carbonate is practically insoluble in water; Calcite has a water solubility of 0.0013 grams per 100 grams in neutral water at 18 °C. Its water solubility is increased in presence of carbon dioxide (carbonic acid) and ammonium salts, and in dilute acidic solutions. Dissolution of calcium carbonate to the extent it is soluble in neutral water generates alkaline aqueous solutions (pH = 8–9) (HSDB, 2006c; Sigma Aldrich, 2012).

Chromium

Chromium in dietary supplements generally takes the form of chromium(III) chloride(i.e., chromic chloride). It forms violet, lustrous, hexagonal crystals with a melting point of 1150 °C. Chromic chloride is only slightly soluble in boiling water, and is insoluble in water, alcoholic solvents, acetone, methanol, and ether. However, addition of a trace amount of chromium(II) dichloride rapidly solubilizes chromic chloride in water and alcohols (HSDB, 2005a).

Cobalt

Cobalt chloride [$CoCl_2$] is a synthetically available form of cobalt used in dietary supplements and generally exists as a blue powder with a slight sharp odor. The melting point of cobalt chloride is 724 °C. The substance is soluble in water (1.16 kg L^{-1} at 0 °C), alcohols, acetone, ether, glycerol, and pyridine (HSDB, 2003a; Sigma Aldrich, 2013).

Copper

Copper (II) sulfate [$CuSO_4$] or its hydrated form, copper (II) sulfate pentahydrate [$(CuSO_4)\bullet 5H_2O$], are the forms most likely to be found in trace mineral supplements. The hydrated form exists as large, blue or ultramarine triclinic crystals, blue granules, or light blue powder. Upon heating to 110 °C, the complex dehydrates and decomposes. Copper sulfate is highly soluble in water (31.6 g/100 mL at 0 °C, 203.3 g/100 mL at 100 °C), somewhat soluble in alcohol (1 g per 500 mL), and practically insoluble in most organic solvents. The pH range (pH = 3.7–4.5) of an aqueous solution (50 g L^{-1}) at 25 °C is somewhat acidic (HSDB, 2001a; Sigma Aldrich, 2012).

Iodine

Potassium iodide and ethylenediamine dihydroiodide are commonly used as synthetic forms of iodine in trace mineral supplements. Potassium iodide, for example, typically exists as colorless crystals or in powder form. The melting point of potassium iodide is 681 °C. It is highly soluble in water (148 g L^{-1} at 25°C) and is slightly soluble in ethanol. The pH of an aqueous solution of potassium iodide ranges from neutral to alkaline (pH = 7–9 at 166 g L^{-1} at 25 °C) (HSDB, 2006a; Sigma Aldrich, 2012).

Iron

Iron supplements typically come in the form of iron(III) sulfate [$Fe_2(SO_4)_3$] (ferric sulfate) and iron(II) sulfate [$FeSO_4$] (ferrous sulfate). Iron(III) sulfate exists as a grayish-white powder or yellow crystals with a decomposition point of 480 °C. While ferric sulfate dissolves only slowly in water alone, it is rapidly soluble in the presence of trace quantities of ferrous sulfate. A 10% solution of ferrous sulfate has an acidic pH of 3.7, and readily oxidizes to ferric sulfate (HSDB, 2005b; HSDB, 2005c).

Magnesium

Mineral supplements may contain magnesium in the form of magnesium sulfate, which is a colorless crystalline solid with a melting point of 1124 °C. It is readily soluble in water (360 g/L at 20 °C), alcohol, and glycerol; moderately soluble in ether (1.16 g per 100 mL at 18 °C); and insoluble in acetone. Aqueous solutions of magnesium sulfate are generally of neutral pH (HSDB, 2003b).

Manganese

Manganese sulfate [$MnSO_4$] and hydrated forms of manganese sulfate [$MnSO_4\bullet xH_2O$] exist as white crystals and a light red powder, respectively. Solid hydrated manganese sulfate has a melting point of 700 °C, and aqueous solutions of the substance are acidic (pH = 3.0–3.5 at 50 g L^{-1} at 20 °C). Manganese sulfate is soluble in water (52 g per 100 mL at 5 ºC), slightly soluble to soluble in alcoholic solvents, and insoluble in ether and other nonpolar (predominantly hydrocarbon-based) organic solvents (HSDB, 2001b; Sigma Aldrich, 2012).

Phosphorus

Tricalcium phosphate is one of the common forms of phosphorus used in mineral supplements. It exists as a white amorphous or crystalline powder with a melting point of 1670 °C. The substance is minimally soluble in water (2.5 mg per 100 g water at 25 °C), practically insoluble in alcohol and acetic acid, but exhibits enhanced solubility in dilute solutions of hydrochloric acid and nitric acid (HSDB, 2003c).

Potassium

Potassium chloride may be used for potassium supplementation in mineral premixes. The substance exists as a white crystalline powder with a melting point of 770 °C. Potassium chloride is readily soluble in water (35.5 g per 100 g water at 25 °C) and glycerol, and insoluble in ether, acetone, and absolute alcohol. Aqueous solutions of potassium chloride generally have a neutral pH (pH = 7) (HSDB, 2007a; Sigma Aldrich, 2013).

Selenium

Selenium supplements typically take the form of sodium selenate [Na_2SeO_4] and sodium selenite [Na_2SeO_3]. Sodium selenate decahydrate generally exists in a white crystalline form, and the substance is soluble in water (58.5 g L^{-1} at 25 °C). Sodium selenite has been listed as white trigonal crystals and a white powder with a melting point of 710 °C. The pentahydrate form of sodium selenite is freely soluble in water to form a slightly alkaline solution (HSDB 2011a; 2011b).

Sodium and Chlorine

Sodium chloride, the principal component of table salt, is typically used for sodium and chlorine fortification of aquatic animal feeds. The substance exists as colorless, transparent crystals or a white, crystalline powder with an observed melting point of 801 °C. Sodium chloride is readily soluble in water (35.7 g per 100 mL at 0 °C) and forms neutral (pH = 6.7 to 7.3) aqueous solutions (HSDB, 2007b; Sigma Aldrich, 2013).

Zinc

Zinc sulfate [$ZnSO_4$] is commonly used for zinc metal supplementation. Its common hydrated form, zinc sulfate heptahydrate [$ZnSO_4 \bullet 7H_2O$] is a crystalline white solid with a melting point/range of > 500 °C (680 °C for the anhydrous form). The substance is highly water-soluble (965 g L^{-1} at 20 °C) and forms mildly acidic aqueous solutions (pH = 4.0–6.0 at 50 g L^{-1} at 20 °C). Anhydrous zinc sulfate forms when its hydrates (mono- and heptahydrate) are heated above 238 °C (HSDB, 2006b; Sigma Aldrich, 2012).

Specific Uses of the Substance:

Although a number of chemical compounds are classified either as macro- or micro-minerals, the dietary importance of a given trace mineral is conditional on the animal species in question. In the case of aquaculture, calcium, phosphorus, magnesium, sodium, potassium, chlorine, chromium, cobalt, copper, iodine, iron, manganese, selenium, and zinc have been identified as essential minerals (NRC, 2011). Further, the National Research Council's (NRC's) Nutrient Requirements for Fish and Shrimp defines all essential mineral compounds as "required nutrients" (NRC, 2011). Accordingly, the aquaculture industry has petitioned the National Organic Standards Board to permit synthetic forms of minerals considered "required" by NRC for use in organic aquatic animal production. The aquaculture industry has also emphasized the importance of well-balanced trace mineral mixes for the health and productivity of finfish and shellfish populations; trace minerals are normally incorporated into conventional fish feed (Gatlin, 2010; Hertrampf, 2003).

A number of synthetic mineral compounds are commonly used in the fortification of conventional as well as organic terrestrial livestock feed. Trace mineral elements, whether naturally occurring in the diet or provided in supplements, are important for the maintenance, growth, and reproduction in the healthy production of beef cattle, swine, and poultry. In beef cattle production, minerals needed in larger amounts include calcium, phosphorus, magnesium, potassium, sodium, chlorine, and sulfur, while iron, zinc, manganese, copper, cobalt, and selenium are needed only in trace amounts (Hale, 2001). Forages and grains are good sources of calcium and phosphorus, respectively. However, the bioavailability of minerals in forage may vary depending on the mineral content of the soil and the level of pasture fertilization.

Mineral premixes are therefore widely used for livestock feed fortification to ensure the adequate intake of minerals (Hale, 2001). Likewise, poultry and swine production uses dietary supplementation of trace mineral compounds (Richards, 2010). Nutritional investigations using these production animals revealed that organic forms of trace minerals (e.g., zinc lysine) exhibit enhanced bioavailability when compared to inorganic forms (e.g., zinc sulfate). The higher bioavailability of these trace mineral forms may allow the producer to achieve similar or improved performance at reduced loadings of trace minerals in feeds (Richards, 2010).

Maintaining the regular intake of minerals through dietary or supplemental means is important for human health. Certain medical conditions may lead to mineral deficiency, making supplementation a potential avenue of mineral intake when dietary sources prove insufficient. For example, supplements may be used to treat iron deficiency resulting from gastrointestinal inflammation (e.g., Crohn's disease or celiac disease) and blood loss (e.g., associated with colorectal cancer), among other conditions (Saunders, 2012). Additionally, food products are commonly fortified with minerals and other essential nutrients to facilitate sufficient public consumption of these compounds. Typical examples of food vehicle-mineral combinations include iodized salt for iodine supplementation, and cereals and grain products for iron and other trace mineral fortification (Saunders 2012; FAO, 1996). Animal feeds may also be useful for increasing the iodine content of animal products consumed by humans (FAO, 1996).

Approved Legal Uses of the Substance:

Conventional Aquaculture and Livestock Feed

The U.S. Food and Drug Administration (FDA) and Departments of Agriculture in respective states regulate conventional aquaculture feeds with advisement from the Association of American Feed Control Officials (AAFCO). To ensure compliance with federal and state requirements, these agencies regularly inspect and analyze formulated fish feed and fish samples (NOAA, undated). All mineral compounds added to animal or fish feed must first be approved by the FDA followed by state regulatory approval in the form of registration with the respective Departments of Agriculture (21 CFR 573; 21 CFR 582). As opposed to other production animals, the FDA has not issued specific recommendations for mineral intake in aquatic animal species. Multiple forms of trace minerals used for supplementation are classified as Generally Recognized as Safe (GRAS) by the FDA and therefore not subject to additional regulatory oversight. The following is a list of common mineral forms added to animal and fish feeds (OMRI, 2009):

- Zinc (zinc sulfate) 21 CFR 582.80, 582.5997
- Manganese (manganese sulfate) 21 CFR 582.80, 582.5461
- Copper (copper sulfate) 21 CFR 582.80
- Iodine (potassium iodide) 21 CFR 582.80, 582.5634
- Iodine (ethylenediamine dihydroiodide, EDDI) 21 CFR 582.80
- Iron (Iron sulfate) 21 CFR 582.80
- Cobalt (cobalt chloride) 21 CFR 582.80
- Magnesium (magnesium sulfate) 21 CFR 582.5443
- Calcium (calcium sulfate) 21 CFR 582.5230

In general, the FDA requires additives including minerals, vitamins, other nutrients, flavorings, preservatives, or processing aids to be generally recognized as safe (GRAS) for their intended use (21 CFR 582 and 584) or be approved as food additives (21 CFR 570, 571, and 573). However, 21 CFR (FDA regulations) does not distinguish between organic and conventional additives. Other restrictions may also apply; for example, the FDA does not permit the use of EDDI as an animal drug and limits the amount fed to 50 mg/head/day in dairy cattle per the Compliance Policy Guide 7125.18 (FDA, 2000). Selenium supplementation typically takes place in the form of sodium selenate and sodium selenite, which are food additives permitted in feed and drinking water of animals (21 CFR 573.90) at specified concentrations. As an example, 21 CFR 573.90 states that the prescribed selenium supplements may be used in beef cattle "at a level not to exceed an intake of 3 milligrams per head per day." In complete feed for chickens, swine, turkeys, sheep, cattle, and ducks, selenium supplement levels may not exceed 0.3 parts per million (21 CFR 573.90).

Organic Livestock Feed

The National Organic Program (NOP) final rule currently allows the use of trace minerals in organic livestock production under 7 CFR 205.603, Synthetic Substances Allowed for Use in Organic Livestock Production, for enrichment and fortification when FDA approved. Further, the USDA organic regulations require producers to meet certain standards for livestock health care practices. As part of this requirement, livestock feed rations must sufficiently meet nutritional requirements, including minerals, vitamins, protein and/or amino acids, fatty acids, energy sources, and fiber (ruminants) (7 CFR 205.238(a)(2)). The USDA organic regulations define livestock to include the following (7 CFR 205.2):

> *any cattle, sheep, goats, swine, poultry, or equine animals used for food or in the production of food, fiber, feed, or other agricultural-based consumer products; wild or domesticated game; or other nonplant life, except such term shall not include aquatic animals for the production of food, fiber, feed, or other agricultural-based consumer products.*

As such, no U.S. federal regulations exist concerning the use of vitamin supplements in the organic production of aquatic animal species.

Food Additives and Dietary Supplements

The National Organic Program (NOP) final rule currently allows nutrient minerals in the organic handling of food for human consumption under 7 CFR 205.605, synthetic substances allowed as ingredients in or on processed products labeled as "organic" or "made with organic (specified ingredients or food group(s))." Organic handlers must also comply with the FDA Nutritional Quality Guidelines for Foods (21 CFR 104.20) in the fortification of processed foods. Listed below are nutrient profiles for selected minerals (Table 2). In contrast to its role in the regulation of drugs and animal feed additives, the FDA does not regulate human dietary supplements containing mineral compounds (FDA, 2005); however, if an unsafe product is marketed, it is the responsibility of the FDA to take any necessary regulatory action and/or ensure the accuracy of the supplement's label (FDA, 2005).

Table 2. FDA Nutrition Quality Guidelines for Foods: Minerals

Mineral	Unit of Measurement	DRV or RDI
Calcium	gram	1.0
Iron	mg	18
Phosphorus	gram	1.0
Magnesium	mg	400
Zinc	mg	15
Iodine	µg	150
Copper	mg	2.0
Potassium	gram	3.5

mg = milligram (gram/1,000); µg = microgram (gram/1,000,000); DRV = Dietary Reference Values; RDI = Reference (Recommended) Daily Intake

Use in Organic Crop Production

According to the National Organic Program (NOP) Final Rule, the following may be used as plant or soil amendments in organic crop production: sulfates, carbonates, oxides, or silicates of zinc, copper, iron, manganese, molybdenum, and cobalt (7 CFR 205.601(j)(6)(ii)). The listed micronutrients may not be used as defoliants, herbicides, or desiccants, and those made from nitrates and chlorides are not allowed. Copper sulfate may also be used for plant disease control if handled in a manner that minimizes accumulation of copper in the soil (7 CFR 206.601(i)(2)).

Action of the Substance:

Dietary intake of nutrient minerals is essential for the health and well being of farmed aquatic species. In general, minerals play key roles in the maintenance of skeletal structures, such as bones and teeth, and osmotic pressure (a type of pressure that regulates the flow of water across biological membranes), thus helping to regulate the exchange of water and solutes (dissolved substances) within the animal body. Trace minerals may also serve as structural components of soft tissues or perform essential functions for the transmission of nerve impulses and muscle contraction. More specifically, trace minerals serve as essential components of many enzymes, vitamins, hormones, and oxygen transport molecules; cofactors in metabolism and catalysis; and enzyme activators.

Iron

Iron is an essential component of the oxygen transport proteins hemoglobin and myoglobin, as well as various enzyme systems including cytochromes, catalases, peroxidases, the enzymes xanthine and aldehyde oxidases, and succinic dehydrogenase. As a component of the respiratory pigments and enzymes involved in tissue oxidation, iron serves a critical function for oxygen and electron transport within the body. In addition to hypochromic microcytic anemia (the production of small red blood cells resulting in reduced cell counts and/or hemoglobin concentrations), reduced growth and feeding efficiency have been observed for various fish species with iron deficiency.

Zinc

Although its specific functions are not always understood, zinc cations are essential components of more than 80 metalloenzymes (enzymes containing tightly bound metal atoms); for example, zinc has been observed in carbonic anhydrase (required for carbon dioxide transport) and superoxide dismutase (required for the regulation of reactive free radicals, or molecules having one or more unpaired electrons), among other important enzymatic systems. More specifically, zinc serves as a cofactor in many enzyme systems, including arginase, enolase, and several peptidases. As an active component or cofactor for many important enzymes, zinc plays a vital role in lipid, protein, and carbohydrate metabolism, particularly in the synthesis and metabolism of nucleic acids and proteins. Zinc may also play a beneficial role in wound healing. In fish, signs of zinc deficiency include reduced growth, anorexia, depressed bone Ca and Zn content, erosion of fins and skin, and mortality.

Manganese

A primary function of manganese in the body is to activate enzymes that mediate phosphate group transfer (i.e., phosphate transferases and phosphate dehydrogenases). Manganese is also an essential component of the enzyme pyruvate carboxylase, which is responsible for the generation of glucose from lactate or amino acids. As a cofactor or component of several key enzyme systems, manganese is essential for bone formation, the regeneration of red blood cells, carbohydrate metabolism, and the reproductive cycle. In fish, manganese deficiency may result in reduced growth, anorexia, cataracts, and short body dwarfism.

Copper

Copper is an essential component of numerous oxidation-reduction (redox) enzyme systems. As examples, copper is observed in cytochrome oxidase, superoxide dismutase, and amine oxidase. As a component of the enzyme ferroxidase, copper is intimately involved with iron metabolism, and therefore hemoglobin synthesis and red blood cell production and maintenance. The formation of melanin, and consequently skin pigmentation, the formation of bone and connective tissue, and maintenance of nerve fibers may also rely on dietary intake of copper. Signs of copper deficiency in common carp include reduced growth and cataracts.

Cobalt

As an integral component of vitamin B_{12} (cyanocobalamin), cobalt is essential for red blood cell formation and the maintenance of nerve tissue. Cobalt may also serve as an activating agent for other enzyme systems. Cobalt deficiency is not commonly observed in aquatic organisms.

Iodine

Iodine is an integral component of the thyroid hormones, thyroxine and tri-iodo-thyronine. Therefore, iodine is essential for regulating the metabolic rate of all body processes. Thyroid hyperplasia (goiter) is the most common sign of iodine deficiency observed in salmonids.

Selenium

Selenium is an essential component of the enzyme glutathione peroxidase, and functions together with vitamin E to protect cellular tissues and membranes against oxidative damage. As a biological antioxidant, dietary intake of selenium mitigates copper-induced oxidative stress. Selenium may also participate in the biosynthesis of ubiquinone (coenzyme Q; involved in cellular electron transport) and influence the absorption and retention of the antioxidant vitamin E. Signs of selenium deficiency in fish include muscular dystrophy, reduced growth, cataracts, anemia, and mortality.

Chromium

Trivalent chromium (i.e., chromium with three binding sites) is an integral component of the glucose tolerance factor, a low molecular weight compound in which trivalent chromium coordinating two nicotinic acid molecules, and acts as a cofactor for the hormone insulin. Chromium is believed to play an important role in the metabolism of cholesterol and amino acids in addition to its vital role in carbohydrate metabolism (i.e., glucose tolerance and glycogen synthesis). Chromium deficiency is not commonly observed in aquatic organisms.

Calcium

Calcium is an essential component of bone, cartilage, and the crustacean exoskeleton. It is also essential for the normal clotting of blood through stimulated release of thromboplastin from the blood platelets. Certain enzymes (e.g., cholinesterase and ATPases) require calcium as an activator. Through enzymatic activation, calcium stimulates muscle contraction (i.e., muscle tone and normal heart beat) and regulates the transmission of nerve impulses from one cell to another. Calcium deficiency may result in anorexia and reduced growth and feeding efficiency in numerous fish species.

Magnesium

Many similarities exist between the biological actions of magnesium and calcium. Magnesium is also an essential component of bone, cartilage, and the crustacean exoskeleton. It is an activator of several key enzymes, including kinases and muscle ATPases. Through its role in enzyme activation, magnesium (like calcium) stimulates muscle and nerve irritability (contraction), is involved in the regulation of intracellular acid-base balance, and plays a vital role in carbohydrate, protein, and lipid metabolism. Signs of magnesium deficiency in fish include reduced growth, sluggishness, anorexia, and poor survival.

Sodium

As the main monovalent (one binding site) ion of extracellular fluids, sodium ions constitute 93% of the ions found in the blood stream. The principal role of sodium in the animal is connected with the regulation of osmotic pressure and maintenance of acid-base balance. In addition, sodium affects muscle irritability (contraction), and plays a specific role in the absorption of carbohydrate. Sodium deficiency in animals, and specifically aquatic organisms, is not generally observed.

Potassium

As the major intracellular (inside cells) cation, potassium ions regulate intracellular osmotic pressure and acid-base balance. Much like sodium, potassium acts as a stimulant for muscle irritability. Potassium is required for glycogen and protein synthesis, as well as the metabolic breakdown of glucose. Potassium deficiency is not generally observed in aquatic organisms.

Chlorine

As with cationic sodium, anionic chloride is a major component of extracellular (outside cells) fluids. Chlorine ions account for about 65% of the total anions in blood plasma and other extracellular fluids within the body, and are essential for the regulation of osmotic pressure and acid-base balance. In addition, chlorine plays a specific role in the transport of oxygen and carbon dioxide in the blood and maintenance of digestive juice pH. No symptoms of chlorine deficiency in aquatic animals have been documented.

Phosphorus

Phosphorus is an essential component of bone, cartilage, and the crustacean exoskeleton. It is also an essential component of phospholipids, nucleic acids, phosphoproteins (e.g., casein), high-energy phosphate esters (e.g., adenosine triphosphate), other biological phosphates, and several key enzymes. Phosphorus therefore plays an essential role in energy and cell metabolism. In addition, inorganic phosphates serve as important buffers to regulate the normal acid/base balance (i.e., pH) of animal body fluids.

Sources: FAO, 1987; NRC, 2011

Combinations of the Substance:

Feeds for aquaculture and terrestrial livestock have similar additive profiles, as the nutrients required by fish for growth, reproduction, and other normal physiological functions are similar to those of land animals. Trace minerals are typically provided in aquaculture feed diets of fish oil, fishmeal, vegetable oil, and plant proteins (e.g., corn, soy) with essential amino acids, antioxidants, vitamins, and certain pigments proven safe and permitted by U.S. FDA regulation (Lovell, 1998; NOAA, undated). Wheat is widely used as a binding agent in feed pellet production (Lovell, 1998).

Excluded materials, such as hormones and antibiotics used to enhance growth rates, are prohibited in conventional aquaculture and therefore are not included in aquatic animal feed premixes containing minerals (NOAA, undated). Although growth hormones are given to terrestrial farmed animals in conventional agriculture, such as cattle and poultry, the U.S. FDA prohibits their use in fish feed. In addition, U.S. law prohibits the use of antibiotics in aquaculture for non-therapeutic purposes (NOAA, undated). Incidentally, the use of growth hormones and antibiotics does not improve growth or efficiency in farmed fish.

In organic and conventional livestock production, trace minerals are combined in feed diets of grains, beans, oilseeds, and other meals with amino acids and various vitamin compounds (Pond et al., 1995). Depending on the raw nutrients available to the animal, individual mineral compounds or a premix of multiple trace minerals is added to feed rations (Hale, 2001). Further, antibiotics are routinely added to grain feed as a growth stimulant in conventional livestock production (Board on Agriculture, 1999).

Mineral supplements for human consumption may be formulated individually or collectively as multivitamin/mineral (MVM) dietary supplements. Major and trace minerals, vitamins, and various herbs are therefore the most common active ingredients in MVM supplements (NIH, 2013; Woodward, undated). MVM tablets and supplements also contain additives that aid in the manufacturing process or alter how the pill is accepted by the body. These additives include fillers that impart bulk to the vitamin pill, such as microcrystalline cellulose, lactose, calcium or malto-dextrin; lubricants, such as magnesium stearate or stearic acid; flow agents, such as silicon dioxide; disintegration agents, such as cellulose gum or starch; cellulose or carnauba wax coatings; and coloring and flavoring agents (Woodward, undated). It should be emphasized that not all of these additives (e.g., stearic acid, malto-dextrin) are allowed in organic handling (7 CFR 205.605–205.606).

Status

Historic Use:

Although the trace mineral requirements for aquatic animals are not fully understood, conventional aquaculture feeds have been fortified with trace minerals and other micronutrients for several decades (Abowei, 2011; NRC, 2011. Commercial fish hatcheries were mostly reliant upon raw meat (i.e., horse meat) as a dietary staple for trout until the end of World War II. In the early 1950s, John Hanson of the New Mexico Game and Fish Department developed the first dry pellet formulations while experimenting with dietary routine (Sigler, 1986). Following the introduction of dry pellets to trout hatcheries, producers observed higher conversion rates of food intake to fish production, which led to the wider adoption of fish pellets in hatcheries across the U.S. (Sigler, 1986). Research into more specific requirements for fats, protein levels, vitamins, amino acids, and other constituents, such as trace minerals, has led to the incorporation of the petitioned minerals and other essential nutrients into many commercial fish feed pellets.

<u>Organic Foods Production Act, USDA Final Rule:</u>

Section 2118 of the Organic Foods Production Act of 1990 (OFPA) provides the guidelines for prohibitions and/or exemptions on the National List of Allowed and Prohibited Substances (7 U.S.C. 6517), and includes minerals at Section 2118(c)(1)(B)(i). Trace minerals are listed as allowed synthetic substances on the National List for use in organic livestock production (7 CFR 205.603(d)(2)) when FDA approved.

Both the NOP and NOSB received correspondences and public comments requesting consideration of adoption of organic standards for the production of aquatic species prior to 2007. To facilitate this work, the NOP created an Aquatic Animal Task Force composed of knowledgeable members of the aquaculture and organic communities. In 2007, the National Organic Standards Board (NOSB) adopted a final recommendation for the addition of Aquaculture Standards to the regulation. This proposal was consistent with the OFPA: § 2102 (11) LIVESTOCK – The term "livestock" means any cattle, sheep, goats, swine, poultry, equine animals used for food or in the production of food, <u>fish used for food</u>, wild or domesticated game, or other non-plant life. From the 7 CFR 205.2, Terms Defined:

> *<u>Livestock</u> Any cattle, sheep, goat, swine, poultry, or equine animals used for food or in the production of food, fiber, feed or other agricultural-based consumer products; wild or domesticated game; or other non-plant life, except such term shall not include aquatic animals or bees for the production of food, fiber, feed, or other agricultural-based consumer products.*

Therefore, the NOSB recommended that the NOP implement a change striking "aquatic animals" from the above definition, which would allow the development of regulations for the organic production of aquatic species. Beyond these recommendations, the NOSB indicated several areas requiring further public comment, including further fact finding on sources of feed for aquatic animals that require a diet that includes fish (NOSB, 2007).

The Aquaculture Working Group provided a recommendation that included regulation related to the feeding of aquatic animals. This recommendation was developed after careful consideration of the nutritional and health needs of aquatic species presented by the Aquaculture Working Group, panelists selected at the Aquaculture Symposium of November 2007, and public comment and statements made by the NOP at the Spring 2008 NOSB meeting. The essence of this recommendation is to allow, by exemption of a prohibited natural material in a proposed new section of the National List, the feeding of fish meal and fish oil from wild caught fish and other wild aquatic animals if produced from environmentally responsible food grade wild caught fisheries. In addition, the aquaculture producer must adhere to a 12 year plan aimed at reducing the consumption of wild fish following the implementation of organic aquaculture regulations. Regarding synthetic substances, a proposed new section of the National List states that aquaculture feeds must be composed of feed ingredients that are certified organic, except that non-synthetic substances and synthetic substances allowed in new sections of the National List may be used as feed additives and supplements (NOSB, 2008).

The Aquaculture Working Group's final recommendation to the NOSB involved the inclusion of Molluscan Shellfish (Bivalves) in the regulatory framework for organic aquaculture. Specifically, a proposed new section of the National List defines relevant terms and describes organic production practices for molluscan shellfish (NOSB, 2009).

To date, the NOP has not implemented the NOSB's recommendations on aquaculture through rulemaking.

<u>International</u>

A number of international organizations specify the application of synthetic trace minerals in organic livestock production. However, only the European Union (EU), Canadian General Standards Board, and the United Kingdom (UK) Soil Association have published standards specifying the use of synthetic minerals in the organic production of aquatic animals. The EU standards constitute binding regulations, while the Canadian standards have not yet been implemented in regulation. Below, international regulations and standards regarding the use of synthetic minerals in any form of organic animal production are summarized.

Canadian General Standards Board

According to the Canadian General Standards Board General Principles and Management Standards (CAN/CGSB-32.310-2006), organic operators may not use "feed and feed additives, including amino acids and feed supplements that contain substances not in accordance with CAN/CGSB-32.311, Organic Production Systems - Permitted Substances Lists" (CAN, 2011a). Minerals are included in the definition of feed additives and therefore subject to regulation. However, the Permitted Substances List (CAN/CGSB-32.311-2006) allows the use of synthetic minerals under certain circumstances: "minerals, trace minerals, elements" may be used for enrichment or fortification of livestock feed, and synthetic nutrient minerals may be used if non-synthetic sources are not commercially available. Under no circumstances should minerals be used to stimulate growth or production (CAN, 2011b). The 2012 Canadian Organic Aquaculture Standard, a non-binding and unregulated version of the official government standards for organic agriculture, considers trace minerals used in aquaculture the same as those used in livestock.

The allowed uses of synthetic minerals in organic livestock production are much broader than those for the organic production of crops. In organic crop production, mineral sulfates produced without the use of sulfuric acid may be used only to correct for mineral deficiencies determined by soil or plant tissue analysis. Further, trace elements (i.e., minerals) may be used to address documented soil and plant deficiencies when documented by soil and plant testing and when derived "from natural sources that are unchelated or chelated by substances listed as allowed" (CAN, 2011b).

Codex Alimentarius

The specific criteria for feedstuffs and nutritional elements section of the standards set forth by the Codex Alimentarius Commission (2012) pertaining to livestock production states that "feedstuffs of mineral origin, trace minerals, vitamins, or provitamins can only be used if they are of natural origin. In case of shortage of these substances, or in exceptional circumstances, chemically well-defined analogic substances may be used" (Codex Alimentarius Commission, 2012). At this time, Codex does not include aquaculture within its organic guidelines.

European Economic Community Council

The European Economic Community (EEC) Council Regulations, EC No. 834/2007 and 889/2008, state that "feed of mineral origin, trace elements, vitamins or provitamins shall be of natural origin. In case these substances are unavailable, chemically well-defined analogic substances may be authorized for use in organic production." Specifically, the following trace elemental compounds are allowed as nutritional additives in the organic production of livestock under Annex VI:

- Iron – Ferrous (II) carbonate, ferrous (II) sulfate, monohydrate and/or heptahydrate, ferric (III) oxide;
- Iodine – Calcium iodate (anhydrous and hexahydrate), sodium iodide;
- Cobalt – Cobaltous (II) sulfate monohydrate and/or heptahydrate, basic cobaltous (II) carbonate monohydrate;
- Copper – Copper (II) oxide, basic copper (II) carbonate monohydrate, copper (II) sulfate pentahydrate;
- Manganese – Manganous (II) carbonate, manganous oxide and manganic oxide; manganous (II) sulfate mono and/or tetrahydrate;
- Zinc – Zinc carbonate, zinc oxide, zinc sulfate mono and/or heptahydrate;
- Molybdenum – Ammonium molybdate, sodium molybdate;
- Selenium – Sodium selenate, sodium selenite.

EEC Council Regulation EC No. 710/2009 allows "feed additives, certain products used in animal nutrition and processing aids...if listed in Annex VI and the restrictions laid down therein are complied with." Therefore, the trace elemental compounds listed above are allowed in the organic production of aquatic animals in addition to terrestrial livestock.

United Kingdom Soil Association Standards

In order to be certified organic by the United Kingdom Soil Association, vitamin and mineral supplements of natural origin must be used in the diets of farmed fish. Organic producers may use vitamin and mineral supplements not of natural origin only with prior approval (Soil Association, 2011).

Japan Ministry of Agriculture, Forestry, and Fisheries

The Japan Ministry of Agriculture, Forestry, and Fisheries Standard for Organic Feed do not specify the allowed or prohibited status of trace minerals in organic livestock or aquatic animal feed. However, the standard permits natural feed additives:

> *Feed additives (except for those produced by using antibiotic and recombinant DNA technology), which are natural substances or those derived from natural substances without being chemically treated. In case of a difficulty to obtain feed additives listed in 8, the use of similar agents to the described food additives are permitted only for supplementing nutrition and effective components in feeds.*

This statement suggests that synthetic minerals may be allowed if naturally derived substitutes are not available (JMAFF, 2005). However, Japan does not currently include aquaculture in its organic guidelines.

International Federation of Organic Agricultural Movements

Within their norms, the International Federation of Organic Agricultural Movements (IFOAM) allows vitamins, trace elements and supplements from natural sources in animal feed. An exception to this rule states that "synthetic vitamins, minerals and supplements may be used when natural sources are not available in sufficient quantity and quality" (IFOAM, 2012). Although trace minerals are not addressed in the section on Aquatic Animal Nutrition (section 6.5), it is implied within the norms that this standard applies to both livestock and aquatic animal production.

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?

(A) The petitioned substances, trace minerals, would fall under the category of minerals. Sulfates of trace elemental species (e.g., iron sulfate and copper sulfate) are necessarily sulfur-containing compounds, although minerals bearing various counter anions (e.g, chloride, carbonate) may also be used as aquaculture and livestock feed supplements. In addition, copper sulfate, a commonly used supplemental form of the trace mineral copper necessarily contains copper.

(B) Since the petitioned trace minerals are not requested for use in a pesticide, they are not, by definition, an inert. The previous paragraph provides sufficient information to determine eligibility of the substance under OFPA; however, the inert status of the substance is briefly described. No synthetic trace mineral compounds are present on the EPA's list of inerts of toxicological concern (List 1). Potassium iodide, sodium selenite, and zinc sulfate (basic and monohydrate) are listed as an inerts of unknown toxicity (List 3). The petitioned trace mineral compounds are not mentioned on EPA List 4 or 40 CFR part 180, pesticide chemical or pesticide chemical residues exempt from tolerance requirements.

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)).

Individual mineral compounds are produced on an industrial scale through chemical synthesis and extraction from either natural or reclaimed sources. Selection of the manufacturing processes typically depends on the available technology, cost of raw materials/chemical feedstocks, availability of mineral-containing reclaimed materials, market prices and size, cost of implementing extraction versus chemical synthetic processes and, to a lesser extent, the overall environmental impact of the production method. A representative sample of common production methods for individual mineral compounds is presented in the following paragraphs.

Metal Sulfates

A number of the petitioned metal sulfates are commercially available and utilized in mineral premixes and other dietary supplements. Copper fortification is most commonly accomplished using copper sulfate pentahydrate ($CuSO_4 \bullet 5H_2O$), which occurs naturally as the mineral chalcanthite. The pentahydrate is made commercially through dissolution of scrap copper in hot concentrated sulfuric acid (generating sulfur dioxide) or oxidation of scrap copper in dilute sulfuric acid (Pimentel, 1981; USDA, 1995a). Similarly, ferrous sulfate [iron(II) sulfate, $FeSO_4$] and its associated hydrates are made through the treatment of iron metal with sulfuric acid (Merck Index, 2006; USDA, 1995b), and the processing of brass scraps with zinc oxide and sulfuric acid provides a means for obtaining zinc sulfate (Moore, 1976). Lead is often added to brass at levels of 2–8%, making it a potential contaminant of mineral supplements (U.S. EPA, 1996). Alternatively, a recent patent describes the production of manganese sulfate monohydrate ($MnSO_4 \bullet H_2O$) involving treatment of low-mid grade manganese dioxide with a sulfur-containing fume (i.e., sulfur dioxide-containing exhaust) (Jiang, 2012).

Although magnesium sulfate ($MgSO_4$) can be produced through recovery of the mineral kieserite (magnesium sulfate monohydrate) or epsomite (magnesium sulfate heptahydrate), commercial forms are generally produced synthetically (Sadan, 1997; HSDB, 2003b). This process begins with ignition of magnesite ore (i.e., magnesium carbonate) or magnesium hydroxide (obtained from seawater) to produce magnesium oxide, which is then reacted with sulfuric acid to afford magnesium sulfate (Kawamura and Rao, 2007). For further details regarding magnesium sulfate production, please see the recent technical evaluation report (USDA, 2011a).

Metal Chlorides and Carbonates

Cobaltous chloride ($CoCl_2$) and chromic chloride ($CrCl_3$) are common forms of cobalt and chromium used in dietary supplements and in the fortification of foods. Anhydrous (water free) chromium(III) chloride is usually prepared by passing a chlorinating agent such as chlorine, sulfur chloride, carbon tetrachloride, phosgene ($COCl_2$), or hydrochloric acid (HCl) and carbon disulfide (CS_2) over hot chromium(III) oxide (i.e., about 600 °C) or an oxide-carbon mixture (Heisig, 1946). The patent literature also describes a procedure for synthesizing cobalt(II) chloride through treatment of trivalent (three binding sites) cobalt with hydrochloric acid (Devuyst, 1982). Nickel electrorefining industries generate black hydrous material byproducts containing cobaltic chloride (trivalent cobalt), which can be treated with hydrochloric acid to generate cobaltous chloride. Overall, the process involves forming an aqueous slurry of a trivalent cobalt compound (i.e., cobaltic hydroxide) and treating the resulting slurry with hydrochloric acid in the presence of an organic reducing agent capable of reducing cobalt from the trivalent to divalent (two binding sites) state (Devuyst, 1982).

A variety of patented methods have been developed for the synthesis of calcium salts, as well as the recovery of dissolved calcium from industrial wastes. One process involves the treatment of an aqueous solution containing calcium hydroxide [$Ca(OH)_2$] having a basic pH (i.e., pH of at least 11.5) with carbon dioxide to form calcium carbonate. An alkaline reagent may be added to maintain a pH for the product mixture of at least 9.5 (Jensen, 2012). Another patent describes the extraction of calcium salts from papermaking sludge or sludge-derived ash accomplished by mixing with a solution of an inorganic or organic acid. Because most non-calcium salts (e.g., those of aluminum, magnesium, and iron) precipitate as hydroxides at lower pH than calcium hydroxide, a caustic or other alkaline reagent is gradually added to precipitate non-calcium salts to facilitate their removal from solution by filtration or centrifugation prior to precipitation of calcium hydroxide. The desired calcium salt is then precipitated through addition of the appropriate acid anion, which is generally accomplished by way of the acid or a salt having greater water solubility than the resulting calcium salt (Klyosov, 1999).

Compounds Containing Anionic Minerals

In contrast to the minerals described above, the synthetic forms of iodide, phosphorus, selenium, and chloride used for fortification of food and feed typically consist of the mineral anion coordinated to a metal cation, such as calcium, potassium, or sodium. Combining a hot aqueous solution of potassium hydroxide (KOH) with molecular iodine (I_2) in slight excess generates a mixture of potassium iodide (KI) and potassium iodate (KIO_3). Treatment of the dried material with charcoal followed by ignition leads to chemical reduction of the iodate to iodide, thus providing an attractive route to the synthetic mineral potassium iodide (Osol, 1975). In addition, potassium iodide can be prepared by reacting hydroiodic acid (HI) with potassium bicarbonate ($KHCO_3$) followed by melting in dry hydrogen as a purification step (Merck Index, 2006). For further details regarding synthetic procedures and purification protocols, please see the recent technical evaluation report (USDA, 2011b).

Phosphate rock and selenium metal are common feedstocks for industrially producing the respective mineral compounds. Various calcium phosphates (i.e., mono-, di-, and tribasic) can be manufactured by treating pulverized phosphate rock with sulfuric acid or phosphoric acid (Merck Index, 2006). The laboratory preparation of calcium dihydrogen orthophosphate (monobasic) involves the reaction of calcium carbonate with two equivalents of phosphoric acid and subsequent crystallization of the desired inorganic product (Jensen, 1953). Phosphate rock defluorination in the presence of phosphoric acid, lime (i.e., calcium oxide or calcium hydroxide), and water vapor in a rotary kiln at elevated temperatures (at least 2500 °F) is a convenient method for the production of tricalcium phosphate (tribasic) with low residual fluorine content and high fertilizer availability (Hollingsworth, 1951). Selenium salts (i.e., sodium selenate and sodium selenite) may be produced from selenious acid, which is generated through the dissolution of metallic selenium in nitric acid (Björnberg, 1987). Treatment of selenious acid with an alkali metal hydroxide and/or an alkali metal carbonate produces alkali metal selenite, which may be oxidized to the corresponding alkali metal selenate using hydrogen peroxide (Björnberg, 1987).

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)).

Commercial forms of mineral compounds are typically generated via chemical synthesis. As described in Evaluation Question #2 above, these chemical processes include acid base reactions, calcification, reaction of substances or elements through mixing, and oxidation-reduction reactions, among other chemical transformations. For example, a number of mineral compounds are manufactured via the reaction of a strong acid (e.g., sulfuric acid, hydrochloric acid, etc.) with a metal oxide or metal hydroxide. In addition, industrial manufacture of these substances generally involves the utilization of processing units, such as mixers, reactors, and kilns.

Natural forms of minerals are not typically included in supplements and fortified foods. However, feedstocks in the chemical process may include natural materials such as rocks in addition to synthetic materials. For example, naturally derived phosphate rocks may be treated with strong acids (such as phosphoric acid) in the production of calcium phosphates. Commercial copper sulfate, on the other hand, is commonly generated via the reaction of scrap metal/material (e.g., brass) with sulfuric acid followed by various separation and purification procedures.

The chemical reactions producing supplemental forms of minerals generally involve reactions of metal oxides and scrap metals with strong acids and bases. As a result of the strong reactivity of the latter chemical reagents, it is unlikely that any residues of the original metal oxide or strong acids/bases remain in the final mineral product. However, other metal-containing impurities originating from the metallic feedstock may be present in minor quantities (Merck Index, 2006). For example, calcium carbonate minerals frequently contain lead (Gulson, 2001; Nriagu, 2007). The concentration of these impurities in the final product depends upon the contamination of the metal feedstock and the manufacturer's purification protocol.

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)).

When used as petitioned, trace minerals from unconsumed feed pellets have the potential to persist in treated bodies of water, ground water, sediments and bioaccumulate in animal tissues. Data regarding the persistence of trace minerals resulting from uses in aquaculture are limited; however, comparisons may be drawn from industrial and agricultural human activities resulting in trace element releases. Investigations of trace element concentrations in the ground water of various districts in India revealed concentrations of all trace minerals at or below permissible limits, with the exception of iron (Jinwal, 2009). The promotion of bacterial growth in waters is the primary adverse environmental effect related to elevated iron concentrations. In another recent study from the Prut river wetland ecosystem of Romania, concentrations of copper and zinc were measured in environmental waters, sediments, and tissues of exposed aquatic organisms (i.e., plants, molluscs, and fish) (David, 2012). Significantly high concentrations (i.e., in excess of maximum admitted concentrations) of both metal species were observed in Prut river water samples due to agricultural and industrial activities. Biological samples from primary producers (i.e., plants) and primary and secondary consumers (i.e., mollusks and fish, respectively) also showed elevated concentrations, but only partial bioaccumulation for copper could be established in this study (David, 2012). It is known that selenium bioaccumulates in living tissues; as examples, the selenium content of human blood is about 1,000 times greater than that found in surface waters and fish meal selenium levels have been observed at levels 50,000 greater than those of seawater (U.S. EPA, 2010).

A subset of the petitioned minerals, including some considered to be heavy metals,have the potential for bioconcentration through interactions with various functional groups of enzymes and proteins within the body (Agarwal, 2009). Persistence of certain trace mineral compounds has been observed in humans; for example, the breast milk of female vineyard workers exposed to copper sulfate contained 6.2 times as much copper as the milk from unexposed workers (HSDB, 2001a). Potassium iodide may also be distributed into human breast milk, although specific examples of its persistence are lacking (HSDB, 2006a). The bioconcentration factors observed for selenium in carp (*Cyprinus carpio*) after 28 days exposed at 10 and 1 μg/L (BCF = 8.1–10 and <85, respectively) are suggestive of low bioconcentration potentials for selenium compounds (HSDB, 2011b). For constant exposures to cobalt chloride, more significant bioconcentration factors of 200–1,000 have been observed (HSDB, 2003a). The selenium content of human blood is roughly 0.2 ppm or about 1,000 times greater than the selenium concentration of surface waters (U.S. EPA, 2010). Overall, the risk of lethal effects from bioconcentration of the petitioned trace elements is considered to be low.

Trace elements released to aquatic systems also have the potential for interaction with soils, sediments, and other organic matter. Copper sulfate released to soils may be partly washed down to lower soil levels, bound by soil components, and/or oxidatively transformed to other copper species (HSDB, 2001a). Selenates (SeO_4^{-2}) are very mobile because of their high water solubility and low tendency to adsorb onto soil particles (HSDB, 2011a), while selenites (SeO_3^{-2}) readily adsorb to soil minerals and organic matter (HSDB, 2011b). Inorganic selenium compounds can be methylated by microorganisms and subsequently volatilized to the atmosphere (HSDB, 2011a; 2011b; U.S. EPA, 2010).

<u>Evaluation Question #5:</u> 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)).

At excessive levels of exposure, many of the trace minerals have the potential for toxicity toward humans, aquatic animals, and terrestrial animals. As a result, the U.S. EPA has established maximum contaminant levels for some minerals due to human toxicity concerns (U.S. EPA, 2012). Instances of copper poisoning in humans have been observed and copper toxicity may be exacerbated by hereditary disorders; for example, Wilson's disease may lead to liver and nervous system disorders due to excess copper levels in the body's tissues (Merck Manual, 2012). Below, a summary of the toxic effects related to excessive amounts of selected trace mineral elements is provided:

- Chromium: Allergic dermatitis;
- Copper: Wilson's disease, copper poisoning;
- Iodine: Hyperthyroidism and hypothyroidism;
- Iron: Hemochromatosis (iron overload leading to abdominal pain, fatigue, darkening of skin color), cirrhosis (scarring of the liver, poor liver function), diabetes mellitus, skin pigmentation;
- Manganese: Toxicity: Neurologic symptoms resembling those of parkinsonism or Wilson's disease;

- Selenium: Hair and fingernail changes, damage to the peripheral nervous system, fatigue and irritability;
- Zinc: RBC microcytosis (unusually small red blood cells), neutropenia (abnormally low levels of certain types of white blood cells), impaired immunity.

In fish, trace mineral toxicity is generally associated with respiratory disruption caused by physical gill clogging. Laboratory investigations of two grades of iron (III) sulfate using brown trout as the model organism indicated that iron is relatively non-toxic to fish (96 h LC_{50} = 28–47 mg L^{-1}) (Dalzell, 1999). Alternatively, copper exhibited pronounced acute toxicity toward rainbow trout, with 96 h LC_{50} values of 20 µg L^{-1} in soft acidic (pH 6.0) water and 520 µg L^{-1} in hard alkaline (pH 8.0) water (Howarth & Sprague, 1978). As with other trace elements, the toxicity of zinc toward fish can vary dramatically depending on fish species and size, temperature, pH, and water hardness. Two competing mechanisms of toxicity were revealed in an experimental study of rainbow trout: as the pH rises, dissolved zinc becomes increasingly toxic, but at higher pH, zinc precipitates from solution as zinc hydroxide ($Zn(OH)_2$), which is of very low toxicity to fish (Bradley & Sprague, 1985). Likewise, earlier studies of acute zinc toxicity in rainbow trout supported a mechanism of fish death involving coagulation or precipitation of mucus on the gills ultimately leading to tissue hypoxia (oxygen deprivation) (Burton, 1972). The observed 96-h LC_{50} of 1.5 mg L^{-1} for zinc with flagfish might be cause for concern in aquatic environments vulnerable to zinc contamination (Spehar, 1976). Although concerns regarding the potential toxicity and contamination of commercial fish feeds have been noted (Kavanagh, 2000), specific examples of toxicity related to trace mineral supplements in feeds are lacking.

In evaluating the potential toxicity of trace minerals, it is important to consider the levels that may be present in growing waters and effluents as a result of their use as petitioned. A simplified calculation was conducted using the concentration of specific trace minerals (copper, iodine, manganese, and zinc) in a sample mineral premix (Exhibit B; Aquaculture Working Group, 2012). In addition, the following assumptions were made: (1) feed supplied at 10% body weight daily (Wurtz, 2001), (2) stocking density = 18 kg fish per m³ water (FOC, 2011), (3) 0.2% mineral premix in manufactured feed pellets, and (4) 20–50% feed wastage. Concentration estimates (µg/L) for these four minerals were determined using the above data, assumed values, and the equations presented below with Table 3.

$$\frac{\mu g\ mineral}{kg\ feed} = \frac{grams\ mineral}{kg\ premix} \times \frac{kg\ premix}{kg\ feed} \times 10^6\,\frac{\mu g}{g}$$

$$\frac{\mu g\ mineral}{L\ water} = \frac{\mu g\ mineral}{kg\ feed} \times \frac{10\ kg\ feed}{100\ kg\ fish} \times \frac{18\ kg\ fish}{m^3\ water} \times \frac{m^3}{1000\ L} \times 0.5\ (fraction\ wasted)$$

Comparison of these effluent concentrations (Table 3) to the aquatic toxicity discussion above and drinking water quality standards for each mineral points to a negligible potential for toxicity under the prescribed use of the substance. It should also be noted that many of the assumed levels were overestimated; for example, most producers seek to optimize feed conversion, thereby minimizing feed wastage (CAN, 2011c; NOAA, undated).

Table 3. Calculated Effluent Levels of Selected Trace Minerals

Trace Mineral	Conc. in Feed (g/kg)	Conc. in Effluent (µg/L) @ 50% waste	Conc. in Effluent (µg/L) @ 20% waste	EPA Secondary Drinking Water Standard (µg/L)[a]
Copper	0.004	0.0036	0.00144	1,000
Iodine	0.025	0.0225	0.009	300[b]
Manganese	0.05	0.045	0.018	50
Zinc	0.2	0.18	0.072	5,000

[a] US EPA, 2013.

[b] ATSDR, 2004. Based on the Minimal Risk Level of 0.01 mg/kg-day and assuming a 70 kg adult consuming 2 L of water per day.

Mineral toxicity has also been observed in terrestrial animals fed excessive amounts of trace mineral supplements. Incidences of iodism (chronic iodine toxicity) in dairy cattle have been associated with feeding excessive iodine as ethylenediamine dihydroioide (EDDI) and other supplemental forms of iodide (Hillman, 1980). Clinic signs of iodine poisoning include nasal and lacrimal discharge, coughing, bronchopneumonia, hair loss, and dermatitis. In addition, signs of magnesium toxicity have been observed in broiler chicks fed corn-soy rations intentionally supplemented with toxic levels of magnesium salts. Specifically, tibiae from magnesium-intoxicated chicks were shortened, thickened, and bowed (Lee, 1980). Overall, it was concluded that increased bodily magnesium concentrations disrupt the calcium to phosphorus mass ratios leading to skeletal deformities.

Dioxins, furans, and other persistent organic pollutants have been observed in commercial trace mineral supplements. Vast quantities of low to mid purity metals are produced annually for applications in the automotive, electronics, and airplane manufacturing industries. Certain members of the animal feed manufacturing industry have suggested that failure to completely remove insulation and other plastics prior to recycling the metals in feed supplements can result in dioxins and polychlorinated biphenyls (PCBs) mixed with the final trace mineral product (Alltech, undated). Dioxin and furan occurrences have been observed in feed supplements containing trace minerals (i.e., copper, zinc, manganese, magnesium, and iron) complexed to polysaccharides for delivery of the trace minerals in animal feeds (Ferrario, 2003). Although specific manufacturing details were not provided, the ingredients for production of the copper supplement (i.e., feed grade kelp, copper sulfate, dextrose monohydrate, silicone dioxide, white mineral oil, and water) were combined, dried at 50 °C and then heated at various temperatures to reproduce production conditions. The results of these studies indicate that both the organic materials and salt content of kelp used in the product are responsible for halogenated dioxin and furan formation; it is unlikely that copper sulfate is involved in the transformation forming halogenated dioxins and furans (Ferrario, 2003).

Trace mineral and vitamin supplements may also suffer from the contamination by toxic heavy metal compounds. In the 1980s, the U.S. FDA cautioned consumers to restrict intake of calcium supplements, which are commonly used in aquaculture feeds, due to elevated concentrations of lead (Nriagu, 2007). Early studies of bone meal and dolomite supplements used for calcium and phosphorus fortification revealed elevated concentrations of lead and cadmium, while more recent studies of calcium supplements derived from calcium carbonate or chelate bound calcium have shown similar heavy metal contaminants (Nriagu, 2007). Although the lead content of calcium supplements available in North America has declined over the past 20 years, metal contamination of dietary supplements is now becoming a concern in other countries. For example, estimates of daily lead intake from Korean calcium supplements ranged from 0.1 μg to 11.35 μg (Nriagu, 2007). Limited information is available concerning the heavy metal content of other trace mineral supplements (e.g., magnesium, iron, zinc, etc); however, existing studies suggest that trace mineral supplements do not contribute significantly to the U.S. FDA's recommended maximum tolerable daily intake of heavy metals (Nriagu, 2007).

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

In the course of production, use, and disposal, mineral compounds may be released to soil and water. As highly water-soluble compounds, most minerals are expected to have some degree of mobility if released to soil and therefore may spread to other soil areas or directly to waterways. Studies have indicated that several polar pharmaceutically active compounds (i.e., drugs, vitamins, minerals, and other supplements) can leach through subsoils into aquifers (HSDB, 2006a). In general, water-soluble minerals do not volatilize from moist or dry soils due to their ionic nature (i.e., polarity) and low vapor pressures, respectively. If released to water, most water-soluble minerals are not expected to adsorb to suspended solids and sediment (HSDB, 2006a), remaining dissolved in solution. Adsorption may occur with less soluble mineral compounds such as tricalcium phosphate and calcium carbonate.

The environmental fate and toxicity of selenium is generally dependent upon whether it is in the biologically active form (U.S. EPA, 2010). If present in alkaline soils and oxidizing conditions, selenium may be sufficiently oxidized (as selenate) to maintain its biological availability. Alternatively, selenium in acidic or neutral soils tends to remain in the relatively insoluble form of selenite, which is not biologically available for plant uptake. Selenium is known to volatilize from soils when converted to volatile selenium compounds (e.g., dimethyl selenide) by microorganisms (U.S. EPA, 2010).

Eutrophication may result from the overload of nutrients in aquatic ecosystems. Notorious among agents of eutrophication are natural and synthetic phosphates included in agricultural fertilizers (NAS, 1969; Wu, 1995) and formerly in detergents. Dietary manipulations, such as reducing total dietary phosphorus or increasing the availability of phosphorus in the diet by adding the enzyme phytase or other additives such as citric acid, are effective for reducing urinary and fecal phosphorus excretions (NRC, 2011). In addition phosphorus and phosphates, vitamins, growth hormones, amino acids, and trace elements may also contribute to eutrophication and the explosive growth of algal species. For example, bacterial growth promotion in waters is the primary adverse environmental effect related to elevated iron concentrations (Jinwal, 2009). However, the inorganic phosphorus and nitrogen inherently present in fish feeds, feces, and other excrements are more likely to result in eutrophication than trace minerals due to the limited concentrations of the latter in commercial feeds. Industrial effluents consisting of trace minerals, however, may indeed contribute to the deleterious growth algal blooms (Jinwal, 2009).

The potential exists for contamination of water and soil resulting from the industrial production of several mineral compounds. Strong acids (e.g., sulfuric acid, nitric acid, and hydrochloric acid) used in the syntheses of numerous minerals may alter the pH of aquatic systems if accidentally released to the environment. Phosgene, a chlorinating agent used in the production of cobaltous chloride ($CoCl_2$), may be hydrolytically transformed to hydrochloric acid on contact with water and exerts additional toxic effects through the acylation of hydroxyl and sulfhydryl groups of proteins in the lungs (HSDB, 2008; IPCS, 1998). Release of caustic alkali hydroxides (e.g., sodium hydroxide, potassium hydroxide) solutions from the manufacturing site may lead to similar environmental impairments as those described for strong acids. In general, chemical manufacturers are required to limit the release of these and related industrial effluents that may lead to environmental contamination.

<u>Evaluation Question #7:</u> 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)).

No direct interactions between trace minerals and other aquatic animal feed additives were identified. For the current petition, trace minerals would be utilized in the manufacture of aquatic animal feed pellets, such as fish and shrimp feed. Aquatic organisms are not currently defined as “livestock” under 7 CFR 205.2 and, as such, it is unlikely that trace minerals petitioned for use in aquatic animal feed would regularly be combined or interact with substances used in organic crop or livestock production. However, the petitioned trace minerals are chemically equivalent to trace minerals that have been used in the fortification of organic livestock feed under 7 CFR 206.603. In the body, trace minerals interact as activators and cofactors in a variety of biological processes including DNA replication, cell signaling, and metabolism. Please see the “action of the substance” section for further details regarding the specific biological functions of the petitioned minerals.

The primary chemical interactions of trace minerals occur physiologically once inside the animal’s body. Some minerals are involved in biochemical reactions that generate essential compounds; for example, dietary iodine acts as an iodide source in the biological synthesis of thyroid hormones. In other cases, minerals interact with enzymes to effect important biochemical transformations and regulate the concentrations of other trace elements (FAO, 1987). An example of this category is the cooperative interaction of copper and ferroxidase, which is intimately involved in iron metabolism, hemoglobin synthesis, and red blood cell production (FAO, 1987). Alternatively, excesses of one particular trace mineral may cause deficiencies in another mineral or lead to toxic effects. Large doses of zinc may interfere with the absorption of copper compounds, while imbalances of the iron/zinc and selenium/zinc ratios may reduce the absorption of dietary zinc (Solomons, 1983; NRC, 2011). In addition, dietary calcium-to-phosphorus ratios in excess of ~2:1 may lead to growth abnormalities in commercial fish and shrimp species (NRC, 2011). It is presumed that the prescribed trace mineral supplementation in aquatic animal feed would be balanced for optimum health of the given farmed aquatic species (NRC, 2011).

Numerous synergistic and antagonistic effects have been observed for the absorption, bioavailability, and action of trace minerals and vitamins (Sandström, 2001; Vannucchi, 1991). The role played by vitamin D in calcium and phosphorus metabolism is a prime example of a synergistic interaction between vitamins and minerals (Vannucchi, 1991). Vitamin C acts as a strong promoter of dietary iron absorption while also counteracting the inhibitory effects of dietary phytate and tannins on iron levels. However, long-term vitamin C supplementation may diminish the absorption of copper, thereby countering the beneficial effect on iron absorption. Further, there is evidence that vitamin C affects the bioavailability of selenium both positively and negatively depending on the dietary conditions (Sandström, 2001). The synergistic interaction of selenium and vitamin E as an oxidant defense system has been observed in a number of species (Vannucchi, 1991). Vitamin A may promote the absorption of iron, thereby indirectly contributing to an increase in hemoglobin levels (Sandström, 2001). On the other hand, sufficient dietary levels of zinc are beneficial for the absorption of vitamin A (Smith, 1980).

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)).

The current petition concerns the use of trace minerals in the feed for organically raised aquatic animal species. Through this specific application, it is unlikely that the petitioned minerals would regularly interact with components of the terrestrial agro-ecosystem. More likely, however, are interactions resulting from the use of synthetic minerals in the organic production of terrestrial livestock (7 CFR 206.603). Synthetic minerals are widely used in conventional and organic livestock production with no reported toxicity observed in non-target wildlife or livestock. Any potential leakage of minerals from aquatic animal feeds near the agro-ecosystem would be neither routine nor widespread.

No studies have been found indicating toxic effects of minerals on soil-dwelling organisms. Virtually all microbial organisms require trace minerals for healthy growth and development, and are able to acquire minerals through the weathering of primary mineral sources in soils (Churchman, 2011). Therefore, minerals are unlikely to exhibit toxicity toward the agro-ecosystem despite their mobility in soils resulting from high water solubility and low tendency to adsorb onto soil particles (HSDB, 2001a, 2011a). Accidental release of chemical reagents during the production process, however, may lead to ecological impairment. Specifically, strong acids (e.g., sulfuric acid) are used in the extraction of trace elements from mineral ores. Improper use or disposal of these chemicals during the production of trace minerals could affect both the pH and chemical composition of the soil, potentially resulting in physiological effects on soil organisms.

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)).

Excessive amounts of artificial or natural nutrients in aquatic systems may lead to damaging eutrophication. Phosphates, which are present in naturally derived and synthetic fertilizers, are particularly potent initiators of eutrophication (NAS, 1969; Wu, 1995). In addition to these substances, vitamins, growth hormones, amino acids, and trace elements may also contribute to eutrophication and the explosive growth of algal species. For example, bacterial growth promotion in waters is the primary adverse environmental effect related to elevated iron concentrations (Jinwal, 2009). However, the inorganic phosphorus and nitrogen inherently present in fish feeds, feces, and other excrements are more likely to result in eutrophication than trace minerals due to the limited concentrations of the latter in commercial feeds. Industrial effluents consisting of trace minerals, however, may indeed contribute to the growth of deleterious algal blooms (Jinwal, 2009).

The potential exists for contamination of water and soil resulting from the industrial production of several mineral compounds. Strong acids (e.g., sulfuric acid, nitric acid, and hydrochloric acid) used in the syntheses of numerous minerals may alter the pH of aquatic systems if accidentally released to the environment. Release of caustic alkali hydroxides (e.g., sodium hydroxide, potassium hydroxide) solutions from the manufacturing site may lead to similar environmental impairments as those described for strong acids. Improper use or disposal of these chemicals during the production of trace minerals could affect both the pH and chemical composition of the soil, potentially resulting in physiological effects on soil organisms. In general, chemical manufacturers are required to limit the release of these and related industrial effluents that may lead to environmental contamination.

The petitioned substances are considered heavy metals, and as such have the potential for bioconcentration through interactions with various functional groups within enzymes and proteins (Agarwal, 2009). Persistence of certain trace mineral compounds, such as copper and iodide, has been observed in humans. Studies of acute zinc and iron toxicity in experimental fish populations supported a mechanism of fish death involving coagulation or precipitation of mucus on the gills ultimately leading to tissue hypoxia. Notwithstanding these observations, the risk of lethal effects from bioconcentration of the petitioned trace elements is considered low.

Potentially hazardous contamination of trace minerals may occur depending on the feedstock source. Certain members of the animal feed manufacturing industry have suggested that failure to completely remove insulation and other plastics prior to recycling the metals in feed supplements can result in dioxins and polychlorinated biphenyls (PCBs) mixed with the final trace mineral product (Alltech, undated). Further, dioxin and furan occurrences have been observed in feed supplements containing trace minerals (i.e., copper, zinc, manganese, magnesium, and iron); however, it was proven unlikely that the trace mineral components of these feeds were responsible for the contamination (Ferrario, 2003). Analytical studies of trace mineral supplements have indicated elevated levels of heavy metals (e.g., lead), but only the levels present in calcium supplements were cause for concern (Nriagu, 2007).

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)).

Environmental concentrations of trace minerals are unlikely to cause adverse health effects in humans. However, improper disposal of supplements containing vitamins or other pharmaceutical compounds, such as multivitamin and minerals supplements, may lead to environmental and toxicological issues. Any observed adverse impacts are typically due to the overload of organic compounds, which are not readily processed in municipal water treatment plants, as opposed to the trace mineral contents (U.S. EPA, 2011). Information regarding the reported effects of the petitioned substances on human health is provided below; however, these effects are not necessarily expected to result from the petitioned uses (i.e., aquatic animal feed supplements) of the substances. Human health effects specifically related to trace minerals in aquatic animal feeds have not been reported.

Copper

Liver damage is the critical adverse effect of copper poisoning for adults. Other adverse effects observed resulting from copper overload include abdominal pain, cramps, nausea, diarrhea, and vomiting. The tolerable upper intake level (UL, maximum level of daily nutrient intake that is likely to pose no risk of adverse effects) for copper is 10 mg per day (Driskell, 2009; Institute of Medicine, 2001).

Chromium

No adverse effects have been convincingly associated with excess intake of chromium from food or dietary supplements, although the risk of chromium toxicity still exists. The UL for chromium was not determined due to the lack of data on adverse effects (Driskell, 2009; Institute of Medicine, 2001).

Iodine

An excess of iodine may lead to elevated serum thyroid stimulating hormone (TSH) concentrations as the critical adverse effect. Acute responses to iodine poisoning include burning of the mouth, throat, and stomach; abdominal pain; fever; nausea; vomiting; diarrhea, weak pulse; cardiac irritability; coma; and cyanosis. Iodine poisoning may also lead to goiter production, increased risk of thyroid papillary cancer, and iodermia (skin irritation/rash similar to acne or hives). The UL for iodine is 1,100 micrograms (1.1 mg) per day (Driskell, 2009; Institute of Medicine, 2001).

Iron

Gastrointestinal side effects are the critical adverse effects of iron poisoning. Other effects include impaired zinc absorption, increased risk for vascular disease and cancer, and systemic iron overload. The UL for iron is 45 mg per day (Driskell, 2009; Institute of Medicine, 2001)

Manganese

Critical adverse effects resulting from manganese poisoning are elevated blood manganese concentration and neurotoxicity. The UL for manganese is 11 mg per day (Driskell, 2009; Institute of Medicine, 2001).

Selenium

Hair and nail brittleness and loss are critical adverse effects of selenium poisoning. Other effects include gastrointestinal disturbances, skin rash, garlic breath odor, fatigue, irritability, and nervous system disorders. The UL for selenium is 400 micrograms (0.4 mg) per day (Driskell, 2009; Institute of Medicine, 2000).

Zinc

No adverse effects for zinc through the consumption of foods have been observed. The influence of excess zinc on copper metabolism may be interpreted as the critical adverse effect of excess zinc. Other effects include epigastric pain, nausea, vomiting, loss of appetite, abdominal cramps, diarrhea, headaches, and immune response impairment. The UL for zinc is 40 mg per day (Driskell, 2009; Institute of Medicine, 2001).

Calcium

The critical adverse health effect of excess calcium is kidney stone formation or milk-alkali syndrome (hypercalcemia and renal insufficiency/failure). Calcium may also affect the absorption of iron, zinc, magnesium, and phosphorus. The UL for zinc is 2.5 g per day (Driskell, 2009; Institute of Medicine, 1997).

Magnesium

Any adverse health effects observed for magnesium generally result from nonfood sources, such as magnesium salts used for pharmacologic purposes. The critical effect is osmotic diarrhea. Other effects of magnesium poisoning include nausea, abdominal cramping, serious neurological and cardiac symptoms, and death. The UL for magnesium is 350 mg per day (Driskell, 2009; Institute of Medicine, 1997).

Potassium

No adverse effects observed from dietary intake of potassium-rich foods. Individuals with impaired urinary potassium secretion may experience adverse effects. The UL for potassium was not determined due to the lack of data of adverse effects (Driskell, 2009)

Sodium

Some individuals are salt-sensitive and exhibit adverse effects of blood pressure following dietary intake of excess sodium. Other adverse effects included cardiovascular abnormalities, increased urinary calcium excretion, osteoporosis, gastric cancer, and asthma. The UL for sodium is 2.3 g per day (Driskell, 2009; Institute of Medicine, 2005).

Chlorine

Sodium chloride constitutes the major contribution to chlorine intake. Because chloride is assumed to be in foods in equimolar quantities with sodium, the UL for chloride (UL = 3.6 g per day) was set based on the UL of sodium above (Driskell, 2009; Institute of Medicine, 2005).

Phosphorus

Hyperphosphatemia (abnormally elevated blood phosphate level) may results from excessive phosphorus intake. Other effects include hypocalcemia (reduced calcium levels), adjustments in calcium-regulating hormones, and calcification of nonskeletal tissues, especially the kidneys. The UL for phosphate is 3–4 grams per day (Driskell, 2009; Institute of Medicine, 1997).

<u>Evaluation Question #11:</u> 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)).

There are no direct substitutes for trace minerals; however, natural, non-synthetic sources of trace mineral compounds do exist. Approximately 20 inorganic mineral elements have been isolated from or identified in biological materials, and are considered essential to the health and well-being of animals, including fish and shrimp. Of these mineral elements, calcium, phosphorus, magnesium, sodium, chloride, potassium, iron, zinc, manganese, copper, iodine, cobalt, chromium, and selenium may be combined in trace mineral premixes for inclusion in aquatic animal feed pellets. Fish and crustaceans readily absorb the petitioned minerals from the gastro-intestinal tract and the surrounding water through gills, fins, and skin. Trace minerals are also present in very small quantities within animal and plant foodstuffs; natural (non-synthetic) sources of the petitioned minerals are identified below:

- **Iron:** Blood meal, kelp meal, coconut meal, meat and bone meal, sunflower seed meal, dried distillers solubles, alfalfa meal, crab meal, condensed fish solubles, fish meal, meat meal, poulty by-product meal, linseed meal dried brewers yeast, dehydrated cane molasses, rice bran, delactose whey powder, and dried poultry manure.
- **Zinc:** Chick hatchery meal, dried Candida yeast, dehydrated fish solubles, dried distillers grains with solubles, dried poultry manure, fish meal, corn gluten meal, poultry and by-product meal, wheat bran, rice mill run, dehydrated cattle manure, wheat middlings, crab meal, sunflower seed meal, dried torula yeast.
- **Manganese:** Kelp meal, rice bran, dehydrated poultry manure, palm kernel, crab meal, wheat bran, wheat germ meal, wheat mill run, wheat middlings, dehydrate cattle manure, corn distillers dried solubles, rye grain, dehydrated cane molasses, dehydrated fish solubles, copra meal, wheat, rapeseed meal, sesame seed meal, linseed meal, brewers dried grains, safflower seed meal, shrimp meal, and oats.
- **Copper:** Condensed fish solubles, corn distillers dried solubles, dehydrated sugar cane molasses, corn distillers grains with solubles, dehydrated poultry manure, dried brewers yeast, crab meal, corn gluten meal, linseed meal, soybean meal, dried brewers grains, wheat mill run, millet, cottonseed meal, wheat middlings, and copra meal.
- **Cobalt:** Copra meal, linseed meal, dried brewers yeast, fish meal, meat meal, cottonseed meal, and soybean meal.
- **Iodine:** All foodstuff of marine origin, and in particular seaweed meals, marine fish, and crustacean meals.
- **Selenium:** Dehydrated fish solubles, fish meal, dried brewers yeast, corn gluten meal, dried torula yeast, rapeseed meal, cottonseed meal, dried brewers grains, wheat bran, wheat middlings, linseed meal, hydrolyzed feather meal, poultry by-product meal, meat meal, and alfalfa.
- **Chromium:** Chick shell meal, shrimp tail meat, Artemia salina, dried brewers yeast, shellfish, liver, poultry by-product meal, fish meal.
- **Calcium:** Limestone, oystershell grit, bone meal, rock phosphate, crab meal, shrimp meal, meat and bone meal, white fish meal, poultry manure, meat meal, brown fish meal, delactose whey powder, dried skim milk, poultry by-product meal, kelp meal, alfalfa meal.
- **Magnesium:** Meat and bone meal, rice bran, kelp meal, sunflower seed meal, wheat bran, wheat mill run, rice polishings, rapeseed meal, shrimp meal, cottonseed meal, linseed meal, poultry manure, crab meal.
- **Sodium:** Kelp meal, condensed fish solubles, dried delactose whey, shrimp meal, white fish meal, meat meal, meat and bone meal.
- **Potassium:** Dehydrated cane molasses, condensed fish solubles, delactose whey powder, alfalfa meal, dried torula yeast, soybean meal, rice bran, dried brewers yeast, dried distillers solubles, wheat bran, cottonseed meal, meat and bone meal, wheat mill run, copra meal, rapeseed meal, peanut meal, sunflower seed meal.
- **Chlorine:** Salt (sodium chloride) and potassium chloride.
- **Phosphorus:** rock phosphate, dicalcium phosphate, bone meal, meat and bone meal, white fish meal, shrimp meal, poultry by-product meal, dried poultry manure, rice bran, rice polishings, wheat bran, wheat mill run, dried brewers yeast, sunflower seed meal, cottonseed meal, rapeseed meal, sesame seed meal, dried delactose whey.

Of all practical animal feed ingredients, fish meal is the richest source of endogenous minerals. From the above discussion, it is apparent that fish meals and other fish products satisfy virtually all protein, oil, and other metabolic requirements while also providing a majority of the required micronutrients. Therefore, diets composed of either whole fish or conventional aquatic animal feed pellets containing fish meals and oils naturally supply many of the trace minerals and other essential nutrients required by farmed aquatic species. A combination of fish meals and oils may be used in combination with plant-based meals to reduce the amount of fish products required for aquatic animal feeds while simultaneously meeting nutritional requirements of the farmed species. Although vitamin and trace mineral premixes are potentially unnecessary to meet nutritional needs, trace minerals are typically included as ingredients in feed pellets for aquatic animals at approximately 0.1–0.2% of the feed pellet mass (Aquaculture Working Group, 2012).

In contrast to some organic nutrients, such as vitamins, inorganic trace mineral compounds are generally considered stable in foods. However, when food is cooked, processed, or stored, minerals may be lost, new compounds formed, the food environment changed, and new external factors introduced (Smith, 1988). Any of these changes may decrease the concentrations of trace mineral compounds in foods and animal feeds. Leaching and separation of trace minerals may occur as a result of the procedures utilized in the processing of aquatic animal feeds (Smith, 1988). Boiling in less water or pressure-cooking, which is commonly used prior to the extrusion process, can increase trace mineral retention. While milling and refining processes are also responsible for trace mineral losses from wheat and other grains, the nutrient profile of grain products may be improved when refinement stages are minimized and whole grains are utilized (Smith, 1988). Data examining mineral stability in extruded foods are scarce; however, studies of vitamin and mineral stability in extruded fortified rice kernels revealed significant decrease in mineral content following extrusion (Hof, 2007). The authors noted that this result might be due to improper sampling rather than the extrusion process.

To mitigate the demands on forage fish, the aquaculture industry is currently relying on fish feeds comprised of plant-based meals, such as soy and corn meals, as replacements for fish-meal-containing feeds (USDA, 2010). This practice helps to reduce the ever-growing demand on forage fish from marine and other aquatic environments. At the same time, these feeds may not be nutritionally complete for most aquatic organisms, and their use necessitates the supplementation of aquatic animal feeds with synthetic essential amino acids, vitamins and minerals (Allen & Steeby, 2011; NOAA, undated). Specifically, plant-based feeds are generally poor sources of minerals and may contain factors (e.g., phytate) that reduce the bioavailability of minerals (NRC, 2011). Therefore, as the aquatic animal feed industry increases its use of plant feedstuffs, the need for mineral supplements is likely to increase.

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

Wild caught fish and shrimp stand out as the leading alternative for naturally sourced seafood when low-impact techniques prioritizing environmental stewardship are properly exercised. This method allows the given aquatic species to forage its own natural food in the environment, thereby avoiding synthetic nutrients intentionally included in manufactured feed products and minimizing the risk of exposure to disease and exogenous chemical substances. However, the term "organic" is solely applied to agriculture, and catching wild animals does not align with the definition of agriculture (Martin, 2006). In the absence of allowed synthetic substances, farmed fish and shellfish industries should strive to replicate natural living conditions and feeding habits of wild aquatic species or use alternative feeds that naturally meet all dietary requirements.

Certain aquatic animal farming practices limit the utilization of commercial feeds, and therefore synthetic trace mineral mixes. Farmed fish and other aquatic organisms forage when reared in natural environments (i.e., marine systems, lakes, ponds, and rivers), obtaining natural sources of vitamins, minerals, and other nutrients (Craig, 2009). Supplemental feeds are only incorporated when the natural supply is inadequate, in which case a combination of multiple naturally derived feeds is utilized to provide a balanced diet. Aside from feeds, many of the required trace minerals are readily absorbed through the gills and flesh of fish and crustaceans when reared in natural environments. Zooplankton present in natural ponds provide many of the micronutrients recommended for aquatic animals albeit in small quantities (Robinson, 2001). However, when fish are reared in high-density indoor systems or confined in cages and cannot forage freely on natural feeds, these organisms must be provided complete diets typically consisting of processed and fortified feeds (Craig, 2009).

Uncertainty exists regarding the necessity of trace mineral fortification in aquatic animal feeds (FAO, 1981; Robinson, 2001). Dietary investigations of *Clarias macrocephalus* (or Broadhead catfish) in Thailand suggest that vitamin and mineral premixes are necessary ingredients in basic artificial feeds constituted from local sources (FAO, 1981). In this study, weight gains and survival rates were approximately double in the test group treated with vitamin and mineral premixes (trace minerals included copper, zinc, iodine, manganese, and iron) as opposed to the groups without premix supplementation. Alternatively, researchers from the Mississippi Agricultural and Forestry Experiment Station posit that supplemental (i.e., synthetic) trace minerals are unnecessary in catfish feeds containing 4–5% or more animal protein (Robinson, 2001). Variability in trace mineral content and dietary requirements is expected depending on the feed material source and fish species under consideration, respectively.

A number of commercial feed alternatives are either available or in development (USDA, 2010; NOAA, undated). Natural feed manufacturers typically utilize milder processing and extrusion conditions such that fewer micronutrients are lost, and synthetic chemicals are not added to these feeds. A combination of plant-based (e.g., soy meal, corn meal, cottonseed, etc.) and animal-based (i.e., fish meal) feeds may adequately meet dietary requirements, thereby precluding the supplementation of synthetic trace mineral compounds in aquatic animal feeds. Due to the rising demand for forage fish and resulting fish meal, many aquaculture professionals are turning to alternative nutrient sources, including soy, corn, and algae (USDA, 2010). A combination of these alternative feeds, fish feeds, and milder pellet manufacturing conditions may provide a balanced diet of required nutrients to aquatic organisms without fortification using synthetic trace minerals.

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