Vitamins

Aquaculture - Aquatic Animals

Identification of Petitioned Substance

This technical report discusses 15 specific vitamins petitioned for use in organic aquatic animal production as a feed premix additive. The scope of vitamin 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 (Aquaculture Working Group, 2012; NRC, 2011). Herein, information is provided about the petitioned vitamins individually and collectively per the availability of information. Individual vitamins potentially exist in a variety of biologically active forms; for the purposes of this discussion, the chemical derivative most likely present in vitamin supplements was chosen. Vitamins C and D are well known examples of vitamins, and a previous technical report evaluated the use of vitamin D_3 as a rodenticide (USDA, 2009). As such, vitamins C and D_3 are discussed as specific examples in portions of this report.

Table 1. Petitioned Vitamins Used in Aquaculture Feed

Common Name	Chemical Name	CAS Number	Trade Names	Other Codes
Vitamin A (Retinyl Acetate, etc.)	(2*E*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-yl acetate	127-47-9	Vitamin A acetate	EINECS: 204-844-2
Vitamin B_1 (Thiamine)	2-[3-[(4-Amino-2-methyl-pyrimidin-5-yl)methyl]-4-methyl-thiazol-5-yl] ethanol	59-43-8	Vitamin B_1 hydrochloride	EINECS: 200-425-3
Vitamin B_2 (Riboflavin)	7,8-Dimethyl-10-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl] benzo[*g*]pteridine-2,4-dione	83-88-5	Riboflavin (B2)	EINECS: 201-507-1
Vitamin B_3 (Niacin)	Pyridine-3-carboxylic acid	59-67-6	Nicotinic Acid	EINECS: 200-441-0
Vitamin B_5 (Pantothenic Acid)	3-[(2,4-Dihydroxy-3,3-dimethylbutanoyl)amino] propanoic acid	137-08-6	D-pantothenic acid hemicalcium salt	EINECS: 205-278-9
Vitamin B_6 (Pyridoxine)	4,5-Bis(hydroxymethyl)-2-methylpyridin-3-ol	58-56-0	Vitamin B_6 hydrochloride	EINECS: 200-386-2
Vitamin B_7 (Biotin)	5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoic acid	58-85-5	Biotin; Coenzyme R; Vitamin H)	EINECS: 200-399-3
Inositol	(1*R*,2*R*,3*S*,4*S*,5*R*,6*S*)-cyclohexane-1,2,3,4,5,6-hexol	87-89-8	*myo*-inositol	EINECS: 201-781-2
Vitamin B_9 (Folic Acid)	(2*S*)-2-[(4-{[(2-amino-4-hydroxypteridin-6-yl)methyl]amino}phenyl)formamido]pentanedioic acid	59-30-3	Folic acid	EINECS: 200-419-0
Choline	(2-hydroxyethyl) trimethylammonium chloride	67-48-1	Choline Chloride	EINECS: 200-655-4
Vitamin C (L-Ascorbic Acid)	(*R*)-3,4-dihydroxy-5-((*S*)- 1,2-dihydroxyethyl)furan-2(5*H*)-one	50-81-7	L-Ascorbic acid	EINECS: 200-066-2
Vitamin D (Cholecalciferol, etc)	(3β,5*Z*,7*E*)-9,10-secocholesta-5,7,10(19)-trien-3-ol	67-97-0	Cholecalciferol (D_3); Vitamin D_3	EINECS: 200-673-2
Vitamin E	(2*R*)-2,5,7,8-Tetramethyl-2-	59-02-9	α-Tocopherol	EINECS:

Common Name	Chemical Name	CAS Number	Trade Names	Other Codes
(Tocopherols)	[(4*R*,8*R*)-(4,8,12-trimethyltridecyl)]-6-chromanol			200-412-2
Vitamin K (menadione sodium bisulfate)	2-methyl-1,4-naphthoquinone sodium bisulfite	130-37-0	Vitamin K_3	EINECS: 204-987-0
Vitamin B_{12} (cobalamin)	α-(5,6-dimethylbenzimidazolyl cyanocobamide	68-19-9	Cyanocobalamin	EINECS: 200-680-0

Summary of Petitioned Use

The petitioner, the Aquaculture Working Group, is requesting the addition of vitamins (i.e., vitamin A, vitamin B_1, vitamin B_2, vitamin B_3, vitamin B_5, vitamin B_6, vitamin B_7, inositol, vitamin B_9, vitamin B_{12}, choline, vitamin C, vitamin D, vitamin E, and vitamin K) to the National List for the fortification of feeds used in organic aquatic animal production. Specifically, the petitioner is seeking the addition of vitamins, including those listed in Table 1, to the National List as Synthetic Substances Allowed for Use in Organic Aquatic Animal Production. Petitioned vitamins would be included as ingredients in feed pellets for aquatic animals at approximately 0.5% to 1.5% of 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 vitamins in organic livestock production for enrichment or fortification, under 7 CFR 205.603, Synthetic Substances Allowed for Use in Organic Livestock Production.

Characterization of Petitioned Substance

Composition of the Substance:

Vitamin premixes used to fortify animal feed are composed of 10–15 essential vitamins, organic chemical compounds not ingested or synthesized in sufficient quantities by a given animal species. Additional vitamins may be individually supplemented in the feed depending on the organism being fed (Sewell, 1993). Traditionally, vitamins are categorized based on their solubility properties: Vitamin C and most of the B-vitamin complex group compounds are water-soluble while vitamins A, D, E, and K are fat-soluble. In Figures 1 and 2, the structures of vitamin D_3 (cholecalciferol) and vitamin C (ascorbic acid) are presented as examples of fat- and water-soluble vitamins, respectively. In contrast to fat-soluble vitamin compounds, the structures of water-soluble vitamins contain multiple polar functionalities and/or exist as the corresponding salt (see Figure 2) (Friedrich, 1988).

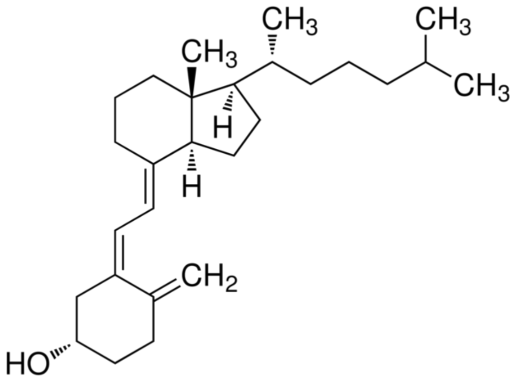

Figure 1. Structure of Vitamin D_3 (cholecalciferol)
Source: ChemIDplus Lite (2012)

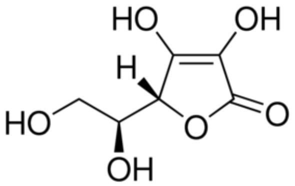

Figure 2. Structure of Vitamin C (Ascorbic Acid)
Source: ChemIDplus Lite (2012)

Source or Origin of the Substance:

Vitamins can be extracted from foods or synthesized by chemical or biofermentation processes. Regarding the former, certain vitamins can be obtained from natural dietary sources in varying quantities. For example, Vitamin C (ascorbic acid) is a major nutritional component of citrus fruits and Vitamin D is a natural constituent nutrient of cold-water fish. Individual vitamin compounds used in vitamin supplements may be generated chemically using synthetic methods, obtained through extraction from biological sources, and/or produced through biological fermentation processes. Regarding the latter category, the patent literature indicates that the last decade has seen the development of a growing number of methods for the fermentative production of individual vitamin compounds utilizing genetically modified microorganisms (GMMs). This report provides information on both the current commercial production methods and an analysis of trends in the application of GMMs in the synthesis of individual vitamins.

Properties of the Substance:

As a result of the structural diversity among the vitamin compounds, there is great variability in the physical and chemical properties of vitamins as a chemical class. Vitamins are organic (i.e., carbon-containing) compounds and are typically grouped depending on their solubility in water vs. organic solvents. The more hydrophilic vitamin compounds tend to have multiple polar functionalities (i.e., hydroxyl groups, amino groups, carboxylic acids, alkoxy groups, and/or salts of carboxylic acids). Due to their enhanced aqueous solubility, molecules not metabolized by the organism are rapidly excreted. Alternatively, more lipophilic vitamins are primarily comprised of aliphatic and aromatic carbon frameworks and are stored in animal fat tissues upon consumption of an excess of the vitamin. As a class of substances, vitamins have a relatively low vapor pressure (HSDB, 2005a; 2006; 2010a).

Vitamin A

Retinyl acetate, a synthetic form of vitamin A commonly used in vitamin supplements and feeds, has been listed as pale yellow crystals and a yellow to yellow-brown viscous oil (HSDB, 2005b). The melting point of crystalline retinyl acetate is listed as 57–58 °C. Most forms of vitamin A are practically insoluble in water or glycerol; however, solubility is observed in alcoholic solvents, chloroform, ether, fats and oils. For example, the solubility of retinyl acetate in absolute ethanol is 25 mg/mL (ChemicalBook, 2010b).

Vitamin C

Vitamin C (L-ascorbic acid) is a colorless crystalline powder or solid. It has a pH of 1.0–2.5 at 176 g/L at 25 °C. The melting point/range of pure L-ascorbic acid is 190–194 °C. L-ascorbic acid is highly soluble in water (solubility of 176 g/L at 20 °C). In addition, L-ascorbic acid exhibits air and light sensitivity and, as an antioxidant, it acts as a strong reducing agent with some organic compounds (Sigma Aldrich, 2012; Fisher Scientific, 2012).

Vitamin D

Vitamin D_3 (cholecalciferol) exists as a white crystalline powder or solid. While cholecalciferol has negligible water solubility (<0.1 g/L at 20 °C), it is soluble in organic hydrocarbon and aromatic solvents. Cholecalciferol is a neutral compound with no acid-base properties. It has a melting point/range of 84–85 °C. Cholecalciferol may react vigorously and exothermically in the presence of strong oxidizing and reducing agents, respectively (ChemicalBook, 2010; Acros Organics, 2009).

Vitamin B_1

Vitamin B_1 (thiamine) and thiamine hydrochloride, a commonly used supplemental form of vitamin B_1, are colorless solids with melting points of 164 and 250 °C (HSDB, 2010c; ChemicalBook, 2010k). One gram of thiamine dissolves in approximately 1 mL water, 18 mL glycerol, 100 mL 95% alcohol, or 315 mL absolute alcohol; thiamine is practically insoluble in ether, benzene, hexane, and chloroform. The pH of 1% wt/vol solution of thiamine in water is 3.13 (HSDB, 2010c). Chemical forms of vitamin B_1 are generally light sensitive and hygroscopic (ChemicalBook, 2010k).

Vitamin B_2

Pure vitamin B_2 (riboflavin) is a solid and may be isolated as orange to yellow needles or crystals. The melting point of vitamin B_2 is approximately 280–290 °C, at which point the substance decomposes. Vitamin B_2 is soluble in sodium chloride solutions and has a solubility of 0.0045 g/100 mL in absolute ethanol at 27.5 °C. Slight solubility has been observed in cyclohexanol, amyl acetate and benzyl alcohol, phenol and vitamin B_2 is insoluble in ether, chloroform, acetone, and benzene. Saturated aqueous solutions of vitamin B_2 have a pH of approximately 6. Vitamin B_2 exhibits light sensitivity and is incompatible with strong oxidizing and reducing agents, bases, calcium and metallic salts (HSDB, 2010b; ChemicalBook, 2010c).

Vitamin B_3

Nicotinic acid, a commercial form of vitamin B_3, exists has a colorless powder with a melting point/range of 236-239 °C. It has a superior water solubility of 15 g/L at 20 °C and 150 g/L at 100 °C as well as ethanol solubility (12.5 g/L at 25 °C) (Sigma Aldrich, 2012). Vitamin B_3 is stable overall, but is incompatible with strong oxidizing agents and may be light sensitive (ChemicalBook, 2010e).

Vitamin B_5

Calcium pantothenate is a common form of vitamin B_5 used for fortification. It has a melting point of 190 °C and water solubility of 50 mg/mL at 25 °C. A concentrated aqueous solution (50 g/L) of vitamin B_5 has a pH of 7–8. The pure substance is stable but may be air or moisture sensitive. Vitamin B_5 is incompatible with strong acids and bases (ChemicalBook, 2010f; Sigma Aldrich, 2012).

Vitamin B_6

Pyridoxine hydrochloride, which is the common supplemental form of vitamin B_6, is typically isolated as a white powder or colorless crystals with a melting point/range of 214–215 °C. Its solubility in water is 0.1 g/mL at 20 °C, and forms acidic solutions in water (pH = 3.2 at 10%weight in volume). In addition, vitamin B_6 exhibits solubility in alcohol (1 g in 90 mL alcohol), but is sparingly soluble in acetone and insoluble in ether and chloroform. The substance is considered to be light sensitive (ChemicalBook, 2010d; HSDB, 2002).

Vitamin B_7

Vitamin B_7 (i.e., vitamin H, biotin) is a colorless crystalline solid with a melting point/range of 231–233 °C, at which point the substance decomposes. It is slightly soluble in organic solvents, such as chloroform and ether. Likewise, it is slightly soluble in aqueous solution (0.2 mg/mL), but its salts are significantly more soluble in water. Additionally, vitamin B_7 is light sensitive, incompatible with strong oxidizing agents, strong acids and bases, and formaldehyde (ChemicalBook, 2010g; HSDB, 2007).

Inositol

Myo-inositol, the biologically prominent form of inositol, is generally isolated as a white powder or crystalline solid. It has a melting point/range of 220–228 °C. The water solubility of *myo*-inositol is 140 g/L at 25 °C, and is likely soluble in some polar organic solvents, such as ethanol and acetone. It is incompatible with strong oxidizing agents and decomposes to carbon monoxide and carbon dioxide (Sigma Aldrich, 2012; Acros Organics, 2011).

Vitamin B_9

Folic acid, the dietary form of vitamin B_9 is a yellow-orange crystalline powder having a melting point of 250 °C. In this form, vitamin B_9 is practically insoluble in water (water solubility = 1.6 mg/L). The pH of a saturated aqueous solution of vitamin B_9 (1 gram per 10 mL suspension) is 4.0–4.8. Vitamin B_9 is incompatible with heavy metal ions, and strong oxidizing and reducing agents. Solutions of vitamin B_9 may be light and heat sensitive (Acros Organics, 2009; ChemicalBook, 2010h).

Vitamin B_{12}

Synthetic vitamin B_{12} is generally isolated as a dark red crystalline solid having a melting point of > 300 °C. Vitamin B_{12} is moderately soluble in water; aqueous solutions of the substance have a neutral pH. Stability is generally observed under standard temperatures and pressures, but decomposition may occur upon exposure to light. Hazardous decomposition products include carbon monoxide, oxides of nitrogen and phosphorus, carbon dioxide, and oxides of cobalt (Acros Organics, 2009).

Choline

Pure choline chloride exists as a white solid. The melting point/range of choline chloride is 302–305 °C at which point the substance decomposes. Choline chloride is readily soluble in aqueous solution (water solubility = 140 g/L). Saturated aqueous solutions of choline chloride (concentration = 140 g/L) exhibit a pH range of 5.0–6.5 at 25 °C. The substance is incompatible with strong oxidizing and reducing agents as well as strong acids and bases (Sigma Aldrich, 2012; ChemicalBook, 2010).

Vitamin E

The most biologically active form of vitamin E is α-tocopherol. It exists as a yellow-brown viscous oil with a melting point/range of 200–220 °C and a density of 0.95 g/mL at 20 °C. As a fat-soluble vitamin, all forms of vitamin E are insoluble in water and soluble in many non-polar organic solvents. Due to its antioxidant properties, vitamin E may also react violently with oxidizing agents. Combustion of vitamin E may lead to the production of carbon oxides (Sigma Aldrich, 2012; ChemicalBook, 2010i).

Vitamin K

Menadione sodium bisulfite, a synthetic form of vitamin K, is a solid material with a melting point/range of 121–124 °C. Due to its ionic nature, vitamin K_3 exhibits water solubility. Combustion of vitamin K_3 may result in the formation of carbon oxides, sulfur oxides, and sodium oxides (ChemialBook, 2008). Vitamin K_1, a natural form of vitamin K, is a viscous liquid having a density of 0.984 g/mL at 25 °C. In contrast to synthetic vitamin K_3, vitamin K_1 is insoluble in aqueous solution and soluble in various non-polar organic solvents (ChemicalBook, 2010l).

Specific Uses of the Substance:

Vitamins are included in nutritional supplements, pest control substances, and feedstock chemicals for research and industrial processes. Green chemistry research has exploited the reactivity of these naturally derived compounds; for example, thiamine salts were developed as catalysts for the Benzoin Condensation, effectively replacing toxic cyanide salts (Jenkins, 2009). Vitamin D_3 has also been developed as an effective rodenticide in gel and pellet baiting products for gophers, mice, rats, and other rodents (ATTRA, 2006).

Although a number of chemical compounds are classified as vitamins, this term is conditional on the particular animal species in question. In the case of aquaculture, the petitioner has identified vitamins A, B_1, B_2, B_3, B_5, B_6, B_7, B_9, B_{12}, C, D, E, K, inositol, and choline as essential vitamins (Aquaculture Working Group, 2012). The National Research Council's (NRC's) Nutrient Requirements for Fish and Shrimp defines all essential vitamin compounds as "required nutrients" (NRC, 2011). Accordingly, the aquaculture industry has petitioned the National Organic Standards Board to permit vitamin compounds considered "required" by NRC for use in organic aquatic animal production. The aquaculture industry has also emphasized the importance of well-balanced vitamin mixes for the health and productivity of finfish and shellfish populations; vitamins are normally incorporated into conventional fish feed (DSM Nutrition, 2011a).

A number of vitamins are commonly used in the fortification of conventional as well as organic terrestrial livestock feed. Of all farmed species, poultry receives the highest proportion of its feed, and therefore vitamins, from manufactured sources (DSM Nutrition, 2011b). The production of poultry, meat and eggs relies on dietary intake of 13 required vitamins (NRC, 1994), many of which are supplied through synthetic sources. Although bacteria in the rumen of beef cattle are able to synthesize sufficient quantities vitamin K and the B vitamins, these animals are typically supplemented with external sources of vitamins A, D, and E (Sewell, 1993). Deficiencies in these required nutrients have been observed for cattle having diets devoid of leafy roughage and/or vitamin fortification of the animal feed. Vitamin A is more heavily fortified in cattle feed than vitamins D and E, with an application rate of 1,000 to 1,500 IU of vitamin A per pound of feed. Muscular injection of vitamin A more efficiently increases liver stores of this vitamin than feed supplementation (Sewell, 1993).

Human dietary supplements generally contain a combination of essential nutrients, including vitamins. Higher intake or topical application (e.g., vitamin A) of certain vitamins is particularly important for post-operative patients. For example, recent scientific literature suggested the intake of 500 mg/day of vitamin C to minimize postoperative oxidative stress (Fukushima, 2010). Additionally, food products are commonly fortified with vitamins and other essential nutrients to facilitate sufficient public consumption of these compounds. Typical examples of food vehicle-vitamin combinations include oils and dairy products for vitamin D supplementation, and cereals and grain products for B complex vitamins and other vitamin fortification (FAO, undated).

Approved Legal Uses of the Substance:

Conventional Aquaculture and Terrestrial 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 vitamin 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). The FDA has not issued specific recommendations for vitamin intake in aquatic animal species; however, the following vitamins used in animal and fish feeds are classified as Generally Recognized As Safe (GRAS) by the FDA and therefore not subject to additional regulatory oversight (OMRI, 2009):

- Vitamin A (vitamin A acetate) 21 CFR 582.5933
- Vitamin B_1 (thiamine hydrochloride) 21 CFR 582.5875
- Vitamin B_2 (riboflavin) 21 CFR 582.5695
- Vitamin B_3 (niacin, nicotinic acid) 21 CFR 582.5530
- Vitamin B_5 (calcium pantothenate) 21 CFR 582.5212
- Vitamin B_6 (pyridoxine hydrochloride) 21 CFR 582.5676
- Vitamin B_7 (biotin) 21 CFR 582.5159
- Vitamin B_{12} (cyanocobalamin) 21 CFR 582.5945
- Vitamin C (ascorbic acid) 21 CFR 582.5013
- Choline chloride 21 CFR 582.5252
- Vitamin D_3 (cholecalciferol) 21 CFR 582.5953
- Vitamin E (α-Tocopherol acetate) 21 CFR 582.5892
- Inositol 21 CFR 582.5370

In general, additives including vitamins, minerals, other nutrients, flavorings, preservatives, or processing aids must 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. With the exception of vitamin K_3 (menadione dimethylpyrimidinol bisulfite), all petitioned vitamins are referenced in 21 CFR 582, GRAS substances. Menadione, a synthetic version of vitamin K, is listed under 21 CFR 573, Food Additives Permitted in Feed and Drinking Water of Animals. Although K_3 is allowed as nutritional supplement in conventional chicken and turkey feed for the prevention of vitamin K deficiency (21 CFR 573.20), it is not approved for use in human or prenatal supplements or any other food products (FDA, 2008).

Organic Livestock Feed

The National Organic Program (NOP) final rule currently allows the use of vitamins in organic livestock production under 7 CFR 205.603, Synthetic Substances Allowed for Use in Organic Livestock Production, for enrichment or 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 meet nutritional requirements, including vitamins, minerals, 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 vitamins 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. The nutrient profiles are provided below (Table 2). In contrast to its role in the regulation of drugs and animal feed additives, the FDA does not regulate human dietary supplements (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: Vitamins

Vitamin	Unit of Measurement	DRV or RDI	Amount per 100 calories
Vitamin A	IU	5,000	250
Vitamin C	mg	60	3
Vitamin D	IU	400	20
Vitamin E	IU	30	1.5
Vitamin B_1 (thiamine)	mg	1.5	0.08
Vitamin B_2 (riboflavin)	mg	1.7	0.09
Vitamin B_3 (niacin)	mg	20	1
Vitamin B_6 (pyridoxine)	mg	2.0	0.1
Vitamin B_9 (folate)	µg	400	20
Vitamin B_{12} (cobalamin)	µg	6.0	0.3
Vitamin B_7 (biotin)	mg	0.3	0.015
Vitamin B_5 (pantothenic acid)	mg	10	0.5

IU = International Unit, unit of activity or potency for vitamins and other substances; 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

Four synthetic vitamin compounds are also allowed for use in organic crop production. Vitamins B_1, C, and E are included on the National List of allowed synthetic substances for use as plant or soil amendments in organic crop production (7 CFR 205.601(j)(8)). In addition, vitamin D_3 (cholcalciferol) is on the National List as an allowed synthetic rodenticide in organic crop production (7 CFR 205.601(g)).

<u>Action of the Substance:</u>

Dietary intake of vitamins is essential for the health and well being of farmed aquatic species. In particular, most vitamins aid in the metabolism of proteins, carbohydrates, and fats while some vitamin compounds have important antioxidant properties. Common signs of vitamin deficiency include anorexia, poor growth, reduced feeding efficiency and, in some cases, mortality. The petitioned vitamins and details regarding their specific functions in fish and shrimp are as follows:

Vitamin A (retinol)

Vitamin A is required for normal vision; in the retina of the eye vitamin A is combined with a specific protein (opsin) to form a visual pigment, which in turn functions in the reception and transmission of light from the eye to the brain. In addition, vitamin A is required for the maintenance of the mucous secreting epithelial tissues of the reproductive tract, skin, bone and gastro-intestinal tract. Reduced growth, exopthalmia (bulging eyes), depigmentation, clouding and thickening of corneal epithelium, degradation of the retina, and anorexia are common symptoms of vitamin A deficiency.

Vitamin B_1 (thiamine)

In the form of its di-phosphate ester (thiamine pyrophosphate, TPP), vitamin B_1 functions as a coenzyme in carbohydrate metabolism. In particular, TPP is involved in formation of acetylcoenzyme A and succinyl coenzyme A via carbon dioxide removal of pyruvic acid and alpha-ketoglutaric acid, respectively. It is also involved in the oxidation of glucose via the pentose phosphate pathway. Symptoms of vitamin B_1 deficiency include anorexia, poor growth, nervous disorders, fin hemorrhage, fading body color, ataxia, and mortality.

Vitamin B_2 (riboflavin)

As a constituent of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), vitamin B_2 functions as a coenzyme for many enzyme oxidases and reductases, and therefore plays an important role in energy metabolism. FMN and FAD facilitate the enzymatic breakdown of energy-yielding nutrients such as fatty acids, amino acids and pyruvic acid. Deficiency may result in anorexia, poor growth, corneal vascularization, cloudy lens, snout erosion, spinal deformities, severe fin erosion, fin hemorrhage, and increased mortality rate.

Vitamin B_3 (nicotinic acid)

A constituent of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), vitamin B_3 functions as a coenzyme for electron transfer in metabolic processes (i.e., hydrogen removal and transport), plays a central role in tissue oxidation and therefore essential for the release of energy from carbohydrates, fats and proteins. Anorexia, poor growth, reduced feed efficiency, dark coloration, erratic swimming, muscle spasms while resting, and edema of the stomach may result from vitamin B_3 deficiency.

Vitamin B_5 (pantothenic acid)

In the form of 3 phospho-adenosine-5-diphospho-pantotheine (commonly known as acetyl coenzyme A), vitamin B_5 functions as a coenzyme and plays a central role in all reactions involving the formation or transfer of a 2-carbon acetyl group. Pantothenic acid is essential for the release of energy from fats and proteins, which are converted to acetyl coenzyme A before being oxidized in the Krebs or tricarboxylic acid cycles. Signs of deficiency include anorexia, reduced growth, gill necrosis/clubbing, anemia, mucous covered gills, sluggishness, eroded skin, skin lesions, abnormal swimming behavior and mortality.

Vitamin B_6 (pyridoxine)

In the form of its phosphate ester (pyridoxal phosphate), vitamin B_6 functions as a coenzyme in nearly all reactions involved in the non-oxidative degradation of amino acids (protein metabolism), which include amino and carboxyl group transformations. It is required for the metabolic breakdown of tryptophan, the synthesis of hemoglobin, acetyl coenzyme A and messenger RNA, and the metabolic release of glycogen from muscle and liver (carbohydrate metabolism). Nervous disorders, hyperirritability, anorexia, rigor mortis, ataxia, edema of peritoneal cavity, erratic/rapid swimming, anemia, and poor growth may result from vitamin B_6 deficiency.

Vitamin B_7 (biotin)

Vitamin B_7 functions as a coenzyme in tissue reactions involving the transfer of carbon dioxide from one compound to another (i.e., carboxylation reactions). For example, as a component of the enzymes pyruvate carboxylase and acetyl coenzyme A carboxylase, B_7 is responsible for the conversion of pyruvic acid to oxaloacetic acid (an intermediate in gluconeogenesis and the Krebs cycle). Signs of vitamin B_7 deficiency include anorexia, reduced growth, poor feed efficiency, lesions in the colon, muscle atrophy, spastic convulsions, thick gill lamellae, and increased mortality.

Vitamin B_9 (folic acid)

In the form of tetrahydrofolic acid, vitamin B_9 functions as a coenzyme for reactions effecting the transfer of one-carbon units (i.e., formyl, methyl, formate and hydroxymethyl units) from one compound to another. For example, tetrahydrofolic acid is involved in the synthesis of hemoglobin, glycine, methionine, choline, thymine (pyrimidine) and purines, and in the metabolism of the amino acids phenylalanine, tyrosine and histidine. Vitamin B_9 deficiency results in anemia, poor growth, anorexia, lethargy, dark coloration, and distended abdomen for salmonids, while multiple fish species (e.g., common carp) exhibit no symptoms.

Vitamin B_{12} (cyanocobalamin)

Vitamin B_{12} is required for normal red blood cell formation and the maintenance of nerve tissue. It is involved in the synthesis of nucleic acids, the recycling of tetrahydrofolic acid, the maintenance of glutathione activity (carbohydrate metabolism), the conversion of methylmalonyl coenzyme A to succinyl coenzyme A (fat metabolism), and in the methylation of homocysteine to methionine (amino acid metabolism). Deficiency may result in anorexia, reduced growth, and poor feed efficiency for salmonids, while some fish species (e.g., common carp) exhibit no symptoms from lack of dietary vitamin B_{12}.

Vitamin C (ascorbic acid)

Vitamin C acts as a physiological antioxidant, facilitating hydrogen transport within the animal cell. It is also required for numerous hydroxylation reactions within the body, including the hydroxylation of the amino acids tryptophan, tyrosine, lysine, phenylalanine and proline. Vitamin C plays a vital role in maintaining the integrity of connective tissue, blood vessels, bone tissue and wound tissue, and is required for the conversion of folic acid into its metabolically active form of tetrahydrofolic acid, for the conversion of tryptophan to serotonin, and for the synthesis of steroid hormones by the adrenal cortex. Reduced growth, impaired collagen formation, scoliosis, internal/fin hemorrhage, distorted/twisted gill filaments, poor wound repair, increased mortality rates, and reduced egg hatchability are commonly observed in the absence of vitamin C.

Vitamin D (cholecalciferol)

Vitamin D plays an essential role in calcium and phosphorus metabolism in animals. In particular, cholecalciferol is required for the absorption of calcium from the gastro-intestinal tract and for the calcification of growing bone tissue. Vitamin D deficiency may result in reduced growth and feeding efficiency, anorexia, tetany (involuntary contraction of muscles), elevated liver/muscle lipid content and plasma T3 levels, increased blood-clotting time, and vascularized tissue.

Vitamin E (tocopherol)

Vitamin E acts as a fat-soluble extracellular and intracellular antioxidant within the animal body. In particular, tocopherols protect the highly unsaturated fatty acids present in cellular and subcellular membranes, and other reactive compounds (i.e., vitamins A and C) from oxidative damage by acting as free radical traps. It has also been suggested that tocopherols play an important role in cellular respiration and in the biosynthesis of DNA and coenzyme Q. Reduced growth, bulging eye-balls, anemia, clubbed gills, ceroid (insoluble polymer of oxidized lipid and protein) deposition in spleen, damage/degeneration of muscle, reduced egg hatching rate/spawning efficiency, and increased mortality may be observed in the absence of vitamin E.

Vitamin K (phylloquinone)

Vitamin K is required for the maintenance of normal blood coagulation by facilitating the production and/or release of various plasma proteins required for blood coagulation, including; prothrombin, proconvertin, plasma thromboplastin, and the Stuart-Prower factor. It has been suggested that vitamin K may play a role in electron transport and oxidative phosphorylation reactions. Deficiency in vitamin K may result in increased blood clotting time, anemia, and hemorrhagic gills, eyes, and vascular tissue.

Inositol

Inositol is an important structural component of skeletal, heart and brain tissue when in the form of *myo*-inositol. Although the physiological role of myo-inositol is still unclear, it is believed to play an important role in the growth of liver and bone marrow cells, liver lipid (cholesterol) transport, and in the synthesis of RNA. No coenzyme function has so far been ascribed to myo-inositol. Reduced growth, distended abdomen, increased gastric emptying timeskin and fin lesions/hemorrhage have been observed in salmonids having inositol deficiency.

Choline

Choline is an essential component of phospholipids and acetylcholine, and as such plays a vital role in the maintenance of cell structure and the transmission of nerve impulses respectively. Choline also acts as a methyl donor in certain methylation reactions (i.e., synthesis of methionine) and in the form of the phospholipid lecithin plays an important role in the transport of lipid within the body. No coenzyme functions have so far been ascribed to choline. A deficiency in choline may result in reduced growth, fatty liver, poor feed efficiency, hemorrhagic kidney and intestine, and mortality.

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. Vitamins are typically provided in aquaculture feed rations of fish oil, fishmeal, vegetable oil, and plant proteins (e.g., corn, soy) with essential amino acids, antioxidants, dietary minerals, 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 vitamins (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 antibiotics does not improve growth or efficiency in farmed fish.

In organic and conventional livestock production, vitamins are combined in feed rations of grains, beans, oilseeds, and other meals with minerals, amino acids, and vitamins (Pond et al., 1995). Depending on the raw nutrients available to the animal, individual vitamins or a premix of multiple vitamins is added to feed rations (Sewell, 1993). Further, antibiotics are routinely added to grain feed as a growth stimulant in conventional livestock production (Board on Agriculture, 1999).

Human vitamin tablets and supplements usually contain additives that aid in the manufacturing process or alter how the pill is accepted by the body. These additives include fillers that impart proper 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. In addition, multivitamins may contain various herbs and essential minerals (Woodward, undated). It should be emphasized that not all of these additives are allowed in organic handling (7 CFR 205.605–205.606).

Status

Historic Use:

The existence and importance of vitamins, a group of compounds considered essential to life, in various natural food products became understood toward the beginning of the 20th century. Vitamin A was discovered between 1912-1914, and the first synthesis of vitamin A was developed in 1947. Vitamin B_2 was discovered in 1926, while other B vitamins niacin, folic acid, and vitamin B_6 were discovered in the mid-1930s. In 1747, naval surgeon James Lindin observed the importance of a nutrient contained within citrus fruits in preventing scurvy; Vitamin C was rediscovered in 1912 and was the first vitamin to be artificially synthesized in 1935. The causal relationship between vitamin D deficiency and incidence of rickets led to the discovery of vitamin D in 1922. In the same year, vitamin E was found as a component nutrient in green leafy vegetables (Obikoya, 2010). The addition of nutrients to specific foods can be an effective way of maintaining and improving the quality of the food supply, and a number of food products are fortified with vitamin compounds. As examples, dairy products are often fortified with vitamin D, while breakfast cereals and other grain products are commonly fortified with B vitamins.

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. Following the introduction of dry pellets to trout hatcheries, producers observed higher conversion rates of food intake to fish production, which lead 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 has led to the incorporation of the petitioned vitamins and other essential nutrients into many commercial fish feed pellets.

Organic Foods Production Act, USDA Final Rule:

Under authority of the Organic Food Production Act (OFPA) of 1990, vitamins are listed as synthetic substances on the National List of Allowed and Prohibited Substances for Use in Organic Livestock Production (7 CFR 205.603(d)(3)).

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 Livestock Committee of 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, fish used for food, wild or domesticated game, or other non-plant life. From the 21 CFR 205.2, Terms Defined:

> *Livestock 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 Livestock Committee 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 Livestock Committee 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 of the Aquatic Animal Task Force 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 and fed in step-wise levels for 12 years following the implementation of this regulation. Regarding synthetic substances, it was stated under § 205.252(e) 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.

International

A number of international organizations specify the application of synthetic vitamins 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 vitamins in the organic production of aquatic animals. The EU standards constitute binding regulations, while the Canadian standards have not yet been implemented into the regulations. Below, international regulations and standards regarding the use of synthetic vitamins 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). Vitamins are included in the definition of feed additives and therefore subject to regulation. From the Permitted Substances List (CAN/CGSB-32.311-2006), vitamins may be used for enrichment or fortification of livestock feed, and synthetic vitamins may be used if non-synthetic sources are not commercially available (CAN, 2011b). Under no circumstances should vitamins 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 vitamins used in aquaculture the same as those used in livestock.

Codex Alimentarius

The specific criteria for feedstuffs and nutritional elements section of the standards set forth by the Codex Alimentarius Commission (2012) pertaining to terrestrial 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).

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, vitamins are allowed nutritional additives for use in animal production under the following conditions:

(1) Vitamins derived from raw materials occurring naturally in feedstuffs;
(2) Synthetic vitamins identical to natural vitamins for monogastric animals and aquatic animals;
(3) Synthetic vitamins A, D, and E identical to natural vitamins for ruminants with prior authorization of the Member States based on the assessment of the possibility for organic ruminants to obtain the necessary quantities of the said vitamins through their feed rations.

EEC Council Regulation EC No. 710/2009 specified the addition of "aquatic animals" to criteria number two for describing the use of synthetic vitamin sources.

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 vitamins 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 vitamins may be allowed if naturally derived substitutes are not available (JMAFF, 2005).

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).

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) Vitamins petitioned for use in organic aquatic animal feed fall under the category of vitamins and minerals. Vitamins B_1 (thiamine) and B_7 (thiamine) are sulfur-containing substances. In addition, the synthetic form of vitamin K, menadione sodium bisulfite, contains a sulfur atom. The other petitioned vitamins do not contain sulfur.

(B) Since the petitioned vitamins 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. Vitamin E and L-ascorbic acid appear on List 4A, minimal risk inert ingredients. Thiamine mononitrate, vitamin A, vitamin B complex, vitamin B_{12} vitamin D3, choline chloride are present on List 4B, minimal risk other ingredients. Biotin, retinol acetate, riboflavin, nicotinic acid, pantothenic acid, vitamin E acetate appear on List 3, inerts of unknown toxicity. Synthetic vitamin K (menadione sodium bisulfite) is not considered to be an inert ingredient, as defined under 7 CRF 205.2 because it is not included in EPA-regulated pesticide products.

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 vitamin compounds are produced on an industrial scale by chemical synthesis or partial synthesis, fermentation and/or by extraction from natural material sources. Selection of the manufacturing processes typically depends on available technology, cost of raw materials/chemical feedstocks, market prices and size, cost of implementing fermentation versus chemical processes (synthesis or extraction) and, to a lesser extent, the overall environmental impact of the production method.

There is a high degree of structural diversity among individual vitamin compounds; as such, a large number of chemical reactions may be applied to the synthesis of vitamins. Chemical synthesis is advantageous for the commercial production of vitamins as it can be carried out in a continuous manner on an industrial scale. However, chemical synthetic processes can become increasingly complex when specific stereoisomers (i.e., enantiomers, diastereomers, etc.) of a given vitamin must be selectively generated in the reaction sequence or isolated from a mixture of stereoisomers. For example, the chemical synthesis of *myo*-inositol, an essential nutrient for many aquatic organisms, suffers from the difficulty of isolating it free of the other eight stereoisomeric forms (Henry, 1996).

While chemical synthesis remains the dominant industrial production method for many vitamins, an increasing number of fermentation processes are being developed for vitamin production (Festel, 2005). Fermentation is an enzymatic process whereby microorganisms convert natural carbon-based nutrients (e.g., glucose, molasses, etc.) to desired compounds. Many recently developed fermentation methods for generating vitamins utilize genetically engineered microorganisms, generating concerns over the use of these vitamin sources in organic food production (Roseboro, 2008). Proponents of fermentative processes cite production cost savings, reduction in waste and energy requirements, and the use of renewable resources (e.g., sugar or plant oil) (Stahmann, 2000).

Extraction from natural sources is widely considering inefficient and low yielding, making this the least utilized method of vitamin production for use in animal feeds and human supplements (Survase, 2006). An extraction method is described below in the context of vitamin E (tocopherol) extraction from various vegetable oils.

The following section provides a detailed discussion of the industrial production methods for petitioned vitamins. A breakdown of the commonly used technologies for the production of a subset of the petitioned vitamin compounds is presented below in Table 3.

Table 3. Technologies Used in the Production of Vitamins

Vitamin	Commercial Methods	Other Methods
Vitamin A	Chemical synthesis	Fermentation, Extraction
Vitamin B_1	Chemical synthesis	Fermentation
Vitamin B_2	Fermentation, Chemical synthesis	N/A
Vitamin B_6	Chemical synthesis	Fermentation
Vitamin B_{12}	Fermentation	N/A
Vitamin C	Chemical synthesis	Fermentation
Vitamin D_3	Chemical synthesis	Extraction
Vitamin E	Extraction, Chemical Synthesis	N/A
Vitamin K	Chemical synthesis	Extraction
Biotin	Chemical synthesis	Fermentation
Folic acid	Chemical synthesis	Fermentation
Niacin	Chemical synthesis	N/A
Pantothenic acid	Chemical synthesis	Fermentation

Source: Festel, 2005

Vitamin A

Vitamin A is produced via a step-wise synthetic procedure. A representative synthetic method involves the reaction of geranial and acetone in the presence of sodium ethoxide and ethanol (i.e., Claisen-Schmidt reaction). The reaction initially forms pseudolonone, which is subsequently transformed to ionone in the presence of boron trifluoride/acetic acid (Solomons, 2000). Two sequential Wittig reactions complete the commercial synthesis of vitamin A acetate (Pommer, 1977). Hoffmann-La Roche employed a related synthetic method for the industrial production of vitamin A (McMurry, 2011).

A 2010 patent was filed for a vitamin A production process using biofermentation with algae or yeast that are genetically modified to enhance the production of geranylgeraniol and farnesol, potential starting materials in the syntheses of vitamins A and E (Maurina-Brunker, 2010).

Vitamin B_1

Commercial production involves a six-step synthetic procedure (Williams, 1936). Beginning with ethyl 3-ethoxypropionate as the feedstock for vitamin B_1 production, the synthetic reactions include (1) formylation using ethyl formate, (2) reaction with acetamidine hydrochloride leading to aminopyrimidine ring formation, (3) replacement of aminopyrimidine hydroxyl group with a chlorine atom (chlorination) using phosphorus(V) oxychloride, (4) replacement of the labile chlorine atom with an amino group using alcoholic ammonia, (5) ammonium salt formation using hydrobromic acid, (6) introduction of the thiazole ring using 4-methyl 5-hydroxyethyl thiazole.

A search of the patent literature revealed two methods for vitamin B_1 (thiamine) production by fermentative methods. The first patent describes the development of mutants of the genus *Saccharomyces Meyen emend Reess* (yeast) for synthesizing vitamin B_1 from sugars and inorganic salts (Silhankova, 1980). A more recent invention provides a method for producing thiamine products using a microorganism of the genus *Bacillus* containing a mutation (i.e., gene deletions or other mutations) that causes it to overproduce and release thiamin products into the medium (Goese, 2012).

Vitamin B_2

As of 2000, chemical production still accounted for a major component of industrial riboflavin synthesis. D-ribose is the chemical feedstock for this method. Reaction of D-ribose with 3,4-xylidine in methanol begins the synthesis, followed by hydrogenation of the intermediate riboside to give *N*-(3,4-dimethylphenyl)-D-1'-ribamine. Subsequent coupling with a phenyl diazonium halogenide provides an azo compound, which is used in a cyclocondensation with barbituric acid to give riboflavin. The final step eliminates aniline, and trace amounts of aniline are commonly found in chemically synthesized riboflavin products (Stahmann, 2000).

Microbial processes are currently replacing chemical riboflavin production methods in industry. Naturally occurring overproducers of riboflavin include hemiascomycetes *Ashbya gossypii* (fungus) and *Candida famata* (yeast). In addition, the Gram-positive bacterium *Bacillus subtilis* overproduces riboflavin upon deregulation of purine synthesis and mutation in flavokinase/FAD-synthase (Stahmann, 2000). Patents describing the use of genetically engineered bacteria, which overexpress the genes of enzymes involved in vitamin B_2 biosynthesis, have been known since the late 1990s. A more recent patent developed a mutant of *Bacillus subtilis* bearing proline anologue resistance resulting in the concomitant enhancement of osmotic pressure resistance and vitamin B_2 production.

Vitamin B_3

Chemical synthesis remains the primary means of producing vitamin B_3. One method for the generation of nicotinic acid involves the oxidation of 3-methylpyridine using nitric acid in air as the oxidizing agent (Friedrich, 1988). Alternatively, the electrochemical oxidation of pi-deficient N-heterocyclic precursor compounds was described as a facile method for the synthesis of niacin in the patent literature; specifically, the electro-oxidative synthesis of niacin from 3-methylpyridine (Toomey, 1993).

Vitamin B_5

Calcium pantothenate is the form of vitamin B_5 commonly employed in vitamin supplements and the fortification of food products. The conventional synthesis of calcium pantothenate involves three sequential chemical operations. Reaction of isobutyraldehyde with formaldehyde and cyanide initially yields racemic pantoyl lactone. The racemic mixture is then subjected to optical resolution using quinine, quinidine, cinchonidine, and/or brucine, providing enantiomerically-enriched D-(–)-pantoyl lactone. Condensation of D-(—)-pantoyl lactone with β-alanine, followed by isolation as the calcium salt affords calcium pantothenate (Vandamme, 1989).

Methods for the fermentative production of vitamin B_5 using genetically modified microorganisms have also been developed. A recent invention utilized *Bacillus subtilis* mutants wherein the gene encoding PanB had been modified to increase production of pantothenic acid (Perkins, 2010). An earlier example developed a process for the fermentative preparation of D-pantothenic acid and its salts (including the commonly used calcium salt) by fermentation of microorganisms from the *Enterobacteriaceae* family having modified glyA genes (Hermann, 2005).

Vitamin B_6

The chemical synthesis of vitamin B_6 begins with reaction of ethoxyacetylacetone and cyanoacetamide in the presence of ethanol and a catalytic amount of piperidine. Treatment of the resulting pyridone with nitric acid in acetic anhydride introduces a nitrogroup, and subsequent reaction with phosphorus pentachloride in chlorobenzene aromatizes the cyclic system via replacement of the ring carbonyl group with a chlorine atom. The nitro and cyano groups are reduced using hydrogen gas over platinum and hydrogen gas over platinum in the presence of palladium charcoal, respectively. Treatment with hydrochloric acid generates the ammonium chloride, and subsequent reaction of the ammonium compound with sulfuric acid and sodium nitrite converts both ammonium chlorides to hydroxyl groups. Reaction of the resulting dihydroxylated pyridine derivative with hydrobromic acid generates the pyridinium bromide, which is converted to the corresponding pyridinium chloride following treatment with an aqueous mixture of silver chloride (Harris, 1939).

As discussed in the patent literature, recombinant microorganisms of the genus *Escherichia* have also been developed for the fermentative production of vitamin B_6. Specifically, these microorganisms carry cloned genes for over-expression of the enzymes involved in the vitamin B_6 biosynthetic pathway. The forms of vitamin B_6 generated using this method include pyridoxol, pyridoxal, and pyridoxamine (Hoshino, 2007).

Vitamin B_7

Current industrial production methods for vitamin B_7 are based on the original total synthesis of Goldberg and Sternbach of Hoffmann-La Roche Inc. The synthesis begins with fumaric acid as the starting material and involves 15 linear synthetic steps. In short, vicinal bromination of fumaric acid followed by diamination with benzylamine, and subsequent treatment with oxalyl chloride provides a dibenzyl imidazolidinone. Reaction of this species with acetic anhydride forms a *meso*-anhydride, which then undergoes acetylation in the presence of zinc, acetic anhydride, and acetic acid. Incorporation of sulfur to give a thiolactone is accomplished through reactions of the core structure with dihydrogen sulfide, potassium hydrosulfide, and zinc/acetic acid. The alkyl chain adjacent to sulfur is inserted using an appropriate Grignard reagent followed by reduction with hydrogen over palladium. Reaction with hydrobromic acid results in cyclization to form a zwitterionic compound. Resolution with silver *d*-camphorsulfonate followed by fractional crystallization leads to enrichment of the desired stereoisomer. Treatment with sodium diethyl malonate followed by hydrobromic acid affords the final product, biotin (Shioiri, 2010).

Not surprisingly, the chemical synthetic production of biotin is both costly and low yielding. Since only one optical isomer of biotin is active, the above and related chemical synthetic methods must separate active and inactive isomers (i.e., resolve stereoisomers) or prepare intermediates that yield only the active isomer. Microbial fermentation methods have been developed to address this issue, as microbes produce only the biologically active isomer of biotin (Cheung, 1994). As an example, a microorganism of the genus *Kurthia* (bacteria) was developed with resistance to biotin antimetabolites (i.e., acidomycin, amiclenomycin, bisnorbiotinol, etc.) and capability of producing d-biotin under aerobic conditions (Hoshino, 2002).

Inositol

Structually, inositol is a sixfold alcohol (polyol) of cylcohexane with formula $C_6H_{12}O_6$. Of its nine possible stereoisomers, *cis*-1,2,3,5-*trans*-4,6-cyclohexanehexol or *myo*-inositol is the most abundant form in nature. While *myo*-inositol can be chemically synthesized, its purification from the other stereoisomeric forms renders this method too expensive. Rather, industrial production of *myo*-inositol is accomplished through hydrolysis of phytic acid, or IP6, derived from plant sources. Some disadvantages to this method include the intensive energy requirement and its production of acidic byproducts that are environmental pollutants (Henry, 1996). For additional details regarding the synthetic procedure, please see the recent technical evaluation report for the use of inositol in organic handling/processing (USDA, 2012a).

Fermentative methods for the production of inositol have also been disclosed. The dephosphorylated (i.e., desired) form of inositol has been recovered from cultures of *Saccharomyces cerevisiae* (yeast) containing a functional stable recombinant DNA sequence that disallows the encoding of a negative regulator of phospholipid biosynthesis and bears multiple copies of an INO1 gene (Henry, 1996). The claims for this method cite reduced energy costs and cleaner inositol production lacking the generation of environmental pollutants. Although various fermentation methods are known, it is unlikely that a commercial-scale process for inositol recovery from yeast cultures has been developed (Makoto Shirai, 1997).

Vitamin B_9

Researchers from the American Cyanamid Company reported the first industrial synthesis of folic acid, a form of vitamin B_9 in 1948. This method of manufacturing vitamin B_9 utilized only halogen free compounds, and began with the reaction of p-aminobenzoyl-L-glutamic acid diethyl ester with 2-hydroxymalondialdehyde yielding p-(2,3-dihydroxy-2-ene-propylideneamino)-benzoic acid diethyl ester. This intermediate was then reacted with triaminopyrimidinone (Angier, 1948). A variation of this method involves the condensation of 2,4,5-triamino-6-hydroxypyrimidine, 1,1,3-trichloroacetone and p-aminobenzoylglutamic acid in a sodium nitrite/sodium acetate solution to give PteGlu, the crude product of folic acid (Miyata, 2001).

More recent developments in the patent literature include improved chemical synthetic processes and fermentative methods of producing folic acid in high purity and yield. Specifically, the former invention presents a novel synthetic strategy for producing folic acid, which utilizes diimine compounds as intermediates (Wehrli, 1995). The latter invention describes the incubation of yeast or bacterial strains having the ability to overproduce folic acid in the culture medium. For this method, yeast strains include *Candida famata, Candida fuilliermondii, Torulopsis petrophilum, Pichia glucozyma, Torulopsis glabrata or Saccharomyces cerevisiae*, and bacterial strains belong to the genus *Bacillus* (Miyata, 2001).

Choline

Chemical synthesis is the method of choice for generating choline derivatives. Industrial production of choline chloride is straightforward, involving the chemical reaction of ethylene oxide, trimethylamine, and hydrochloric acid (Choline Chloride, 2012). Recently developed methods include a patented process for generating a variety of choline salts from inexpensive, impure, halogen-free sources of choline (Lustig, 2012). For variations of this synthetic procedure, please see the recent technical evaluation report for use of choline in organic handling/processing (USDA, 2012b).

Vitamin C

Hoffmann-La Roche company synthesizes vitamin C from glucose through a five-step route. Glucose is first reduced to sorbitol using hydrogen and a transition metal catalyst. The microorganism *Acetobacter suboxydans* is then employed to oxidize sorbitol since no chemical oxidant is selective enough to oxidize only one of the six hydroxyl groups in sorbitol. Subsequent treatment with acetone and an acid catalyst converts four of the other hydroxyl groups into acetal linkages; the remaining hydroxyl group is chemically oxidized to the corresponding carboxylic acid through reaction with aqueous sodium hypochlorite (bleach). Hydrolysis with acid removes the two acetal groups and leads to an internal esterification yielding vitamin C (McMurry, 2011).

More recently developed synthetic strategies for producing vitamin C have also been described in the patent literature. One example involves the esterification of 2-keto-L-gulonic acid with a subsequent lactonization step and crystallization to form vitamin C (Fur, 1995). A related invention utilizes a similar synthetic process wherein L-ascorbic acid is produced in high yield through conversion of an aqueous solution of 2-keto-L-gulonic acid in the presence of an acid catalyst (Arumugam, 2003).

Recently a breakthrough fermentative method of vitamin C synthesis was disclosed, effectively transforming a 3-5 step chemical synthesis into a one-pot process (Festel, 2005). The patent literature also reveals a number of fermentative methods utilizing genetically modified microorganism for the overproduction of vitamin C (Beuzelin-Ollivier, 2012; Berry, 2001). It seems likely that vitamin C producing industries will be shifting toward fermentative methods using genetically modified microorganisms due to the increasing global demand for vitamin C.

Vitamin D

The commercial manufacturing process of vitamin D_3 mimics the biosynthesis of the cholecalciferol in animals. Cholesterol extracted from the lanolin of sheep wool is commonly used as the chemical feedstock (Norman, 2011). In the Windaus oxidation procedure, 3-hydroxy protected cholesterol is oxidized to form the 7-keto cholesteryl acetate. This intermediate is then reduced to the 7-hydroxycholesterol with aluminum isopropylate in isopropyl alcohol. The 3,7-dihydroxycholesterol is benzoylated followed by dehydration of the 3,5-dibenzoate at elevated temperatures to furnish 7-dehydrocholesterol benzoate. Crystalline 7-dehydrocholesterol is then dissolved in organic solvent and irradiated with UV light to generate cholecalciferol (Feldman, 2011). Following further purification and crystallization, cholecalciferol can be formulated for use in dairy milk and animal feed supplements (Norman, 2011).

Vitamin E

Synthetic vitamin E (α-tocopherol) is not identical to the form that occurs in nature; rather, it is a mixture of eight stereoisomers collectively known as all-rac-alpha-tocopherol, consisting of four 2R- and four 2S-isomers (Survase, 2006). Alternatively, a natural mixture of tocopherols can be extracted from vegetable oil sources (Vandamme, 1992). Extraction of tocopherols from vegetable oils typically involves a series of neutralization and separation stages following contact of the tocopherol containing substance with a caustic aqueous methanol solution and various aliphatic hydrocarbon solvents (Swanson, 1991).

Genetically modified organisms are potentially used in the production of vitamin E. Members of the organic community have voiced concerns over the use of vitamin E containing oils originating from genetically modified organisms, particularly soybeans (Roseboro, 2008). In addition, a 2010 patent was filed for a vitamin E production process using biofermentation with algae or yeast that are genetically modified to enhance the production of farnesol and geranylgeraniol, potential starting materials in the syntheses of vitamins E and A (Maurina-Brunker, 2010).

Vitamin K

Both natural (i.e., vitamin K_1 and K_2) and synthetic (vitamin K_3, etc.) versions of vitamin K may be used as supplements in animal feeds. Oxidation of the requisite naphthalene derivative to a 1,4-napththoquinone is likely the first step in commercial synthesis of natural K_1 and K_2 as well as K_3, an inexpensive form of vitamin K commonly used in the supplementation of pet food and livestock feeds (Braude, 1953). Subsequent alkylation of the 1,4-naphthoquinone leads to generation of the biologically active vitamin K derivative (Büchi, 1987).

Vitamin B_{12}

Microorganism fermentation is the exclusive commercial method of synthesizing vitamin B_{12}. Species of *Pseduomonas* or *Propionibacterium* have been used for both complete and partial anaerobiosis. However, the primary industrial organisms are *Pseudomonas denitrificans* and *Propionibacterium shermanii*. Genetic modification of *P. denitrificans* increased production of vitamin B_{12} by approximately 100% (El-Mansi, 2007).

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)).

The synthetic forms of vitamins categorized as required in aquatic animal feed are petitioned for inclusion on the National List. Synthetic forms of the following vitamins are likely derived through a combination of chemical synthetic and fermentation methods: Vitamins A, B_1, B_3, B_5, B_6 B_7, B_9, C, D, E, K, choline and inositol. Vitamin E (tocopherols) is typically extracted from natural materials (e.g., vegetable oils) using aliphatic hydrocarbon solvents and acid-base extraction methods. In contrast, commercial production of vitamin B_2 (riboflavin) and B_{12} (cobalamin) is performed exclusively using biological fermentation. Please see Evaluation Question #2 for details regarding methods for manufacturing synthetic forms of the petitioned vitamins. Sources and availability of natural forms of vitamin substances are discussed in further detail in Evaluation Question #11.

Vitamins produced through biological fermentation may be considered non-synthetic or synthetic, depending on the feedstocks, fermentation organisms used, and processing aids used. Alternatively, chemical synthesis and extraction techniques are typically considered chemical processes due to the application of chemical reagents in these methods. In the case of chemical synthesis, the chemical structures of natural feedstock chemicals are necessarily modified in the process of generating the desired vitamin compound.

__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)).

The petitioned substances, synthetic vitamins used as nutritional supplements in aquatic animal feed, may enter the aquatic environment through their production, usage, and disposal. Non-synthetic vitamins exist naturally in the environment as they are commonly found in plants, animal tissues, and products derived from plant and animal sources. Please see Evaluation Question #11 for more information regarding natural sources of non-synthetic vitamins.

Open and closed aquatic systems have inherently different levels of risk pertaining to eutrophication. Water system carrying capacities depend upon tidal flushing, current, and assimilative capacities of the water body to pollutants, making closed aquatic systems especially susceptible to eutrophication (Wu, 1995). Stated another way, closed systems are more vulnerable due to the continuous input of fish feeds, the lack of fresh water inflow, and slow nutrient cycling. Alternatively, the risk of large-scale eutrophication from open system marine fish farming is low (Wu, 1995). Ultimately, the persistence of the given vitamin compound may not be of paramount concern when there is a continuous supply of the nutrient in question. Any adverse effects due to the persistence of vitamins will be more severe in closed aquatic systems.

Organic and inorganic nutrients present in aquatic animal feed are largely introduced into the environment through excess feed, aquatic organisms excretion, feces production, and respiration (Wu, 1995). Although producers generally seek to minimize feed wastage, the accumulation of aquaculture feed containing synthetic vitamins in waters has raised environmental concerns. Results from various studies indicate that a large proportion of animal feed nutrients introduced into the environment have the ability to accumulate in bottom sediments (Wu, 1995). This phenomenon may lead to high sediment oxygen demand, anoxic sediments, production of toxic gases, and a decrease in benthic diversity. In particular, laboratory studies suggest that the accumulation of these nutrients, including vitamins, may encourage the growth of algal blooms and red tide species (Wu, 1995). The potential for environmental impairments resulting from the accumulation of vitamins in aquatic systems is further discussed in Evaluation Question #6.

While the effects of vitamins on aquatic environments are not well established, the half-lives of vitamins in oxic (i.e., oxygen rich) environments are believed to be short, and accumulation of vitamins in the environment is highly unlikely (Wu, 1995). Scientific studies revealed a half-life of less than seven days for the breakdown of biotin in seawater versus one to two months in fish farm sediments (Wu, 1995). In addition, the half-life for vitamin C in surface water and at a meter depth exposed to continuous sunlight was reported as 3.5 and nine hours, respectively (HSDB, 2010a). The Henry's Law constant for vitamin D_3 points to volatilization half-lives of seven hours and ten days from a model river and model lake, respectively. However, volatilization from water surfaces is attenuated by adsorption to suspended solids and sediment in the water column, giving an estimated volatilization half-life of 85 years for cholecalciferol from a model pond if adsorption is considered (HSDB, 2006). It is unlikely that the latter process (i.e., volatilization) is chemically significant for the degradation of vitamin compounds. Overall, vitamins should not be considered persistent in marine environments, as these compounds readily decompose under oxic conditions.

Literature information regarding the potential for bioconcentration of individual vitamins in aquatic organisms is limited. In general, lipid-soluble vitamins are chemically predisposed to accumulate in an organism's fatty tissues, while water-soluble vitamins are more readily excreted. However, both ascorbic acid and cholecalciferol have bioconcentration factors (BCFs) of three, indicating that the potential for bioconcentration in aquatic organisms is low. A number of vitamins, including cholecalciferol and ascorbic acid, contain chromophores that absorb wavelengths of >290 nm and therefore may be susceptible to degradation in water or soil upon exposure to sunlight (HSBD, 2005; 2006; 2010).

Differences are observed in soil mobility based on the vitamin's solubility properties. Lipid-soluble cholecalciferol is expected to be immobile in soil. Based on its Henry's Law constant and vapor pressure, volatilization of cholecalciferol from moist soil may occur, but volatilization from dry soil surfaces is not expected (HSBD, 2006). Alternatively, the water-soluble vitamins folic acid and ascorbic acid are highly mobile in soils, existing almost exclusively as the corresponding anion in the environment. These water-soluble vitamins are not expected to volatilize from dry or moist soils due to their low vapor pressure and the fact that anions do not generally volatilize, respectively (HSBD, 2005; 2010).

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

The potential for toxicity is generally dependent upon the vitamin's solubility properties, amount left unconsumed, and amount excreted by the farmed aquatic animals, when used as petitioned. As water-soluble vitamins, thiamine (B_1), riboflavin (B_2), pyridoxine (B_6), pantothenic acid (B_5), nicotinic acid (B_3), biotin (B_7), folic acid (B_9), cobalamin (B_{12}), inositol, choline, and ascorbic acid (C) are rapidly depleted in the absence of regular dietary intake and appreciable quantities of these vitamins do not build up in the animal body. In contrast, the lipid-soluble vitamins retinol (A), cholecalciferol (D), tocopherols (E), and phylloquinone (K) are readily absorbed from the gastrointestinal tract in the presence of fat, and stored in the animal's fatty tissues whenever dietary intake exceeds metabolic demands for the vitamin compound. Hypervitaminosis, increasing vitamin storage to the extent that a toxic condition is produced, is therefore most commonly associated with the fat-soluble vitamins (FOA, 1987).

Limited scientific information exists regarding the toxicity of synthetic vitamins petitioned for use in aquatic animal feed. No reports of acute or chronic toxicity related to large quantities of the water-soluble vitamins B vitamin complex, vitamin C, choline, inositol or lipid-soluble vitamins D and E have been observed in aquaculture studies. NRC (2011) considers the following vitamin levels safe in feeds for fish species, including channel catfish, rainbow trout, pacific salmon, common carp, and tilapia:

- Vitamin A: 1,000 – 4,000 IU/kg in a diet containing 3.0 – 3.6 kcal/g;
- Vitamin D: 500 – 2,400 IU/kg in a diet containing 3.0 – 3.6 kcal/g;
- Vitamin E: 50 – 100 IU/kg in a diet containing 3.0 – 3.6 kcal/g;
- Vitamin K: Quantity not determined or not tested;
- Vitamin B_2: 4 – 9 mg/kg in a diet containing 3.0 – 3.6 kcal/g;
- Vitamin B_5: 10 – 30 mg/kg in a diet containing 3.0 – 3.6 kcal/g;
- Vitamin B_3: 10 – 28 mg/kg in a diet containing 3.0 – 3.6 kcal/g;
- Vitamin B_{12}: 0.01 (estimated) mg/kg in a diet containing 3.0 – 3.6 kcal/g;
- Choline: 400 – 1,000 mg/kg in a diet containing 3.0 – 3.6 kcal/g;
- Vitamin B_7: 0.15 – 1.0 mg/kg in a diet containing 3.0 – 3.6 kcal/g;
- Vitamin B_9: 1.0 – 2 mg/kg in a diet containing 3.0 – 3.6 kcal/g;
- Vitamin B_1: 0.5 – 1.0 mg/kg in a diet containing 3.0 – 3.6 kcal/g;
- Vitamin B_6: 3 – 6 mg/kg in a diet containing 3.0 – 3.6 kcal/g;
- Myoinositol: 300 – 440 mg/kg in a diet containing 3.0 – 3.6 kcal/g;
- Vitamin C: 25 – 50 mg/kg in a diet containing 3.0 – 3.6 kcal/g.

Source: NRC, 2011

Concerns have been noted regarding the use of synthetic vitamin K in aquatic animal feed human supplements, and pet food products. In certain cases, high dietary levels of menadione sodium bisulfite (synthetic vitamin K_3) of 2,4000 mg/kg had no adverse effects on growth, survival, blood coagulation or the number of erythrocytes of young brook trout (*Salvelinus fontinalis*) (DSM, 2011c). In another study, small Atlantic salmon (*Salmo salar*) were fed a ration supplemented with 30 mg/kg menadione sodium bisulfite (K_3) or the molar equivalent of natural phylloquinone (K_1) (Grisdale-Helland, 1991). After 28 weeks, the fish that were fed the K_3 ration displayed reduced growth and increased mortality compared with animals fed an equivalent quantity of natural vitamin K_3. Other factors, such as reduced absorption efficiency of K_3 versus K_1, oxidation of K_3, and/or leaching of K_3 from the feed pellet, may also contribute to the reduced performance of fish fed vitamin K_3 instead of natural K_1 (Grisdale-Helland, 1991).

Synthetic menadione (vitamin K_3) and its derivatives have also been linked to health issues in humans and are considered controversial ingredients in pet foods. Menadione may promote oxidative damage to cell membranes through interfering with the function of glutathione, an important biological antioxidant compound. When injected in infants, menadione has induced liver toxicity, jaundice, and hemolytic anemia (Higdon, 2004). For these reasons, menadione is no longer used to treat vitamin K deficiency, no tolerable upper level (UL) of intake has been established for menadione, and no FDA-approved prescription or over-the-counter drugs containing menadione are currently available (FDA, 2012).

Vitamin A, another example of a lipid soluble vitamin, has also generated toxicity concerns. However, data sources pertaining to vitamin A toxicity in aquaculture are lacking, and the available literature information concerns human toxicity associated with abuse of vitamin A supplements and diets extremely high in preformed vitamin A. For example, it is stated that human consumption of 25,000–50,000 IU per day for periods of several months or more may produce a number of adverse effects (Hathcock, 1990). Vitamin A, along with other lipid-soluble vitamins D, E, and K, has the potential for bioaccumulation in aquatic species. Although the potential bioaccumulation of these vitamins may be of concern to humans consuming farmed aquatic animals, no health reports to this effect were found.

Finally, vitamin D_3 (cholecalciferol) exhibits toxicity when used in high quantities as a rodenticide (USDA, 2009). If ingested in sufficiently high doses, vitamin D_3 can result in hypercalcemia from mobilization of calcium from the bone matrix into blood plasma, which leads to metastatic calcification of soft tissues (U.S. EPA, 2011a). Rodenticides containing vitamin D_3 have been used to control various species of rats, mice, and other rodents. Vitamin D_3 rodenticides have exhibited the potential for acute and chronic toxicity in some non-target organisms, including the federally endangered salt marsh harvest mouse (SMHM, *Reithrodontomys raviventris*). Although possible, it is unlikely that the low concentrations of vitamin D_3 used in aquaculture would lead to toxic effects in SMHM. Further, the U.S. EPA has indicated no potential for adverse effects to terrestrial invertebrates, terrestrial plants, or aquatic wildlife resulting from vitamin D_3 exposure (U.S. EPA, 2011a; 1984). Toxicological studies in birds have indicated that vitamin D_3 is of low toxicity (U.S. EPA, 1984).

The potential for the occurrence of residues of synthetic materials (i.e., solvents, reagents) used in the production or extraction of a substance in the final product depends on how rigorously the manufacturer purifies the compound following the synthetic procedure. While most manufacturers utilize quality assurance protocols to ensure purity, concerns regarding the quality and purity of specific vitamin compounds have been noted (Balchem, 2010). Likewise, the fermentative production of vitamins presents a slight risk of product contamination from genetic material in the fermentation broth and any chemicals used during processing. Technical information regarding the contamination of foods and supplements with GMOs is scarce. The Commission for European Communities regulates and requires labels for foods containing GMOs; however, foods subjected to GMO processing aids are neither regulated nor require labeling (European Commission, 2006).

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

In the course of production, use, and disposal, vitamins may possibly be released to soil and water. Water-soluble vitamins, such as vitamin C, are expected to have slight to high mobility if released to soil and therefore may spread to other soil areas and waterways (HSDB, 2005a; 2010a). Water-soluble vitamins are also unlikely to volatilize from moist or dry soils due to their high polarity and low vapor pressures, respectively. If released to the water, most of the water-soluble vitamins are not expected to adsorb to suspended solids and sediment (HSDB, 2010a). Others, such as folic acid, may adsorb to solids and sediments (HSDB, 2005a). For many of these chemical species, the presence of functional groups that hydrolyze means hydrolysis is expected to be an important environmental fate, while volatilization of these chemicals from water surfaces is less likely. Most water-soluble vitamins have low bioconcentration factors (BCFs) suggesting minimal potential for bioconcentration in aquatic organisms (HSDB, 2005a; 2010a).

Lipid soluble vitamins, such as cholecalciferol, are generally less polar than water-soluble vitamins, making soil mobility unlikely (HSDB, 2006). In a similar sense, it is unlikely that lipid-soluble vitamins would volatilize from dry soil based on their relatively low vapor pressures. Lipid-soluble vitamins are essentially insoluble in water and will adsorb preferentially to sediments and other suspended solids present in the water column (HSDB, 2006). Most lipid-soluble vitamins lack functional groups that hydrolyze under environmental conditions, making hydrolysis an unlikely environmental breakdown process. Overall, the observed BCFs are low, suggesting that lipid-soluble vitamins do not pose a significant risk of bioconcentration in aquatic organisms (HSDB, 2006).

The potential exists for environmental contamination resulting from the industrial production of several vitamin compounds. In particular, materials safety data sheets for several feedstock chemicals and other chemical reagents used in the synthesis of calcium pantothenate (vitamin B_5) and biotin (vitamin B_7) indicate the potential for ecological damage if accidentally released into the environment. Isobutyraldehyde and cyanide salts used in the synthesis of calcium pantothenate as well as ethylene oxide used for choline chloride generation have shown toxicity toward fish and aquatic invertebrates. Further, hydrogen sulfide, which is used in the synthesis of biotin, is toxic to fish at low doses, and is therefore listed as very toxic to aquatic life. Strong acids (e.g., nitric acid, hydrochloric acid) used in the syntheses of numerous vitamins may alter the pH of aquatic systems if accidentally released to the environment. Strong acids and bases are also utilized in the extraction of tocopherols from vegetable oils, and may lead to environmental impairment if accidentally released or improperly handled. Many of the feedstock chemicals and reagents used in vitamin synthetic procedures are considered petrochemicals or may be obtained from genetically modified organisms (GMOs). Acetone, for example, is a commonly used chemical reagent derived from petroleum as well as from GMOs such as corn.

Waste streams resulting from the fermentative production of vitamins may also pose risks to the environment. In general, the EPA assumes “no control features for the fermentor offgases, and no inactivation of the fermentation broth for the liquid and solid waste releases,” suggesting that environmental exposure to these waste streams is likely (EPA, 1997). However, lacking are specific examples of environmental damage resulting from exposure to DNA from genetically modified microorganisms used in food and food additive production. Some potential risks to the environment include the transfer of novel genes into crops, poisoned wildlife, and the creation of new and more potent viruses, in addition to a host of unknown risks (UCS, 2002).

The use of vitamins and other chemicals in aquaculture has raised environmental concerns. Chemical nutrients, such as vitamins, present in aquatic animal feed are largely introduced into the environment through excess feed, aquatic organisms excretion, feces production, and respiration (Wu, 1995). Some of these organic and inorganic nutrients have a propensity to accumulate in the bottom sediments, which may lead to high sediment oxygen demand, anoxic sediments, production of toxic gases, and a decrease in benthic diversity (Wu, 1995). However, it is unlikely that vitamins are primarily responsible for environmental impairment due to their short half-lives in aquatic systems. Rather, laboratory studies suggest that a continuous supply of vitamins may provide nutritional support to any algal blooms and red tides that develop due to eutrophication (Wu, 1995; NAS, 1969). Once algal proliferation commences, available vitamins derived from fish feeds may support the growing population. In particular, unicellular photosynthetic algae require nutritional intake of vitamin B_1 (thiamine), B_7 (biotin), and B_{12} (cobalamin) (NAS, 1969). Therefore, a deficiency of these vitamins, as well as other macro- and micronutrients, can be a limiting growth factor for environmentally beneficial and deleterious algae. This particular environmental issue is most directly mitigated through the application of responsible aquatic animal husbandry practices emphasizing environmental stewardship.

Overall, accidental release of small amounts of vitamins into the environment is not assumed to pose any significant risk. Material safety data sheets for many synthetic vitamins, including vitamins C and D, advise that containers holding synthetic vitamins be “suitable” and closed containers for disposal. No further disposal instructions are provided (Sigma Aldrich, 2012; Acros Organics, 2009). However, release of large amounts of vitamins into the environment may result in eco-toxic events, such as the promotion of algal blooms and red tides.

<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 vitamins and other aquatic animal feed additives were identified. For the current petition, vitamins 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 vitamins 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 vitamins are chemically equivalent to vitamins that have been used in the fortification of organic livestock feed under 7 CFR 205.603 since 2001. In the body, vitamins interact as coenzymes and cofactors in a variety of biological processes including respiration, metabolism, and cellular growth and differentiation. Please see the "action of the substance" section for further details regarding the specific biological functions of the petitioned vitamins.

The primary chemical interactions of vitamins occur physiologically once inside the animal's body. Some vitamins are involved in biochemical reactions that generate essential compounds; for example, choline acts as a methyl donor in the biological synthesis of methionine. In other cases vitamins interact with one another to effect important biochemical transformations, such as the cooperative interaction of riboflavin and pyridoxine that is responsible for converting tryptophan to nicotinic acid (FAO, 1987). Alternatively, excesses of one particular vitamin may cause deficiencies in another vitamin or lead to toxic effects. As an example, it has been shown that large doses of vitamin A may interfere with the absorption of vitamin K when taken at excessively high doses (Chandler, 2011). It is presumed that the prescribed vitamin supplementation in aquatic animal feed would be balanced for optimum health of the given farmed aquatic species (NRC, 2011).

Excessive vitamin loadings can also lead to synergistic and/or antagonistic effects for the absorption and bioavailability of minerals and other trace nutrients (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. 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 vitamin E and selenium as an oxidant defense system has been observed in a number of species (Vannucchi, 1991). Vitamin A may also increase iron absorption, thereby indirectly contributing to an increase in hemoglobin levels (Sandström, 2001). On the other hand, sufficient dietary levels of zinc are necessary for 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 vitamins in the feed for organically raised aquatic animal species. Through this specific application, it is unlikely that the petitioned vitamins would regularly interact with components of the terrestrial agro-ecosystem. More likely, however, are interactions resulting from the use of synthetic vitamins in the organic production of terrestrial livestock (7 CFR 206.603). Synthetic vitamins are widely used in conventional and organic livestock production with no reported toxicity observed in non-target wildlife or livestock. Any potential leakage of vitamins from aquatic animal feeds near the agro-ecosystem would be neither routine nor widespread.

No studies have been found indicating toxic effects of vitamins on soil-dwelling organisms. Some bacteria, for example, do not require growth factors such as vitamins, while other bacterial strains (e.g., *Lactobacillus*) require vitamins and other nutrients in order to grow (Todar, 2012). Therefore, despite the fact that some water-soluble vitamins have the potential for high soil mobility, vitamins are unlikely to exhibit toxicity toward the agro-ecosystem (HSDB, 2005a; 2010a). Accidental release of chemical reagents during the production process, however, may lead to ecological impairment. Specifically, strong acids and bases are used in the synthetic or extraction process of vitamin compounds. Improper use or disposal of these chemicals during the production of vitamins 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)).

Limited information is available regarding the environmental toxicity of vitamin compounds. Lipid-soluble vitamins are virtually insoluble in water and are most likely to adsorb to suspended solids and sediments (HSDB, 2006). Some water-soluble vitamins, such as vitamin C, are unlikely to adsorb to surfaces, while others, such as folic acid, are more likely to adsorb sediments and suspended solids (HSDB, 2005a; 2010a). It is unlikely that any of the petitioned vitamins would bioaccumulate in aquatic life (HSDB, 2005a; 2006; 2010a).

Based on their chemical properties, water-soluble vitamins may exhibit some level of soil mobility ranging from low to high (HSDB, 2005a; 2010a). Lipid-soluble vitamins, however, are unlikely to show any soil mobility (HSDB, 2006). Data regarding the biodegradation of vitamins in soil are not available.

Water-soluble vitamins are not expected to volatilize from moist or dry soils, while volatilization of lipid-soluble vitamins from moist soils may be an important fate process. If released to the ambient atmosphere, vitamins are expected to remain as particular matter due to their vapor pressures and may be removed from the air by wet and dry deposition (HSDB, 2005a; 2006; 2010a). Photolysis (i.e., photochemical degradation) from direct sunlight is likely to occur because most vitamins can absorb light at wavelength of 290 nm or greater (HSDB, 2005a; 2006; 2010a).

The production methodologies used to synthesize vitamin compounds pose potential risks to the environment. If released, strong acids and bases may affect the pH and chemical composition of soils and aquatic ecosystems. Likewise, accidental release of toxic organic (e.g., isobutyraldehyde and ethylene oxide) and inorganic (e.g., cyanide salts and hydrogen sulfide) may present toxicological issues for terrestrial and aquatic organisms. The ecological risks associated with the chemical production of vitamin compounds are generally low when manufacturers exercise prudent standard operating procedures.

Specific chemical forms of certain vitamins may pose toxicological risks. Synthetic vitamin K_3 (menadione) may promote oxidative damage to cell membranes through interfering with the function of glutathione, an important biological antioxidant compound. When injected in infants, vitamin K_3 has induced liver toxicity, jaundice, and hemolytic anemia (Higdon, 2004). No FDA-approved prescription or over-the-counter drugs containing menadione are currently available; only discontinued menadione drug products are listed (FDA, 2012). Vitamin D_3 (cholecalciferol) is used in a rodenticide, exhibiting toxicity in both target and non-target rodent species, including the federally endangered salt marsh harvest mouse. However, the U.S. EPA has indicated no potential for adverse effects to birds, terrestrial invertebrates and plants, or aquatic wildlife resulting from vitamin D_3 exposure (U.S. EPA, 2011a; 1984).

Overloading aquatic ecosystems with nutrients, such as vitamins, could potentially lead to depletion of the dissolved oxygen content and eutrophication. This is commonly manifested through occurrences of algal blooms and red tides, fish kills, and overall loss of biodiversity from the aquatic system. Following established aquatic animal husbandry practices with regard to feeding intervals and volumes can be helpful in mitigating potential environmental impairments from nutrient overload.

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)).

As with prescription and over-the-counter medications, improper disposal of vitamins and other supplements may lead to environmental and toxicological issues. Excessive use of vitamin supplements and aquatic animal feeds fortified with vitamins may lead to similar environmental issues when these nutrients enter lakes, rivers, and streams, which are often used as sources for community drinking water. Water treatment plants are typically not equipped to routinely remove these types of organic compounds, and overloads of these substances may lead to toxic effects (EPA, 2011). The petitioned substances are intended for use in aquatic animal feed and therefore are not meant for human consumption. However, these substances are chemically identical to vitamin compounds incorporated into human supplements. 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.

Vitamin A:

Liver abnormalities are critical adverse effects of vitamin A poisoning for adults. In the case of women of childbearing age, teratogenicity (potential to cause malformations of an embryo or fetus) becomes the critical effect. Other adverse effects include nausea, vomiting, headache, increased cerebrospinal fluid pressure, vertigo, blurred vision, muscular incoordination, bulging, fontanel in infants, nervous system changes, and bone and skin abnormalities. The tolerable upper intake level (UL, maximum level of daily nutrient intake that is likely to pose no risk of adverse effects) for preformed vitamin A (i.e., retinol) is 3,000 micrograms per day (Driskell, 2009; Institute of Medicine, 2001).

Vitamin B_1*:*

No adverse effects have been observed relating to the consumption of foods or dietary supplements containing vitamin B_1 (thiamine). There have been occasional reports of anaphylaxis to parenteral thiamin as well as pruritus due to allergic sensitivity to thiamine injection. UL for thiamine was not determined due to lack of data of adverse effects (Driskell, 2009; Institute of Medicine, 1998).

Vitamin B_2*:*

No adverse effects have been observed relating to the consumption of foods or dietary supplements containing vitamin B_2 (riboflavin). UL for thiamine was not determined due to lack of data of adverse effects (Driskell, 2009; Institute of Medicine, 1998).

Vitamin B_3*:*

No adverse effects have been observed relating to the consumption of naturally occurring vitamin B_3 (niacin) in foods. One form of vitamin B_3, nicotinic acid, is associated with vasodilation (flushing) and gastrointestinal effects. Another common form, nicotinamide, does not appear to be associated with these flushing effects. Hepatic toxicity has been reported in patients medically treated with vitamin B_3. The UL of vitamin B_3 (35 mg/day) is based on the flushing effects observed with nicotinic acid (Driskell, 2009; Institute of Medicine, 1998).

Vitamin B_5*:*

No adverse effects have been associated with high intakes of vitamin B_5 (pantothenic acid) from foods or supplements. UL for thiamine was not determined due to lack of data of adverse effects (Driskell, 2009; Institute of Medicine, 1998).

Vitamin B_6*:*

The consumption of vitamin B_6 (pyridoxine) from food sources is not associated with adverse health effects. The critical adverse effect from high supplemental intake is neuropathy, a collection of disorders that occur when nerves of the peripheral nervous system are damaged. UL for vitamin B_6 is 100 mg/day (Driskell, 2009; Institute of Medicine, 1998).

Vitamin B_7*:*

No adverse effects have been observed relating to the consumption of foods or dietary supplements containing vitamin B_7 (biotin). UL for vitamin B_7 was not determined due to lack of data of adverse effects (Driskell, 2009; Institute of Medicine, 1998).

Inositol:

Humans are able to synthesize inositol in the body from glucose. Inositol consumption from the average diet is about one gram daily, obtained in high quantities from cereals and legumes. Although no acute or chronic toxic effects are known, diarrhea has been noted with the intake of very high doses of inositol (Inositol Toxicity, 2010). Rather, inositol deficiency may potentially lead to more severe human health issues, including eczema, constipation, eye problems, hair loss, and elevated cholesterol levels (Haas, 2006).

Vitamin B_9:

No adverse effects have been associated with the consumption of vitamin B_9 (folate) at levels naturally present in foods or in fortified foods. However, excess vitamin B_9 has been shown to precipitate or exacerbate neuropathy in vitamin B_{12}-deficient individuals. UL for vitamin B_9 is 1,000 micrograms per day (Driskell, 2009; Institute of Medicine, 1998).

Choline:

The critical adverse effect of excess dietary choline is hypotension (low blood pressure). Incidence of a fishy body odor as well as nausea and diarrhea are secondarily considered effects. UL for choline is 3.5 g/day (Driskell, 2009; Institute of Medicine, 1998).

Vitamin C:

Excess vitamin C intake is associated with osmotic diarrhea and gastrointestinal disturbances as the primary adverse effects. Other possible effects include increased oxalate excretion and kidney stone formation, increased uric excretion, pro-oxidant effects, rebound scurvy, increased iron absorption leading to iron overload, reduced vitamin B_{12} and copper levels, increased oxygen demand, and erosion of dental enamel. UL for vitamin C is 2,000 mg/day (Driskell, 2009; Institute of Medicine, 2000).

Vitamin D:

Hypercalcemia is the primary adverse effect for excess vitamin D intake. In addition, anorexia, nausea, vomiting, increased thirst and urination, metastatic calcification of soft tissues (i.e., kidneys, blood vessels, heart, and lungs), and renal disorders may develop due to vitamin D poisoning. UL for vitamin D is 50 micrograms or 2,000 IU per day (Driskell, 2009; Institute of Medicine, 1997).

Vitamin E:

Adverse effects have not been observed from the consumption of vitamin E naturally occurring in foods. High intakes of vitamin E from fortified foods, dietary supplements, or pharmacologic agents have resulting in an increased tendency to hemorrhage as the primary adverse health effect. This anticoagulant effect can be particularly severe for individuals deficient in vitamin K, including those taking coumarin drugs. UL for vitamin E in the α-tocopherol form is 1,000 mg/day (Driskell, 2009; Institute of Medicine, 2000).

Vitamin K:

Consumption of foods or dietary supplements containing natural forms of vitamin K (vitamin K_1 and K_2) is not associated with any adverse health effects. Due to the lack of data regarding the adverse health effects, a UL for vitamin K intake has not been determined (Driskell, 2009; Institute of Medicine, 2001). However, synthetic vitamin K (vitamin K_3 or menadione) may promote oxidative damage to cell membranes through interfering with the function of glutathione, an important biological antioxidant compound. When injected in infants, vitamin K_3 has induced liver toxicity, jaundice, and hemolytic anemia (Higdon, 2004).

Vitamin B_{12}:

No adverse effects have been observed relating to the consumption of foods or dietary supplements containing vitamin B_{12} (cobalamin). UL for vitamin B_{12} was not determined due to lack of data of adverse effects (Driskell, 2009; Institute of Medicine, 1998).

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

There are no direct substitutes for vitamins; however, natural, non-synthetic sources of vitamin compounds do exist. Approximately 15 vitamins have been isolated from biological materials, and the essentiality of the individual compounds for use as vitamins depends on the animal species, growth rate of the animal, feed composition, and synthesizing capacity of the gastrointestinal tract of the animal. Aquatic animal species, and most animals in general are incapable of synthesizing the majority of vitamins at a rate sufficient to meet metabolic requirements. Vitamins are present in very small quantities within animal and plant foodstuffs; natural (non-synthetic) sources of the 15 petitioned vitamins are identified below:

- **Vitamin A:** Exists only in animal tissues in the form of retinol (vitamin A_1: mammals and marine fish) or 3,4-dehydroretinol (vitamin A2: freshwater fish); however, a vitamin A precursor is found in plant tissues in the form of the carotenoid pigments. Rich dietary sources of retinol include fish liver oils, animal liver meals, carrots, spinach, and watercress.
- **Vitamin B_1:** Dried brewers yeast, wheat middlings, wheat mill run, rice bran, rice polishings, dried torula yeast, groundnut (peanut) meal, wheat bran, barley, dried fish solubles, cottonseed meal, soybean meal, linseed meal, dried distillers solubles, broad beans, lima beans, dried delactose whey, glandular meals (liver/kidney), green leafy crops, outer coat or germ of cereals.
- **Vitamin B_2:** Dried torula yeast, dried brewers yeast, liver and lung meal, dried delactose whey, chicken egg white, dried skim milk, dried distillers solubles, safflower seed meal, dried fish solubles, alfalfa meal, poultry by-product meal, fish meal, meat meal, meat and bone meal, groundnut meal, rapeseed meal, green vegetables, germinated cereal grains.
- **Vitamin B_3:** Rice polishings, dried torula yeast, dried brewers yeast, rice bran, wheat bran, dried fish solubles, sunflower seed meal, groundnut meal, rapeseed meal, liver and lung meal, dried distillers solubles, wheat meal run, fish meal, wheat middlings, safflower seed meal, corn gluten meal, meat and bone meal, meat meal, dried brewers grains, poultry by-product meal, sorghum, alfalfa meal, barley grain, dried cane molasses, rice mill run, green leafy vegetables.
- **Vitamin B_5:** Dried brewers yeast, dried torula yeast, dried delactose whey, dried fish solubles, whole hens eggs, rice polishings, groundnut meal, sunflower seed meal, wheat bran, safflower meal, dried skim milk, alfalfa meal, dried cane molasses, rice bran, what middlings, wheat mill run, dried distillers solubles, fish meal, soybean meal, linseed meal, sorghum, maize, cottonseed meal, poultry by-product meal, oats, glandular meals (liver/kidney), green leafy chops.
- **Vitamin B_7:** Dried brewers yeast, dried torula yeast, dried distillers solubles, rapeseed meal, safflower seed meal, sunflower seed meal, whole hens eggs, rice polishings, dried brewers grains, liver and lung meal, rice bran, dried delactose whey, cottonseed meal, groundnut meal, soybean meal, dried skim milk, alfalfa meal, oats, sorghum, dried blood meal, dried fish solubles, fish meal, wheat bran, wheat mill run, legumes, green vegetables.
- **Inositol:** Animal tissues (skeletal, brain, heart, liver), dried brewers yeast and fish meal. In plant tissues, inositol exists as phytic acid (inositol hexaphosphate); rich dietary sources include cereal grains and legumes.
- **Vitamin B_9:** Dried torula yeast, dried brewers yeast, dried brewers grains, alfalfa meal, full-fat soybeans, liver, lung and kidney meal, wheat germ meal, rapeseed meal, rice bran, linseed meal, sunflower seed meal, cottonseed meal, whole hens eggs, dried distillers solubles, wheat bran, wheat mill run, safflower seed meal, dried delactose whey, mushrooms, fruits (lemons, strawberries, bananas), and dark green leafy vegetables.
- **Choline:** Rapeseed meal, poultry by-product meal, shrimp meal, liver and lung meal, dried fish solubles, dried distillers solubles, dried brewers yeast, sunflower seed meal, dried delactose whey, brown fish meal, dried torula yeast, wheat germ meal, white fish meal, safflower seed meal, cottonseed meal, soybean meal, meat meal, meat and bone meal, groundnut meal, whole hens eggs, wheat bran, dried brewers grains, wheat middlings, linseed meal, sesame meal, alfalfa meal, barley, rice bran, rice polishings, wheat mill run, and oats.
- **Vitamin C:** Citrus fruits, black currants, green leafy vegetables, green peppers, cauliflower, watercress, green cabbage, strawberries, green cabbage, potatoes, fresh insects, and glandular meals (liver/kidney).
- **Vitamin D:** Fatty fish (bloater, herring, kipper, mackerel, pilchard, salmon, sardines, tuna), fish liver oils (e.g., cod liver oil), fish meal and roe, animal liver meals and oils, and egg yolks.
- **Vitamin E:** Alfalfa meal, wheat germ meal, whole chicken eggs, rice polishings, rice bran, wheat middlings, dried brewers grains, dried distillers solubles, barley grain, full fat soybean meal, maize grain, what mill run, corn gluten meal, wheat bran, rye grain, sorghum, fish meal, oats, sunflower seed meal, cottonseed meal, virtually all vegetable oils, and green leafy chops.
- **Vitamin K:** Alfalfa meal, fish meal, beef and pork liver meal, and green leafy vegetables (e.g., spinach, kale, cabbage, pine needles, nettles).

- **Vitamin B_{12}:** Animal by-products, liver, kidney, heart, muscle meats, fish meals, shellfish, meat and bone meal, condensed fish solubles, and poultry by-product meal.

Sources: FAO, 1987; Ensminger, 1994

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 vitamins. Therefore, diets comprised of either whole fish or conventional aquatic animal feed pellets containing fish meals and oils naturally supply many of the vitamins and other essential nutrients required by farmed aquatic species. However, some nutrients may be lost as a result of aquatic animal feed processing and extrusion (Riaz, 2009). To ensure a balanced diet for the farmed species, vitamin premixes are included as ingredients in feed pellets for aquatic animals at approximately 0.5 to 1.5% of the feed pellet mass (Aquaculture Working Group, 2012). When possible, a diet comprised of forage fish is the most natural means of simultaneously incorporating required proteins, oils, vitamins, and other nutrients into the diets of carnivorous and omnivorous fish species (NOAA, undated).

The aquaculture industry is striving to mitigate the demands on forage fish while continuing to produce feeds that support excellent growth at competitive prices. To accomplish these goals, fish feeds that include higher percentages of plant based proteins and lower percentages of proteins from fish meal are being increasingly used in aquatic animal production (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).

In terrestrial organic livestock production, the NOP requires the producer to "provide livestock with a total feed ration composed of agricultural products, including pasture and forage, that are organically produced and handled by operations certified to the NOP" (7 CFR 205.237). This requirement necessarily excludes the use of genetically modified organisms in livestock feed. The NOP has not yet promulgated regulations regarding the use of organically vs. conventionally grown feed ingredients in aquaculture. However, the NOSB proposal § 205.252 (j)(6) states that the producer shall not "use any genetically modified organism, or any organism produced by any other excluded method…as a feed ingredient" (NOSB, 2008). Since USDA organic standards require organic feed, nonorganic/GMO soy cannot be fed to livestock (aquatic or terrestrial).

Differences among innate feeding habits (i.e., carnivorous, omnivorous, herbivorous) of the farmed species may require the incorporation of multiple feed sources in order to avoid the use of synthetic vitamins in aquatic animal feeds, especially when plant-based meals are the predominant feed source. As mentioned above, the high temperatures and pressures of the feed extrusion process lead to decomposition of many sensitive vitamins, prompting feed manufacturers to fortify feeds with vitamins and other nutrients (Riaz, 2009). Information from the scientific literature suggests that milder extrusion conditions (i.e., high moisture content, low residence time, low temperature) typically improve nutritional quality, as compared to traditional extrusion methods (i.e., low moisture, temperatures ≥ 200 °C) (Singh, 2007).

<u>Evaluation Question #12:</u> 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 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 vitamins. 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 should be utilized to provide a balanced diet. In addition, zooplankton present in natural ponds provides most of the recommended micronutrients, including vitamins (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).

A number of commercial feed alternatives are either available or in development (USDA, 2010; NOAA, undated). The manufacture of feeds utilizing mild extrusion conditions maintains a higher percentage of bioavailable vitamins in the feed. Natural feed manufacturers typically use milder processing conditions such that fewer nutrients 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 vitamins in these feeds. Further, it has been suggested that many aquatic animal feeds are unnecessarily fortified with vitamins (Robinson, 2001). 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). Only through a combination of these alternative feed sources and mild processing conditions can nutritional requirements be met without the use of synthetic vitamins.

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