Tocopherols

Aquaculture - Aquatic Animals

Identification of Petitioned Substance

Chemical Names:
Tocopherols
5,7,8-Trimethyltocol
5,8-Dimethyltocol
7,8-Dimethyltocol
8-Methyltocol

Other Names:
Mixed tocopherols
Vitamin E

Trade Names:
Decanox™
Naturox™

CAS Numbers:
1406-66-2 (tocopherols)
59-02-9 (vitamin E/alpha-tocopherol)
148-03-8 (beta-tocopherol)
54-28-4 (gamma-tocopherol)
119-13-1 (delta-tocopherol)

Summary of Petitioned Use

The petitioner is requesting the addition of tocopherols to the National List of Allowed and Prohibited Substances (hereafter referred to as the National List) as a synthetic substance allowed for use in organic aquatic animal production. The petitioner reports that tocopherols are currently being used as a feed antioxidant in the nonorganic production of aquatic animals and are mixed with fish oil in the amount of 0.2% and above; with fish meal in the amount of 0.03% to 0.06%; and with various other feed ingredients such as nutritional pigments, grains, and lipids (Aquaculture Working Group, 2012).

Synthetic tocopherols are currently permitted for specific uses in organic livestock production and organic handling. Tocopherols are not specifically named in the National List as synthetic feed additives allowed for use in organic livestock production. However, mixed tocopherols are a source of vitamin E. Vitamins (used for enrichment or fortification when FDA approved) are included on the National List as synthetic ingredients allowed as feed additives in organic livestock production (7 CFR 205.603[d][3]). Tocopherols derived from vegetable oil are allowed for use as ingredients in or on processed products labeled as "organic" or "made with organic (specified ingredients or food group[s])" when rosemary extracts are not a suitable alternative (7 CFR 205.605[b]).

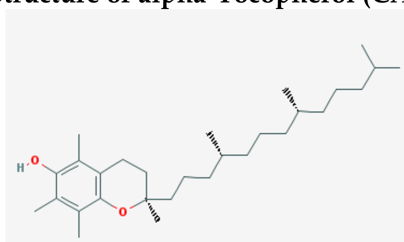
Characterization of Petitioned Substance

__Composition of the Substance:__
Tocopherols are a group of lipophilic phenolic antioxidants that occur naturally in a variety of plant species. Rich sources of naturally-occurring tocopherols include cereal grains, oilseeds, nuts, and vegetables (Burdock, 1997). The term "tocopherols" refers to structurally similar compounds that occur in nature in four forms: alpha-, beta-, gamma-, and delta-tocopherol (CIR, 2002). Tocopherols that are derived from plant products are often referred to as "mixed tocopherols" because the mixture contains all four forms of tocopherol (CIR, 2002). The different forms of tocopherol vary in the number and position of the methyl groups attached to the chromanol ring (IOM, 2000; Burdock, 1997). The molecular structures and chemical formulas of the tocopherol compounds are shown in Figures 1–4.

The proportion of each of the tocopherol compounds present in a mixed tocopherols product is a reflection of the tocopherol profile of the particular vegetable oil(s) used to isolate the tocopherols (EFSA, 2008). A typical mixed tocopherols product consists primarily of gamma-tocopherol, followed by delta- or alpha-tocopherol, with beta-tocopherol representing the lowest proportion in the mixture (CIR, 2002; EFSA, 2008; Organic Technologies, 2009; BASF, undated).

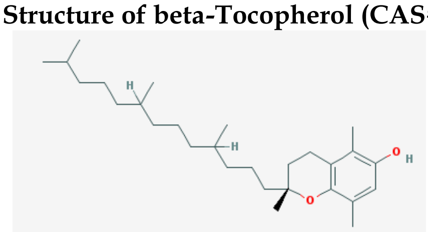
Mixed tocopherols for use as antioxidants in foods or animal feeds are manufactured in liquid and powder forms. The tocopherols available in liquid form are diluted in a vegetable oil and may be mixed with certain additives to enhance their effectiveness, such as rosemary extract, ascorbyl palmitate or ascorbic acid, lecithin, and/or citric acid (Pokorny et al., 2001; Lampi et al., 2002). For example, the petitioner provided a material safety data sheet (MSDS) for a product called Naturox® IPO Liquid (Kemin Industries, Inc.) which lists organic sunflower oil, lecithin, and rosemary extract as components of the mixed tocopherols formulation (Kemin Industries, Inc., 2008). The Joint Expert Committee on Food Additives (JECFA) specification for the food additive "mixed tocopherols concentrate" states that it may contain an edible vegetable oil added to adjust the required amount of total tocopherols (JECFA, 2006). Powdered forms of mixed tocopherols contain a carrier such as tapioca starch, gum acacia, and/or maltodextrin (Organic Technologies, 2009; NOSB, 1995). No additional sources were found that discuss possible additives to commercially-produced tocopherols for use as antioxidants in food or feed, including aquaculture feed products.

Figure 1. Molecular Structure of alpha-Tocopherol (CAS# 59-02-9; $C_{29}H_{50}O_2$)



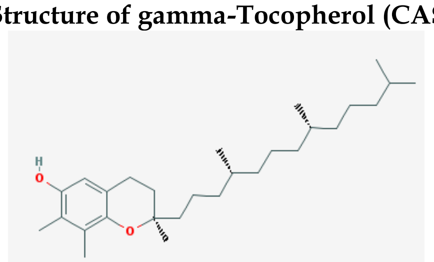
Source: PubChem Compound, 2013

Figure 2. Molecular Structure of beta-Tocopherol (CAS# 148-03-8; $C_{28}H_{48}O_2$)



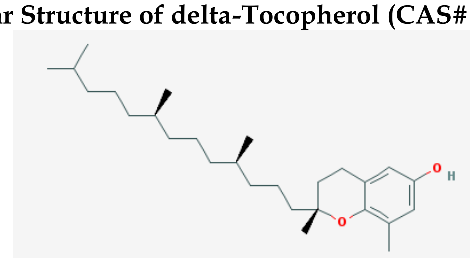
Source: PubChem Compound, 2013

Figure 3. Molecular Structure of gamma-Tocopherol (CAS# 54-28-4; $C_{28}H_{48}O_2$)



Source: PubChem Compound, 2013

Figure 4. Molecular Structure of delta-Tocopherol (CAS# 119-13-1; $C_{27}H_{46}O_2$)



Source: PubChem Compound, 2013

Source or Origin of the Substance:

Tocopherols for use as antioxidants in foods or animal feeds are commonly extracted from the distillate obtained in the deodorization of vegetable oils (e.g., soybean, canola, sunflower, corn, cottonseed) (Burdock, 1997). Tocopherols are separated from the other compounds in the oil distillate by multiple extraction and refining steps. These steps can include vacuum distillation, extraction with organic solvents, molecular distillation, crystallization, and standardization of the additive with vegetable oil (Burdock, 1997; EFSA, 2008; Organic Technologies, 2009; EFSA, 2012; Aquaculture Working Group, 2012). The total tocopherol content of the resulting product is usually 30–80% (Burdock, 1997). The powdered form of tocopherols is produced by spray-drying the liquid tocopherol oils onto a carrier or mixture of carriers (NOSB, 1995; Organic Technologies, 2009).

Properties of the Substance:

The liquid form of tocopherols is described as a light brown to red viscous liquid with the odor of vegetable oil (JECFA, 2006). It is insoluble in water but miscible with oils and fats. The powdered form of tocopherols is described as a light tan to off-white powder that is water dispersible (Organic Technologies, 2009; ADM, 2013).

Specific Uses of the Substance:

Tocopherols are intended to be used in organic aquaculture as an antioxidant added to aquatic animal feed (Aquaculture Working Group, 2012). Tocopherols are mixed with fish oil, fish meal, and other feed ingredients to prevent oxidation of the polyunsaturated fatty acids present in the lipids and thereby protect the nutritional value of the feed. Polyunsaturated fatty acids are very susceptible to autoxidation when exposed to oxygen in the atmosphere (Tacon, 1992). During the process of lipid autoxidation, toxic degradation products are formed in the feed that may cause pathological changes in the fish (Hardy and Roley, 2000). Furthermore, oxidation destroys essential fatty acids in the feed, and consuming oxidized lipids may have deleterious effects on tissue levels of vitamins C and E. Finally, oxidation of the lipids in fish meal generates heat that is sometime sufficient to cause spontaneous combustion of feeds (Hardy and Roley, 2000).

Tocopherols are also used as an antioxidant additive in terrestrial livestock feed, human food, dietary supplements, and pet food (ADM, 2013; BASF, undated; Organic Technologies, 2009). As a chemical preservative, tocopherols are permitted by the U.S. Food and Drug Administration (FDA) in every human food category (21 CFR 182.3890).

The petitioner reports that: "It is understood that mixed tocopherols are in regular use as antioxidants in fish meal as a feed ingredient in organic poultry production" (Aquaculture Working Group, 2012). Evidence of this usage was found through a feed manufacturer's website (Fertrell, undated). Fertrell® (Bainbridge, PA) produces a fish meal product designed for organic poultry producers, and the company's website states that it contains Naturox™, which is a mixed tocopherols formulation manufactured by Kemin Industries, Inc. (Kemin Industries, Inc., 2013).

No sources were identified that discussed if mixed tocopherols are currently being used as a source of vitamin E supplementation in terrestrial livestock feed. Vitamin E content of the feeds may be provided by alpha-tocopherol, an ester of alpha-tocopherol, or possibly mixed tocopherols.

Typical usage levels of tocopherols in foods and feeds vary from about 150–450 parts per million (ppm) (Lampi et al., 2002). Concentrations of 2,000 ppm may be necessary for oils containing highly polyunsaturated fatty acids (such as fish oil) (BASF, undated). According to the petitioner, tocopherols are added to fish oil at levels of 2,000 ppm and above and to fish meal at levels of 300–600 ppm (Aquaculture Working Group, 2012).

Tocopherols are also used in a wide variety of cosmetic formulations functioning as an antioxidant to protect the formulation and/or a skin conditioning agent (CIR, 2002). The usage levels are reported to be ≤5% in such products (CIR, 2002).

Approved Legal Uses of the Substance:

Tocopherols are affirmed as generally recognized as safe (GRAS) by the FDA when used as chemical preservatives (21 CFR 182.3890) or nutrients (21 CFR 182.8890) in food for human consumption in accordance with good manufacturing practice. Their use is 0.03% in animal fats; however, a 30% concentration of tocopherols in vegetable oils shall be used when added as an antioxidant to products designated as ''lard'' or ''rendered pork fat.'' For meat products, levels are not to exceed 0.03% based on the total fat content and are not to be used in combination with other antioxidants. Levels of 0.03% or 0.02% (when in combination with other antioxidants) are used in poultry products (9 CFR 424.21).

Tocopherols are also affirmed as GRAS by the FDA when used as chemical preservatives (21 CFR 582.3890) and nutrients and/or dietary supplements (21 CFR 582.5890) in animal feeds in accordance with good manufacturing or feeding practice.

Action of the Substance:

Tocopherols are added to foods or animal feeds to help prevent oxidation of the fatty acids present in the lipid components of the food. Polyunsaturated fatty acids are the least stable components of lipids and readily react with oxygen in the air (Pokorny, 2007). Saturated fatty acids are oxidized as well, but at higher temperatures. Oxidation begins when oxygen is converted to highly reactive free radicals by metal catalysis or exposure to light (Hardy and Roley, 2000). The free radicals attack fatty acids through the addition of oxygen atoms along their carbon chains. Upon oxidation, the fatty acids form more free radicals that start a chain reaction of further oxidation leading to oxidative rancidity of the food or feed. The addition of tocopherols at the optimum concentration in the food or feed can prevent oxidative rancidity. Tocopherols are sacrificial antioxidants because they donate their phenolic hydrogen atoms to free radicals thereby converting them to stable and nonreactive forms. This prevents free radicals from attacking the fatty acids in the food or feed (Hardy and Roley, 2000).

Combinations of the Substance:

Tocopherols in liquid form are diluted in a vegetable oil and may be mixed with certain additives to enhance their effectiveness, such as rosemary extract, ascorbyl palmitate or ascorbic acid, lecithin, and/or citric acid (Pokorny et al., 2001; Lampi et al., 2002). Powdered forms of mixed tocopherols contain a carrier such as tapioca starch, gum acacia, and/or maltodextrin (Organic Technologies, 2009; NOSB, 1995).

Vegetable oil and rosemary extract are agricultural products that are not specifically named in the National List. Vitamin C, which may be derived from ascorbyl palmitate or ascorbic acid, is allowed for use in organic livestock production as a feed additive (7 CFR 205.603[d][3]). Lecithin (de-oiled) is included on the National List as a nonorganically-produced agricultural product allowed as an ingredient in or on processed products labeled as "organic," (7 CFR 205.606[p]) and is permitted only when an organic form is not commercially available. Citric acid (produced by microbial fermentation of carbohydrate substances) is included in the National List as a nonagricultural substance allowed as an ingredient in or on processed products labeled as "organic" or ''made with organic (specified ingredients or food group[s])'' (7 CFR 205.605[a]). Tapioca starch and gum acacia are agricultural products not specifically mentioned in the National List. Maltodextrin is a synthetic substance derived from a starch and is not included in the National List.

Status

Historic Use:

Tocopherols were first used as antioxidants in food in 1949 (Burdock, 1997). Today they are used in a variety of processed foods and animal feeds. Their use in organic production in the United States dates back to the 1995 approval of tocopherols for use as a food antioxidant in organic handling (NOSB, 1995). Tocopherols have been used in cosmetic formulations for many years (CIR, 2002).

Organic Foods Production Act, USDA Final Rule:

Mixed tocopherols contain alpha-tocopherol, a form of vitamin E. Vitamins (used for enrichment or fortification when FDA approved) are included on the National List as synthetic ingredients allowed as feed additives in organic livestock production (7 CFR 205.603[d][3]).

Tocopherols (derived from vegetable oil when rosemary extracts are not a suitable alternative) are included on the National List as a synthetic nonagricultural substance allowed as an ingredient in or on processed products labeled as "organic" or "made with organic (specified ingredients or food group[s])" (7 CFR 205.605[b]).

International:

Canadian General Standards Board (CGSB) Permitted Substances List

Tocopherols and mixed natural concentrates (derived from vegetable oil when rosemary extracts are not a suitable alternative) are included on the CGSB Permitted Substances List as a nonorganic ingredient classified as a food additive (CGSB, 2011). Tocopherols are not specifically permitted by the CGSB for use as antioxidants in organic livestock production. Antioxidants for use in livestock feed must be from nonsynthetic sources only (water, alcohol, acid and base extracts permitted by the standard only) (CGSB, 2011).

The new CGSB Organic Aquaculture Standards published in May 2012 do not specifically list tocopherols for use as feed additives in organic aquaculture (CGSB, 2012). Antioxidants are permitted as feed additives from non-synthetic sources only (water, alcohol, acid and base extracts permitted by CAN/CGSB-32.310 and CAN/CGSB-32.311 only). Synthetic sources are permitted when legally required.

CODEX Alimentarius Commission, Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (GL 32-1999)

Tocopherols (mixed natural concentrates) are permitted for use in organic processed plant products by the CODEX Alimentarius Commission (2001). Tocopherols are not specifically permitted for use in organic livestock feed by the CODEX Alimentarius Commission; only antioxidants from natural sources are allowed.

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

Tocopherol-rich extract (as an antioxidant for fats and oils) is listed as a substance permitted in the European Union for use in the preparation of organic foodstuffs of plant and animal origin (Commission of the European Communities, 2008). "Tocopherol-rich extracts of natural origin used as an antioxidant" are permitted as a feed additive in organic livestock production.

In August 2009, detailed rules on organic aquaculture animal and seaweed production were published in the Official Journal of the European Union (Commission of the European Communities, 2009). "Tocopherol-rich extracts of natural origin used as an antioxidant" remained listed as substances permitted as a feed additive for all organic animals. In addition, all "natural antioxidant substances" are listed as substances permitted in feed specifically for aquaculture animals.

Japan Agricultural Standard (JAS) for Organic Production

"Mix tocopherol" is listed by the JAS for Organic Production as a substance permitted for use in organic processed foods of plant and animal origin (Japanese Ministry of Agriculture, Forestry and Fisheries, 2012). In the case of processed foods of animal origin, their use is limited to processed meat products. Tocopherols are not specifically listed by the JAS for Organic Production for use in organic livestock production. Feed additives are only permitted for use in organic livestock production if they are natural substances or derived from natural substances without being chemically treated (Japanese Ministry of Agriculture, Forestry and Fisheries, 2012).

International Federation of Organic Agriculture Movements (IFOAM)
Tocopherols (mixed natural concentrates) are listed by IFOAM as food additives permitted for use in organic processed foods (IFOAM, 2012). Tocopherols are not specifically listed by IFOAM for use in organic livestock production. However, all preservatives (except when used as a processing aid) are prohibited in the diet of organic livestock.

The 2012 IFOAM Norms for Organic Production and Processing also include organic aquaculture production standards. The standards for organic aquaculture feed are the same as those for organic livestock feed. Tocopherols are not specifically mentioned. Preservatives (except when used as a processing aid) are prohibited in the diet of organic aquaculture animals (IFOAM, 2012).

Naturland – Association for Organic Agriculture (Germany)
According to the Naturland Standards for Organic Aquaculture published in 2012, synthetic feed additives are not permitted in organic aquaculture feed. However, "natural antioxidants (e.g., tocopherol)" may be added to the feed upon approval by Naturland (Naturland, 2012).

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

<u>Evaluation Question #1: Indicate which category in OFPA that the substance falls under:</u> (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) Tocopherols possess vitamin E activity. The isomer with the highest vitamin E activity is alpha-tocopherol. Therefore, tocopherols can be considered part of the vitamin category.
(B) Tocopherols are not classified by the EPA as an inert of toxicological concern. Vitamin E (d-alpha tocopherol) is exempt from a requirement of a tolerance per 40 CFR 180.910.

<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)).

Processes used to manufacture tocopherol products are described by several sources that are summarized here. The petitioner indicated that mixed tocopherols are extracted from soybean oil using solvent extraction. Hexane was reported as a commonly-used solvent, and other solvents may include ethanol, isopropanol, acetone, isopentane, isohexane, and trichloroethylene (Aquaculture Working Group, 2012).

The 1995 Technical Advisory Panel (TAP) Report for Tocopherols, which reviewed the use of tocopherols as a food antioxidant, states that tocopherols are made via vacuum steam distillation of edible vegetable oil products (NOSB, 1995). The European Food Safety Authority (EFSA) also reports that mixed tocopherols are obtained via vacuum steam distillation of edible vegetable oil products (EFSA, 2008). The raw material used for the manufacturing of tocopherols is reported to be a byproduct of vegetable oil refining (e.g., deodorizer distillate). Common vegetable oils being used include soybean, rapeseed, sunflower, corn, and cottonseed oils. The vegetable oil byproduct undergoes a combination of purification and distillation steps to produce the mixed tocopherols material. The stereochemistry of the tocopherol compounds is reportedly preserved so that the mixed tocopherols are identical to the various forms of tocopherols found in the natural source material (EFSA, 2008).

Burdock (1997) reports that tocopherols are extracted from vegetable oil deodorizer distillate. Deodorizer distillate, obtained from the deodorization process of vegetable oil refining, is a complex mixture containing many compounds including tocopherols, tocotrienols, sterols, esters of sterols, free fatty acids, and mono-, di- and triglycerides. The other compounds can be separated from tocopherols through a series of steps that may include esterification with a lower alcohol followed by washing and vacuum distillation, or by saponifiction or fractional liquid-liquid extraction (Burdock, 1997). The tocopherols can be further purified using one or more of the following processes: molecular distillation, extraction, and/or crystallization.

In a 2012 document, EFSA reports that tocopherols are produced from vegetable oils through a series of extraction steps that include crystallization, multiple distillations, and, finally, a standardization of the additive with vegetable oil (EFSA, 2012). JECFA (2006) also reports that a vegetable oil may be added to the purified tocopherols mixture in order to adjust the required amount of total tocopherols in the product. The final product may also be mixed with certain additives to enhance the effectiveness of tocopherols, such as rosemary extract, ascorbyl palmitate or ascorbic acid, lecithin, and/or citric acid (Pokorny et al., 2001; Lampi et al., 2002). Powdered tocopherol products are produced by spray-drying the liquid tocopherol product onto a carrier or mixture of carriers such as tapioca starch, gum acacia, or maltodextrin (Organic Technologies, 2009; NOSB, 1995).

<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)).

The available sources discussed below all indicate that tocopherols for use as antioxidants in food or feed are derived from plant products, and therefore the compounds originate from naturally occurring biological processes. Tocopherols can be extracted from vegetable oils in several different ways. All of the methods found in the literature involve chemical processes. At the end of the process used to extract and purify tocopherols, the compounds remain in the same form as in the naturally occurring source materials.

Tocopherols are extracted from a natural material (vegetable oil) through many different steps. Most of the available sources indicate that a byproduct of vegetable oil refining (deodorizer distillate) is commonly used as the raw material for the manufacturing of tocopherols. Deodorizer distillate is reported to be an important commercial source of tocopherols (Verleyen et al., 2001). Deodorization is the final step in the chemical refining of edible vegetable oils. It is a steam distillation process used to remove undesirable ingredients to produce oil with characteristic mild odor and flavor (Medina-Juarez and Gamez-Meza, 2011).

The extraction of tocopherols from vegetable oil byproducts may include one or more of the following chemical processes: esterification, saponification, solvent extraction, and/or crystallization using a solvent (see the response to Evaluation Question #2). Physical separation methods may also be used during the extraction of tocopherols, and these include various distillation steps.

Esterification is a chemical process that can be used to prepare the deodorizer distillate for easier separation of the tocopherols. The tocopherol compounds themselves are not meant to be esterified in this step, although they may react to a limited extent (Barnicki et al., 1996). Rather, esterification is used to convert the volatile alcohols in vegetable oils into less volatile fatty acid esters (Ogbonna, 2009). The tocopherols can then be separated from the other compounds with different boiling points using distillation at different temperatures. One example of this is explained in U.S. Patent No. 5,512,691 (Barnicki et al., 1996). According to this document, esterification occurs when the distillate is heated under high pressure. An acid may be added as a catalyst (e.g., butyl stannoic acid, zinc acetate, phosphoric acid, dibutyl tin oxide, or other mild mineral acids), and additional C_{10}-C_{22} fatty acids may be added to the solution (Barnicki et al., 1996). Nagao et al. (2005) report that a lipase such as *Candida* sp. lipase may be used as a catalyst during esterification instead of an acid. During the reaction steps, the sterols present in the distillate react with the free fatty acids to form sterol esters; the alcohol moieties react to form fatty acid esters and waxes; and the mono- and di- fatty acid glycerides are converted to triglycerides (Barnicki et al., 1996). The unchanged tocopherols are then separated from these compounds through a series of distillations.

Saponification is another chemical process that can be used to prepare the deodorizer distillate for easier separation of the tocopherols. One example of this is explained in U.S. Patent Application No. US 2008/0015367 A1 (Dobbins et al., 2008). According to this document, the phytosterol fatty acid esters present in the deodorizer distillate can be saponified with potassium hydroxide forming a solvent medium of methanol, water, and the potassium soaps of fatty acids. The tocopherols remain unsaponified and can be recovered via acidification of the mixture with a dilute aqueous solution of a mineral acid followed by separation of the water-immiscible mixture and fractional distillation (Dobbins et al., 2008).

The petitioner indicated that mixed tocopherols are extracted from soybean oil using solvent extraction. Hexane was reported as a commonly used solvent, and other solvents may include ethanol, isopropanol, acetone, isopentane, isohexane, and trichloroethylene (Aquaculture Working Group, 2012). Ogbonna (2009) reported that various organic solvents such as hexane are traditionally used during the extraction of tocopherols from plant products.

No sources were identified that discuss whether the synthetic materials used in the extraction of tocopherols remain in the final product in any significant amounts.

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

Tocopherols are produced by all plant tissues, algae, and some cyanobacteria (DellaPenna and Pogson, 2006). They are naturally part of the aquatic environment, and low levels of tocopherols are present in the tissues of fish and other aquatic animals (Polat et al., 2012). The European Food Safety Authority has stated that the use of tocopherol-rich extracts in animal nutrition will not result in a substantial increase in the concentration of tocopherols in the environment (EFSA, 2012).

Tocopherols are easily oxidized in the presence of light or metals or when exposed to high temperatures or alkaline pH conditions (Lampi et al., 2002). Oxidative degradation of tocopherols results in the formation of tocopheroxides, tocopherol quinones, and tocopherol hydroquinones (Gregory, 1996). Further oxidation and rearrangement reactions can lead to the formation of many other compounds. Pokorny (2007) stated that, by reaction with free radicals, tocopherols are converted to quinones, spirodimers, copolymers with oxidized lipids, and various other compounds. Quinones are a group of compounds that are ubiquitous in nature, and they naturally occur in plants, fungi, and bacteria (Monks and Jones, 2002). Tocopherol spirodimer is a major product of tocopherol oxidation in vivo and is found in animal tissues (Al-Malaika, 2004).

No sources were identified that discuss the possible persistence of tocopherols or its breakdown products in the environment.

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

Tocopherols are a natural part of the diet of humans and animals. The various forms of tocopherols are not interconvertible within the human body, and alpha-tocopherol is the only form that has vitamin E activity for humans (IOM, 2000). The Recommended Dietary Allowance for vitamin E (as alpha-tocopherol) set by the Institute of Medicine (IOM, 2000) ranges from 6 mg/day for young children to 15 mg/day for adults (19 mg/day for lactating women). The Tolerable Upper Intake Level (UL) for alpha-tocopherol ranges from 200 mg/day for young children to 1,000 mg/day for adults. The UL is the highest level of total daily alpha-tocopherol intake that is likely to pose no risk of adverse health effects in almost all individuals. The UL applies to all stereoisomers of alpha-tocopherol (IOM, 2000).

There is no evidence of adverse effects resulting from the consumption of alpha-tocopherol naturally occurring in foods (IOM, 2000). Excess intake of alpha-tocopherol in humans from supplementation, fortification of foods, or pharmacological use might increase the risk of bleeding (by reducing the blood's ability to form clots after a cut or injury) or hemorrhagic stroke (IOM, 2000; Office of Dietary Supplements, 2011). However, a clear causal relationship has not yet been established (IOM, 2000). Other side effects of excessive alpha-tocopherol intake have been reported in various studies and include fatigue, emotional disturbances, thrombophlebitis, breast soreness, creatinuria, altered serum lipid and lipoprotein levels, gastrointestinal disturbances, and thyroid effects (IOM, 2000). However, none of these reported effects have been consistently observed or shown in controlled studies.

Animal studies demonstrate that alpha-tocopherol is not mutagenic, carcinogenic, or teratogenic (IOM, 2000). However, a large study supported by the National Institutes of Health (NIH) concluded that vitamin E supplementation increased the occurrence of prostate cancer by 17% in men who received the vitamin E supplement alone versus those who received a placebo. No increase in prostate cancer was observed when vitamin E and selenium supplements were taken together (Klein et al., 2011). The vitamin E supplement used in this study was 400 international units (IU)/day of all-rac-alpha-tocopherol acetate, which is equivalent to 180 mg/day of natural alpha-tocopherol (using calculations provided in IOM, 2000).

In regard to the other forms of tocopherols (beta, gamma, and delta forms), the IOM reports that little information is available on the possible adverse effects to humans resulting from ingestion of amounts that exceed the levels normally found in foods. All forms of tocopherols are absorbed into the body following ingestion; therefore, all forms could contribute to vitamin E toxicity (IOM, 2000).

Vitamin E toxicity may be caused by antagonism with the function of other fat-soluble vitamins (EFSA, 2008). Very high doses of vitamin E in animal studies have shown impaired bone mineralization, reduced liver storage of vitamin A, and hemorrhagic effects. These effects could be corrected in animals by increasing the dietary supplements of the appropriate fat-soluble vitamin (i.e., vitamin D for impaired bone mineralization, vitamin A for reduced liver storage of vitamin A, and vitamin K for hemorrhagic effects) (EFSA, 2008).

No sources were identified that discuss toxic effects resulting from the breakdown products of tocopherols or resulting from contaminants in commercially-produced tocopherols. No sources were identified that discuss the possible persistence and areas of concentration of tocopherols or its breakdown products in the environment.

<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)).

No sources were identified that discuss whether the manufacture, use, misuse, or disposal of tocopherols results in any environmental contamination.

As described in the response to Evaluation Question #3, organic solvents and other chemicals may be used in the commercial extraction of tocopherols from vegetable oil. If these chemicals are released into the environment through waste streams, then environmental contamination could occur.

<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 sources were identified that discuss any known chemical interactions between tocopherols and other substances used in organic crop or livestock production or handling. In general, tocopherols are incompatible with strong oxidizing agents and alkali compounds (ADM, 2009). Because they are added to foods and feeds as antioxidants, by definition, they are not considered stable in those products (EFSA, 2012).

<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)).

No sources were identified that discuss any negative effects of tocopherols on biological or chemical interactions in the aquatic agro-ecosystem, including nontarget aquatic organisms, physical water conditions, endangered species, or biodiversity.

Tocopherols are one of the most important lipid-soluble antioxidants for living things (Häubner, 2010). Tocopherols can only be produced by photosynthetic organisms, including phytoplankton (microalgae) in marine and freshwater ecosystems. Tocopherols are transported up the aquatic food chain and are essential to the health of fish species (Häubner, 2010). The most biologically active tocopherol in fish is alpha-tocopherol (Häubner, 2010). In animal tissues, alpha-tocopherol functions primarily to stabilize membranes. It also ensures the best utilization of lipids in the diet and helps maintain body stores of essential fatty acids (Häubner, 2010). The vitamin E (alpha-tocopherol) requirements of many fish have been studied in aquaculture and are generally in the range of 20-50 mg/kg of dry feed, although some fish have higher requirements (~100 mg/kg of dry feed) (Sargent et al., 2002; Halver, 2011).

Alpha-tocopherol is the major naturally occurring tocopherol in the lipids of marine fish, however beta- and gamma- tocopherols are also absorbed and deposited in fish tissues (Sargent et al., 2002). Excessive intake of tocopherols above the vitamin E requirement of fish could result in hypervitaminosis E, a condition of high storage levels of the vitamin in the fish which could result in toxic symptoms such as poor growth, toxic liver reaction, and death (De Silva et al., 2012; Halver, 2002). No sources were identified that specifically discussed the possibility of hypervitaminosis E caused by the use of tocopherols as antioxidants in aquaculture 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)).

It is unlikely that the use of tocopherols as an antioxidant in aquatic animal feed would be harmful to the environment. Tocopherols are produced by all photosynthetic organisms, they are naturally part of the aquatic environment, and low levels of tocopherols are present in the tissues of fish and other aquatic animals (Häubner, 2010; Polat et al., 2012). The European Food Safety Authority has stated that the use of tocopherol-rich extracts in animal nutrition will not result in a substantial increase in the concentration of tocopherols in the environment (EFSA, 2012).

As described in the response to Evaluation Question #3, organic solvents and other chemicals may be used in the commercial extraction of tocopherols from vegetable oil. If these chemicals are released into the environment through waste streams, then environmental contamination could occur. However, no sources were identified that discussed environmental contamination resulting from the manufacturing of tocopherols. As discussed in the response to Evaluation Question #5, no sources were identified that discussed the possible persistence and areas of concentration of tocopherols or its breakdown products in the environment.

<u>Evaluation Question #10:</u> 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)).

No sources were identified that discuss adverse effects upon human health from the use of tocopherols as an antioxidant in aquatic or terrestrial animal feed. It is unlikely that the use of tocopherols as an antioxidant in aquatic animal feed would be harmful to human health. Tocopherols are a natural part of the human diet, with a large proportion of intake coming from tocopherols naturally present in vegetable oils (IOM, 2000). Tocopherols are affirmed as GRAS by the FDA when used as chemical preservatives or nutrients in food for human consumption in accordance with good manufacturing practice (21 CFR

182.3890, 182.8890). In 2012, the European Food Safety Authority published a scientific opinion of the use of tocopherols as antioxidants in feed for all animal species with no minimum and maximum content. The scientific panel concluded that: "Tocopherol-rich extracts and all-rac-alpha-tocopherol at use levels are safe for all animal species and the consumer. No concern for user safety is expected from the use of tocopherol-rich extracts and all-rac-alpha-tocopherol in feed" (EFSA, 2012).

The tocopherol level found in the flesh of a fish is related to the fish's total dietary intake of tocopherols (Sargent et al., 2002). The use of tocopherols as an antioxidant or vitamin supplement in aquatic animal feed will possibly increase tocopherol levels in those fish that consume the feed. It is unlikely that an increase in the flesh tocopherol levels caused by the addition of tocopherols in fish feed will cause adverse effects in humans who consume those fish. No sources were identified that discuss this possibility. Reports of adverse effects of tocopherols in humans are limited to studies and cases involving supplementation with high levels of alpha-tocopherol, the most biologically active form of tocopherol in humans.

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)).

Natural antioxidants that may be used in place of tocopherols in aquatic animal feed include rosemary extract, lecithin, and ascorbic acid (Hamre et al., 2010; Southgate, 2012; Hardy and Burrows, 2002). Very few sources were identified that discussed the commercial use and efficacy of these antioxidants in aquaculture feeds. The available information is summarized below.

The main active components of rosemary extracts are phenolic compounds including carnosic acid, carnosol, and rosmarinic acid (Hamre et al., 2010). The antioxidant activity of a commercially-available conventional rosemary extract, Herbalox® (Kalsec, Kalamazoo, MI), was tested in an experimental fish feed study conducted by researchers from the Norwegian National Institute of Nutrition and Seafood Research (Hamre et al., 2010). Rosemary extract was added to the fish feed at concentrations ranging from 120 mg/kg to 6,000 mg/kg. The results showed that rosemary extract was an effective antioxidant when added at levels of 1,500 to 6,000 mg/kg in the feed. The addition of 6,000 mg/kg rosemary extract was more effective than the control antioxidant blend containing the synthetic antioxidant ethoxyquin (1,200 mg/kg) along with mixed tocopherols (700 mg/kg), ascorbic acid (1,000 mg/kg), and a phosphate mix (850 mg/kg). It was also more effective than mixed tocopherols alone added at 350 to 1,400 mg/kg in the feed. The authors of this study stress that it is difficult to predict the effectiveness of antioxidants in different feed types, and it is necessary to study them in the specific systems in which they are going to be used (Hamre et al., 2010).

According to the petitioner, rosemary imparts an undesirable taste to fish; therefore, rosemary extracts can only be used in small amounts in fish feed (Aquaculture Working Group, 2012). No additional sources were identified that discussed this issue.

Lecithin can be used as a supplemental source of phospholipids in aquatic animal feeds (ADM, 2003). According to Archer Daniels Midland Company (Decatur, IL), a manufacturer of conventional lecithin products, lecithin also acts as a natural antioxidant in the feed (ADM, 2003). No additional sources were identified that discussed the use of lecithin as an antioxidant in aquatic animal feed (except in combination with tocopherols or other synthetic antioxidants).

Ascorbic acid (vitamin C) has antioxidant properties due to its ability to scavenge free radicals and inactivate metal ions (Hamre et al., 2010). The antioxidant activity of ascorbic acid in an experimental fish feed was tested in the Hamre et al. (2010) study. Other antioxidants studied included rosemary extract, mixed tocopherols, ascorbyl palmitate, citric acid, and spermine. Crystalline ascorbic acid was added to the fish feed at concentrations ranging from 500 to 2,000 mg/kg. The results showed that ascorbic acid was an effective antioxidant at all concentrations studied, and that ascorbic acid was more effective than mixed tocopherols (added at 350 to 1,400 mg/kg) under the specific conditions of this experiment. The authors concluded that ascorbic acid and rosemary extract were the most effective of the natural antioxidants studied and that ascorbyl palmitate, citric acid, and spermine had only minor antioxidant effects (Hamre et al., 2010).

Many other substances have shown promise in laboratory studies as possible natural replacements for synthetic antioxidants used to preserve fish oil and fishmeal. However, no evidence was found of their use in commercial aquaculture. These natural antioxidants include boldine (an extract from the boldo tree, Valenzuela et al., 1991); hard winter wheat extracts (Yi et al., 2002); red algal extracts (Athukorala et al., 2003); Chardonnay grape and black raspberry seed extracts (Luther et al., 2007); *Vitis vinifera* grape extracts (Pazos et al., 2005); green tea extracts (Wanasundara and Shahidi, 1998); oregano (Tsimidou et al., 1995); brown seaweed extracts (*Ecklonia cava*, *Hizikia fusiformis*) (Heo et al., 2003; Senevirathne et al., 2006; Siriwardhana et al., 2004); tannic acid (Maqsood and Benjakul, 2010); and extracts of the *Salvia plebia* herb (Jiang and Wang, 2006).

Nonsynthetic substances (not prohibited under 7 CFR 205.604) may be used as feed additives in organic livestock production provided that all agricultural ingredients included in the list of ingredients for such additives have been produced and handled organically (7 CFR 205.237). The National Organic Program (NOP) guidance document, "Evaluating Allowed Ingredients and Sources of Vitamins and Minerals for Organic Livestock Feed" provides further clarification on the agricultural, nonsynthetic, and synthetic ingredients permitted in organic livestock feed (NOP, 2013).

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

No sources were identified that discussed alternative practices that would make the use of an antioxidant unnecessary in aquatic animal feed. Tucker (2012) reports that antioxidants are necessary in aquaculture feeds because the oxidative rancidity of lipids in feeds can affect the survival and growth of fish.

References

ADM (Archer Daniels Midland Company). 2003. Lecithin in Aquaculture. Available online at http://www.adm.com/en-US/products/Documents/ADM-Europe-Lecithin-for-Aquaculture.pdf

ADM (Archer Daniels Midland Company). 2009. Decanox™ MTS-90G Material Safety Data Sheet. Provided as Appendix A to Aquaculture Working Group (2012).

ADM (Archer Daniels Midland Company). 2013. Decanox MTS-30 P Product Details page. Available online at http://www.adm.com/_layouts/ProductDetails.aspx?productid=493

Al-Malaika, S. 2004. Perspectives in stabilisation of polyolefins. In: Long Term Properties of Polyolefins *Advances in Polymer Science* 169: 121-150.

Aquaculture Working Group. 2012. Petition for Listing Tocopherols for Aquatic Animals on National List of Approved and Prohibited Substances. Available online at http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5098474

Athukorala, Y., Lee, K., Shahidi, F., Heu, M.S., Kim, H.-T., Lee, J.S., Jeon, Y.J. 2003. Antioxidant efficacy of extracts of an edible red alga (*Grateloupia filicina*) in linoleic acid and fish oil. *Journal of Food Lipids* 10: 313–327.

Barnicki, S.D., Sumner, C.E., Jr., Williams, H.C. 1996. Process for the production of tocopherol concentrates. US Patent Number 5,512,691.

BASF, undated. Covi-Ox® T-70 Product Datasheet. Available online at http://www.brenntagspecialties.com/en/downloads/Products/Food/BASF_Food/Vitamins/BASF_Covi_Ox_T_70_PDS.PDF

Burdock, G.A. 1997. Tocopherols. In: *Encyclopedia of Food and Color Additives, Volume III*. CRC Press, Boca Raton, FL, pp. 2801-2803.

CGSB (Canadian General Standards Board). 2011. Organic Production Systems Permitted Substances List. CAN/CGSB-32.311-2006. Amended June 2011. Available online at http://www.tpsgc-pwgsc.gc.ca/ongc-cgsb/programme-program/normes-standards/internet/bio-org/permises-permitted-eng.html

CGSB (Canadian General Standards Board). 2012. Organic Aquaculture Standards. CAN/CGSB-32.312-2012. Available online at http://www.techstreet.com/cgi-bin/detail?doc_no=can_cgsb%7C32_312_2012;product_id=1831710

CIR (Cosmetic Ingredient Review). 2002. Final Report on the Safety Assessment of Tocopherol, Tocopheryl Acetate, Tocopheryl Linoleate, Tocopheryl Linoleate/Oleate, Tocopheryl Nicotinate, Tocopheryl Succinate, Dioleyl Tocopheryl Methylsilanol, Potassium Ascorbyl Tocopheryl Phosphate, and Tocophersolan. *International Journal of Toxicology* 21: 51-116.

CODEX Alimentarius Commission. 2001. Guidelines for the production, processing, labelling and marketing of organically produced foods (*GL 32 – 1999, Rev. 1 – 2001*). Joint FAO/WHO Food Standards Programme. Available online at ftp://ftp.fao.org/docrep/fao/005/Y2772e/Y2772e.pdf.

Commission of the European Communities. 2008. Commission Regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. Official Journal of the European Union. Available online at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:250:0001:0084:EN:PDF

Commission of the European Communities. 2009. Commission Regulation (EC) No 710/2009 of 5 August 2009 amending Regulation (EC) No 889/2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007, as regards laying down detailed rules on organic aquaculture animal and seaweed production. Official Journal of the European Union. Available online at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:204:0015:0034:EN:PDF

DellaPenna, D., Pogson, B.J. 2006. Vitamin synthesis in plants; Tocopherols and carotenoids. *Annual Review of Plant Biology* 57: 711-738. Available online at http://www.ask-force.org/web/Golden-Rice/DellaPenna-Vitamin-Synthesis-2006.pdf

De Silva, S., Turchini, G., Francis, D. 2012. Ch. 8 Nutrition. In: *Aquaculture: Farming aquatic animals and plants, Second Edition* (Eds. J.S. Lucas and P.C. Southgate). Wiley-Blackwell, Chichester, West Sussex, UK, pp. 164-187.

Dobbins, T.A., Wiley, D.B., Dobbins, D.C. 2008. Process for isolation phytosterols and tocopherols from deodorizer distillate. US Patent Application Publication Number US 2008/0015367 A1. Available online at http://www.freepatentsonline.com/20080015367.pdf

EFSA (European Food Safety Authority). 2008. Opinion on mixed tocopherols, tocotrienol tocopherol and tocotrienols as sources for vitamin E added as a nutritional substance in food supplements. Scientific Opinion of the Panel on Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food. *The EFSA Journal* (2008) 640, 1-34. Available online at http://www.efsa.europa.eu/de/efsajournal/doc/640.pdf

EFSA (European Food Safety Authority). 2012. Scientific Opinion on the safety and efficacy of tocopherol-rich extracts of natural origin, tocopherol-rich extracts of natural origin/delta rich, synthetic tocopherol for all animal species. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). The EFSA Journal 2012;10(7):2783. Available online at http://www.efsa.europa.eu/en/efsajournal/doc/2783.pdf

Fertrell. undated. Fish Meal with Naturox product webpage. Available online at http://www.fertrell.com/fishmealwnaturox.htm

Gregory, J.F. 1996. Ch. 8 Vitamins. In: *Food Chemistry Third Edition* (Ed. O.R. Fennema). Marcel Dekker, NY, NY.

Halver, J.E. 2002. Ch. 2 The Vitamins. In: *Fish Nutrition Third Edition* (Eds. J.E. Halver and R.W. Hardy). Academic Press, San Diego, CA, pp. 62-143.

Halver, J.E. 2011. Latest facts for fish and shrimp feed formulations (NRC bulletin on Nutrient Requirements for Fish and Shrimp). 2011 Northwest Fish Culture Conference Presentations. Available online at http://www.rmpc.org/2011-nwfcc-presentations.html

Hamre, K., Kolas, K., Sandnes, K. 2010. Protection of fish feed, made directly from marine raw materials, with natural antioxidants. *Food Chemistry* 119: 270-278.

Hardy, R.W., Burrows, F.T. 2002. Ch. 9 Diet Formulation and Manufacture. In: *Fish Nutrition Third Edition* (Eds. J.E. Halver and R.W. Hardy). Academic Press, San Diego, CA, pp. 506-601.

Hardy, R.W., Roley, D.D. 2000. Lipid oxidation and antioxidants In: *The Encyclopedia of Aquaculture* (Ed. R.R. Stickney). New York, NY, John Wiley and Sons, pp. 470-476.

Häubner, N. 2010. Dynamics of astaxanthin, tocopherol (Vitamin E) and thiamine (Vitamin B_1) in the Baltic Sea ecosystem. Bottom-up effects in an aquatic food web. Acta Universitatis Upsaliensis. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 762. 47 pp. Upsala. Available online at http://uu.diva-portal.org/smash/record.jsf?pid=diva2:346646.

Heo, S.-J., Jeon, Y.-J., Lee, J., Kim, H.T., Lee, K.-W. 2003. Antioxidant effect of enzymatic hydrolyzate from a kelp, *Ecklonia cava*. *Algae* 18(4): 341-347. Available online at http://testj.kofst.or.kr/Upload/files/TEST/12Soo-Jin%20Heo-%EC%82%AC.pdf

IFOAM (International Federation of Organic Agriculture Movements). 2012. The IFOAM Norms for Organic Production and Processing. Version 2012. Available online at http://www.ifoam.org/about_ifoam/standards/norms.html

IOM (Institute of Medicine). 2000. DRI DIETARY REFERENCE INTAKES for Vitamin C, Vitamin E, Selenium, and Carotenoids. A Report of the Panel on Dietary Antioxidants and Related Compounds, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington, D.C. Available online at https://download.nap.edu/catalog.php?record_id=9810

Japanese Ministry of Agriculture, Forestry and Fisheries. 2012. Organic JAS Standards and Technical Criteria. Available online at http://www.maff.go.jp/e/jas/specific/criteria_o.html.

Jiang, A.-L., Wang C.-H. 2006. Antioxidant properties of natural components from *Salvia plebeia* on oxidative stability of ascidian oil. *Process Biochemistry* 41(5): 1111-1116.

JECFA (Joint FAO/WHO Expert Committee on Food Additives). 2006. Tocopherol Concentrate, mixed. Monograph 1. Available online at http://www.fao.org/ag/agn/jecfa-additives/details.html;jsessionid=5AA5B3321733596253BD521F4B633EE8?id=451

Kemin Industries, Inc. 2008. Material Safety Data Sheet for Naturox® IPO Liquid. Provided as Appendix B to Aquaculture Working Group, 2012.

Kemin Industries, Inc. 2013. Naturox™ product webpage. Available online at http://www.kemin.com/products/naturox

Klein, E.A., Thompson, I.M., Tangen, C.M. et al. 2011. Vitamin E and the risk of prostate cancer: The selenium and vitamin E cancer prevention trial (SELECT). *The Journal of the American Medical Association* 306(14): 1549-1556. Available online at http://jama.jamanetwork.com/article.aspx?articleid=1104493 http://jama.jamanetwork.com/article.aspx?articleid=1104493#ref-joc15117-18

Lampi, A.-M., Kamal-Eldin, A., and Piironen, V. 2002. Ch. 1 Tocopherols and Tocotrienols from Oil and Cereal Grains. In: *Functional Foods, Biochemical and Processing Aspects, Volume 2* (Eds. J. Shi, G. Mazza, M. Le Maguer). CRC Press, Boca Raton, FL, pp. 1-38.

Luther, M., Parry, J., Moore, J., Meng, J., Zhang, Y., Cheng, Z., Yu, L. 2007. Inhibitory effect of Chardonnay and black raspberry seed extracts on lipid oxidation in fish oil and their radical scavenging and antimicrobial properties. *Food Chemistry* 104(3): 1065–1073

Maqsood, S., Benjakul, S. 2010. Comparative studies of four different phenolic compounds on in vitro antioxidative activity and the preventive effect on lipid oxidation of fish oil emulsion and fish mince. *Food Chemistry* 119: 123-132.

Medina-Juárez, L.A., Gámez-Meza, N. 2011. Ch. 25 Effect of Refining Process and Use of Natural Antioxidants on Soybean Oil. In: *Soybean - Biochemistry, Chemistry and Physiology* (Ed. Prof. Tzi-Bun Ng) ISBN:978-953-307-219-7, InTech, Available online at http://www.intechopen.com/books/soybean-biochemistrychemistry-and-physiology/effect-of-refining-process-and-use-of-natural-antioxidants-on-soybean-oil

Monks, T.J., Jones, D.C. 2002. The metabolism and toxicity of quinones, quinonimines, quinone methides, and quinone-thioethers. *Current Drug Metabolism* 3: 425-438.

Nagao, T., Kobayashi, T., Hirota, Y., Kitano, M., Kishimoto, N., Fujita, T, Watanabe, Y., Shimada, Y. 2005. Improvement of a process for purification of tocopherols and sterols from soybean oil deodorizer distillate. *Journal of Molecular Catalysis B: Enzymatic* 37(1–6): 56–62.

Naturland. 2012. Naturland Standards for Organic Aquaculture. Available online at http://www.naturland.de/standards.html#c1855

NOP (National Organic Program). 2013. Evaluating Allowed Ingredients and Sources of Vitamins and Minerals for Organic Livestock Feed. Guidance NOP 5030. Effective Date: February 28, 2013. Available online at http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5102733

NOSB (National Organic Standards Board). 1995. TAP Report Tocopherols. Available online at http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5088849

Office of Dietary Supplements. 2011. National Institutes of Health, Dietary Supplement Fact Sheet: Vitamin E. Reviewed June 24, 2011. Available online at http://ods.od.nih.gov/factsheets/VitaminE-QuickFacts

Ogbanna, J.C. 2009. Microbiological production of tocopherols: current state and prospects. *Applied Microbiology and Biotechnology* 84(2): 217-225.

Organic Technologies. 2009. TC-45P Natural Mixed Tocopherols (45% Powder) product details. Available online at http://www.organictech.com/Portals/0/pdf/TC-45P%20Rev%20B%2011.10.09.pdf

Pazos, M., Gallardo, J.M., Torres, J.L., Medina, I. Activity of grape polyphenols as inhibitors of the oxidation of fish lipids and frozen fish muscle. 2005. *Food Chemistry* 92: 547-557.

Pokorny, J., Yanishlieva, N., Gordon, M. (Eds.). 2001. Antioxidants in Food: Practical Applications. CRC Press, Boca Raton, FL.

Pokorny, J. 2007. Ch. 11 Antioxidants in Food Preservation. In: *Handbook of Food Preservation, Second Edition* (Ed. M.S. Rahman). CRC Press, Boca Raton, FL, pp. 259-286.

Polat, A., Özogul, Y., Kuley, E., Özogul, F., Özyurt, G. and Şimsek, A. (2012), Tocopherol content of commercial fish species as affected by microwave cooking. *Journal of Food Biochemistry* Early View (online version of record published before inclusion in an issue). DOI: 10.1111/j.1745-4514.2011.00635.x.

PubChem Compound. 2013. PubChem Compound search. Accessed online at http://pubchem.ncbi.nlm.nih.gov/

Sargent, J.R., Tocher, D.R., Bell, J.G. 2002. Ch. 4 The Lipids. In: *Fish Nutrition Third Edition* (Eds. J.E. Halver and R.W. Hardy). Academic Press, San Diego, CA, pp. 182-259.

Senevirathne, M., Kim, S.-H., Siriwardhana, N., Ha, J.-H., Lee, K.-W., Jeon, Y.-J. 2006. Antioxidant potential of *Ecklonia cava* on reactive oxygen species scavenging, metal chelating, reducing power and lipid peroxidation inhibition. *Food Science Technology and International* 12: 27-38. Available online at http://nando.startlogic.com/store/Research/Studys/Antioxidant_Potential_10B7A8.pdf

Siriwardhana, N., Lee, K.-W., Kim, S.-H., Ha, J.-H., Park, G.-T., Jeon, Y.-J. 2004. Lipid peroxidation inhibitory effects of *Hizikia fusiformis* methanolic extract on fish oil and linoleic acid. *Food Science and Technology International* 10: 65-72.

Southgate, P.C. 2012. Ch. 9 Foods and Feeding. In: *Aquaculture: Farming aquatic animals and plants, Second Edition* (Eds. J.S. Lucas and P.C. Southgate). Wiley-Blackwell, Chichester, West Sussex, UK, pp. 188-213.

Tacon, A.G.J. 1992. Nutritional fish pathology: Morphological signs of nutrient deficiency and toxicity in farmed fish. FAO Fisheries Technical Paper 330. Food and Agriculture Organization of the United Nations, Rome, 1992. Available online at http://www.fao.org/docrep/003/T0700E/T0700E00.HTM

Tsimidou, M., Papavergou, E., Boskou, D. 1995. Evaluation of oregano antioxidant activity in mackerel oil. *Food Research International* 28: 431-433.

Tucker, J. 2012. Ch.18 Marine Fish. In: *Aquaculture: Farming aquatic animals and plants, Second Edition* (Eds. J.S. Lucas and P.C. Southgate). Wiley-Blackwell, Chichester, West Sussex, UK, 384-444.

Valenzuela, A., Nieto, S., Cassels, B.K., Speisky, H. 1991. Inhibitory effect of boldine on fish oil oxidation. *Journal of the American Oil Chemists Society* 68: 935-937.

Verleyen, T., Verhe, R., Garcia, L., Dewettinck, K., Huyghebaert, A., De Greyt, W. 2001. Gas chromatographic characterization of vegetable oil deodorizer distillate. *Journal of Chromatography* 921: 277-285.

Wanasundara, U.N., Shahidi, F. 1998. Antioxidant and pro-oxidant activity of green tea extracts in marine oils. *Food Chemistry* 63: 335-342.

Yi, L., Haley, S., Perret, J., Harris, M. 2002. Antioxidant properties of hard winter wheat extracts. *Food Chemistry* 78: 457-461.