Vitamins

Livestock

Identification of Petitioned Substance

This technical report discusses 15 vitamins currently allowed for use in organic livestock production for fortification and enrichment. The scope of vitamin compounds presented in this report is reflective of vitamins defined as "required nutrients" by the National Research Council's (NRC's) Nutrient Requirements of cattle, sheep, swine and poultry. Herein, information is provided about the 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. Nutrient Vitamins for Livestock

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

Common Name	Chemical Name	CAS Number	Trade Names	Other Codes
Vitamin E (Tocopherols)	(2*R*)-2,5,7,8-Tetramethyl-2-[(4*R*,8*R*)-(4,8,12-trimethyltridecyl)]-6-chromanol	59-02-9	α-Tocopherol	EINECS: 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 National Organic Program (NOP) final rule currently allows the use of vitamins, as feed additives, in organic livestock production under 7 CFR §205.603(d)(3) in amounts needed for adequate nutrition and health maintenance (7 CFR §205.237). In crop production, vitamins B_1, C and E are allowed as plant or soil amendments (7 CFR 205.601(j)(8)) and vitamin D_3 may be used as a rodenticide (7 CFR 205.601(g)). Synthetic sources of vitamins are also allowed in processed products labeled as “organic” or “made with organic (specified ingredients or food group(s))” (7 CFR 205.605(b)). This technical report provides targeted technical information regarding the identity and dietary requirements of various vitamin species for the production of cattle, sheep, swine, poultry and other livestock. In addition, this review addresses the potential toxicity or environmental impact of vitamins, as well as the availability of alternatives and compatibility of synthetic vitamins in organic production. The compiled technical information will be used in the National Organic Standard’s Boards review of supplemental vitamins in livestock production under the sunset process.

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 Figure 1, 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 (Friedrich, 1988).

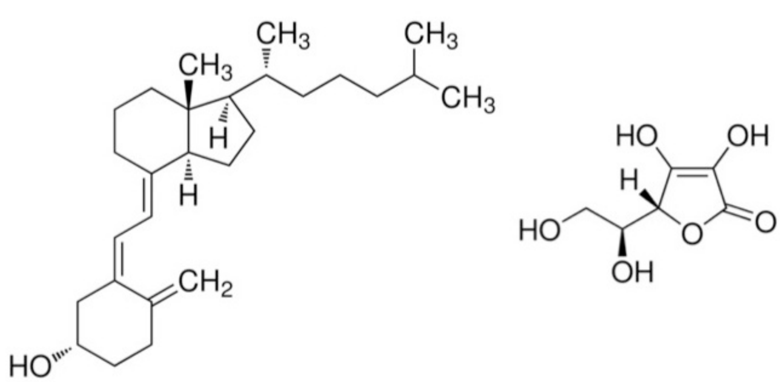

Figure 1. Structures of Vitamin D_3 (left) and Vitamin C (right).

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 (acetic ester of retinol) is a synthetic form of vitamin A commonly used in vitamin supplements and processed foods. The substance has been physically described as a crystalline pale yellow solid 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 ºC. 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, 2015; 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 ºC. 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 saline (aqueous 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 as 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, 2014). 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, 2015).

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, 2014; 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, 2014; 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, 2014; 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). This section summarizes the available information regarding the fortification of animal feed with vitamin premixes in conventional and organic livestock production, as well as the occurrence of vitamins in dietary supplements for human consumption. Vitamins are commonly supplemented by injection (vitamins A, D and E); fortification of grain mixes or silage-based rations to ensure each animal receives some vitamins each day; and free choice supplementation through free choice mineral supplements, protein licks/blocks or in salt/mineral/vitamin mixes (Alberta, 2015).

Ruminants such as cattle and sheep typically produce adequate amounts of the water-soluble B-vitamin complex and fat-soluble vitamin K, making supplementation with these vitamins unnecessary (Adams, 2010; Gadberry, undated). Indeed, vitamin supplementation is generally not as critical as mineral supplementation for ruminants grazing actively growing forages (Parish & Rhinehart, 2008). Although bacteria in the rumen of these animals 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 are commonly observed in animals provided diets devoid of leafy roughage and/or vitamin fortification of the animal feed. Young animals or animals under stress with low levels of fermentation in the rumen can be deficient in B vitamins (Adams, 2010); incorporation of B-vitamin supplements may be required in these situations.

Vitamin A is the vitamin that is most likely to be deficient for beef cattle. The liver can store large amounts of vitamin A, and stores will generally last from two to four months following extended time periods grazing green forage. Because of these factors, vitamin A deficiency in much of the United States is most likely to occur during the latter portion of the wintering period when animals have been fed stored hay for several months, or during an extended period of drought (Gadberry, undated). 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). Another resource indicated that the dietary requirements for vitamin A are 1,270 IU/pound dry feed for pregnant beef heifers and cows and 1,770 IU/pound dry feed for lactating cows. Supplementation with vitamin A can be given either in the diet or by injection (Gadberry, undated). Injections are considered more effective than providing vitamin A through the diet in situations of extreme vitamin deficiency (Parish & Rhinehart, 2008).

With their ruminant digestive system, sheep are able to generate many of the required vitamins from the raw materials consumed in their diet. They efficiently produce all B-vitamins, and vitamins A and E are readily generated inside the body from compounds found in green forage. Vitamin A can be stored in the liver for two to three months after sheep have consumed green forage over an extended period of time (Wahlberg & Greiner, 2006). Accordingly, no supplemental vitamins are needed when ruminants such as sheep and cattle are eating fresh pasture or well-made hay. When sheep are feeding on forage that is old, weathered, mature or otherwise low in the vitamin A precursor compound, this vitamin should be added to the mineral mixture used to fortify animal feed. For example, supplementation is important when feeding sheep and other ruminants fibrous materials that may have inadequate concentrations of vitamin A, such as corn silage, corn stalks and straw. Most commercial mineral premixes for sheep designed for free-choice feeding will contain added A, D and E (Wahlberg & Greiner, 2006).

In contrast to ruminants, swine and poultry must obtain a greater number of vitamins through the diet. Of all farmed species, poultry receives the highest proportion of its feed, and therefore vitamins, from manufactured sources (DSM, 2011a). 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. For swine, the fat-soluble vitamins A, D, E and K, as well as specific B-vitamins that may be deficient in corn- or milo-based diets (i.e., pantothenic acid, riboflavin, niacin, choline and vitamin B_{12}) are generally included in vitamin premixes for supplementation of feed sources. Research has also indicated that additions of folic acid and biotin may improve sow and litter performance when added to gestation and lactation diets (NCSU, undated). In addition to the fat-soluble and water-soluble vitamins, fortification of swine animal feed with choline chloride is recommended to avoid possible choline deficiency in growing-finishing pigs being fattened for slaughter (NCSU, undated).

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:

Vitamins are legally allowed for use as feed additives for animal production, supplements for human consumption, and soil/plant amendments in crop production. This section summarizes the legal uses of various vitamin compounds according to relevant federal regulations.

Conventional and Organic Livestock Feed

The U.S. Food and Drug Administration (FDA) enforces provisions of the Federal Food, Drug and Cosmetic Act (FFDCA) associated with additives used in animal feed and food for human consumption. According to the FFDCA, any substance that is added or expected to directly or indirectly become a component of animal food must be used according to the relevant food additive regulation unless the substance is generally recognized as safe (GRAS) under 21 CFR 582 and 584 for that use pattern (FDA, 2014a). In addition, substances listed as FDA-approved food additives (21 CFR 570, 571, and 573) may also be incorporated into animal feeds. The following synthetic compounds used as vitamins in animal supplements are classified as GRAS by the FDA and therefore not subject to additional regulatory oversight (OMRI, 2013):

- 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

With the exception of vitamin K_3 (menadione dimethylpyrimidinol bisulfite), all of the fat- and water-soluble vitamins commonly included in animal feed supplements 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 573.620), it is not approved for use in human or prenatal supplements or any other food products (FDA, 2014b).

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 "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" (7 CFR 205.2):

Human 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; 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, 2014c).

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 all animals, including livestock. 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 functions of vitamins currently included in vitamin premixes for cattle, sheep, swine and poultry are as follows:

Vitamin A (retinol)

This fat-soluble vitamin is essential for vision, reproduction, growth and maintenance of epithelial tissue, and mucous secretions. 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), degradation of the retina, and anorexia are common symptoms of vitamin A deficiency. In vitamin A deficient poultry, egg production drops markedly, hatchability decreases, and embryonic mortality with incubated eggs increases.

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 decreased appetite, anorexia, poor growth, neuromuscular disorders, and ataxia.

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, spinal deformities, and increased mortality rate. In poultry, egg production is affected, and riboflavin-deficient eggs do not hatch.

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. Loss of appetite, anorexia, poor growth, reduced feed efficiency, 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 decreased food consumption, anorexia, reduced growth, anemia, sluggishness, 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, dermatitis, hyperirritability, anorexia, ataxia, anemia, and retarded 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, and increased mortality. In poultry, biotin deficiency may result in dermatitis of the feet and the skin around the beak and eyes similar to that observed in pantothenic acid deficiency.

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, and lethargy.

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. Poor feathering, nervous disorders and reduced egg hatchability are observed in deficient poultry.

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, poor wound repair, and increased mortality rates are commonly observed in the absence of vitamin C.

Vitamin D (cholecalciferol)

Vitamin D_3 plays an essential role in calcium and phosphorus metabolisms and is necessary for proper bone growth and ossification in animals. In particular, cholecalciferol is required for the absorption of calcium and phosphorus from the gastro-intestinal tract and for the calcification of growing bone tissue (i.e, deposition in the bone matrix). Stiff joints, irritability, anorexia, convulsions, brittle bones, decreased appetite, digestive problems, labored breathing, and weakness are deficiency signs in livestock. Laying hens fed a vitamin D-deficient diet show loss of egg production within two to three weeks, and deteriorated shell quality is observed in severe cases.

Vitamin E (tocopherol)

Vitamin E acts as a fat-soluble extracellular and intracellular antioxidant that prevents the formation of peroxides that can damage tissues 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. Its function is related to that of selenium, which detoxifies peroxides once they are formed. Reduced growth, bulging eye-balls, anemia, damage/degeneration of muscle, 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. Impairment of blood coagulation and prolonged blood clotting time are the major clinical signs of vitamin K deficiency.

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.

Data Sources: FAO, 1987; Bermudez & El-Begearmi, 2012; Gadberry, undated; Parish & Rhinehart, 2008; Cromwell, 2011; Adams, 2010; Stewart, 2013

Combinations of the Substance:

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 may be added to feed rations (Sewell, 1993). Antibiotics are routinely added to grain feed as a growth stimulant in conventional livestock production; however, this practice is not permitted under the USDA organic regulations (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 maltodextrin; 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.

Organic Foods Production Act, USDA Final Rule:

Vitamins are included in Section 2118 of the Organic Foods Production Act of 1990 (OFPA). Specifically, the OFPA states that the National List may allow the use of substances that would otherwise be prohibited under organic regulations (i.e., synthetics) if the substance contains an active ingredient in 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" (OFPA 2118(c)(B)(i)).

The NOP final rule currently allows the use of vitamins in the organic production livestock (e.g., cattle, sheep, swine and poultry) under 7 CFR 205.603(d)(3) according to the nutritional requirements of livestock feed (7 CFR 205.237). In crop production, vitamins B_1, C and E are allowed as plant or soil amendments (7 CFR 205.601(j)(8)) and vitamin D_3 may be used as a rodenticide (7 CFR 205.601(g)). Synthetic sources of vitamins are also allowed in processed products labeled as "organic" or "made with organic (specified ingredients of food group(s))" and intended for human consumption (7 CFR 205.605(b)).

International

Several international organizations have provided guidance on the fortification of feed for organic livestock production with synthetically produced vitamins. Among these are regulatory agencies (Canada, Japan and the EU) and independent standards organizations (Codex and IFOAM). International organic regulations and standards concerning vitamins are described in the following subsections.

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 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 (CAN, 2012).

Codex Alimentarius Commission

The Codex Guidelines for the Production, Processing, Labeling and Marketing of Organically Produced Foods (CAC GL 32-1999) provides criteria for feedstuffs and nutritional elements. Specifically, the section of these guidelines pertaining to livestock production states that "feedstuffs of mineral origin, trace minerals, vitamins, or provitamins can only be used if they are of natural origin. In case of shortage of these substances, or in exceptional circumstances, chemically well-defined analogic substances may be used" (Codex, 2013).

European Union

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

Nature identical synthetic vitamins may be used in the production of non-herbivores without permission, while producers of herbivores must seek approval to use nature identical synthetic vitamins A, D and E. Regarding the latter group, the operator must demonstrate nutritional deficiency of the animals' feed. Soil Association standards do not permit the use of concentrated vitamins and minerals to encourage early maturity or high levels of production (Soil Association, 2014).

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 feed materials. 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, 2012).

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, 2014).

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 currently allowed for use as supplements in organic animal feed fall under the category of vitamins and minerals; thus, these synthetic substances are eligible for consideration under OFPA. Vitamins B_1 (thiamine) and B_7 (biotin) are sulfur-containing substances.

(B) Since the vitamins under consideration are not used in pesticide formulations, they are not, by definition, inert ingredients. 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 US EPA 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.

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

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 manufacturing 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 subsections summarize common manufacturing methods used for vitamins. Processes reviewed in this section are provided as examples, and should not be considered the sole manufacturing procedures used for vitamin compounds. A breakdown of the commonly used production technologies for a subset of 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	Commercial Methods	Other Methods
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, which resulted in decreased susceptibility of the organisms to osmotic dehydration and increased vitamin B_2 production (Lee, 2006).

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).

The chemical synthetic production of biotin is both costly and low yielding. Since only one optical isomer of biotin is biologically 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 Folic acid

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). The available information suggests that many vitamin C producing industries will ultimately shift toward fermentative methods using genetically modified microorganisms due to the increasing global demand for vitamin C and cost saving potential of these developing technologies (Festel, 2005).

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 crop materials, 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)).

According to USDA organic regulations, the NOP defines synthetic as "a substance that is formulated or manufactured by a chemical process or by a process that chemically changes a substance extracted from naturally occurring plant, animal, or mineral sources" (7 CFR 205.2). The following vitamin supplements are likely derived synthetically or 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. 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 synthetic 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)).

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 process, while volatilization of these chemicals from water surfaces is less likely. Most water-soluble vitamins have low bioconcentration factors (BCFs) suggesting minimal potential for bioaccumulation in aquatic organisms (HSDB, 2005a; 2010a).

Fat-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 fat-soluble vitamins would volatilize from dry soil based on their relatively low vapor pressures. Fat-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 bioaccumulation in aquatic organisms (HSDB, 2006).

Erosion of soils contaminated with animal feed and manure will increase the rate at which phosphates, nitrates and other nutrients enter streams, rivers, lakes and coastal regions (Muir, 2012). Ultimately, the persistence of the given vitamin compound may not be of paramount concern when there is a continuous supply of nutrients from the animal feed or other agricultural activities. Laboratory-scale aquaculture studies have suggested that the accumulation of nutrients, including vitamins, in bottom sediments may encourage the growth of algal blooms and red tide species (Wu, 1995).

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).

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 in livestock is generally dependent upon the vitamin's solubility properties and amount consumed. 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 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, in cattle and other livestock is therefore most commonly associated with the fat-soluble vitamins A and D (Parish & Rhinehart, 2008; NIH, 2015a; NIH, 2015b).

Limited technical information exists regarding the toxicity of vitamins to livestock. Reports of acute or chronic toxicity related to the dietary intake of water-soluble vitamins B vitamin complex, vitamin C, choline, inositol or lipid-soluble vitamins D and E are not readily available. Vitamin A toxicity is rare in practical feeding scenarios for beef cattle. Rumen microorganisms can break down vitamin A, which helps to prevent toxic effects associated with excess vitamin A intake (Parish & Rhinehart, 2008). Symptoms of vitamin D toxicity in cattle and other mammals include calcification of soft tissues, bone demineralization, decreased appetite and weight loss (Parish & Rhinehart, 2008). There is less toxicity risk with vitamin E than with vitamins A and D for livestock fed supplemental sources of vitamins. The National Research Council (NRC) considers the following vitamin concentrations in feed—based on a daily digestive energy intake of 6,450 kcal/day—safe for gestating and lactating swine:

- Vitamin A: 3,760 – 7,520 IU/day (1.3 – 2.6 mg/day retinyl acetate);
- Vitamin D: 376 IU/day (9.4 μg/day cholecalciferol);
- Vitamin E: 83 IU/day (83 mg/day dl-α-tocopherol acetate);
- Vitamin K (menadione): 1.0 mg/day;
- Vitamin B_7 (Biotin): 376 mg/day;
- Choline: 2.35 g/day;

- Vitamin B_9 (Folacin): 2.44 mg/day;
- Vitamin B_3 (Niacin): 18.8 mg/day;
- Vitamin B_5 (Pantothenic acid): 22.6 mg/day;
- Vitamin B_2 (Riboflavin): 7.1 mg/day;
- Vitamin B_1 (Thiamine): 1.9 mg/day;
- Vitamin B_6 (Pyridoxine): 1.9 mg/day;
- Vitamin B_{12} (Cobalamin): 28 μg/day.

Data Sources: NRC, 2012; Cromwell, 2011; de Lange, 2013

Concerns have been noted regarding the use of synthetic vitamin K in livestock products, human supplements, and pet foods. 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, 2011b). 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. The bulk of 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 fatty tissues. Although the potential bioaccumulation of these vitamins may be of concern to humans consuming farmed animal meats, no health reports to this effect were identified.

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 specific use pattern for vitamin D_3 in organic livestock production 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).

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)).

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 vitamins synthesized for supplements and feed fortification are derived from petroleum products or genetically modified crop materials. Acetone, for example, is a commonly used chemical reagent derived from petroleum as well as genetically modified 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 highly likely (EPA, 1997). However, lacking are specific examples of environmental damage resulting from exposure to recombinant 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, the creation of new and more potent viruses, as well as unanticipated health risks (UCS, 2002).

There is a slight risk of environmental contamination directly associated with the use of vitamins in organic livestock production. Chemical nutrients, such as vitamins, present in livestock feeds could be introduced to aquatic environments through accidental spills or leaching of nutrients from manure. 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 in eutrophic water bodies (Wu, 1995; NAS, 1969). Once algal proliferation commences, available vitamins may therefore 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.

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, 2015; Acros Organics, 2009). However, release of large amounts of vitamins—particularly the combination of these vitamins with nutrients in animal feed and manure—into the environment may result in eco-toxic events, such as the promotion of algal blooms and red tides (Wu, 1995; Muir, 2012).

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

No direct chemical interactions between vitamins and other additives used in organic crop or livestock production were identified. 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 reviewed 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 terrestrial animal feed will be balanced for optimum health of the particular animal (NRC, 1994; 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).

<u>Evaluation Question #8:</u> 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)).

This technical evaluation report concerns the use of synthetic vitamins in feed for organically raised livestock, such as cattle, sheep, swine and poultry. It is highly likely that small amounts of the supplemental vitamins used in animal feeds would regularly interact with components of the terrestrial agro-ecosystem through animal waste and other releases. Synthetic vitamins are widely used in conventional and organic livestock production with no reported toxicity observed in non-target wildlife or livestock.

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. Reports of large-scale environmental releases or contamination associated with the industrial production of vitamins were not identified during the review of vitamin supplements used in animal feed.

<u>Evaluation Question #9:</u> 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 reviewed 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).

Commercially available forms of supplemental vitamins used in livestock feed pose a slight toxicological risk to overexposed non-target organisms. 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).

Aquatic ecosystems are particularly sensitive to the introduction of nutrients from nearby agricultural operations. Releasing excessive amount of agricultural materials—including phosphate and nitrate fertilizers, feed materials and manure—to waterways can encourage the growth of algae (algal bloom) and other aquatic plants and ultimately oxygen depletion in the affected water zone (Wu, 1995; NAS, 1969). The occurrence of eutrophication due to agricultural activities is generally associated with runoff of phosphate and nitrate fertilizer from soils rather than the introduction of vitamins in animal feed (NAS, 1969). Depending on the contamination level, eutrophication can be manifested as occurrences of algal blooms and red tides, fish kills, and overall loss of biodiversity from the aquatic system (Wu, 1995). Only accidental spills containing large quantities of vitamins in feed have the potential to encourage eutrophication in receiving waters. As such, typical use of vitamin premixes for fortification of feed materials in organic livestock production should not directly contribute to adverse impacts in aquatic ecosystems.

Industrial 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.

As with prescription and over-the-counter medications, improper disposal of vitamins and other supplements may lead to environmental and toxicological issues. 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). Supplemental vitamins are intended for use in livestock production and therefore should not routinely enter waterways. In the event of large accidental releases, however, human exposure to these substances could occur.

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)).

In addition to being essential nutrients, vitamins are generally considered non-toxic and safe for human consumption at levels typically ingested through the diet and dietary supplements taken according to label directions. This response provides technical information regarding reported human health effects associated with direct consumption of vitamins included in this review. Supplementation of animal feeds with vitamins is unlikely to result in excessive vitamin intake for humans; hence, the agricultural use pattern for vitamins under review should not adversely impact human health.

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 essential 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. Microorganisms within the rumen of cattle, sheep and other ruminants produce sufficient amounts of the B-vitamins and vitamin K for the host animal. However, ruminants still require supplementation with vitamin A and, to a lesser extent, vitamins D and E. Other livestock, including swine and poultry are incapable of synthesizing the majority of water- and fat-soluble 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 most commonly recognized vitamins are provided:

- **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. Of specific relevance to livestock, large amounts of the vitamin A precursor (beta-carotene) are typically found in green, growing forages and freshly-stored green forages, with significantly lower concentrations in drought-stricken forage and hay that has been stored for prolonged periods of time.
- **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 D is found in sun-cured forages and is also synthesized in the skin of animals exposed to sunlight (Gadberry, undated).
- **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 E is found in forages, but it may be destroyed during sun-curing and long-term storage.

- **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.

Data Sources: FAO, 1987; Ensminger, 1994; Gadberry, undated; Adams, 2010

Raising livestock without the use of synthetic vitamins premixes may be possible depending on the animal species being raised, local weather conditions and nutritional quality of available feeds. Based on the naturally occurring sources of vitamins A, D and E, it is unlikely that ruminants such as cattle and sheep will require year-round supplementation of feed rations with vitamin premixes. When fresh green forage and periods of sunlight exposure are reduced, however, supplementation of feed or injection with synthetic sources of vitamins A and D are commonly required for ruminants raised in most regions of the United States (Gadberry, undated). Further, providing non-ruminants such as poultry and swine with natural forage materials may reduce or, during certain periods of the year, eliminate the need for grains and processed feeds fortified with vitamin premixes. Although pigs have limited ability to utilize pasture roughage, operators providing excellent quality forage can reduce swine consumption of processed grain by 30 to 60% (Schivera, 2015a). Additional information related to the provision of high quality forage in cattle, swine and poultry is reviewed in Evaluation Question #12.

Poultry and other livestock animals receiving the highest percentage of their diets from manufactured feed sources typically require some form of vitamin supplementation to maintain a healthy diet. For example, a recent report indicates that certified organic corn meal, sorghum grain, and mechanically-extruded soybean meal may be provided to organically produced swine (Shurson, 2013). Essential vitamins readily decompose during feed storage or are lost as a result of animal feed processing and extrusion (Riaz, 2009). Specifically, 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 key nutrients (Riaz, 2009). As such, organic feed mixes for broiler chickens typically contain peas, wheat, barley, linseed meal (extruded), corn, camelina meal (extruded) and fish meal in combination with FDA-approved version of synthetic vitamins, including vitamin E, niacin, vitamin A, d-calcium pantothenate, riboflavin, biotin, vitamin D_3, menadione sodium bisulfite, thiamine mononitrate, pyridoxine hydrochloride, vitamin B_{12} and folic acid (Scratch and Peck Feeds, 2014). Open literature reports indicate that milder extrusion conditions—high moisture content, low residence time, low temperature—typically improve the nutritional quality of processed feeds when compared to feeds produced according to traditional extrusion methods involving low moisture and temperatures in excess of 200 ^{o}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)).

Modern agricultural practices at large-scale operations have increased the prominence of vitamin and mineral supplementation because of the changes in feeding, housing and management systems. The amount of available forage directly affects the need for supplemental feeding, and thus fortification of these feed materials. Reducing the number of grazing animals can lessen the demand for supplemental feeding for the remaining livestock (Hammack & Gill, 2012). Taking swine production as a specific example, several common practices have resulted in the supplementation of feed materials with synthetic vitamins (NCSU, undated). Pigs raised in confinement are sometimes denied free access to soils and grazing crops, which naturally provide many of the vitamins and minerals needed for proper nutrition. Although confinement practices are not permitted in organic production, other agricultural trends are relevant to both conventional and organic livestock producers. The increased use of slotted floors may prevent the recycling of feces rich in B-vitamins and vitamin K, nutrients that are synthesized by microorganisms in the large intestine of some animals (NCSU, undated). In addition, feeding practices that provide less diverse protein sources limit the variety of vitamins and minerals available in the diet of raised animals. More artificial nutrients are also required when swine producers seek to decrease the weaning period of pigs. Lastly, swine feeds are commonly fortified to compensate for potentially low bioavailability of nutrients in heat-dried grains and feed ingredients (NCSU, undated).

Producing nutritionally balanced livestock requires operators to provide all of the good quality forage that animals desire, and dietary supplementation with nutrients that may be deficient. Therefore, animal nutrition is related to good forage management, including proper fertilization, growing mixtures of grasses and legumes, maintaining forage at a nutritional stage of growth and providing the forage in adequate quantities (Wahlberg & Greiner, 2006). Livestock producers are encouraged to provide fresh forage whenever possible, and not to use feeds excessively exposed to sunlight, heat and air; heavily processed feeds; or feed materials stored for long periods of time. Regarding the latter issue, exposure of cut hay—including baled hay, silage, round bale silage—to the air for 60–90 days results in destruction of the fat soluble vitamins (Alberta, 2015). Pasture can be beneficial for all livestock, and may even reduce the amount of vitamin-supplemented mash and grain required for poultry. In fact, free-range chickens receive large amounts of protein and essential nutrients from the insects they eat while pecking at the soil in pasture areas (Schivera, 2015b). For cattle, vitamin A deficiency is highly probable when cattle are fed diets primary consisting of bleached pasture or hay during drought conditions (Parish & Rhinehart, 2008). Therefore, in more extreme conditions (e.g., drought), supplementation with synthetic vitamins may be unavoidable for organic livestock producers maintaining large animal herds.

While unlikely to obviate the need for synthetic vitamins supplements, the combination of alternative natural materials and cultural practices may allow conventional and organic livestock producers to provide vitamin-fortified processed animal feed less often and in smaller quantities.

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