Vitamins B_1, C and E

Crops

Identification of Petitioned Substance

Chemical Names:
Vitamin B_1: 2-[3-[4-Amino-2-methyl-pyrimidin-5-yl)methyl]-4-methyl-thiazol-5-yl] ethanol
Vitamin C: (*R*)-3,4-dihydroxy-5-((*S*)-1,2-dihydroxyethyl)furan-2(5*H*)-one
Vitamin E: (2*R*)-2,5,7,8-Tetramethyl-2-[(4*R*,8*R*)-(4,8,12-trimethyltridecyl)]-6-chromanol

Other Name:
Thiamine (Vitamin B_1)
Ascorbic Acid (Vitamin C)
Tocopherols (Vitamin E)

Trade Names:
Dyna-Gro, Rhizo Gel (B_1 products); Thiamine hydrochloride, L-Ascorbic acid, α-Tocopherol

CAS Numbers:
67-03-8 (Thiamine hydrochloride)
50-81-7 (L(+)-Ascorbic acid)
59-02-9 ((+)-α-Tocopherol)

Other Codes:
200-641-8 (Thiamine hydrochloride)
200-066-2 (L(+)-Ascorbic acid)
200-412-2 ((+)-α-Tocopherol)

Summary of Petitioned Use

The National Organic Program (NOP) final rule currently allows the use of vitamins B_1, C and E as plant or soil amendments in organic crop production under 7 CFR §205.601(j)(8). Synthetic forms of vitamins—including vitamins B_1, C and E—are also permitted in other areas of organic production and handling. In addition to crop applications, nutrient vitamins are also allowed as feed additives in organic livestock production under 7 CFR §205.603(d)(3) in amounts needed for nutrition and health maintenance (7 CFR 205.237). Similarly, synthetic sources of vitamins may also be incorporated into processed products labeled as "organic" or "made with organic (specified ingredients or food group(s))" (7 CFR 205.605(b)). This report provides targeted technical information on vitamins B_1, C and E for the National Organic Standards Board's review of these substances under the sunset process.

Characterization of Petitioned Substance

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

Vitamins B_1, C and E are naturally occurring compounds synthesized in small amounts by various plants and microorganisms, including bacteria and fungi. At the molecular level, water-soluble vitamins in this group (i.e., vitamins B_1 and C) consist of carbon and hydrogen as well as several electronegative atoms, including nitrogen, oxygen, sulfur and chlorine. The abundance of polar functionality—amino groups, hydroxyl groups, and positively or negatively charged atoms—imparts enhanced water solubility to vitamins B_1 and C. Vitamin E, on the other hand, is composed predominantly of non-polar carbon-carbon and carbon-hydrogen bonds, and contains only one polar functionality (i.e., hydroxyl group) on the substituted benzopyran subunit. Vitamin E is classified as a fat-soluble vitamin. Figure 1 below provides two-dimension representations of the molecular structures for vitamins B_1, C and E.

<u>Source or Origin of the Substance:</u>

Vitamins can be extracted from foods or synthesized by chemical or biofermentation processes. Regarding the former method, 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 green leafy vegetables. Vitamins B_1 and C are produced on an industrial scale using chemical synthesis, while commercially available sources of vitamin E are obtained through a combination of extraction and chemical synthesis (Festel, 2005). The available patent literature indicates tremendous growth in the development of fermentative methods for the production of vitamin compounds utilizing genetically modified microorganisms (GMMs) over the past decade. See the response to Evaluation Question #2 for detailed information regarding currently utilized industrial production techniques and an analysis of trends in the application of GMMs in the synthesis of vitamins B_1, C and E.

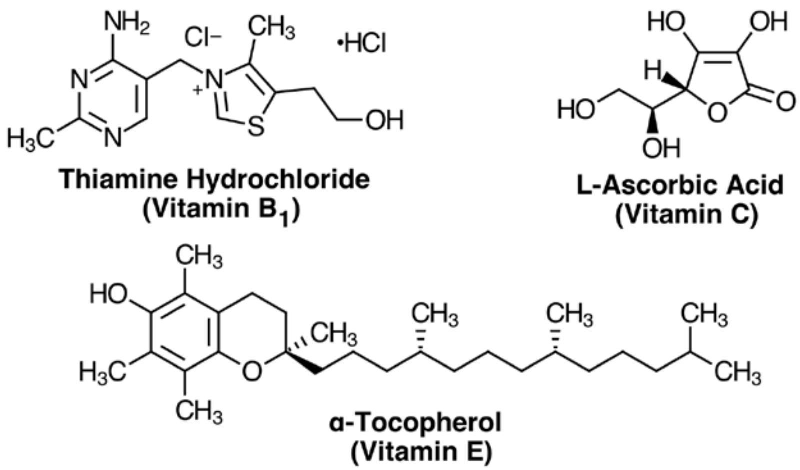


Figure 1. Water-soluble vitamin B_1 and C contain polar functionalities, while fat-soluble vitamin E contains largely non-polar chemical bonds.

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

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-based) compounds and are typically grouped depending on their solubility in water vs. organic solvents. The more hydrophilic vitamin compounds (vitamin B_1 and C) tend to have multiple polar functionalities, such as hydroxyl groups, amino groups, carboxylic acids, alkoxy groups, and/or salts of carboxylic acids and amines. Due to their enhanced aqueous solubility, vitamin B_1 and C molecules not absorbed or metabolized by the organisms are rapidly excreted in biological fluids. Alternatively, more lipophilic (fat-soluble) vitamins such as vitamin E are primarily composed of aliphatic carbon frameworks and may be stored in fatty tissues upon excessive consumption of the vitamin. As a class of substances, vitamins have relatively low vapor pressures (HSDB, 2010a; HSDB, 2010b). Details regarding the physical and chemical properties for vitamins included in this review (i.e., vitamins B_1, C and E) are provided in the following subsections.

Vitamin B_1

Vitamin B_1 (thiamine) and thiamine hydrochloride, a commonly used industrial form of vitamin B_1 are colorless solids with melting points of 164 and 250 ºC (HSDB, 2010a; ChemicalBook, 2010a). One gram of thiamine dissolves in approximately 1 mL water, 18 mL glycerol, 100 mL 95% alcohol, or 315 mL absolute alcohol (ethanol); thiamine is practically insoluble in non-polar organic solvents such as diethyl ether, benzene, hexane and chloroform. The pH of a 1% weight/volume solution of thiamine in water is 3.13 (HSDB, 2010a). Chemical forms of vitamin B_1 are generally light sensitive and hygroscopic (i.e., readily absorb moisture from the atmosphere) (ChemicalBook, 2010a).

Vitamin C

Pure vitamin C (L-ascorbic acid) is a colorless crystalline powder or solid at room temperature. Aqueous solutions of vitamin C have 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 ºC. L-ascorbic acid is highly soluble in water (solubility = 176 g/L at 20 ºC). 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 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 ºC and a density of 0.95 g/mL at 20 ºC. 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, 2010b).

Specific Uses of the Substance:

Synthetic forms of vitamins B_1, C and E enjoy a variety of uses ranging from the stimulation of crop growth and plant protection to supplementation in livestock feeds and human dietary supplements. This section summarizes the uses of these specific vitamin compounds, with a focus on their application in organic and conventional crop production.

Root Stimulation Using Vitamin B_1

Vitamin B_1 is included in several commercially available root stimulator products to help nursery-grown mature plants, seedlings and cuttings become established when transplanted to native soil (Schalau, 2010). According to the Organic Materials Review Institute (OMRI) Generic Materials List, growth regulators for plants include non-synthetic plant hormones such as gibberellic acid, indole acetic acid (IAA) and cytokinins. Synthetic forms of vitamin B_1 are also approved for use in organic crop production as a growth regulator for plants. Organically approved growth stimulator products containing vitamin B_1 were not identified on the OMRI product list (OMRI, 2013).

Nursery operators and farmers commonly use vitamin B_1 root stimulator products to prevent transplant shock and stimulate the growth of new roots when planting trees, shrubs, roses and other plants (Cox, 2010). Many conventional products marketed as root stimulators list vitamin B_1 as an essential ingredient; however, these products also contain auxins—such as the synthetic plant hormone indole butyric acid (IBA) and naphthylacetic acid (NAA)—as well as various fertilizer compounds (Schalau, 2010). The bulk of the available scientific data suggests that IBA and NAA contribute to root regeneration of transplanted trees by suppressing crown growth to effectively redirect resources to developing roots. Conversely, the root growth claims associated with vitamin B_1 are largely unsubstantiated and generally based on tissue culture research in which plant tissues are propagated using sterile conditions and artificial growth media (Schalau, 2010; Kontaxis & Cox, 1984). According to Cox (2010), "several studies using intact mums, apple trees, orange trees, pine, tomato, beans, pepper, corn, pear, watermelon and squash have failed to demonstrate that vitamin B_1 treatments provide any type of growth response." For example, Kontaxis & Cox (1984) transplanted 19-day old seedlings of tomato, green pepper, squash, watermelon, sweet corn, snap bean and pole bean, applying thiamine hydrochloride solutions to the soil of treatment groups at planting and once several days later. No difference was observed between vitamin B_1-treated and control plants in terms of vigor, size, color or root development at 18 to 29 days after first treatment.

Plant Protection and Growth Stimulation Using Vitamins C and E

Practical information regarding the use of vitamins C and E in modern crop production from agricultural extension service websites and related resources are generally unavailable. Therefore, specific use information for these two synthetic substances in crop production is based largely on information derived from the peer-reviewed scientific literature.

The antioxidant qualities of vitamins E and C have been successfully employed to promote growth/yield and protect plants from oxidative stress due to salinity. El-Tohamy & El-Greadly (2007) found that foliar applications of vitamin E (0.1 mL/L) to 20-day-old bean plants resulted in statistically significant increases in growth, yield, bean pod quality, natural plant growth hormone levels, as well as chlorophyll and mineral (nitrogen, phosphorus and potassium) contents in leaves. The antioxidant properties of vitamin E have also been utilized in the treatment of Jonagold apple trees, which resulted in a 6-fold increase in the fruits within 48 hours following application to leaf discs (Noga & Schmitz, 2000). Hussein et al. (2007) demonstrated that foliar application of vitamin E (200 ppm) to cowpea plants under salinity stress significantly improve plant health parameters, including plant height and dry weight. Vitamin E produced within the plant (i.e., endogenous vitamin E) has also been found to play a key role in low-temperature adaptation and nutrient transport in the phloem (Maeda, 2006). Research conducted using live apple seedlings and trees found that foliar applications of vitamin E in glycerol and water (0.25%) protected the leaves of apple seedlings as well as flowers of orchard trees against freezing injury (Albrecht, 2004).

Recent research indicates that application of vitamin C to maize and kidney bean plants are protective of changes in growth and metabolic activity associated with salt-induced stress (Hassanein, 2009; Salama, 2014). According to Hassanein et al. (2009), application of vitamin C as a shoot spray or grain soaking "did not only alleviate the inhibitory effect of salinity stress on the biosynthesis of photosynthetic pigments, but also induced a significant stimulatory effect greater than observed in the corresponding controls." The potential antioxidant mechanisms of vitamins C and E associated with plant protection and enhanced growth are described below in "Action of the Substance." A strong body of evidence for commercially grown plants indicates that vitamin C applied as an aqueous spray solution of ascorbate salts (e.g., potassium ascorbate) moves into leaf cells and increases the tolerance of these cells to the damaging effects of smog in some plants (Freebairn, 1960). Indeed, a more recent study demonstrated that application of foliar sprays containing vitamin C at 300 mg/L to broad beans minimizes the adverse effects associated with exposure to the oxidant ozone and other air pollutants, such as nitrogen oxides and sulfur dioxide (Ali & Musallam, 2007). Application of foliar sprays containing vitamin B_{12} (50 ppm), folic acid (50 ppm) and vitamin C (500 ppm) to wheat at 30 and 60 days after planting encouraged plant growth, grain yield and mineral contents in leaves and grains relative to control plants (Mohamed, 2013).

Patents have been developed based on the ability of antioxidants such as vitamins C and E to strengthen the defense systems of treated plants. Indeed, one disclosed method involves the application of vitamin C and E to plants as either soil drenches or foliar sprays to increase resistance to pests and pathogens associated with common plant diseases (Norris, 1991). Likewise, Abdel-Kader et al. (2012) used ascorbic acid as a resistance chemical inducer in combination with biological control agents (e.g., *Bacillus subtilis*) to reduce the incidence and severity of foliar plant disease, such as powder/downy mildews of cucumber, cantaloupe and pepper as well as early and late blights of tomato.

Vitamins B_1, C and E as Nutrient Vitamins in Livestock Production and Dietary Supplements

Organically- and conventionally-raised livestock are regularly provided with synthetic forms of water-soluble (B-complex and C) and fat-soluble vitamins to supplement the nutrient loads naturally found in the diet. 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 are able to generate virtually all of the require B-complex vitamins from the raw materials in their diet; nutrient premixes for ruminants generally contain vitamins A, D and E (Wahlberg & Greiner, 2006). In contrast, swine and poultry must obtain a greater number of vitamins through the diet. Because of this limitation and the nutritional variability of natural feed materials, many livestock producers supplement feed sources with vitamins A, D, E and K as well as specific B-vitamins that may otherwise be deficient in feed (NRC, 1994; NCSU, undated).

Human dietary supplements generally contain a combination of essential nutrients, including synthetic vitamins such as thiamine, ascorbic acid and tocopherols. Higher intakes of vitamins can be particularly important for recovery following surgical operations. Indeed, recent scientific research suggests that intake of 500 mg/day of the antioxidant vitamin C is associated with reduced postoperative oxidative stress (Fukushima, 2010). Additionally, food products are commonly fortified with vitamins B_1, C and E and other essential nutrients to facilitate sufficient public consumption of these vital compounds (FDA, 2015).

Approved Legal Uses of the Substance:

Vitamins B_1, C and E are legally allowed for use as feed additives for animal production and supplements for human consumption. This section summarizes the legal uses of these specific vitamin compounds according to relevant federal regulations.

Ingredients in 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 forms of vitamins B_1, C and E are classified as GRAS by the FDA and therefore are not subject to additional regulatory oversight (OMRI, 2013):

- Vitamin B_1 (thiamine hydrochloride) 21 CFR 582.5875
- Vitamin C (ascorbic acid) 21 CFR 582.5013
- Vitamin E (α-tocopherol acetate) 21 CFR 582.5892

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 1). 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, 2014).

Table 1. FDA Nutrition Quality Guidelines for Foods: Vitamins B_1, C and E

Vitamin	Unit of Measurement	DRV or RDI	Amount per 100 calories
Vitamin C	mg	60	3
Vitamin E	IU	30	1.5
Vitamin B_1 (thiamine)	mg	1.5	0.08

IU = International Unit, unit of activity or potency for vitamins and other substances; mg = milligram (gram/1,000); DRV = Dietary Reference Values; RDI = Reference (Recommended) Daily Intake

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

Modes of action have not been clearly elucidated for vitamin B_1, C and E as plant and soil amendments. Indeed, many argue that soil applications of vitamin B_1 provide no benefit for root growth, and the proposed plant protection mechanisms for vitamins C and E are based primarily on correlations and established antioxidant pathways for these compounds in animals and plants. This section summarizes plausible mechanisms for vitamins B_1, C and E as used in crop production.

Root stimulant substances generally work by stimulating the root to produce its own natural rooting hormones to help increase lateral root growth and root mass (Smith, 2015). However, several sources have indicated that application of vitamin B_1 to the root systems of whole plants does not actually stimulate root growth following transplantation (Schalau, 2010; Kontaxis & Cox, 1984). The claims associating vitamin B_1 with enhanced root growth are likely based on tissue culture studies in which vitamin B_1 is commonly included as an ingredient of the growth media (Schalau, 2010). It is therefore difficult to describe the mode of action for vitamin B_1 root growth stimulants in the absence of significant *in vivo* results correlating vitamin B_1 soil applications with enhanced root growth.

L-ascorbic acid (vitamin C) serves a predominantly protective role in both plants and animals. The naturally occurring substance functions as a major oxidation/reduction buffer and as a cofactor for enzymes involved in regulating photosynthesis, hormone biosynthesis and regenerating other antioxidant species (Gallie, 2013). It also regulates cell division and growth and is involved in signal transduction. Foliar sprays and soil drenches of aqueous vitamin C solutions (exogenous sources) can help plants cope with moderate fluctuations in the endogenous levels of this essential plant nutrient. As a powerful antioxidant, vitamin C defends vulnerable plant tissues against the damaging oxidative effects of ozone by neutralizing free radical species (Burkey, 2003). Researchers have found that plants capable of moving greater quantities of vitamin C into the space surrounding cell walls (apoplasts) have a better change of detoxifying ozone (Burkey, 2003). Scientific results supporting the protective effects of plant and soil amendments containing vitamin C have been disclosed in peer-reviewed journal articles and patents (Freebairn, 1960; Norris, 1991).

The stimulatory effects of vitamin E applications on plant height, number of leaves and branches, and yield of various bean species has generally been attributed to the antioxidant properties of vitamin E (El-Tohamy, 2007). While vitamin C is highly water-soluble and generally resides in aqueous compartments outside of cell walls and membranes, vitamin E is a low molecular weight lipophilic (fat-soluble) antioxidant that accumulates within cell membranes (El Bassiouny, 2005). Thus, vitamin E can be characterized as a highly effective antioxidant at the membrane site (Hess, 1993). The concerted actions of fat-soluble vitamin E at the surface of membranes and water-soluble vitamin C within aqueous extracellular spaces provides plant tissues with multiple layers of protection from oxidative damage associated with exposure to ozone, as discussed above for vitamin C. Abiotic stressors—including high-intensity light, salinity, drought and low temperatures—are commonly associated with higher levels of oxidative stress in plants. The tocopherols comprising vitamin E can efficiently quench singlet oxygen (and other reactive oxygen species), scavenge various radicals such as lipid peroxy radicals, and thereby terminate lipid peroxidation chain reactions associated with oxidative stress (Maeda, 2006).

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

Products marketed as root stimulators often list vitamin B_1 in combination with synthetic plants hormones known as auxins as well as various fertilizer compounds (Schalau, 2010). Common auxins include indole butyric acid (IBA) and 1-naphthaleneacetic acid (NAA). For example, Dyna-Grow K-L-N Rooting Concentrate is a vitamin-hormone solution containing vitamin B_1 and both of the synthetic rooting hormones, IBA and NAA, with claims of promoting vigorous root growth in trees, foliage and flowering plants (Hydro Galaxy, 2015). Synthetic plant hormones are not approved for use in organic production; however, naturally occurring auxins such as indole acetic acid (IAA) and other plant growth hormones such as cytokinins and gibberellic acid may be used in combination with vitamin B_1 to regulate plant growth in organic crop production (OMRI, 2013). The prevalent combination in commercially available products of vitamin B_1 with synthetic substances prohibited in organic production suggests that organic operators must obtain technical forms of vitamin B_1 (e.g., thiamine hydrochloride) lacking synthetic hormones for use as a rooting agent.

Less is known regarding materials typically applied with vitamins C and E for plant protection in organic or conventional crop production. Albrecht et al. (2004) demonstrated the synergistic effect of mixtures containing the antioxidant α-tocopherol (vitamin E) and the cryoprotector glycerol against freezing injury in the leaves and flowers of apple trees. In addition, Norris (1991) described how co-application of vitamins C and E to the surface of plants confers enhanced resistance to environmental stresses associated with pest insects and pathogenic microorganisms. The defensive response elicited through application of vitamins C and E is systemic in nature; treatment of one portion of a plant initiates a defensive response throughout the plant (Norris, 1991).

Status

<u>Historic Use:</u>

In 1995, the National Organic Standards Board (NOSB) recommended addition of vitamins B_1, C and E to the National List as approved plant and soil amendments in organic crop production (USDA, 1995). Researchers observed a positive correlation between vitamin B_1 application and the rate of root growth in plant tissue culture studies conducted in the late 1930s (Bonner & Greene, 1938). Around this time, naturally occurring plant growth regulators known as auxins were isolated and found to stimulate cell elongation in roots and stem tissue (Chalker-Scott, undated; Hoffman, 2010). Manufacturers have therefore co-formulated plant hormones (e.g., synthetic auxins) and vitamin B_1 in root stimulation products since the late 1940s (Chalker-Scott, undated). No historical information relevant to the current review was identified regarding the use of vitamins C and E in organic or conventional crop production.

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 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 B_1, C and E as plant or soil amendments in organic crop production under 7 CFR §205.601(j)(8). Synthetic forms of vitamins—including vitamins B_1, C and E—are also permitted in other areas of organic production and handling. In addition to crop applications, nutrient vitamins are also allowed as feed additives in organic livestock production under 7 CFR §205.603(d)(3) in amounts needed for nutrition and health maintenance (7 CFR 205.237). Similarly, synthetic sources of vitamins may also be incorporated into processed products labeled as "organic" or "made with organic (specified ingredients or food group(s))" (7 CFR 205.605(b)).

International

Only Canadian organic regulations mention the use of synthetic vitamin compounds in organic crop production. According to Section 4.2 of the Canadian General Standards Board's Permitted Substances List, synthetic forms of vitamins B_1, C and E are allowed soil amendments and crop nutrition agents in organic agriculture (CAN, 2011). The following international standards and regulations were consulted but did not contain guidance on the use of synthetic vitamins in organic crop production: Codex Alimentarius Commission Guidelines for the Production, Processing, Labeling and Marketing of Organically Produced Foods (CAC/GL 32-1999; Codex, 2013), European Commission Regulation Number 889/2008 (EC, 2008), the IFOAM Norms for Organic Production and Processing (IFOAM, 2014), and the Japanese Agricultural Standards for Organic Plants (Notification Number 1605; JMAFF, 2012).

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

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

(A) Vitamins B_1, C and E currently allowed for use as plant and soil amendments in organic crop production fall under the category of vitamins and minerals; thus, these synthetic substances are eligible for consideration under OFPA. Vitamin B_1 (thiamine) is a sulfur-containing compound.

(B) The previous paragraph provides sufficient information to determine eligibility of the substance under OFPA; however, the inert status of vitamins B_1, C and E is briefly described. Vitamin E and L-ascorbic acid (vitamin C) appear on US EPA List 4A, minimum risk inert ingredients. Thiamine mononitrate and vitamin B complex are present on List 4B, minimal risk other ingredients. Vitamin E acetate (a synthetic form of vitamin E) is included on List 3, inerts of unknown toxicity.

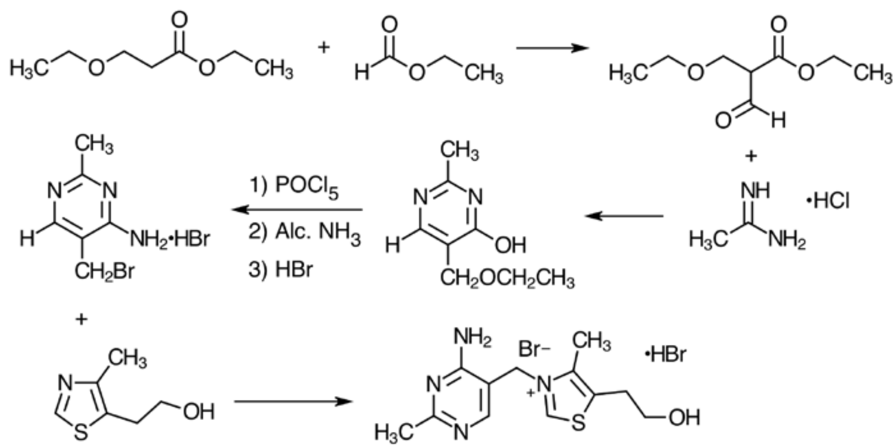
Evaluation Question #2: Describe the most prevalent processes used to manufacture or formulate the petitioned substance. Further, describe any chemical change that may occur during manufacture or formulation of the petitioned substance when this substance is extracted from naturally occurring plant,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

Individual vitamin compounds are produced on an industrial scale by chemical synthesis or partial synthesis, fermentation and/or by extraction from natural material sources. Selection of the manufacturing processes typically depends on available technology, cost of raw materials/chemical feedstocks, market prices and size, cost of implementing fermentation versus chemical processes (synthesis or extraction) and, to a lesser extent, the overall environmental impact of the production method. Chemical synthesis is the primary production method for the three vitamins included in this technical review. Extraction of tocopherols (vitamin E) from plant oils is also commonly utilized, and laboratory-scale fermentation processes have been developed for both vitamin B_1 and C (Festel, 2005).

The following subsections summarize common manufacturing methods used in the commercial production of vitamins B_1, C and E. Processes reviewed in this section are provided as examples, and should not be considered the sole manufacturing procedures used for these vitamin compounds.

Vitamin B_1

Commercial production involves a six-step synthetic procedure (Williams & Cline, 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.



Scheme 1. The commercial production of thiamine salts (e.g., thiamine hydrobromide) involves a multi-step synthetic sequence (*Adapted from* Williams & Cline, 1936).

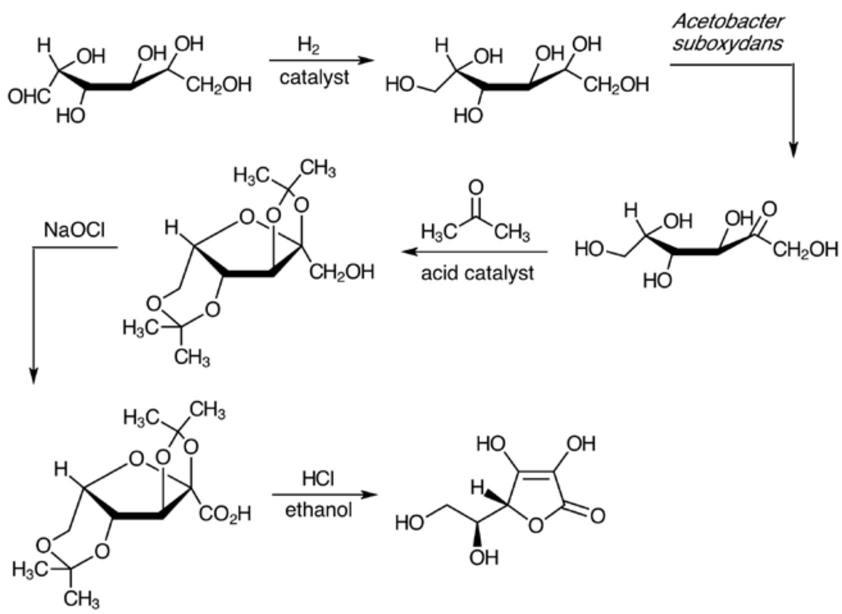
A search of the patent literature revealed two methods for vitamin B_1 (thiamine) production by fermentative methods. The first patent describes the development of mutants of the genus *Saccharomyces Meyen emend Reess* (yeast) for synthesizing vitamin B_1 from sugars and inorganic salts (Silhankova, 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 thiamine products into the medium (Goese, 2012).

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).



Scheme 2. The Hoffman-La Roche vitamin C commercial production involves five synthetic steps (*Adapted from* McMurry, 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).

<u>Evaluation Question #3:</u> 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). Vitamins B_1 and C are likely derived synthetically or through a combination of chemical synthesis and fermentation methods. Alternatively, vitamin E (mixture of tocopherols) is typically extracted from natural materials (e.g., vegetable oils) using aliphatic hydrocarbon solvents and acid-base extraction methods. Chemical synthesis and extraction techniques are typically considered chemical processes due to the application of synthetic chemical reagents in these methods. Vitamins produced through biological fermentation may be considered non-synthetic or synthetic, depending on the nutrient feedstocks, fermentation organisms, and processing aids used during production. Based on the NOP definitions and the predominant manufacturing processes, it is reasonable to conclude that vitamins B_1, C and E are synthetic substances. The NOSB classified vitamins B_1, C and E as synthetic following the 1995 TAP review; hence, these vitamins were included on the list of synthetic substances allowed for use in organic crop production (7 CFR 205.601).

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 B_1, C and E will be released to soil and water. This section summarizes information concerning the environmental fate and persistence of vitamins B_1, C and E in terrestrial and aquatic environments.

Water-soluble vitamins, such as vitamin B_1 and C, are expected to have slight to high mobility if released to soil and therefore may spread to other soil areas and waterways (HSDB, 2010a). In contrast to vitamin C, vitamin B_1 is more likely to adsorb to clay-based soils and other soil types with significant amounts of organic matter (Schmidhalter, 1994). Water-soluble vitamins are unlikely to volatilize from moist and dry soils due to their high polarity and low vapor pressures, respectively (HSDB, 2010a; HSDB, 2010b). Vitamins B_1 and C are not expected to persist in the environment; for example, vitamin C exhibited a biodegradability of 97% after 5 days and 100% after 15 days when applied to activated sludge (IPCS, 2014). If released to water, vitamin C is not expected to adsorb to suspended solids and sediments (HSDB, 2010b), while vitamin B_1 may exhibit low to moderate adsorption in aquatic environments (HSDB, 2010a). For many water-soluble vitamins, the presence of functional groups that hydrolyze means hydrolysis is expected to be an important degradation process. In contrast, volatilization of vitamins C and B_1 from water surfaces is less likely. Water-soluble vitamins generally have low bioconcentration factors (BCFs), suggesting minimal potential for bioaccumulation in aquatic organisms (HSDB, 2010a).

Fat-soluble vitamin compounds—such as the mixture of tocopherols comprising vitamin E—are less polar than water-soluble vitamins and thus practically insoluble in water. Because of this property, vitamin E is unlikely to be mobile in soils (HSDB, 2006). It is unlikely that fat-soluble vitamin E would volatilize from dry soil based on its relatively low vapor pressure (1.4×10^{-8} mm Hg at 25 ^{o}C). If released to water, fat-soluble vitamin E will adsorb preferentially to sediments and other suspended solids present in the water column due to its solubility properties. The synthetic acetate esters of tocopherols used in many commercial supplements readily hydrolyze to liberate the biologically active tocopherol species (van Henegouwen, 1995); however, these free tocopherols that comprise vitamin E lack hydrolysable functional groups. Thus, hydrolysis is not expected to be the dominant degradation process for vitamin E. The bioconcentration factor (BCF) for the acetic ester of vitamin E is 3.2, indicating significant accumulation in organisms is not expected (BASF, 2005). As components of highly biodegradable natural oils (e.g., soybean oil), it is unlikely that tocopherols comprising vitamin E will persist in the environment due to use of the substance to protect plant tissues and promote plant growth in crop production.

Erosion of soils containing fertilizers and other plant growth substances will increase the rate at which phosphates, nitrates and nutrients such as vitamins enter streams, rivers, lakes and coastal regions (Muir, 2012). Ultimately, the persistence of the given vitamin compound may not be of paramount concern for situations in which there is a continuous supply of nutrients resulting from intensive 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).

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)).

It is unlikely that the use of vitamins B_1, C and E as seed treatments, soil applications, and foliar sprays is harmful to treated plants or non-target plants and animals. Vitamins are generally considered non-toxic essential nutrients for terrestrial and aquatic organisms. Indeed, plants and microorganisms naturally produce vitamin B_1, C and E for various biological functions. Based on the low hazard profile of these substances, the potential for toxicity in any exposed organism is dependent upon the solubility properties of the vitamin and the amount of exposure. In animals, the water-soluble vitamins (B_1 and C) are rapidly depleted in the absence of regular dietary intake, and appreciable quantities of these vitamins do not accumulate in the animal body. Fat-soluble vitamin E is readily absorbed from the gastrointestinal tract and stored in the animal's fatty tissues when dietary intake exceeds metabolic demands for the vitamin.

Animals excrete vitamins B_1 and C in urine and generally do not exhibit symptoms of toxicity following ingestion of these substances. Based on our review of the scientific literature and the 1998 Institute of Medicine chapter for thiamine, there are no reports of adverse effects from consumption of excess vitamin B_1 through ingestion of food and supplements. A tolerable upper intake level (UL) was not established for thiamine due to the lack of hazard data necessary to conduct a quantitative risk assessment (Institute of Medicine, 1998). There have been occasional reports of serious and even fatal anaphylactic reactions to the administration of thiamine through routes other than the gastrointestinal tract. Allergic sensitivity and itching were observed in 12 of 989 patients receiving intravenous injections of thiamine hydrochloride at relatively high doses of 100 mg/day. These effects are irrelevant for setting a UL because the route of exposure is not reflective of typical dietary intake for vitamin B_1 (Institute of Medicine, 1998).

Humans often take vitamin C supplements in large amounts because many people believe vitamin C is non-toxic and beneficial to health. There is no evidence suggesting that vitamin C is carcinogenic or teratogenic (i.e., causes developmental malformations) or that it causes adverse reproductive effects (i.e., fertility issues). Reviews of high vitamin C intakes have indicated low toxicity; however, adverse effects have been reported after ingestion of doses exceeding three grams per day (Institute of Medicine, 2000). The available data indicate that the human body does not continue to absorb dietary vitamin C at intakes above 200 mg/day, suggesting that overload of vitamin C is unlikely in most humans. Very high intakes of vitamin C may lead to diarrhea and other gastrointestinal disturbances, increased oxalate excretion and kidney stone formation, increased uric acid excretion, pro-oxidant effects, systemic conditioning ("rebound scurvy"), increased iron absorption leading to iron overload, reduced vitamin B_{12} and copper status, increased oxygen demand, and erosion of dental enamel (Institute of Medicine, 2000). Excessively high vitamin C doses (e.g., up to 10 grams per day) may also interfere with the healthy antioxidant-prooxidant balance in the body (Johnson, 2014a). Daily intake below the upper limit (UL = 2,000 mg/day) is not associated with toxic effects in healthy adult humans (Institute of Medicine, 2000; Johnson, 2014a).

There is no evidence of adverse effects from consumption of vitamin E at levels naturally occurring in foods. Instead, excessive intake of α-tocopherol in humans from supplementation, fortification of foods, or pharmacological might reduce the coagulation properties of blood. This anticoagulant effect at high doses could increase the risk of severe bleeding—reducing the blood's ability to form clots after a cut or injury—or hemorrhagic stroke (Institute of Medicine, 2000). Animal studies have demonstrated that α-tocopherol is not mutagenic, carcinogenic or responsible for fetal malformations (i.e., teratogenic). Occasionally, muscle weakness, fatigue, nausea, and diarrhea occur at typical doses of α-tocopherol ranging from 400 to 800 mg/day (Johnson, 2014b). Bleeding is uncommon unless the dose it greater than 1,000 mg/day or the patient takes oral coumarin or warfarin, which are commonly prescribed as anticoagulant agents. Based on the hemorrhagic potential of vitamin E alone, an upper limit of 1,000 mg/day has been established for any form of α-tocopherol in adults (Institute of Medicine, 2000; Johnson, 2014b).

The available hazard assessments for vitamins B_1, C and E pertain to effects associated with large doses of vitamin supplements without consideration of metabolites. No sources were identified that discuss toxic effects resulting from the breakdown products of either native vitamins B_1, C, and E or synthetic forms of these vitamins commonly found in supplements and fortified foods. Further, no sources were identified that discuss the possible persistence and areas of concentration of these vitamins compounds or their breakdown products in the environment. Foliar residues of vitamin compounds could present moderate toxicological risks for animals if target vegetation treated at excessively high application rates is ingested very soon after treatment. However, the modest foliar application concentrations (e.g., 300 mg/L for vitamin C) and rapid dissipation rates for these substances suggests that non-target animals are not at risk of poisoning related to the current use pattern of vitamins in organic crop production.

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

The potential exists for environmental contamination resulting from the industrial production of several vitamin compounds. Strong acids (e.g., hydrochloric acid and hydrobromic acid) used in the syntheses of vitamins B_1 and C 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. In addition, organic solvents used in the commercial extraction of tocopherols from vegetable oil could result in environmental contamination if released into the environment through waste streams. Many of the vitamins synthesized for supplements and feed fortification are derived from petroleum products or genetically modified crop materials. For example, acetone (CH_3COCH_3) used in the commercial synthesis of vitamin C is a high volume production chemical 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 crop production. Chemical nutrients, such as vitamins, present in crop/soil amendments and livestock feeds could be introduced to aquatic environments through accidental spills or leaching of nutrients from treated soils. 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 the primary drivers of 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), among other B-vitamins (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 releases of small amounts of vitamins into the environment are not assumed to pose any significant risk. Material safety data sheets for many synthetic vitamins, including vitamin C, advise that containers holding synthetic vitamins be "suitable" and closed containers for disposal. No further disposal instructions are provided (Sigma Aldrich, 2015).

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

No direct chemical interactions between vitamins and other additives used in organic crop or livestock production were identified. When ingested, 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 in humans. 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 animal species (Vannucchi, 1991). When vitamin E intercepts a radical to reduce a reactive oxygen species, a tocopherol radical is formed; this radical can be reduced by ascorbic acid (vitamin C) to return vitamin E to its biologically active state (Institute of Medicine, 2000). According to material safety data sheets, the antioxidants vitamin C (L-ascorbic acid) and vitamin E (tocopherols) are incompatible with strong oxidizing agents and alkali compounds (Sigma Aldrich, 2015; Sigma Aldrich, 2014).

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

This technical evaluation report concerns the use of synthetic vitamins B_1, C and E as growth regulators and plant protectants in organic crop production. It is highly likely that small amounts of these vitamins would regularly interact with components of the terrestrial agro-ecosystem through runoff from treated plants, leaching from soil, and accidental spills of vitamin solutions. Vitamins B_1, C and E—in addition to numerous other vitamin species—are widely used in conventional and organic agriculture 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, 2010a; HSDB, 2010b). Accidental release of chemical reagents during industrial production, however, may lead to ecological impairment. Specifically, strong acids and bases are used in the synthetic or extraction processes of vitamins B_1, C and E compounds (see response to Evaluation Question #2 for details). 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.

Evaluation Question #9: Discuss and summarize findings on whether the use of the petitioned substance may be harmful to the environment (7 U.S.C. § 6517 (c) (1) (A) (i) and 7 U.S.C. § 6517 (c) (2) (A) (i)).

It is unlikely that the use of vitamins B_1, C and E as growth regulators and plant protectants in organic crop production would be harmful to the environment. All photosynthetic organisms produce vitamins B_1, C and E, and these nutrients are naturally part of the agro-ecosystem—from plant and animal tissues to the soils supporting symbiotic, vitamin-producing microorganisms. Based on the presumably low application rates and rapid dissipation, vitamin treatments as foliar applications, seed coatings and soil drenches should not result in a substantial increase in the concentration of vitamins in the environment.

Strong acids (e.g., hydrochloric acid and hydrobromic acid) used in the syntheses of vitamins B_1 and C 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. Organic solvents used in the commercial extraction of tocopherols from vegetable oil could also result in environmental contamination if released into the environment through waste streams. However, no sources were identified that discussed environmental contamination resulting from the manufacturing, transport or use of vitamins B_1, C and E.

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 summarizes the available technical information regarding reported human health effects associated with direct consumption of vitamins included in this review. Treatment of crops and soils with vitamins B_1, C and E is unlikely to result in excessive vitamin intake for humans; hence, the agricultural use pattern for these three vitamin species should not adversely affect impact human health.

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 thiamine as well as pruritus due to allergic sensitivity to thiamine injection. A tolerable upper intake level (UL, maximum level of daily nutrient uptake that is likely to pose no risk of adverse effects) was not determined for thiamine due to the lack of data of adverse effects (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 E

Adverse effects have not been observed form the consumption of vitamin E naturally occurring in foods. High intakes of vitamin E from fortified foods, dietary supplements, or pharmacologic agents have resulted 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 a-tocopherol form is 1,000 mg/day (Driskell, 2009; Institute of Medicine, 2000).

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)).

Vitamins are available in a variety of natural plant and animal materials. In addition, various naturally occurring, non-synthetic and synthetic substances applied as plant and soil amendments may perform similar functions to those for vitamins B_1, C and E described in this report. The following sections provide details regarding the natural availability of vitamins and alternative substances for root growth stimulation (vitamin B_1) and antioxidant plant defense (vitamins C and E).

Natural Sources of Vitamins B_1, C and E

There are no direct substitutes for essential vitamins; however, plants readily synthesize vitamins B_1, C and E within their roots and leaves for distribution throughout the entire plant. For example, plants generate vitamin B_1 in the leaves and transport the compound to various plant tissues, including the root zone (Cox, 2010). Mycorrhizal fungi also secrete vitamin B_1 for plant uptake through the roots (Strzelczyk & Leniarska, 1985). Vitamins B_1, C and E are present in very small quantities within animal and plant foodstuffs. Natural sources of these vitamins include the following:

- **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 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 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, wheat mill run, corn gluten meal, wheat bran, rye grain, sorghum, fish meal, oats, sunflower seed meal, cotton seed meal, virtually all vegetable oils, and green leafy chops.

Alternative Substances for Vitamins B_1, C and E in Crop Production

Agricultural extension specialists generally recommend that conventional crop producers employ products containing synthetic plants hormones or methods of stimulating the production of these hormones within plants to enhance root growth in transplants and cuttings. Common synthetic auxins include indole butyric acid (IBA) and naphthalene acetic acid (NAA). IBA is one of the most common auxin formulations, and has been found to increase the number of roots and rooting percentage as well as regenerate roots in transplanted trees (Chalker-Scott, undated). Neither synthetic IBA nor NAA is approved for use in organic crop production (Schalau, 2010). Willow tea, made by soaking stems of the willow tree in warm water, is often recommended for use as a natural source of the IBA hormone to encourage new cuttings to take root (OSU, 2015); however, technical literature validating or refuting these claims is unavailable. A search of the OMRI generic materials database indicates that nonsynthetic plant hormones such as gibberellic acid, indole acetic acid (IAA) and cytokinins may be applied to organic crops as plant growth regulators (OMRI, 2013). Plant hormones regulate the cell division and cell elongation in general, but each group of plant hormones performs specialized functions (Pederson, 2007). For example, auxins stimulate root growth, gibberellins control flowering, and abscisic acid inhibits the effects of other hormones to reduce growth during times of plant stress (Schalau, 2005; Whiting, 2010).

Strategies encouraging auxin formation within plants and applying natural substances/organisms containing these hormones for root growth stimulation is most compatible with organic production. Specifically, germinating seeds, fungi and algae extract may serve as natural auxin sources. Cuttings have been enclosed with germinating seeds to stimulate root growth for several centuries because seeds naturally produce auxins such as IAA when they germinate (Centeno & Gómez-del-Campo, 2008). A commercial extract of macerated cereal seeds known as Terrabal Organico™—which contains soluble proteins, amino acids, vitamins, nitrogen, phosphorus and potassium—has been effectively used to increase the percentage of rooted cuttings in organic crop production (Centeno & Gómez-del-Campo, 2008). Just as soil fungi naturally synthesize vitamin B_1, these same microorganisms generate auxins as well as other essential plant nutrients, such as proteins, carbohydrates, lipids, minerals and other vitamins (Centeno & Gómez-del-Campo, 2008). Encouraging the health of existing soil fungi and supplementing soils with exogenous sources of beneficial fungi that release plant nutrients and growth factors to the soil may naturally stimulate root growth in transplanted crops. Algae also contain IAA, proteins, lipids and carbohydrates; for example, the Sm-6 Organico™ product is a mixture of *Ascophyllum nodosum*, *Fucus serratus*, *Laminaria hyoborea*, and *Laminaria digitata* algae dry used to encourage root development in organic cuttings (Centeno & Gómez-del-Campo, 2008). According to Centeno & Gómez-del-Campo (2008), the organic products Terrabal (macerated cereal seeds) and Sm-6 (mixture of algae dry extract) produced the highest rooting percentages in propagation studies of organic olive plants (*Olea europea* L. cv. Cornicabra).

A search of the Arbico Organics catalog revealed several naturally derived, OMRI-listed substances marketed to stimulate root growth. Pumice is a lightweight volcanic rock that is widely used in growing media such as soil and potting mixes to aid in aeration (i.e., allow airflow around plant roots), moisture retention and to loosen heavy, clay-based soils (Arbico Organics, 2015a). The commercial product Humboldt Roots is a concentrated liquid root stimulant that can be mixed with and applied along with other organic fertilizers. It is derived from *Ascophyllum nodosum* seaweed and potassium humate, and contains humic acid lignite and extract of *Quillaja saponaria*, commonly known as Soapbark (Arbico Organics, 2015a). In addition, Superzyme® Biological Browth Factor Powder 1-0-4 is an organic fertilizer containing plant food extracted from the fermentation of protein and *Bacillus spp.*, *Pseudomonas putida* and *Trichoderma spp.* used to optimize absorption of water and nutrients (Arbico Organics, 2015a). Hydrolyzed seaweed contains a wide range of micronutrients and plant-growth chemicals and hormones (e.g., auxins and gibberellins) (Gush, 2011). In fact, OMRI-listed products such as Maxicrop Soluble Seaweed Powder are marketed for "strengthening cuttings, bare root trees and shrubs with an application before and after transplant" (Arbico Organics, 2015a). Mycorrhizal fungi that form symbiotic relationships with the rooting systems of plants are also included in commercial formulations such as the Arbico Organics™ Root Maximizer – Mycorrhizal Fungi product, which is designed for transplanted trees, vines and shrugs as well as small transplants/seedlings and cuttings (Arbico Organics, 2015b).

No natural substances were identified as alternatives for the antioxidants vitamins C and E in organic crop production. However, the utility of external sources of these substances is uncertain due to the paucity of literature describing practical applications of these substances in agricultural settings.

<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)).

From irrigation intensity to fertilization schedules, cultural practices can greatly influence crop growth and development as well as the concentration of vitamins in plant tissues, including roots, leaves and fruit. This response provides information regarding alternative methods for encouraging root growth in transplants and cuttings and naturally increasing the vitamin content of plant tissues.

Vitamin B_1

Most agricultural experts agree that vitamin B_1 treatments do not reduce transplant shock or stimulate new root growth in transplanted crops and plant cuttings grown outside of the laboratory (i.e., tissue culture studies). Because beneficial soil fungi and bacteria associated with plant roots produce vitamin B_1 (Schalau, 2010), crop producers can foster the production of vitamin B_1 in the soil area by encouraging the growth and productivity of beneficial soil microorganisms. Reducing fertilizer use is one potential strategy, since fertilizers can have negative impacts on beneficial soil microorganisms such as mycorrhizal fungi, bacteria and protozoa (Schalau, 2010). Application of fertilizers at the time of planting is therefore not recommended by the University of Arizona agricultural extension. Proper irrigation has been shown to help plant root systems become established successfully in more arid climates (Schalau, 2010). Operators are encouraged to ensure that the applied irrigation not only saturates the root ball of a transplant, but also some of the adjacent native soil. Proper saturation of the surrounding native soil encourages new roots to expand and colonize a greater soil volume, which helps the plant better utilize available soil nutrients and water resources (Schalau, 2010). Overall, the available literature does not support the premise that foliar and soil applications of vitamin B_1 are responsible for root stimulation in transplanted crops (Schalau, 2010; Cox, 2010; Chalker-Scott, Undated; Kontaxis & Cox, 1984).

Other factors that influence rooting in cuttings of herbaceous plants include health and condition of the parent plant, timing, the rooting medium, water, light, and temperature. Using only vigorous, healthy plants that are free of diseases and infestations as sources of cuttings can increase the likelihood of successful rooting and plant propagation (Guse & Larsen, 2001). Early morning is typically the best time to take cuttings from health plants because the plant is fully turgid (Evans & Blazich, 2015). The supporting medium should provide the plant cutting with physical support, an adequate supply of oxygen and water to the root zone, and proper drainage. In general, the best media for rooting cuttings combines two materials: one that retains moisture and another with large pore spaces, which results in good aeration and drainage (Guse & Larsen, 2001). Light is necessary for photosynthesis to provide the energy required to form tissues that become roots and shoots; however, most thin-leaved plants react best if they are never placed in direct sunlight (Guse & Larsen, 2001). Shading materials are commonly used to reduce natural sunlight by 30 percent in areas with higher light intensity (Hamilton & Midcap, 2003). For optimum rooting, it is best to maintain a temperature that encourages growth processes but does not cause excessive moisture loss and wilting. The use of heating cables beneath the rooting medium allows the root zone to be kept five to ten degrees warmer than air temperature, thus encouraging rooting without stressing higher plant materials (Guse & Larsen, 2001).

Vitamins C and E

Light and average temperature have a strong influence on the chemical composition of horticultural crops (Lee & Kader, 2000). While light is not essential for the synthesis of ascorbic acid in plants, the amount and intensity of light during the growing season positively influences the amount of ascorbic acid formed. For plant fruits, it has been observed that outside fruit exposed to maximum sunlight contain higher amounts of vitamin C than inside and shaded fruit on the same plant (Lee & Kader, 2000). In general, lower light intensity during growth translates to lower ascorbic acid content of plant tissues. Temperature also influences the composition of plant tissues during growth and development. Using citrus as an example, fruit grown under cool temperatures (i.e., 20–22 °C day, 11–13 °C night) generally contain more vitamin C than fruit harvested from hotter areas with temperatures of 30–35 °C during the day and 20–25 °C at night (Lee & Kader, 2000). Crops grown in milder climates with plenty of sunshine—such as the coastal areas of central California—are less likely to require exogenous sources of vitamin C based on this analysis of sunlight and temperature. In the absence of practical information from extension services, it remains uncertain whether the foliar and soil applications of vitamin C are essential for crop production.

Certain cultural practices may enhance or diminish the levels of vitamin C in plant materials (Lee & Kader, 2000). In one study, application of gibberellins was beneficial to green tea quality, increasing the concentration of vitamin C by 18%. The application of increasing amounts of nitrogen fertilizer typically correlates with decreasing vitamin C levels in fruits and vegetables (Yasuor, 2013). In fact, the results of one study indicated that increasing the amount of nitrogen fertilizer from 80 to 120 kg per hectare decreased vitamin C by 7% in cauliflower (Lee & Kader, 2000). Alternatively, application of potassium fertilizer was found to increase ascorbic acid content. Stefanelli et al. (2010) also found that the intensive fertilizer and water use associated with industrial agriculture generally results in lower concentrations of essential nutrients—such as flavonoids, carotenoids, glucosinolates and ascorbic acid (vitamin C)—in fruit and vegetable crops. Indeed, less frequent irrigation can increase the concentrations of dietary fiber, vitamin C, protein, calcium and other nutrients in several crops, including leeks and broccoli (Lee & Kader, 2000). Based on this information, horticultural crops grown under lower nitrogen supply and less frequent irrigation may be preferred due to the high concentrations of vitamin C and low concentrations of nitrate.

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